ISSN 0971-6831
Online ISSN 0974-4541

Pest Management in Horticultural Ecosystems

Volume 28 Number 2 December 2022

Association for Advancement of Pest Management in Horticultural Ecosystems (AAPMHE)
ICAR-Indian Institute of Horticultural Research
Hesaraghatta Lake P.O., Bengaluru - 560089, India
(NAAS 2023 Rating : 5.05)

Pest Management in Horticultural Ecosystems

(An international bi-annual scientific journal, published by the Association for Advancement of Pest Management in Horticultural Ecosystems (AAPMHE), Bengaluru, India)

All papers and editorial matters are to be addressed to the Chief Editor, Division of Crop Protection, ICAR - Indian Institute of Horticultural Research, Bengaluru-560 089, India. E-mail: **editorpmhe@gmail.com**

Disclaimer : Mention of any product or material does not mean endorsement by AAPMHE or Editors. Authors are responsible for the technical veracity of contents.

***Pest Management in Horticultural Ecosystems* is widely abstracted**

Membership fees

Membership	India (₹)	SAARC Countries (US $)	Other Countries (US $)
Annual	1,000	50	100
Life	5,000	200	500
Patron	10,000	-	-
Institutions (Annual only)	2,000	-	200

All payments should be made through Online Bank Transfer

(Name : AAPMHE ; Central Bank of India A/c No : 1805602914; IFSC Code : CBIN0283973)

Correspondence relating to membership of the association and business matters concerning the Journal to be addressed to The Secretary, AAPMHE, Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake, P.O., Bengaluru- 560 089, India. E-mail : **aapmhe@gmail.com**

Acknowledgement: **AAPMHE acknowledges the financial support from the Indian Council of Agricultural Research, New Delhi.**

COVER PHOTOS : Invasive Bondar's Nesting Whitefly (BNW), *Paraleyrodes bondari* Peracchi on coconut
(Photo Courtesy: Drs. P. V. Raghuteja & N. B. V. Chalapathi Rao
Dr. YSRHU - Horticultural Research Station (HRS), Ambajipeta, Andhra Pradesh, India

Journal Website : www.aapmhe.iihr.res.in

ISSN 0971- 6831
Online ISSN 0974-4541

Pest Management in Horticultural Ecosystems

Volume 28 Number 2 December, 2022

Association for Advancement of Pest Management in Horticultural Ecosystems (AAPMHE)

Division of Crop Protection
ICAR- Indian Institute of Horticultural Research
Hesaraghatta Lake P.o., Bengaluru - 560 089, India
Website : wwwaapmhe.in, Phone : + 91 8023086100, Ext -305
E-mail : editorpmhe@gmail.com

CONTENTS 28 (2)

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 1-14 (2022)

DOI Number: 10.5958/0974-4541.2022.00031.5

REVIEW ARTICLE

Status and scope of entomopathogenic fungus, *Beauveria bassiana* in sustainable pest management : A review

M. C. KEERTHI[1], K. DARSHAN[2], L. MANJUNATHA[1] and P.V. RAMI REDDY[1]*

[1]Division of Crop Protection, ICAR- Indian Institute of Horticultural Research, Bengaluru – 560089, India

[2]ICFRE-Tropical Forest Research Institute (TFRI), Jabalpur, MP, India

***E-mail:** pvreddy2011@gmail.com

ABSTRACT: Biopesticides are biological alternatives to synthetic pesticides and environmentally sustainable pest management tools. The demand for biopesticides in agriculture is rising due to increased awareness among farmers about the safety of biopesticides to human health and environment. As a result, the global consumption of biopesticides has steadily increased. The anamorphic hyphomycete, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), is a well-recognized cosmopolitan microbial agent known to infect broader insect groups. This paper provides an overview of current knowledge on isolation, culturing, identification, mode of action, the genes contributing to virulence and formulation of *B. bassiana*. The fungus can develop three distinct infective units *viz*., aerial conidia, blastospores, and submerged conidia. *Beauveria bassiana* grows as a white mold in culture media and generates many dry, powdery conidia in unique white spore balls on most standard culture media. The *B. bassiana* can be isolated through the galleria bait technique and use of specific media. The molecular characterization of *B. bassiana* can be confirmed using the gene sequences of the nuclear intergenic region (bloc), beta-tubulin (bt), and ITS region. Understanding the potential factors of genetic variation on the virulence of *B. bassiana* and its insect-fungus interactions will improve usage of this fungus as a cost-effective and sustainable mycoinsecticide.

Keywords: *Beauveria bassiana*, biopesticide, entomopathogenic fungi, mycoinsecticide, endophyte, safety, ecofriendly, pathogenicity.

INTRODUCTION

Entomopathogenic fungi (EPF) are natural biocontrol agents with global distribution. Selective nature of infection, and their safety to environment combined with simple mass production techniques, have made EPF an effective and viable alternative to synthetic insecticides (Rani *et al*., 2021). The empirical and unilateral use of chemicals to control pests failed to provide a long lasting solution (Archana *et al*., 2022). Pest resurgence and upsets in the natural balance due to the poisons used against them clearly show that a rapid and drastic change is necessary to achieve control of pests in an ecologically and economically satisfactory manner. Implementing fundamental ecological principles in managing pest problems is the most effective approach to significantly reduce the use of insecticides, with some agroecosystems even being able to eliminate their usage (Deguine *et al*., 2021). In the present review, we attempted to provide an overview of current knowledge on isolation, culturing, identification, mode of action, the genes contributing to virulence in entomopathogenic fungi and formulation of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), a widely used biopesticide in agriculture.

History, natural occurrence, geographical distribution and host range of *Beauveria bassiana*

Microbial biopesticides have had considerable success in controlling crop pests. Among EPF's *Beauveria* sp. is the most commonly reported natural enemy of insects causing regular epizootics (Roberts and St. Leger, 2004). Currently, 16 species are included in the genus, *Beauveria*. Rehner *et al*. (2011) recognized 12 species of *Beauveria*, *i.e*., *B. bassiana*, *B. brongniartii*, *B. caledonica*, *B. amorpha*, *B. asiatica, B. australis*, *B. kipukae*, *B. pseudobassiana*, *B. varroae*, *B. sungii*, *B. malawiensis* and *B. vermiconia*. Later 4 more species were described, *i.e., B. lii* (Zhang *et al*., 2012), *B. sinensis* (Chen *et al*., 2013), *B. rudraprayagi* (Agrawal *et al*., 2014) and *B. hoplocheli* (Robène *et al*., 2015). However, only two species, *B. bassiana* and *B. brongniartii*, were most studied and commercially exploited for pest management.

The anamorphic hyphomycete, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), is a well-recognized cosmopolitan microbial agent known to infect broader insect groups. *Beauveria bassiana* was one of the most

intensively studied fungal entomopathogens from which thousands of isolates have been collected from different parts of the world (Rehner *et al*., 2011). The history of research on *B. bassiana* started in the early nineteenth century. In 1835, the Agostino Bassi, an entomologist discovered the causal agent of pebrine disease that turned legions of Italy's silkworms into white mummies (Lord, 2005). The fungus was subsequently named after Bassi by Vuillemin. The characteristic appearance of a white powdery layer on the cadavers gave rise to the descriptor white muscardine disease. One of the first and most prominent early attempts to extensively use *Beauveria* was made in the mid-1800s in the US Midwest to control chinch bugs, *Blissus leucopterus* (Lord, 2005).

Beauveria bassiana is a generalist entomopathogen with a broad ecological host range of over 700 arthropod species, covering most orders of the class Insecta (Feng *et al*., 1994). However, *B*. *brongniartii* (Saccardo) Petch has a more restricted host range, mainly infecting coleoptera and the other seven orders in the field. For several species, such as *B*. *vermiconia* or *B*. *caledonica*, the number of strains available in collections needs to be larger to conclude their host range (Rehner *et al*., 2011). To date, the species *B*. *hoplocheli* has only been isolated in natural conditions from the white grub, *Hoplochelus marginalis* (Fairmaire) (Coleoptera: Scarabaeidae) (Robène *et al*., 2015). Many studies have compared the virulence of several strains of *Beauveria* spp. on a given insect host, especially strains of *B*. *bassiana* (Quesada-Moraga *et al*., 2003). Few works have studied the physiological host range of *Beauveria* spp. strains by comparing their pathogenicity and virulence on several insect species. For example, 43 *B*. *bassiana* strains collected worldwide exhibited a substantial variation in virulence against eight lepidopteran species (Wraight *et al*., 2010). Twenty-nine genetically diverse *B*. *bassiana* strains were pathogenic to nine insect species from five orders, with significantly different levels of virulence (Uma Devi *et al*., 2008). Despite a few preliminary studies, the physiological host range of many species of *Beauveria*, excluding *B*. *bassiana* and *B*. *brongniartii*, has not been investigated extensively.

Cultural, morphological and molecular identification of *B. bassiana*

The taxonomic hierarchy of *Beauveria bassiana* is as follows kingdom- Fungi, division- Ascomycota, class- Sordariomycetes, order- Hypocreales, and Family- Cordycipitaceae. Under varying nutritional and climatic conditions, *B. bassiana* can develop three distinct infective units: aerial conidia, blastospores, and submerged conidia. *B. bassiana* is the anamorphic stage (asexually reproducing form) of *Cordyceps bassiana*. Potato Dextrose Agar (PDA) and sporulation media (SM) can be utilized for the growth and multiplication of *B. bassiana* at two different temperatures (25 ^{0}C and 28 ^{0}C) with a relative humidity (RH) of 65-70 % for 10 days. *B. bassiana* grows as a white mold in culture media. It generates many dry, powdery conidia in unique white spore balls on most standard culture media. Each spore ball is made up of a group of conidiogenous cells. *B. bassiana* conidiogenous cells are short and oval, with a slender apical projection known as a rachis. The rachis elongates after each conidium is produced, resulting in a long zig-zag extension. Conidia are single-celled, haploid, and hydrophobic organisms.

The microscopic observation (100× magnification) of morphological characteristics is the most widely used criterion for characterizing EPF during their asexual stages. It requires adequate observation of both conidia and conidiogenous cells. Commonly two methods are employed for microscopic observation, *i.e.,* the whole mount method, a straightforward and rapid method used for observing fungi under a light microscope. The disruption of conidiogenous cells and dehiscence of conidia are also widespread while preparing the slide. However, it can be avoided with the slide culture preparation method, but the culture must be incubated long enough to develop conidiogenesis for examination **(**Senthilkumar *et al*., 2021**)**. Based on microscopic observation, hyphae branched and formed conidiogenous cells and single cell *B. bassiana* conidium is round and tends to be oval with hyaline color.

Molecular characterization has become essential to confirm the identity of the EPF, *Beauveria spp*. using molecular detection tools. The molecular characterization can be confirmed using the gene sequences of the nuclear intergenic region (bloc), beta-tubulin (bt), and ITS region. Molecular identification can be achieved by isolating fungal DNA from the pure cultures and re-isolated on PDA media, as reported by Liu *et al*. (2013). Later the species of *Beauveria* was confirmed at the molecular level through the amplification, sequencing, and phylogenetic analysis of the internal transcribed spacer (ITS) sequence of 5.8S rDNA of the fungus (Sharma *et al*., 2015). Universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) are employed for amplifying a partial sequence of ITS1-5.8S rDNA-ITS4 (Kimaru *et al*., 2018). For *Beauveria* isolates, approximately 1500-bp segments of bloc gene region amplified by the primer pairs of B5.1F (5′-CGACCCGGCCAACT ACTTTGA-3′) and B3.1R (5′-GTCTTCCAGTACCA CTACGCC-3′) as described by Rehner *et al*. (2006).

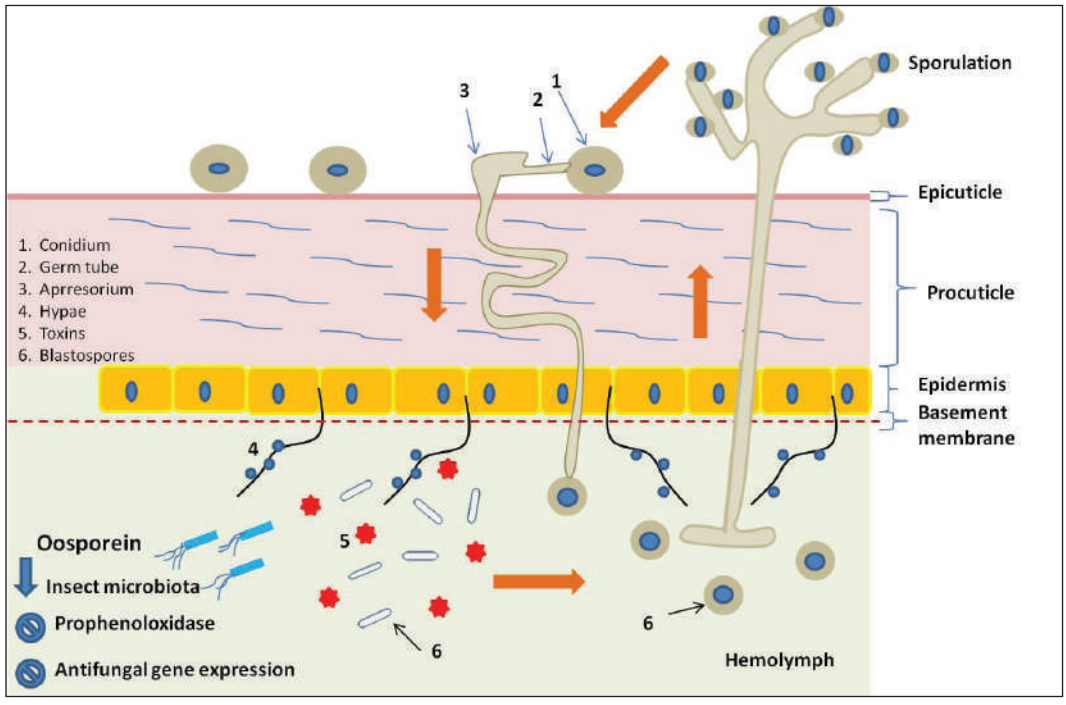


Fig 1. A schematic view of *Beauveria bassiana* pathogenesis

Isolation of EPF from soil and infected insects

Two methods commonly employed for EPF isolation from the soils are (1) baiting the environment with a susceptible insect host, *i.e.,* the Galleria Bait technique, or (2) the use of selective media (Sharma *et al*., 2021).

Isolation from insects

EPF was isolated from dead insects using direct isolation techniques and incubated on the prepared PDA plate at 28 ^{0}C for one week (Parker *et al*., 2003). All dead insects were placed in 9-cm plastic Petri dishes on a sterilized paper towel moistened with a solution of 0.001 g/L of penicillin G and 0.005 g/L of streptomycin sulfate. The Petri dishes were sealed with Parafilm and held at 26^{0}C for 4 weeks. Unopened Petri dishes were examined daily for the presence of fungal outgrowth. The isolate was subcultured several times to obtain a pure culture (Awan *et al.,* (2021).

Isolation of *Beauveria* spp. from soil

Galleria Bait Technique

Isolation of EPF using selective media manipulates the saprotrophic ability of EPF. To manipulate the fungi's ability to infect the host, *Galleria* Bait Technique was commonly used (Zimmermann, 1986). The EPF was isolated from soil using *Galleria mellonella* L. (greater wax moth) larvae. Place four *G. mellonella* larvae in a plastic container containing a soil sample; seal the containers with perforated lids and hold them at room temperature. Place the three to five *G. mellonella* larvae in containers with sterile soil (negative control), no soil (negative control), or sterilized soil to which fungi obtained from one plate of each of the three known EPF cultures were added (positive controls). Examine the containers every other day, and collect dead larvae. Surface-sterilize the cadaver for 3 min in a 1% sodium hypochlorite solution and rinse in sterile distilled water, plate, and incubate at 27°C in a humidity chamber at 100% RH to permit the growth of fungi (Brownbridge *et al*., 1993).

Isolation of *Beauveria* spp. from soil using selective media

Soil is the primary source of the EPF (Sanchez-Pena *et al*., 2011). Insect bait is a susceptible detection method, and EPF can be selectively isolated. However, some insect species may select for specific fungal pathogens, and challenging to quantify inoculum levels. Although the isolated fungi must be evaluated for their pathogenicity to target insects, by contrast, selective media have some advantages for the mass collection of positive EPF and quantitative data. Therefore, various selective media have been developed for the mass collection of EPF from soil (Meyling, 2007). A selective medium is available for the recovery of *B. bassiana,* having been developed by Veen and Ferron (1966) to isolate *B. tenella (B. bassiana,* fide de Hoog, 1972) from natural sources. Using a selective medium, *B. bassiana* was isolated from elm trees' bark and soil (Doberski and Tribe, 1980). For the success of

Table 1. Efficacy of *Beauveria bassiana* against different insect pests

Target pests	Percent efficacy (strain)	Study location	Authors
Sitophilus granarius L. (Coleoptera: Curculionidae)	93.66%	Turkey (Laboratory)	Ak (2019)
Callosobruchus maculatus F. (Coleoptera: Chrysomelidae)	100 % mortality (TR-217)	Turkey (Laboratory)	Ozdemir *et al* (2020)
Frankliniella occidentalis (Thysanoptera: Thripidae)	69%–96% (RSB)	China (Laboratory)	Gao *et al*. (2012)
Thrips tabaci (Thysanoptera: Thripidae)	83%–100% (SZ-26)	China (Laboratory)	Wu *et al.* (2013)
Rhynchophorus ferrugineus (Oilv.) (Coleoptera: Curculionidae)	Up to 90 %	Egypt (Field)	Sewify *et al*. (2009)
Cosmopolites sordidus (Germar, 1824) (Coleoptera: Curculionidae)	54% to 66% (IBCB 74, IBCB 87 and IBCB 146)	Brazil (Laboratory)	Almeida *et al*. (2009)
Polyphylla fullo (L.) (Coleoptera: Scarabaeidae)	71.6 to 79.8% (PPRI5339)	Turkey (Laboratory)	Erler and Ates, (2015)
Thaumastocoris peregrinus Carpintero & Dellapé (Hemiptera: Thaumastocoridae)	37 to 80.1%	Brazil (Laboratory)	Lorencetti *et al*. (2018)
Helicoverpa armigera, *Spodoptera litura* *Earias vittella* (Lepidoptera: Noctuidae)	*H. armigera* (86.67%), *S. litura* (86.67%) and *E. vittella* (73.33%)	India (Laboratory)	Karthikeyan and Elvanarayanan (2011)
Plutella xylostella Linn.	72.64 %	India (Field)	Kamal *et al*. (2018)

EPF-based commercial bio-pesticides, conidia production is crucial. The biphasic growth culture method involving liquid- and solid-state culture is mainly used to produce EPF.

During the isolation of the fungus, one gram of a given soil sample and 10 ml of the sterilized distilled water were mixed in 15 ml test tubes, which were vortexed for 10 min to obtain a homogenous solution. Then, a serial dilution from 10^{-1} to 10^{-7} for each soil sample was prepared to isolate a single colony of fungi. The 1 ml of the soil extracts spread on a selective medium SDA (Sabouraud Dextrose Agar) containing 0.2 µg/ml dodine (N-dodecylguanidine monoacetate), 100 µg/ml chloramphenicol, and 50 µg/ml streptomycin sulfate) and incubated at 28 °C for 2 weeks (Goettel and Inglis, 1997). At the end of the incubation period, growing single colonies were transferred to other SDA plates to get pure cultures. Store all purified fungal isolates in 20% glycerol at − 20 °C. Veen's medium is designed to maximize recovery of naturally occurring *Beauveria* sp.

Pathogenesis and mechanism of *B. bassiana* against plant diseases

The EPF encounters several host obstacles in each generation to produce enough viable infectious spores to perpetuate healthy populations. They would first come close to a susceptible host, then stick to and puncture the host's cuticle. They must subsequently overpower and avoid host immunological systems to receive nutrients and grow. The EPF causes infection at low conidia concentrations, which can be as low as one or two conidia per host (Oduor *et al*., 1997). The adhesion of spores to the host's epicuticle, followed by germinating and pre-penetration proliferation, is a crucial stage of pathogenicity (Ortiz-Urquiza and Keyhani, 2013). Most

EPF has hydrophobic conidia, which allow quick adhesion to the waxy epicuticle. Hydrophobin proteins that form enclose and protect layers on the conidial surface increase adhesion of conidia in *B. bassiana* (Holder *et al*., 2007). Immediately after the initial contact, secrets sticky lime (Boucias and Pendland, 1991).

During the pre-germination stage, moist conidia release proteases, probably for nutrient absorption and invasion (Qazi and Khachatourians, 2007). *B. bassiana* has at least 16 fungal enzymes involved in the oxidative breakdown and assimilation of epicuticle lipids (Pedrini, 2022). The process of infection of arthropods and fungal invasion, attachment to hosts and penetration of the cuticle, virulence enzymes associated with EPF and interaction with the host immune response are well described by Sharma and Sharma (2021) and Chandler (2017). As shown in Figure 1, most pathogenic fungi infect insects through the epidermis and then multiply in the Hemolymph system. The figure 1 shows that the fungal infection cycle not only depends on the successful penetration of the epidermis but also requires a dimorphic transition in vivo, *i.e.,* the transformation of conidia into hyphae. Chitinase, lipases, and proteases are the most important enzymes produced by *B. bassiana*. However, different studies have determined that it can produce other enzymes, such as amylase, asparaginase, cellulase and galactosidase (Petlamul and Boukaew, 2019). Various studies have reported the presence of beauvericin, bassianolide, bassiacridin, and oosporein toxins in *B. bassiana* culture supernatants (Ortiz-Urquiza *et al*., 2010).

Genes involved in virulence and production of toxins-Molecular approaches to improve their virulence

Most microbial biopesticides are found in the microbiome of the agricultural fields, where they are in combination with both pathogenic and beneficial organisms. These fungal biopesticides bio-actively deter harmful insect pests (Archana *et al*., 2022). Their action is often parasitic or may secrete bioactive metabolites like enzymes, *i.e.,* contingent on both the pesticidal fungus applied and the targeted pest. e.g., *B. bassiana* germinates, grows and spreads its spores in the targeted insect body, colonization by degradation, draining nutrients and releasing toxins causing its death (Raya-Díaz *et al*., 2017). Manifold reports have shown the importance of virulence genes to understand better the infection mechanisms deployed by EPF. The implication of various virulence genes directly involved in biocontrol mechanisms are presented in Table 2.

Over the past decade, immense advances in molecular biology and genetic techniques have helped in the understanding of the life history as well as the genetic mechanisms of fungal virulence of *B. bassiana* for a robust and sustainable solution to arthropod pests. Rapid progress in understanding the genetics that constitutes virulence in insects can be made due to the recent availability of the whole genome sequence of *B. bassiana* (Xiao *et al*., 2012). In general, the host–fungus biological interactions are more prominent in the host insect and can be further magnified for research purposes (Joop and Vilcinskas, 2016). Many of the genes that were functionally analyzed thus far involve general biological processes (e.g., conidiation, stress response) that pleiotropically affect virulence.

Studies on comprehensive information of genetic variation and identification of virulence variants and their evolutionary dynamics help to understand their mechanism of inhibition. Knock-out mutant approaches are crucial and will play an essential role in verifying the action of the candidate genes. Valero-Jiménez *et al*. (2016) sequenced the genomes of five isolates of *B. bassiana* with low/high virulence against mosquitoes. Understanding the potential factors of genetic variation on the virulence of *B. bassiana* and its insect-fungus interactions will improve our methods to use this fungus as a cost-effective and sustainable mycoinsecticide. However, do these genetic mutations play a role in virulence, or how does it regulate virulence in biological processes, which is exciting and will need further study (Zhang *et al*., 2020).

***B. bassiana* as an endophyte**

The fungal entomopathogens are found naturally as an endophyte (Vega, 2018) and also colonize plants *via* seed dressings, seed soaking, foliar sprays, and soil drenching (Tefera and Vidal, 2009). They protect their host plant against disease pathogens by enhancing plant growth through plant disease antagonism and rhizosphere colonization. Colonization by fungal endophytes may be systemic, localized or partitioned within plant parts. The artificial introduction of *B. bassiana* as an endophyte has been successful in maize, coffee, banana, broad beans, cotton, the common bean and tomato (Behie *et al*., 2015).

Interaction of EPF with environmental factors

In general, fungi inhabiting higher latitudes experience a wider range of temperatures due to seasonality (Wielgolaski and Inouye, 2003). Thus, abiotic stressors (mainly temperature) at higher latitudes may predominantly drive population genetics and adaptability of EPF. In temperate regions, EPF must adapt to a broad range and greater climatic intensities (Maggi *et al*., 2013; Wang *et al*., 2017), whereby abiotic factors primarily influence generalist pathogen's survival

Table 2. Different virulence genes of *B. bassiana* involved in biological processes of inhibition

***B. bassiana* Genes**	**Gene encoded proteins**	**Genes involved in biological functions**	**References**
hyd1 and *hyd2*	Hydrophobin gene	**Host adherence:** Involved in surface hydrophobicity, adhesion, virulence and composition of the rodlet layer	Zhang *et al*., (2011b)
Bbhog1 & *Bbmpk1,*	MAP kinase gene	**Germination and penetration peg development:** Required for conidial adhesion, appressorium formation and penetration	Zhang *et al*., (2009)
Bbcyp52x1	cytochrome P450 gene	**Enzymatic degradation of the waxy layer:** Act as fatty acid hydroxylase activity	Zhang *et al*., (2012)
Pr1, Pr2	Subtilisin/ trypsin-like protease	**Cuticle degradation**: Cuticle-degrading proteases are important for cuticle breakdown	Pedrini (2022)
Putative gene clusters (BbbeaS BbbslS; BbtenS	Non-ribosomal peptide synthase (NRPS) protein; beauvericin synthetase gene (*BbbeaS*); bassianolide synthetase gene (*BbbslS*); tenellin synthetase gene	**Role in biosynthesis and production of metabolites / toxins** *viz.*, tenellin, beauvericin, Oosporein, and bassianolide role in virulence	Zhang *et al*., (2020);
Bbpks11, Bbpks15, BbopS1	Polyketides synthetases (PKS); Oosporein polyketide synthase (*BbopS1*)	Important functions in fungal asexual development and cell wall integrity and **virulence function**. *BbopS1* directly participate in the evasion of insect immunity	Pedrini (2022)
Bbchit1, Chi1, Chi2, ChsA2	Endo chitosanase and Chitinase D	**Chitin-degrading enzymes for lysis** of insect chitin	Dionisio *et al*., (2016).
Bbmtd, Bbmpd	Mannitol dehydrogenase	Involved in mannitol biosynthesis for growth & colonization. Also involved in **multi-stress tolerance**	Wang *et al*., (2012)
Mdr1, Mrp1, Pdr1, Pdr2, Pdr5	ABC transporters	**Interactions with the insect immune system** for multi drug resistance	Song *et al*., (2013)
Bbac	cAMP signaling/ adenylate cyclase gene	**Regulatory functions**: Regulating multi-stress responses (osmolarity, oxidation, cell wall damage and different chemicals) and helps in conidiation	Wang *et al*., (2014a)
BbGPCR3, Bbrgs1	G-protein coupled receptor (GPCR)	**Role in Hyphal extrusion and conidiation:** Involved in conidiation (transition to blastospore), conidial viability, nutrient sensing and thermo/ stress tolerance	Fang *et al*. (2008)

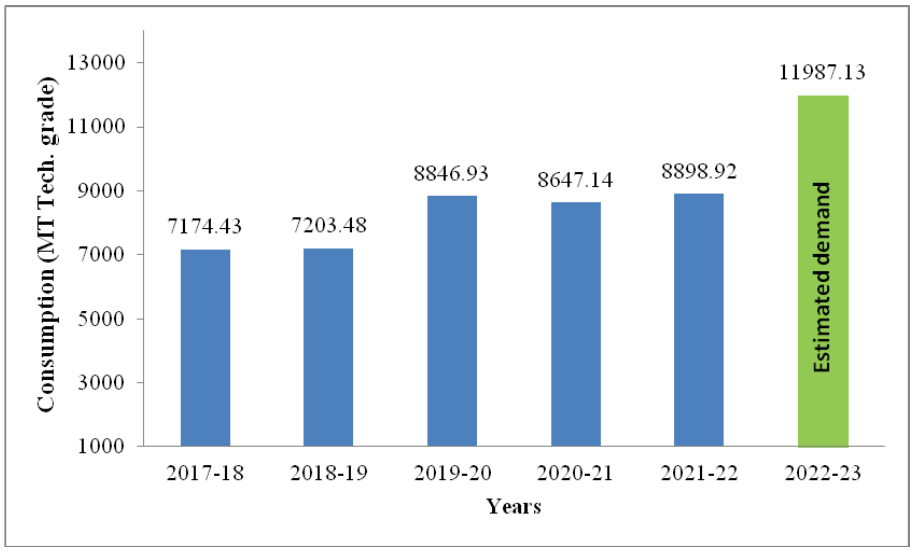


Fig 2. Consumption of biopesticides in India during last 5 years and estimated demand during 2022- 23 (Source: DPPQS, Ministry of Agriculture & Farmers Welfare, Government of India).

(Lennon *et al*., 2012). Phylogenetic *B. bassiana* cluster by habitat type more at seasonally variable high latitudes (Ormond *et al*., 2010). However, one study found no seasonal effect in regions of sub-tropical climates (Garrido-Jurado *et al*., 2015). Phylogenetically structured investigations suggest *B. bassiana* adapts gene regulation to environmental conditions, with habitat adaptation driving population dynamics (Bidochka *et al*., 2002; Xiao *et al*., 2012). The optimal temperature for growth and virulence against insect hosts of *Beauveria* species is generally between 25 and 30 °C (Luz and Fargues, 1997; Devi *et al*., 2005). However, significant variation exists in a fungal pathogen species' thermal preference and their effects on potential hosts due to the environment in which the pathogens evolved (Alali *et al*., 2019), and individual strains can differ in their thermal optima (Alali *et al*., 2019).

The sub-tropical *B. bassiana* strains collected from hotter areas of Syria demonstrated more remarkable thermo-tolerant ability than the outlier collected from a site experiencing lower temperatures (Alali *et al*., 2019). Regarding virulence against insects, temperate isolates of *B. bassiana* were significantly more effective against the elm bark beetle (*Scolytus scolytus* F.) at low temperatures (2 to 6 °C) (Doberski, 1981). The strains of *B. bassiana* are sensitive to ultraviolet radiation, prompting UV protectant use in oil-based field sprays (Kumar *et al*., 2018). UV tolerance often varies among isolates from different latitudes (Fernandes *et al*., 2008) and habitat types (Bidochka *et al*., 2001).

Development of the entamopathogenic fungus in liquid and solid cultures

Microorganism-based bio-pesticide forms the most substantial portion of bio-pesticide products. Worldwide attention has been focused on the mass manufacturing of promising EPFs like *Metarhizium anisopliae*, *Beauveria bassiana, Verticillium* sp., *Trichoderma* sp., *Chaetomium* sp., *Aspergillus* sp., and *Hirsutella sp* for crop protection against various pests. *B. bassiana* has also been found to be one of the potential biocontrol agents effectively used in IPM because of its wide natural distribution and ability to control aphids, lepidopteron larvae and other pests (Abidin *et al*., 2017). The fungus, *Beauveria bassiana* was cultured with excellent results on a medium consisting of yeast extract, *i.e.,* Sabouraud-dextrose-yeast extracts (Ramle *et al*., 2005). Several approaches have been made to increase the effectiveness of *B. bassiana* with suitable mass-production techniques for commercial formulation. Among them, the most common and inexpensive techniques used for cultivation are either a surface culture with a solid substrate or a submerged culture with a liquid medium (Fang *et al*., 2000).

Solid-type microbial culture has a relatively long preservation time due to the hydrophobic nature of conidia. It is suitable for making oil formulation but requires an extended activation time. On the other hand, the liquid-type microbial culture is disadvantaged in making oil formulations due to less viability during storage. It can also be grown by following a biphasic system, in which the fungus is first grown under submerged conditions to produce metabolic active blastospores (hydrophilic) and then allowed to conidiate (hydrophobic) in solid-state conditions (Lopez-Perez *et al*., 2015). To ensure the effective implementation of

potential micro-organisms, solid substrate fermentation is one of the proper methods for mass production of *B. bassiana*. Gola *et al*. (2019) made innovative attempts to produce three stable formulations of *Beauveria bassiana* targeted against multimetal (Cu, Cr, Cd, Ni, Zn, and Pb) containing synthetic wastewater. The micro-granules, myco-tablets, and myco-capsules formulations can potentially remediate multimetal-containing wastewater. It will also help extend the formulation's shelf life at ambient temperature and solve the problem of storability and transportation.

Fate and behaviour in the environment and effect on non-target organisms including humans

A widespread application entomopathogenic fungus in various crop protection systems raises the concern of potential adverse effects on non-target organisms like human health, earthworms, pollinator and other beneficial arthropods. When *B. bassiana* was tested for pathogenicity against the adults of *Folsomia fimetaria*, *Hypogastrura assimilis*, and *Proisotoma minuta*, no strains of *B. bassiana* were found to be toxic (Zimmermann, 2007).

B. bassiana has been extensively used in agricultural practices in various Asian countries since the past century. The critical issue microbial ecologists raise is that host specificity is a strain-specific trait. A difference was observed between the physiological and ecological host range of *B. bassiana* strains isolated from different parts of the world. The ecological host range shows the susceptibility of insects under natural or field conditions, while the physiological host range demonstrates which insects can be infected in the laboratory. *B. bassiana* has a diverse range of hosts, yet data suggests that using it can have little effect on beneficial organisms (Zimmermann, 2007).

The safety of *B. bassiana* to humans was cautiously evaluated before its registration as a biocontrol agent. In minor cases, some workers involved in the mass production of *B. bassiana* exposed to high spore concentrations likely had allergies. Besides allergy, there are some cases where Mycotic keratitis has been linked to *Beauveria bassiana* in humans and other mammals. The genus *Beauveria* is not mentioned in the medical charts of rare but crucial fungal infections (Zimmermann, 2007).

Formulations of *B. bassiana* and their compatibility with insecticides

Different formulations of *B. bassiana* have been tested against house flies (bait, encapsulation, and emulsion), whiteflies (oil, talc, and crude), and other agricultural pests (Prithiva *et al*., 2017; Saeed *et al*., 2017). The results found that oil formulation (45.86 %), followed by talc (29.62 %) and crude formulations (21.63 %) were most effective against whitefly on tomato. Similarly, oil and water-based formulations of *B. bassiana* were suitable for application to control coffee berry borer, *Hypothenemus hampei*. Ritu *et al*. (2012) studied the different formulations (Bentonite oil-based liquid formulation (BOBLF), oil-based liquid formulation (OBLF), and Carrier-based powder formulation (CBPF) of *Beauveria bassiana* tested against larvae of *Helicoverpa armigera*. It was found that the bentonite-based liquid formulation exhibited the highest efficacy at the optimum concentration (60%).

Pesticides can be substituted by biopesticides (Rani *et al*. 2021; Archana *et al*. 2021). Various biologically derived compounds had pesticide action against insect pests and diseases (Shivakumara *et al*., 2022, Darshan *et al*., 2020). The successful formulation depends on its compatibility with other insecticides used in pest management programs. Many research groups have checked the compatibility of *B. bassiana* with several pesticides at different concentrations. Various parameters were studied, like conidial germination, vegetative growth, and fungus sporulation. Alizadeh *et al*. (2007) reported that *B. bassiana* (isolate DEBI008) was compatible with imidacloprid and showed synergistic interaction. However, flufenoxuron was highly incompatible and inhibited conidial germination significantly.

The combination of compatible insecticides and synergistic bioagents at lower doses can help manage the pest sustainably with a low risk of resurgence. Abidin *et al*. (2017) reported the compatibility of *B. bassiana* with various insecticides. Imidacloprid (77.72%) and deltamethrin (76.02%) were compatible and showed the highest vegetative growth and conidial germination. The combined applications (Beta cypermethrin (10%) with *B. bassiana PfBb* (1×10^7), imidacloprid (0.5 x DF) with *B. bassiana*) showed effective pesticidal action on insects than applications of insecticides alone (Chen *et al*., 2021). *B. bassiana* was also shown good compatibility with acaricides formulation like Avermectin and pyrethroids (De Olivera and Neves, 2004). In summary, a detailed compatibility evaluation of insecticides with biocontrol agents is required for simultaneous usage in integrated pest management programs. Knowledge of this will facilitate the choice of entomopathogenic fungi and pesticides used in a cocktail for crop protection.

Demand and production needs of *B. bassiana*

According to the DPPQS (Directorate of Plant Protection, Quarantine and Storage, Ministry of Agriculture, Gov. of India), 361 biocontrol laboratories

and units are working in India. However, only a few of them are involved in the production. They can meet the demand of less than 1% of the cropped area. A wide gap can only be bridged by setting up more units for Bio-pesticides production. However, data suggests that in India, the consumption of biopesticides has increased in the last few decades. Data obtained from DPPQS suggested that the all-India consumption of biopesticides gradually increased for five years, and the estimated demand for 2022-23 was 11987.13 MT Tech. Grade (Fig. 2).

Currently, there are 970 biopesticides products registered with the Central Insecticides Board and Registration Committee (CIBRC) for all types of usage of biopesticides in India. Among which 107 products are *B. bassiana.* Currently, CIBRC recommends the use of *B. bassiana* against different insect pests like cotton bollworm complex, rice leaf folder, *Cnaphalocrosis medinalis*; Diamondback moth, *Plutella xylostella* on cabbage; chickpea pod borer, *Helicoverpa armigera*; Fruit borer and spotted bollworm on Okra; *Helicoverpa armigera* on Tomato. Further, the *B. bassiana* recommended (ad-hoc) for the management strategies for invasive thrips (*Thrips parvispinus*) in Chilli and Fall Armyworm, *Spodoptera frugiperda* in Maize (DPPQS, 2022).

REFERENCES

Abidin, A. F., Ekowati, N. and Ratnaningtyas, N. I. 2017. Compatibility of insecticides with entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*. *Scripta Biologica*, **4**(4): 273-279.

Agrawal, Y., Mual, P. and Shenoy, B. D. 2014. Multi-gene genealogies reveal cryptic species *Beauveria rudraprayagi* sp. nov. from India. *Mycosphere,* **5:** 719-736.

Ak, K. 2019. Efficacy of entomopathogenic fungi against the stored-grain pests, *Sitophilus granarius* L. and *S. oryzae* L. (Coleoptera: Curculionidae). *Egyptian Journal of Biological Pest Control*, **29**(1): 12.

Alali, S., Mereghetti, V., Faoro, F., Bocchi, S., Al Azmeh, F. and Montagna, M. 2019. Thermotolerant isolates of *Beauveria bassiana* as potential control agent of insect pest in subtropical climates. *PLoS ONE,* 14:e0211457. doi: 10.1371/journal.pone.0211457.

Alizadeh, A., Samih, M. A., Khezri, M. A. and Riseh, R. S. 2007. Compatibility of *Beauveria bassiana* (Bals.) Vuill. with several pesticides. *International Journal of Agriculture and Biology*, **9** (1): 31-34.

Almeida, A. M. B., Batista Filho, A., Tavares, F. M. and Leite, L. G. 2021. Seleção de isolados de *Beauveria bassiana* para o controle de Cosmopolites sordidus (Germar, 1824) (Coleoptera: Curculionidae). *Arquivos do Instituto Biológico*, **76**: 489-493.

Archana, H. R., Darshan, K., Lakshmi, M. A., Ghoshal, T., Bashayal, B. M. and Aggarwal, R. 2022. Biopesticides: A key player in agro-environmental sustainability. In Trends of Applied Microbiology for Sustainable Economy, Academic Press. 613-653.

Awan, U. A., Xia, S., Meng, L., Raza, M. F., Zhang, Z. and Zhang, H. 2021. Isolation, characterization, culturing, and formulation of a new *Beauveria bassiana* fungus against *Diaphorina citri*. *Biological Control*, **158**: 104586.

Behie, S.W., Jones, S. J. and Bidochka, M. J. 2015. Plant tissue localization of the endophytic insect pathogenic fungi *Metarhizium* and *Beauveria*. Fungal ecology, **13**: 112-119.

Bidochka, M. J., Kamp, A. M., Lavender, T. M., Dekoning, J. and De Croos, J. N. 2001. Habitat association in two genetic groups of the insect-pathogenic fungus *Metarhizium anisopliae*: uncovering cryptic species? *Applied and Environmental Microbiology*, **67**: 1335–1342. doi: 10.1128/AEM.67.3.1335-1342.2001.

Boucias, D. G. and Pendland, J. C. 1991. Attachment of mycopathogens to cuticle: the initial event of mycosis in arthropod hosts. In: Cole, G.T., Hoch, H.C. (Eds.), the Fungal Spore and Disease Initiation in Plants and Animals. Plenum, New York, USA, 101-128.

Brownbridge, M., Reay, S. D., Nelson, T. L. and Glare, T. R. 2012. Persistence of *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte following inoculation of radiata pine seed and seedlings. *Biological Control*, **61**:194-200.

Chandler, D. 2017. Basic and Applied Research on Entomopathogenic Fungi. *Microbial Control of Insect and Mite Pests*, 69-89.

Chen, M. J., Huang, B., Li, Z. Z. and Spatafora, J. W. 2013. Morphological and genetic characterisation of Beauveria sinensis sp. nov. from China.

Mycotaxon, **124**: 301-308.

Chen, X. M., Wang, X. Y., Lu, W. and Zheng, X. L. 2021. Use of *Beauveria bassiana* in combination with commercial insecticides to manage *Phauda flammans* (Walker) (Lepidoptera: Phaudidae): Testing for compatibility and synergy. *Journal of Asia-Pacific Entomology*, **24** (2): 272-278.

Darshan, K., Aggarwal, R., Bashyal, B. M., Singh, J., Shanmugam, V., Gurjar, M. S. and Solanke, A.U. 2020. Transcriptome profiling provides insights into potential antagonistic mechanisms involved in *Chaetomium globosum* against *Bipolaris sorokiniana*. *Frontiers in Microbiology*, **11**: 578115. doi.org/10.3389/fmicb.2020.578115.

De Olivera, R. C. and P. M. O. J. Neves .2004. Biological control compatibility of *Beauveria bassiana* with acaricides. *Neotropical Entomology*, **33**: 353-358.

Devi, K. U., Sridevi, V., Mohan, C. M. and Padmavathi, J. 2005. Effect of high temperature and water stress on in vitro germination and growth in isolates of the entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuillemin. *Journal of invertebrate pathology,* **88**: 181-189.

Dionisio, G., Kryger, P. and Steenberg, T. 2016. Label-free differential proteomics and quantification of exoenzymes from isolates of the entomopathogenic fungus *Beauveria bassiana*. *Insects*, **7**(4): 54.

Doberski, J. W and Tribe, H.T. 1980. Isolation of entomogenous fungi from elm bark and soil with reference to ecology of *Beauveria bassiana* and *Metarhizium anisopliae*. *Transactions of the British Mycological Society*, **74**(1): 95-100.

Doberski, J. W. 1981. Comparative laboratory studies on three fungal pathogens of the elm bark beetle Scolytus scolytus: effect of temperature and humidity on infection by *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces farinosus*. *Journal of invertebrate pathology,* **37**: 195–200. doi: 10.1016/0022-2011(81)90075-6.

DPPQS, 2022. https://ppqs.gov.in/statistical-database. Accessed on 04.01.2023.

Erler, F. and Ates, A.O. 2015. Potential of two entomopathogenic fungi, Beauveria bassiana and Metarhizium anisopliae (Coleoptera: Scarabaeidae), as biological control agents against the June beetle. *Journal of Insect Science*. **15**(1):44–49. https://doi.org/10.1093/jisesa/iev029.

Fang, W., Scully, L. R., Zhang, L., Pei, Y., Bidochka, M. J. 2008. Implication of a regulator of G protein signalling (BbRGS1) in conidiation and conidial thermotolerance of the insect pathogenic fungus *Beauveria bassiana*. *FEMS microbiology letters*, **279**: 146–156.

Feng, K. C., Liu, B. L. and Tzeng, Y. M. 2000. *Verticillium lecanii* spore production in solid-state and liquid-state fermentation. *Bioprocess Engineering*, **23**: 25-29.

Feng, M., Poprawski, T. and Khachatourians, G. G. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. *Biocontrol Science and Technology*, **4** (1): 3-34.

Fernandes, E. K., Rangel, D. E. N., Moraes, Á. M. L., Bittencourt, V. R. E. P. and Roberts, D. W. 2008. Cold activity of *Beauveria* and *Metarhizium*, and thermotolerance of *Beauveria*. *Journal of invertebrate pathology,* **98**: 69–78. doi: 10.1016/j.jip.2007.10.011.

Gao, Y., Reitz, S. R., Wang, J., Xu, X. and Lei, Z. 2012. Potential of a strain of the entomopathogenic fungus *Beauveria bassiana* (Hypocreales: Cordycipitaceae) as a biological control agent against western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Biocontrol Science and Technology*. **22** (4): 491-495. https://doi.org/10.1080/09583157.2012.662478.

Garrido-Jurado, I., Fernandez-Bravo, M., Campos, C. and Quesada-Moraga, E. 2015. Diversity of entomopathogenic Hypocreales in soil and phylloplanes of five Mediterranean cropping systems. *Journal of invertebrate pathology,* **130**, 97–106.

Goettel, M. S. and Inglis, G. D. 1997. Fungi: Hyphomycetes. Manual of Techniques in Insect Pathology (ed. by L.A. Lacey), Academic Press, San Diego, USA. 213-249.

Gola, D., Kaushik, P., Mishra, A. and Malik, A. 2019. Production and shelf life evaluation of three different formulations of *Beauveria bassiana* in terms of multimetal removal. *Biotechnology Research and Innovation*, **3**(2): 242-251.

Holder, D. J., Kirkland, B. H., Lewis, M. H. and Keyhani, N. O. 2007. Surface characteristics of the entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana*. *Microbiology* **153**: 3448–3457.

Joop, G. and Vilcinskas, A. 2016. Co-evolution of parasitic fungi and insect hosts. *Zoology*, **119**: 350–358. 10.1016/j.zool.2016.06.005.

Kamal, T., Sobita, S. and Apoorv. 2018. Effect of *Beauveria bassiana* against diamondback month (*Plutella xylostella* Linn.) on cabbage (*Brassica oleracea* var *capitata*). Environment and Ecology, **36** (1A): 232–236.

Karthikeyan, A. and Selvanarayanan, V. 2011. In vitro efficacy of *Beauveria bassiana* (Bals.) Vuill. and *Verticillium lecanii* (Zimm.) Viegas against selected insect pests of cotton. The Recent Research in Science and Technology, **3** (2): 142–143.

Kimaru, S. K., Monda, E., Cheruiyot, R. C., Mbaka, J. and Alakonya, A. 2018. Morphological and molecular identification of the causal agent of anthracnose disease of avocado in Kenya. *International Journal of Microbiology*, 1-10.

Kumar, K. K., Sridhar, J., Murali-Baskaran, R. K., Senthil-Nathan, S., Kaushal, P. and Dara, S. K. 2018. Microbial biopesticides for insect pest management in India: current status and future prospects. *Journal of invertebrate pathology,* **165:** 74–81.

Lawrence, A. A. and Khan, A. 2002. Comparison of the pathogenicity of the entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* to *Callosobruchus maculatus*. *International pest control*, **44**(3): 125-127.

Lennon, J. T., Aanderud, Z. T., Lehmkuhl, B. K. and Schoolmaster, D. R. Jr. 2012. Mapping the niche space of soil microorganisms using taxonomy and traits. *Ecology*, **93**, 1867–1879.

Liu, D. 2011. Molecular detection of human bacterial pathogens. CRC press. 1278.

Lopez-Perez, M., Rodriguez-Gomez, D. and Loera, O. 2015. Production of conidia of *Beauveria bassiana* in solid-state culture: Current status and future perspectives. *Critical Reviews in Biotechnology,* **35**(3): 334–341.

Lord, J. C. 2005. From Metchnikoff to Monsanto and beyond: the path of microbial control. *Journal of invertebrate pathology,* **89**:19–29.

Luz, C. and Fargues, J. 1997. Temperature and moisture requirements for conidial germination of an isolate of *Beauveria bassiana*, pathogenic to *Rhodnius prolixus*. *Mycopathologia,* **138**: 117–125. doi: 10.1023/A:1006803812504.

Maggi, O., Tosi, S., Angelova, M., Lagostina, E., Fabbri, A. A. and Pecoraro, L. 2013. Adaptation of fungi, including yeasts, to cold environments. *Plant biosystems,* **147**: 247–258.

Meyling, N. V. 2007. Methods for isolation of entomopathogenic fungi from the soil environment-laboratory manual. 18.

Oduor, G. I., Yaninek, J. S., De Moraes, G. J. and Van Der Geest, L. P. S. 1997. The effect of pathogen dosage on the pathogenicity of *Neozygites floridana* (Zygomycetes: Entomophthorales) to *Mononychellus tanajoa* (Acari: Tetranychidae). *Journal of invertebrate pathology,* **70**: 127–130.

Ormond, E. L., Thomas, A. P., Pugh, P. J., Pell, J. K. and Roy, H. E. 2010. A fungal pathogen in time and space: the population dynamics of *Beauveria bassiana* in a conifer forest. *FEMS microbiology ecology,* **74**: 146–154.

Ortiz-Urquiza, A. and Keyhani, N. O. 2013. Action on the surface: entomopathogenic fungi versus the insect cuticle. *Insects*, 4: 357–374.

Ozdemir, I. O., Tuncer, C., Erper, I. and Kushiyev, R. 2020. Efficacy of the entomopathogenic fungi; *Beauveria bassiana* and *Metarhizium anisopliae* against the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae). *Egyptian Journal of Biological Pest Control*, **30** (1): 1-5.

Parker, B. L., Skinner, M., Costa, S. D., Gouli, S., Reid, W. and El-Bouhssini, M. 2003. Entomopathogenic fungi of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae): collection end characterization for development. *Biological Control*, **27**: 260–272

Pedrini, N. 2022. The Entomopathogenic Fungus *Beauveria bassiana* Shows Its Toxic Side within Insects: Expression of Genes Encoding Secondary Metabolites during Pathogenesis. Journal of Fungi, 8(5), 488.

Petlamul, W. and Boukaew, S. 2019. Optimisation and stabilisation of cellulase and xylanase production by *Beauveria bassiana*. *Environment Asia*, **12**(1): 11–19.

Prithiva, J. N., Ganapathy, N. and Jeyarani, S. 2017. Efficacy of different formulations of *Beauveria bassiana* (Bb 112) against Bemisia tabaci on tomato. *Journal of Ent`omology and Zoology Studies,* **5** (4): 1239-1243.

Qazi, S. S. and Khachatourians, G. G. 2007. Hydrated conidia of *Metarhizium ansiopliae* release a family of metalloproteases. *Journal of invertebrate pathology,* **95**: 48–59.

Quesada-Moraga, E. and Vey, A. 2003. Intra-specific variation in virulence and in vitro production of macromolecular toxins active against locust among *Beauveria bassiana* strains and effects of in vivo and in vitro passage on these factors. *Biocontrol Science and Technology*, **13**(3): 323-340.

Ramle, M., Hisham, H., Wahid, M. B., Kamarudin, N. and Ahmad, A. S. R. 2005. Mass production of *Beauveria bassiana* using solid fermentation and wet harvesting methods. Proceeding of the PIPCO. 928-943.

Rani, A. T., Kammar, V., Keerthi, M. C., Rani, V., Majumder, S., Pandey, K. K. and Singh, J. 2021. Biopesticides: An Alternative to Synthetic Insecticides. In Microbial Technology for Sustainable Environment. Springer, Singapore. 439-466.

Raya-Díaz, S., Sánchez-Rodríguez, A. R., Segura-Fernández, J. M., Del Campillo, M. D. C. and Quesada-Moraga, E. 2017. Entomopathogenic fungi-based mechanisms for improved Fe nutrition in sorghum plants grown on calcareous substrates. *PLoS One,* 12:e0185903.

Rehner, S.A., Minnis, A.M., Sung, G.H., Luangsa ard, J.J., Devotto, L. and Humber, R. A. 2011. Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. *Mycologia*, **103**: 1055–1073.

Ritu, A., Anjali, C., Nidhi, T., Sheetal, P. and Deepak, B. 2012. Biopesticidal Formulation of *Beauveria bassiana* Effective against Larvae of *Helicoverpa Armigera*. *Journal of Biofertilizers and Biopesticides*, **3**(3): 1-3.

Robène, I., Jouen, E., Pastou, D., Payet Hoareau, M., Goble, T., Linderme, D., Lefeuvre, P., Calmès, C., Reynaud, B., Nibouche, S. and Costet, L. 2015. Description and phylogenetic placement of Beauveria hoplocheli sp. nov. used in the biological control of the sugarcane white grub, Hoplochelus marginalis, in Reunion Island. *Mycologia,* **107**: 1221–1232.

Roberts, D. W. and St. Leger, R. J. 2004. *Metarhizium* spp., cosmopolitan insect pathogenic fungi: mycological aspects. *Advances in Applied Microbiology*, **54**: 1-70.

Saeed, M. B., Laing, M. D., Miller, R. M. and Bancole, B. 2017. Ovicidal, Larvicidal and insecticidal activity of strains of *Beauveria bassiana* (Balsamo) Vuillemin against the cigarette beetle, *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae), on rice grain. *Journal of stored products research*, **74**: 78-86.

Sanchez-Pena, S.R., Lara, J.S.J. and Medina, R.F. 2011. Occurrence of entomopathogenic fungi from agricultural and natural ecosystems in saltillo, méxico, and their virulence towards thrips and whiteflies. *Journal of Insect Science*, **11**(1): 1–10. doi:10.1673/031.011.0101.

Senthilkumar, M., Amaresan, N. and Sankaranarayanan, A. 2021. Plant-microbe interactions. springer protocols handbooks. 296.

Sewify, G. H., Belal, M. H. and Al-Awash, S. A. 2009. Use of the Entomopathogenic fungus, *Beauveria bassiana* for the biological control of the red palm weevil, *Rhynchophorus ferrugineus* Olivier. *Egyptian Journal of Biological Pest Control*, **19**(2):157–163.

Sharma, L., Bohra, N., Rajput, V. D., Quiroz-Figueroa, F. R., Singh, R. K. and Marques, G. 2020. Advances in Entomopathogen Isolation: A Case of Bacteria and Fungi. *Microorganisms*, 9(1), 16. doi:10.3390/microorganisms9010016.

Sharma, R. and Sharma, P. 2021. Fungal entomopathogens: a systematic review. *Egyptian Journal of Biological Pest Control*, **31**(1):57. doi:10.1186/s41938-021-00404-7.

Sharma, R., Polkade, A. V. and Shouche, Y. S. 2015. 'Species concept' in microbial taxonomy and systematics. *Current science*, **108** (10): 1804-1814.

Shivakumara, K. T., Keerthi, M. C. and Polaiah, A. C. 2022. Efficacy of different biorational insecticides against *Aphis nerii* Boyer de Fonscolombe

(Hemiptera. Aphididae) on Gymnema sylvestre (R. Br) under laboratory and field conditions. *Journal of Applied Research on Medicinal and Aromatic Plants,* **28**: 100358.

Song, T.T., Zhao, J., Ying, S.H. and Feng, M.G. 2013. Differential contributions of five ABC transporters to multidrug resistance, antioxidant and virulence of *Beauveria bassiana*, an entomopathogenic fungus. *PLoS ONE,* 8, e62179.

Tefera, T. and Vidal, S. 2009. Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus *Beauveria bassiana*. *BioControl,* **54**: 663–669.

Toscan Lorencetti, G. A., Potrich, M., Mazaro, S. M., Lozano, E. R., Barbosa, L. R., Menezes, M. J. S. and Gonçalves, T. E. 2018. *Beauveria bassiana* Vuill. and Isaria sp. efficiency for *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae). *Ciencia Florestal*. **28**(1): 403–411. https://doi.org/10.5902/1980509831612.

Uma Devi, K., Padmavathi, J., Uma Maheswara Rao, C., Khan, A.A.P. and Mohan, M. C. 2008. A study of host specificity in the entomopathogenic fungus *Beauveria bassiana* (Hypocreales, Clavicipitaceae). *Biocontrol Science and Technology*, **18**(10): 975-989.

Valero-Jiménez, C.A., Faino, L., Veld, D.S.I.T., Smit, S., Zwaan, B. J. and Kan, J.A.L.V. 2016. Comparative genomics of *Beauveria bassiana*: uncovering signatures of virulence against mosquitoes. *BMC Genomics*, **17**(1):986.

Veen, K. H. and Ferron, P. 1966. A selective medium for isolation of Beauveria tenella and of Metarrhizium anisopliae. *Journal of Invertebrate Pathology*, **8**: 268-269.

Vega, F. E. 2018. The use of fungal entomopathogens as endophytes in biological control: a review. *Mycologia,* 110: 4–30. doi: 10.1080/00275514.2017.1418578.

Vercambre, B., Goebel, O., Riba, G., Marchal, M., NeuveÂglise, C. and Ferron, P. 1994. Success in biological control of a soil pest, *Hoplochelus marginalis*, in Reunion Island: choice of a suitable fungus. VIth International Colloquium on Invertebrate Pathology and Microbial Control; Dec 7-9; Montpellier, France. 283-288.

Vilas Boas, A. M., Oliveira, J. V., Campos, A. L., Andrade, R. M. and Silva, R. L. X. 1996. Pathogenicity of wild strains and mutants of *Metarhizium anisopliae* and *Beauveria bassiana* to Callosobruchus maculatus (Fab. 1792) (Coleoptera, Bruchidae). *Arquivos de Biologia e Tecnologia*, **39**(1): 99-104.

Wang, J., Zhou, G., Ying, S. H. and Feng, M. G. 2014. Adenylate cyclase orthologues in two filamentous entomopathogens contribute differentially to growth, conidiation, pathogenicity, and multistress responses. Fungal Biology, **118**: 422– 431.

Wang, M., Tian, J., Xiang, M. and Liu, X. 2017. Living strategy of cold-adapted fungi with the reference to several representative species. *Mycology,* **8**: 178–188.

Wang, Z. L., Lu, J. and Feng, M. G. 2012. Primary roles of two dehydrogenases in the mannitol metabolism and multi-stress tolerance of entomopathogenic fungus *Beauveria bassiana*. *Environmental Microbiology*, **14**: 2139–2150.

Wielgolaski, F. E. and Inouye, D. W. 2003. "High latitude climates," in Phenology: An Integrative Environmental Science, ed M.D. Schwartz, Dordrecht: Springer, 175–194.

Wraight, S. P., Ramos, M. E., Avery, P. B., Jaronski, S. T. and Vandenberg, J. D. 2010. Comparative virulence of Beauveria bassiana isolates against lepidopteran pests of vegetable crops. *Journal of Invertebrate Pathology*, **103**(3):186-199.

Wu, S., Gao, Y., Xu, X., Zhang, Y., Wang, J., Lei, Z. and Smagghe, G. 2013. Laboratory and greenhouse evaluation of a new entomopathogenic strain of *Beauveria bassiana* for control of the onion thrips *Thrips tabaci*. *Biocontrol science and technology*. **23**(7): 794-802. https://doi.org/10.1080/09583157.2013.794896.

Xiao, G., Ying, S. H., Zheng, P., Wang, Z. L., Zhang, S. and Xie, X.Q. 2012. Genomic perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. *Scientific reports*, **2**: 483. doi: 10.1038/srep00483.

Zhang, L. B., Tang, L., Guan, Y. and Feng, M. G. 2020. Subcellular localization of Sur7 and

its pleiotropic effect on cell wall integrity, multiple stress responses, and virulence of *Beauveria bassiana*. *Applied Microbiology and Biotechnology*, **104**(15): 6669-6678.

Zhang, S., Widemann, E., Bernard, G., Lesot, A., Pinot, F., Pedrini, N. and Keyhani, N.O., 2012. CYP52X1, representing new cytochrome P450 subfamily, displays fatty acid hydroxylase activity and contributes to virulence and growth on insect cuticular substrates in entomopathogenic fungus *Beauveria bassiana*. *Journal of Biological Chemistry*, **287**(16): 13477-13486.

Zhang, S., Xia, Y.X., Kim, B. and Keyhani, N.O. 2011. Two hydrophobins are involved in fungal spore coat rodlet layer assembly and each play distinct roles in surface interactions, development and pathogenesis in the entomopathogenic fungus, *Beauveria bassiana*. *Molecular microbiology*, **80**(3): 811-826.

Zhang, S. L., He, L.M., Chen, X., Hueng, B. 2012. *Beauveria lii* sp. nov. isolated from *Henosepilachna vigintioctopunctata*. *Mycotaxon,* **121**: 199–206.

Zhang, Y., Zhao, J., Fang, W., Zhang, J., Luo, Z., Zhang, M., Fan, Y. and Pei, Y. 2009. Mitogen-activated protein kinase hog1 in the entomopathogenic fungus *Beauveria bassiana* regulates environmental stress responses and virulence to insects. *Applied and Environmental Microbiology*, 75: 3787–3795.

Zimmermann, G. 1986. The Galleria bait method for detection entomopathogenic fungi in soil. *Journal of Applied Entomology*, **102**: 213–215.

Zimmermann, G. 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology*, **17**(6): 553-596.

MS Received: 18 November 2022
MS Accepted : 23 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 15-20 (2022)

DOI Number: 10.5958/0974-4541.2022.00032.7

Management of legume pod borers on Yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* L.)

K. RAJASHEKHARAPPA*, S. AMBARISH, NUZHAT BANU DIDGUR and RAMESH MARADI

Department of Agricultural Entomology, College of Agriculture, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga-577204, Karnataka, India

***E-mail:** rajashekarappak@uahs.edu.in

ABSTRACT: Field experiments were carried out at Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India, during *kharif* 2020 and 2021 on management of legume pod borers on Yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* L.). Results revealed that chlorantraniliprole 18.5 SC @ 0.4 ml/L was effective in reducing pod borers (89.11 %) and was on par with the spinetoram 11.7 SC @ 0.4 ml/L (88.17 %), followed by other chemicals evaluated. However, the pod borers populations were reduced in all the treatments compared to the control. The highest marketable pod yield was recorded in the chlorantraniliprole 18.5 SC @ 0.4 ml/L (24.23 t/ha) and it was closely on par with the spinetoram 11.7 SC @ 0.4 ml/L (24.06 t/ha). The efficacy and pod yield was superior in chlorantraniliprole 18.5 SC but, after analyzing the C: B ratio, spinetoram 11.7 SC recorded the highest C: B ratio of 1: 4.71) as compared to the chlorantraniliprole 18.5 SC (1: 4.49). Hence, spinetoram 11.7 SC @ 0.4 ml per L was superior in pod borers reduction, increase in yield and C: B ratio.

Keywords: Chlorantraniliprole, *Helicoverpa armigera, Lampides boeticus, Maruca vitrata,* spinetoram, yardlong bean

INTRODUCTION

Yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis* L.) is an important leguminous vegetable crop grown all over the country. It is also known as asparagus bean, string bean, snake bean and vegetable cowpea (Purseglove, 1977). In India, Kerala state contributes a major share accounting for nearly 90 percent in both area and production, followed by Karnataka and Tamil Nadu. The area of yard long beans in India is about 18,560–20,160 ha (Saurabh *et al.,* 2018). It is a highly nutritive vegetable containing a good amount of digestible protein (23.5-26.3 %) both in pods and in leaves (Ano and Ubochi, 2008). It can be used as fodder, vegetable, and green manure crop. The cultivation of this crop encounters various problems, including pest management (Rashid, 1993). About 150 species of insect pests are known to attack beans in India, of which about 25 species are reported to be serious (Srivastava, 1987). In Karnataka, a total of four species of insects (*Spodoptera litura, Maruca vitrata, Liriomyza trifolii* and *Aphis fabae*) and one mite pest (*Tetranychus urticae*) is causing a major serious problem (Manjesh *et al.,* 2017). Flower and pod-feeding lepidopterans cause severe yield losses to edible legumes, particularly in tropical and sub-tropical zones (Rouf and Sardar, 2011). Lepidopteran borers *viz.,* spotted pod borer, *Maruca vitrata* (Fabricius), gram pod borer, *Helicoverpa armigera* (Hubner) and blue butterfly, *Lampides boeticus* (Linnaeus) cause severe loss in yardlong bean (Didgur, 2022).

To overcome the loss, minimize the pest's attack and increase the ultimate production of the yardlong bean, the farmers are using the insecticides indiscriminately. In India, the scientific information on the management of pod borers in yardlong beans is limited. Hence, the present study was initiated to find out the effective insecticide fit into integrated pest management modules for the effective management of pod borers and increase in the yield of yardlong beans.

MATERIALS AND METHODS

A field experiment was conducted during *kharif* 2020 and 2021 at Zonal Agricultural and Horticultural Research Station (ZAHRS), Bavikere, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences (KSNUAHS), Shivamogga (13° 42′ N and 75° 51′ E), Karnataka, India. Yardlong bean variety 'Arka Mangala' was used for the experiment. The crop was sown by dibbling with a spacing of 120 x 30 cm. Gap filling and thinning were done to maintain the optimum plant density. The crop was raised by following a package of practices released by KSNUAHS, Shivamogga, except plant protection measures for pod borers.

Seven insecticides *viz*., lambdacyhalothrin 5 EC, flubendiamide 39.35 SC, chlorantraniliprole 18.5 SC, indoxacarb 15.8 EC, spinetoram 11.7 SC, malathion 50 EC and azadirachtin 5 EC were evaluated. The experiment included eight treatments and three replications, including an absolute control. The treatments were imposed at 45

Table 1. Bio-efficacy of different insecticides against legume pod borers infesting yardlong bean during 2020

Treatment	Dosage (ml/L)	Number of larvae per five plants										Mean	Per cent reduction
		First Spraying					Second Spraying						
		DBS	3 DAS	7 DAS	10 DAS	14 DAS	DBS	3 DAS	7 DAS	10 DAS	14 DAS		
T1-Lambdacyhalothrin 5EC	1.0	11.53 (3.47)	5.20 (2.39)^{bc}	3.41 (1.98)^{cd}	1.78 (1.51)^{bcd}	3.22 (1.93)^{cd}	7.22 (2.78)^{c}	5.14 (2.38)^{cd}	3.69 (2.05)^{cd}	1.24 (1.32)^{c}	2.94 (1.85)^{c}	3.33	75.39
T2-Flubendiamide 39.35 SC	0.20	10.58 (3.33)	2.95 (1.86)^{d}	1.32 (1.35)^{f}	0.64 (1.07)^{e}	1.53 (1.42)^{ef}	6.30 (2.61)^{c}	3.88 (2.09)^{de}	2.36 (1.69)^{d}	0.70 (1.10)^{c}	2.02 (1.59)^{cd}	1.93	85.76
T3-Chlorantraniliprole 18.5SC	0.40	11.15 (3.41)	3.29 (1.95)^{d}	1.08 (1.26)^{f}	0.57 (1.04)^{e}	1.12 (1.27)^{f}	4.49 (2.23)^{e}	2.65 (1.77)^{e}	0.21 (0.84)^{e}	0.00 (0.71)^{d}	0.55 (1.03)^{e}	1.18	91.24
T4-Indoxacarb 15.8 EC	0.60	11.38 (3.45)	4.09 (2.14)^{cd}	2.48 (1.73)^{de}	1.37 (1.37)^{cde}	2.27 (1.67)^{de}	6.24 (2.60)^{cd}	4.87 (2.32)^{cd}	2.97 (1.86)^{d}	0.96 (1.21)^{c}	1.97 (1.57)^{d}	2.62	80.61
T5-Spinetoram 11.7 SC	0.40	12.06 (3.54)	3.11 (1.90)^{d}	1.57 (1.44)^{ef}	0.98 (1.22)^{de}	1.37 (1.37)^{f}	4.85 (2.31)^{de}	2.98 (1.87)^{e}	0.67 (1.08)^{e}	0.54 (1.02)^{cd}	0.95 (1.20)^{e}	1.52	88.75
T6-Malathion 50 EC	2.00	10.06 (3.25)	6.51 (2.65)^{b}	4.18 (2.16)^{bc}	2.48 (1.73)^{bc}	4.05 (2.13)^{bc}	9.04 (3.09)^{b}	6.56 (2.66)^{bc}	4.82 (2.31)^{bc}	3.64 (2.03)^{b}	4.42 (2.22)^{b}	4.58	66.10
T7-Azadirachtin 5 EC	0.50	11.30 (3.44)	6.95 (2.73)^{b}	5.24 (2.40)^{b}	3.05 (1.89)^{b}	4.79 (2.30)^{b}	9.75 (3.20)^{b}	7.78 (2.88)^{b}	5.46 (2.44)^{b}	3.69 (2.05)^{b}	5.01 (2.35)^{b}	5.25	61.19
T8-Control	0.0	11.32 (3.44)	12.20 (3.56)^{a}	12.62 (3.62)^{a}	13.13 (3.69)^{a}	13.42 (3.73)^{a}	13.35 (3.72)^{a}	14.15 (3.83)^{a}	14.64 (3.89)^{a}	14.27 (3.84)^{a}	13.71 (3.77)^{a}	13.52	75.39
CD (p=0.05)		NS	0.43	0.33	0.40	0.27	0.28	0.41	0.36	0.32	0.28	-	-
CV (%)		7.58	10.27	9.75	13.83	7.91	5.79	9.70	10.45	11.12	8.34	-	-

NS-Non significant; Figures within the parentheses indicates √x+0.5 transformed values; Mean followed by the same letter do not differ significantly by DMRT (P=0.05); DBS= Day before spray; DAS= Day after spray

and 60 days after sowing the crop using a knapsack sprayer fitted with a hollow cone nozzle. The observations were recorded on five randomly selected plants on a day before spraying and 3, 7, 10 and 14 days after spraying by counting the number of larvae per plant and the data were expressed as the number of larvae per five plants. Totally three species of pod borers were observed *viz.,* spotted pod borer, *Maruca vitrata* (Fabricius), gram pod borer, *Helicoverpa armigera* (Hubner) and blue butterfly, *Lampides boeticus* (Linnaeus). All the three species data was averaged, subjected to square root transformation, and analyzed statistically. The results were interpreted at a five percent significance level using ICAR WASP (Web Agri Stats Package) 2.0 software. Percent reduction over untreated control was calculated, and the data for two years was pooled for a better interpretation of a valid conclusion.

RESULTS AND DISCUSSION

During 2020, the mean data of first and second spray indicated that, the lowest mean larval population of 1.18 larvae per five plants was recorded in chlorantraniliprole 18.5 SC @ 0.4 ml/L followed by spinetoram 11.7 SC @ 0.4 ml/L (1.52 larvae/5 plants) and flubendiamide 39.35 SC @ 0.2 ml/L (1.93 larvae/5 plants). The highest larval population of 13.52 larvae per plant was recorded in the control plot. However, in all the treatments larval population was reduced when compared to the control. Chlorantraniliprole 18.5 SC recorded 91.24 per cent reduction followed by spinetoram 11.7 SC (88.75 %) and flubendiamide 39.35 SC (85.76 %). The lowest percent reduction (61.19 %) was recorded in Azadirachtin 5 EC, followed by Malathion 50 EC (66.10 %).

In 2021, the same trend was recorded as in the case of 2020. The mean data of first and second spray showed that, the lowest mean larval population of 1.33 larvae per five plants was recorded in chlorantraniliprole 18.5 SC @ 0.4 ml/L followed by spinetoram 11.7 SC @ 0.4 ml/L (1.63 larvae/5 plants) and flubendiamide 39.35 SC @ 0.2 ml/L (2.2 larvae/5 plants). The highest larval population of 12.40 larvae per 5 plants was recorded in the control plot. However, in all the treatments larval population was reduced when compared to the control. Chlorantraniliprole 18.5 SC recorded 88.11 per cent reduction followed by spinetoram 11.7 SC (85.35 %) and flubendiamide 39.35 SC (80.30 %). The lowest percent reduction (52.79 %) was recorded in Azadirachtin 5 EC, followed by Malathion 50 EC (60.25 %).

The pooled mean data (Table 3) of pod borers in yardlong beans didn't vary significantly one day before spraying (DBS) (10.08 to 11.58 pod borers/5 plants), indicating the uniform distribution of pod borers throughout the experimental plot. All the molecules tested proved their superiority in significantly suppressing the pod borers population compared to untreated control up to 14 days of the first and second application of insecticides. The lowest number of the pod was recorded in chlorantraniliprole 18.5 SC @ 0.4 ml/L, which was found to be far with spinetoram 11.7 SC @ 0.4 ml/L. The next best chemical in reducing pod borers population was flubendiamide 39.35 SC @ 0.2 ml/L; it was found to be on par with the indoxacarb15.8 EC @ 0.6 ml/L, followed by lambda-cyhalothrin 5 EC @ 1.0 ml/L. The least reduction in pod borers during all the observations was recorded in azadirachtin 5 EC @ 0.5 ml/L, followed by Malathion 50 EC @ 2.0 ml/L. However, the highest number of pod borers per five plants was observed in the untreated control (Table 3).

The mean population of first and second sprays indicated that lowest number of pod borers per five plants was recorded in the chlorantraniliprole 18.5 SC @ 0.4 ml/L (1.34) and it was found to be on par with the spinetoram 11.7 SC @ 0.4 ml/L (1.46). The next best chemical was flubendiamide 39.35 SC @ 0.2 ml/L (2.06), Indoxacarb 15.8 EC @ 0.6 ml/L (2.66), lambdacyhalothrin 5 EC @ 1.0 ml/L (3.48), Malathion 50 EC @ 2.0 ml/L (4.51) and azadirachtin 5 EC @ 0.5 ml/L (5.25). The highest mean number of pod borers was recorded in the untreated control (12.33 pod borers/5 plants) (Table 3). The chemical, chlorantraniliprole 18.5 SC @ 0.4 ml/L and spinetoram 11.7 SC @ 0.4 ml/L recorded 89.11 and 88.17 per cent reduction, respectively over untreated control. Next best treatment was flubendiamide 39.35 SC @ 0.2 ml/L (83.29 %) followed by Indoxacarb 15.8 EC @ 0.6 ml/L (78.45%) and lambdacyhalothrin 5 EC @ 1.0 ml/L (71.76%). The lowest per cent reduction of pod borers over untreated control was observed in the treatment of azadirachtin 5 EC @ 0.5 ml/L (57.40 %) followed by malathion 50 EC @ 2.0 ml/L (63.46 %) (Table 3).

The marketable pod yield of yardlong beans recorded in all the chemicals treated plots varied between 17.38 to 24.23 tonnes/ha. The highest yield (24.23 t/ha) was recorded in the chlorantraniliprole 18.5 SC @ 0.4 ml/L, which was found to be on par with the spinetoram 11.7 SC @ 0.4 ml/L (24.06 t/ha). The lowest green pod yield (12.11 t/ha) was recorded in the untreated control (Table 3). The efficacy and yield were superior in chlorantraniliprole 18.5 SC treatment, but the B:C ratio of spinetoram 11.7 SC was highest (4.71) compared to the chlorantraniliprole 18.5 SC (1: 4.49) because of the high cost of the chemical. Hence, spinetoram 11.7 SC @ 0.4 ml per L was found to be superior in pod borer management in yardlong bean with respect to the high

Table 2. Bio-efficacy of different insecticides against legume pod borers infesting yardlong bean during 2021

Treatment	Dosage (ml/L)	Number of larvae per five plants										Mean	Per cent reduction
		First spraying					Second spraying						
		DBS	3 DAS	7 DAS	10 DAS	14 DAS	DBS	3 DAS	7 DAS	10 DAS	14 DAS		
T1-Lambdacyhalothrin 5EC	1.0	10.06 (3.25)	5.82 (2.51)^{c}	4.15 (2.16)^{c}	2.96 (1.86)^{c}	3.27 (1.94)^{c}	7.95 (2.91)^{abcd}	5.08 (2.36)^{cd}	3.24 (1.93)^{c}	2.23 (1.65)^{d}	2.35 (1.69)^{d}	3.64	67.36
T2-Flubendiamide 39.35 SC	0.20	9.74 (3.20)	3.69 (2.05)^{de}	2.19 (1.64)^{de}	1.58 (1.44)^{de}	1.76 (1.50)^{de}	7.27 (2.79)^{bcd}	3.53 (2.01)^{e}	1.77 (1.51)^{d}	1.47 (1.40)^{e}	1.56 (1.44)^{e}	2.20	80.30
T3-Chlorantraniliprole 18.5SC	0.40	9.91 (3.23)	2.62 (1.77)^{f}	1.35 (1.36)^{f}	0.92 (1.19)^{e}	1.19 (1.30)^{e}	5.92 (2.53)^{cd}	2.54 (1.74)^{f}	0.92 (1.19)^{e}	0.50 (1.00)^{g}	0.56 (1.03)^{g}	1.33	88.11
T4-Indoxacarb 15.8 EC	0.60	9.81 (3.21)	4.02 (2.13)^{d}	2.43 (1.71)^{d}	1.76 (1.50)^{d}	1.94 (1.56)^{d}	6.74 (2.69)^{bcd}	4.73 (2.29)^{d}	2.72 (1.79)^{c}	1.83 (1.53)^{de}	2.12 (1.62)^{de}	2.69	75.83
T5-Spinetoram 11.7 SC	0.40	9.73 (3.20)	2.88 (1.84)^{ef}	1.52 (1.42)^{ef}	1.10 (1.27)^{de}	1.31 (1.34)^{de}	5.59 (2.47)^{d}	2.91 (1.85)^{ef}	1.44 (1.39)^{de}	0.92 (1.19)^{f}	0.97 (1.21)^{f}	1.63	85.35
T6-Malathion 50 EC	2.00	10.09 (3.25)	6.80 (2.70)^{bc}	4.90 (2.32)^{bc}	3.65 (2.04)^{bc}	3.76 (2.06)^{bc}	8.42 (2.99)^{abc}	5.95 (2.54)^{bc}	4.11 (2.15)^{b}	3.01 (1.87)^{c}	3.24 (1.93)^{c}	4.43	60.25
T7-Azadirachtin 5 EC	0.50	10.17 (3.27)	8.00 (2.92)^{b}	5.78 (2.51)^{b}	4.57 (2.25)^{b}	4.70 (2.28)^{b}	8.67 (3.03)^{ab}	6.37 (2.62)^{b}	4.43 (2.22)^{b}	4.06 (2.13)^{b}	4.17 (2.16)^{b}	5.26	52.79
T8-Control	-	11.83 (3.51)	12.26 (3.57)^{a}	12.55 (3.61)^{a}	12.76 (3.64)^{a}	12.99 (3.67)^{a}	10.99 (3.39)^{a}	10.46 (3.31)^{a}	12.32 (3.58)^{a}	12.68 (3.63)^{a}	13.21 (3.70)^{a}	12.40	0.0
CD (p=0.05)		NS	0.27	0.21	0.25	0.23	0.48	0.24	0.19	0.17	0.18	-	-
CV (%)		6.31	6.45	5.99	7.83	6.83	9.78	6.08	5.89	5.70	5.78	-	-

NS-Non significant; Figures within the parentheses indicates √x+0.5 transformed values; Mean followed by the same letter do not differ significantly by DMRT (P=0.05); DBS= Day before spray; DAS= Day after spray

Table 3. Bio-efficacy of different insecticides against legume pod borers infesting yardlong bean (Pooled data of two seasons)

Treatment	Dosage (ml/L)	Number of larvae per five plants										Per cent reduction	B:C ratio
		First Spraying					Second spraying						
		DBS	3 DAS	7 DAS	10 DAS	14 DAS	DBS	3 DAS	7 DAS	10 DAS	14 DAS		
T1-Lambdacyhalothrin 5EC	1.00	10.80 (3.36)	5.51 (2.45)^{c}	3.78 (2.07)^{c}	2.37 (1.69)^{cd}	3.25 (1.94)$_{bcd}$	7.75 (2.87)^{bc}	5.11 (2.37)$_{c}$	3.47 (1.99)^{cd}	1.74 (1.50)^{c}	2.64 (1.77)^{d}	71.76	4.10
T2-Flubendiamide 39.35 %SC	0.20	10.16 (3.26)	3.32 (1.95)$_{de}$	1.76 (1.50)^{e}	1.11 (1.27)^{ef}	1.65 (1.46)^{de}	7.28 (2.79)^{cd}	3.71 (2.05)$_{d}$	2.07 (1.60)^{e}	1.09 (1.26)^{d}	1.79 (1.51)^{e}	83.29	4.55
T3-Chlorantraniliprole 18.5SC	0.40	10.44 (3.31)	2.87 (1.83)^{e}	1.30 (1.34)^{e}	0.84 (1.16)^{f}	1.21 (1.31)e	6.32 (2.61)^{d}	2.76 (1.81)$_{e}$	0.80 (1.14)^{f}	0.46 (0.98)^{e}	0.70 (1.12)^{f}	89.11	4.49
T4-Indoxacarb 15.8 EC	0.60	10.60 (3.33)	4.05 (2.13)^{d}	2.46 (1.72)^{d}	1.57 (1.44)^{de}	2.11 (1.61)$_{cde}$	7.16 (2.77)^{cd}	4.80 (2.30)$_{c}$	2.84 (1.83)^{de}	1.39 (1.38)^{cd}	2.04 (1.59)^{e}	78.45	4.40
T5-Spinetoram 11.7 SC	0.40	10.99 (3.39)	3.09 (1.89)^{e}	1.46 (1.40)^{e}	0.95 (1.21)^{ef}	1.28 (1.33)^{e}	6.39 (2.62)^{d}	2.78 (1.81)$_{e}$	0.83 (1.15)^{f}	0.52 (1.01)^{e}	0.76 (1.12)^{f}	88.17	4.71
T6-Malathion 50 EC	2.00	10.08 (3.25)	6.66 (2.67)$_{bc}$	4.54 (2.24)^{c}	3.07 (1.89)^{bc}	3.90 (2.10)^{bc}	8.73 (3.04)^{b}	6.26 (2.60)$_{b}$	4.47 (2.23)^{bc}	3.33 (1.96)^{b}	3.83 (2.08)^{c}	63.46	3.89
T7-Azadirachtin 5 EC	0.50	10.74 (3.35)	7.48 (2.82)^{b}	5.51 (2.45)^{b}	3.81 (2.08)^{b}	4.75 (2.29)^{b}	9.21 (3.12)^{b}	7.07 (2.75)$_{b}$	4.95 (2.33)^{b}	3.87 (2.09)^{b}	4.59 (2.26)^{b}	57.40	3.72
T8-Control	0.0	11.58 (3.48)	12.23 (3.57)^{a}	12.59 (3.62)^{a}	12.94 (3.67)^{a}	13.21 (3.70)^{a}	11.34 (3.44)^{a}	11.81 (3.51)$_{a}$	12.13 (3.55)^{a}	11.98 (3.53)^{a}	11.74 (3.50)^{a}	0.0	2.69
CD (p=0.05)		-	0.24	0.19	0.27	0.52	0.25	0.21	0.25	0.17	0.25		-
CV (%)		6.39	5.83	5.41	8.79	15.42	5.08	5.11	7.39	5.79	5.88		-

NS-Non significant; Figures within the parentheses indicates √x+0.5 transformed values; Mean followed by the same letter do not differ significantly by DMRT (P=0.05); DBS= Day before spray; DAS= Day after spray

B:C ratio. These results are also in conformity with the findings of Sontakke and Amrita (2022), who reported that chlorantraniliprole 18.5SC @ 30 g a.i. per hectare was superior in reducing the *Lampides boeticus* in yard long beans. Similarly, the highest grain yield of redgram was obtained using chlorantraniliprole 18.5 SC (Sreekanth *et al.,* 2015). The same results were also obtained by Mohanraj *et al.,* (2012) and Sapkal *et al.,* (2018). Didgur (2022) reported that spinetoram 11.7 SC was the best chemical in reducing *Maruca vitrata*, *Lampides boeticus* and increase in green pod yield in yardlong beans, but chlorantraniliprole 18.5 SC was found to be superior in reducing *Helicoverpa armigera.* In our study, chlorantraniliprole 18.5 SC was proved to be the best insecticide in reducing the pod borers. But, because of the high cost of this chemical, the B:C ratio was low; hence spinetoram 11.7 SC was found to be superior concerning profit. The results of the present investigation indicated that spinetoram 11.7 SC @ 0.4 ml per L was found to be best in pod borer management.

ACKNOWLEDGEMENT

The authors acknowledge the Director of Research, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India for providing facilities and support.

REFERENCES

Ano, A. O. and Ubochi, C. I. 2008. Nutrient composition of climbing and prostrate vegetable cowpea accessions. *The African Journal of Biotechnology*, **7**: 3795-3798.

Didgur, N. B. 2022. Studies on legume pod borer complex on Yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* L.). M. Sc. (Agri.) *Thesis*. Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India.

Manjesh, M., Nagarajappa, A., Srinivasa, V., Sharanabasappa and Girijesh, G. K. 2017. Pest incidence on Yardlong bean grown under protected and open field conditions, *Pest Management in Horticultural Ecosystem*, **23**: 27-30.

Mohanraj, A., Bharathi, K. and Rajavel, D. S. 2012. Evaluation of chlorantraniliprole 20% SC against pests of black gram. *Pestology*, **36** (4): 39-43.

Purseglove, J. W. 1977. Tropical crops: Dicotyledons. London: Longman Group Ltd.

Rashid, M. A. 1993. Shabji Biggan (in Bengali), Bangla Academy, Dhaka, Bangladesh.

Rouf, F. M. A. and Sardar, M. A. 2011. Effect of crude seed extract of some indigenous plants for the control of legume pod borer (*Maruca vitrata* F.) on country bean. *Bangladesh Journal of Agricultural Research,* **36**: 41-50.

Sapkal, S., Sonkamble, M. and Gaikwad, B. 2018. Bioefficacy of newer insecticides against tomato fruit borer, *Helicoverpa armigera* (Hubner) on tomato, *Lycopersicon esculentum* (mill) under protected cultivation. *International Journal of Chemical Studies*, **6**(4): 3326-3330.

Saurabh, T., Devi, S. and Deepanshu, T. 2018. Evaluation trial in yard long bean (*Vigna unguiculata* ssp. *Sesquipedalis* (L.) Verdic.) in Allahabad agro-climatic condition. *Global Journal of Bio Science and Biotechnology*, **7**(3): 447-450.

Sivastava, B. K. 1987. Pests of pulses. In: Entomology in India. Entomological Society of India, IARI, New Delhi, **16**: 83-91.

Sontakke, B. P. and Amrita, V. S. 2022. Field efficacy of insecticides against blue butterfly *Lampides boeticus* (l.) on yard long bean. *Indian Journal of Entomology*, e21099 DoI: 10.5958/IJE.2021.125.

Sreekanth, M., Lakshmi, M. S. and Rao, Y. 2015. Efficacy and economics of certain new generation novel insecticides against legume pod borer, *Maruca vitrata* (Geyer) on pigeonpea (*Cajanus cajan* L.). *Journal of Applied Biology and Biotechnology*, **24**: 7-10.

MS Received: 10 May 2022
MS Accepted : 24 July 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 21-26 (2022)
DOI Number: 10.5958/0974-4541.2022.00033.9

Impact of different pest management modules on the major insect pests and their predators on tomato

JAYDEEP HALDER*, SUJAN MAJUMDER and K. K. PANDEY

ICAR- Indian Institute of Vegetable Research, Varanasi-221305, Uttar Pradesh, India

***E-mail:** jaydeep.halder@gmail.com

ABSTRACT: Effect of three different pest management modules were evaluated against the major insect pests of tomato under Varanasi, Uttar Pradesh, India. Among the three tested modules, integrated pest management module (Module 3) comprised spraying of imidacloprid 17.8 % SL @ 0.33 ml/L, spiromesifen 22.9% SC @ 1.25 ml/L, indoxacarb 14.5% SC @ 0.8 ml/L, cyantraniliprole 10.26% OD @ 1.8 ml/L, chlorantraniliprole 18.5% SC @ 0.35 ml/L; Neem oil (0.5%) + *Lecanicillium lecanii* @ 2.5 g/L; and Neem oil (0.5%) + *Beauveria bassiana* @ 2.5 g/L and Neem seed kernel extract (NSKE) @ 4 ml/L from 30 DAT onwards to till 100 DAT at 10 days intervals each harboured lowest fruit damage (10.48%) along with maximum percent reduction over control (69.54). Integrated pest management module also registered lowest sucking pest population *viz*., leaf hoppers (0.19 leaf^{-1}), whiteflies (0.23 leaf^{-1}) and aphids (0.83 leaf^{-1}) than the other pest management modules with maximum per cent reductions over control of 77.10, 79.46 and 67.83, respectively. The numbers of predatory mirid bugs and polyphagous spiders were also higher in this module. Furthermore, the highest healthy fruit yields (513.7 q ha^{-1}) were recorded from the integrated pest management module. In terms of return, maximum net profit of ₹83875 was obtained from module 3 *i.e.,* integrated pest management module with highest cost benefit ratio of 1:4.13 followed by biointensive pest management module (1:3.92).

Keywords: Tomato, fruit borers, sucking pests, predators, pest management modules, economics

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family, is one of the world's most widely culticvated vegetable crops. It is one of the popular vegetable crops with higher contents of vitamins A, B and C including calcium and carotene (Bose and Som, 1990). In India, tomato was grown in 0.789 million ha of land with an annual production of 19759000 metric tons and productivity of 25.04 metric tons ha^{-1}during 2017-18 (Anonymous, 2018). India remains far behind many other countries in terms of productivity, which is fairly poor due to pest infestation, which is a major barrier in fulfilling the productivity potential of tomato. Several insect pests attack the crop throughout its growth period, including tomato fruit borer (*Helicoverpa armigera* (Hübner), *Spodoptera litura* Fabricius and *Tuta absoluta* Meyrick), Whitefly (*Bemisia tabaci* (Gennadius)), leaf hoppers (*Amrasca biguttula biguttula* Ishida) andAphids (*Aphis gossypii* Glover) are important in the region (Rai *et al*., 2014; Halder and Rai, 2021).

To control these nefarious tomato insects pests that cause significant harm, farmers of the region frequently rely on the use of chemical pesticides. It is not unusual for tomato growers to apply 10-15 chemical sprays per season, which are often needless and unjustifiable, especially when there is no discernible gain in yield (Roy *et al*., 2017). The desire for a faster control strategy against these pests, as well as the desire for higher yields, has resulted in the indiscriminate, injudicious, unnecessary, and excessive use of chemical pesticides, which has resulted in problems such as pesticide resistance, resurgence of target sucking insects accompanied by secondary pest outbreaks, residues problems in food and beverages, adverse effects on human health, and massive killing of non-target organisms (Halder *et al*., 2019, 2021).

The development of an appropriate Integrated Pest Management (IPM) package for ecofriendly insect pest management for sustainable tomato production is urgently needed. Furthermore, there is no information on the creation of such modules for the comprehensive management of insidious insect pests in a larger region in tomato. Numerous pest management measures for tomato crops have been devised, however they have generally been dealt with in isolation and individually. The combination of all pest management measures has the potential to reduce the use of harmful chemical pesticides to a greater level.

MATERIALS AND METHODS

Study area

The field experiments were carried out at experimental farm of Indian Council of Agricultural Research-Indian Institute Vegetable Research (ICAR-IIVR), Varanasi (82°52' E longitude and 25°12' N latitude), Uttar Pradesh, India during *rabi* season (September, 2020 to March, 2021) of 2020-21. The experimental site comes under

the alluvial zone of Indo-Gangetic plains having soils silt loam in texture and low in organic carbon (0.43%) and available nitrogen (185 kg ha^{-1}).

Raising of the crops

Seeds of tomato (*cv*. Kashi Aman) are sown in fine-tilth nursery beds during the last week of September, 2020. The tomato seedlings were transplanted at spacing at 60 × 40 cm (row to row and plant to plant) during last week of October in a large plot size of 20 × 15 m^2 for each module. As such four such plots were prepared. From each plot, five fixed spots (5 x 4 m each, four in corners and one in centre of plot) were selected randomly considering one spot as one replication. Thus five replications were maintained for each module and flatbed system of cultivation was followed. The recommended doses of N, P, K fertilizers (100:60:60) and FYM 15-20 t ha^{-1} were applied. N, P and K were supplied through urea, single super phosphate and muriate of potash, respectively. Half of the nitrogen was applied at the time of sowing as basal dose and the rest half was equally split at vine development stage and at flower initiation stage. The full doses of both phosphorus and potassium were given at the time of final land preparation. Hand weeding and irrigations were provided as required and usual crop husbandry measures were undertaken except plant protection measures for insect pest management.

Pest management modules details

Module 1: Biointensive pest management module (BIPM)

- Spraying of *Lecanicillium* (= *Verticillium*) *lecanii* @ 5 g/lit at 30 days after transplanting (DAT)
- Spraying of Azadirachtin 300 ppm @ 5 ml/L at 40 DAT
- Spraying of *Beauveria bassiana*@ 5 g/L at 50 DAT
- Spraying of *Bacillus thuringiensis*@ 2 g/L at 60 DAT
- Spraying of *Lecanicillium lecanii* + Neem oil (1:1 ratio) @ 2.5 g/L + 2.5 ml/L at 70 DAT
- Spraying of *Beauveria bassiana* + Neem oil (1:1 ratio) @ 2.5 g/L + 2.5 ml/L at 80 DAT
- Spraying of *Lecanicillium lecanii* + Neem oil (1:1 ratio) @ 2.5 g/L + 2.5 ml/L at 90 DAT
- Spraying of Neem seed kernel extract (NSKE) @ 4 ml/L at 100 DAT

Module 2: Chemical pest management module (CPM)

- Spraying of Imidacloprid 17.8% SL @ 0.33 ml/L at 30 DAT
- Spraying of Spiromesifen 22.9% SC @ 1.25 ml/L at 40 DAT
- Spraying of Thiamethoxam 25% WG @ 0.4 g/L at 50 DAT
- Spraying of Indoxacarb 14.5% SC @ 0.8 ml/L at 60 DAT
- Spraying of Chlorantraniliprole 18.5% SC @ 0.3 ml/L at 70 DAT
- Spraying of Cyantraniliprole 10.26% OD @ 1.8 ml/L at 80 DAT
- Spraying of Novaluron10% EC @ 1.5 ml/L at 90 DAT
- Spraying of Chlorantraniliprole 18.5% SC @ 0.35 ml/L at 100 DAT

Module 3: Integrated pest management module (IPM)

- Spraying of Imidacloprid 17.8% SL @ 0.33 ml/L at 30 DAT
- Spraying of Spiromesifen 22.9% SC @ 1.25 ml/L at 40 DAT
- Spraying of Indoxacarb 14.5% SC @ 0.8 ml/L at 50 DAT
- Spraying of Cyantraniliprole 10.26% OD @ 1.8 ml/L at 60 DAT
- Spraying of Chlorantraniliprole 18.5% SC @ 0.35 ml/L at 70 DAT
- Spraying of *Beauveria bassiana* + Neem oil (1:1 ratio) @ 2.5 g/L + 2.5 ml/L at 80 DAT
- Spraying of *Lecanicillium lecanii* + Neem oil (1:1 ratio) @ 2.5 g/L + 2.5 ml/L at 90 DAT
- Spraying of Neem seed kernel extract (NSKE) @ 4 ml/L at 100 DAT

Module 4: Untreated control Data recording

For fruit borer damage, periodically fruits were harvested from the entire plots and cumulative per cent fruit damage by all the three borers (*H. armigera*, *S. litura* and *T. absoluta*) on tomato was calculated.

Similarly, the populations of leaf hoppers, aphids, and whiteflies were determined by counting the insects (including nymphs and adults for leaf hoppers, aphids and only adults for whiteflies) from three leaves (top, middle, and bottom region) sampled from each plant. As such twenty such plants were taken from each plot and expressed as number of sucking pests (leaf hoppers /

Table 1. Effect of different pest management modules against major insect pests in tomato

Treatments	Fruit damage (%)			Whitefly / leaf			Leaf hoppers / leaf			Aphids/ leaf		
	Before spray	After spray	PROC	Before spray	After spray	PROC	Before spray	After spray	PROC	Before spray	After spray	PROC
M1= Biointensive pest management module	--	13.65	60.33	1.69	0.35	68.75	1.05	0.32	61.45	2.46	1.07	58.53
M2= Chemical pest management module	--	17.99	47.72	1.57	0.23	79.46	1.17	0.23	72.29	2.61	0.96	62.79
M3= Integrated pest management module	--	10.48	69.54	1.36	0.23	79.46	1.08	0.19	77.10	2.39	0.83	67.83
Control	--	34.41	--	1.53	1.12	--	1.12	0.83	--	2.87	2.58	--
SEm(±)	--	1.84	--	--	0.10	--	--	0.07	--	--	0.13	--
LSD 5%	--	3.87	--	--	0.22	--	--	0.16	--	--	0.27	--

whitefly) leaf^{-1} plant^{-1}. The observations were recorded at weekly interval in each plot of different modules including untreated control. In case of predator population, number of predators present on tomato ecosystem *i.e.*, number of spiders and lady bird beetles (grubs/pupae/adults) were counted per plant during the month of February – March, 2021.

Two prominent polyphagous predators *viz*., spiders and mirid bugs (*Nesiodiocoris tenuis* (Reuter) (Hemiptera: Miridae) were recorded during the observation. Numbers of these predators per plant were noted and twenty plants from each pest management modules were taken. As regards the yield, different pickings made separately from entire plot after maintaining the waiting period from each module were added and converted to hectare basis.

Statistical analysis

The data were subjected to Analysis of Variance (ANOVA) with least significant difference (p=0.05) as test criterion using SAS software (version 9.3). The yield data were converted to hectare basis and the economics calculated.

RESULTS AND DISCUSSION

Effect of different pest management modules on major insect pests and its associated predators were presented in table 1. All the treatments were statistically significant than the untreated control plots. It is evident that lowest fruit damage (10.48%) was recorded from the integrated pest management (IPM) module with maximum percent reduction over control (69.54) followed by biointensive pest management (BIPM) module with 13.65% fruit damage and 60.33 PROC. It may be noted that all the three species *viz*., *H. armigera, S. litura* and *T. absoluta* were available as fruit borers on tomato during the observation. Similarly, population of adult whitefly, vector of dreaded *tomato leaf curl virus* (TLCV), was recorded and minimum whitefly population (0.23 whitefly leaf^{-1} and 79.46 PROC) was registered from the plots treated with integrated and chemical pest management modules. Similarly, the lowest leaf hopper population, comprising both nymphs and adults, was seen in integrated pest management module (0.19 leaf hoppers leaf^{-1}) which was statistically at par with the chemical pest management module. Population of polyphagous aphid *A. gossypii* was comparatively higher than the other two sucking pests of tomato *i.e*., whiteflies and leaf hoppers during January – March. Among the three tested pest management modules, maximum aphid population (1.07 leaf^{-1}) was noticed in biointensive pest management module whereas lowest (0.83 leaf^{-1}) was in integrated pest management module (Table 1).

In addition, the population of associated beneficial fauna *viz*., polyphagous spiders and mirid bugs available in tomato ecosystem was also recorded. The spiders mainly lynx and jumping spiders were seen during the study. The spider population was available almost throughout the crop growth period from 30 DAT whereas population of predatory mirid bug (*Nesiodiocoris tenuis* (Reuter)) were recorded during February – March coinciding with the sucking pest incidence on tomato and retreating winter in the region. Populations of these

Table 2. Effect of IPM modules on predators and benefit cost ratio

Treatment	Spider / plant			Mirid bug/Plant			Yield of healthy fruits (q/ha)	C:B Ratio
	Before spray	After spray	PROC*	Before spray	After spray	PROC*		
M1= BIPMM	2.21	1.73	18.39	1.62	1.04	33.76	461.6	1:3.92
M2= CPMM	2.11	0.51	72.17	1.81	0.61	61.15	502.1	1:3.10
M3= IPMM	2.06	1.29	39.15	1.67	0.93	40.76	513.7	1:4.13
Control	2.35	2.12	--	1.93	1.57	--	409.5	--
SEm (±)	--	0.12	--	--	0.06	--	--	--
LSD 5%	--	0.2	--	--	0.14	--	--	--

* PROC= Per cent reduction over control

duo predators were most abundant (2.12 spiders plant^{-1} and 1.57 mirid bugs plant^{-1}) in untreated control plots. Amongst the treatments, highest predator populations were noted in biointensive pest management module (1.73 spiders plant^{-1} and 1.04 mirid bugs plant^{-1}). Interestingly, the chemical pest management module harboured lowest spiders (0.51 spiders plant^{-1}) and mirid bugs (0.61 mirid bugs plant^{-1}) population with maximum percent predator reduction over control.

The yields of tomato were computed for each of the pest management modules by periodical harvesting of the tomato fruits (Table 2). Maximum fruit yield was obtained from the integrated pest management module (513.7 q ha^{-1}) followed by chemical (502.1 q ha^{-1}) and biointensive pest management module (461.6 q ha^{-1}) whereas minimum healthy fruit yield was in untreated control plots (409.5 q ha^{-1}). The cost benefit (C: B) ratio of each module was also calculated and presented in table 2. The integrated pest management module had the highest C:B ratio of 1:4.13 followed by biointensive pest management module (1:3.92).

The integrated pest management module registered lowest fruit damage against tomato fruit borers. In tomato fruit settings generally started from 50 DAT. To address these fruit borers, tomato fruit borer specific insecticide *viz*., Indoxacarb 14.5 SC, Cyantrailiprole 10.26% OD, Chlorantarliprole18.5% SC etc were added in the IPM module in addition to the biopesticides like *Beauveria bassiana*, *Lecanicillium lecanii* and neem based biopesticides. Indoxacarb belongs to the oxadiazines group and act on voltage-dependent sodium channel blockers (Koadnadarm *et al*., 2010; Banik and Halder, 2013; IRAC, 2022). Similarly, Cyantrailiprole and Chlorantarliproleare the new group of insecticides belongs to the diamides group which specifically act on insect ryanodine receptor modulators and thereby inhibit nerve and muscle actions (IRAC, 2022). The module 3 *i.e*., Integrated Pest Management module includes systemic insecticides like Imidacloprid 17.8% SL and Spiromesifen 22.9% SC targeting the sucking pests like whiteflies, leaf hoppers and aphids infesting tomato. Modes of actions of these molecules are completely different from each other. Spiromesifen being a tetronic and tetramicacid derivatives act as inhibitors of acetyl CoA carboxylase apart from affecting insect lipid biosynthesis and growth regulation. In enigma, the neonicotinoid insecticide Imidacloprid interferes the nicotinic acetylcholinereceptor (nAChR) as competitive modulators (Banik and Halder, 2013; IRAC, 2022). Integrated pest module also included biopesticides coinciding with fruit harvesting. The entomopathogenic fungi *Beauveria bassiana, Lecanicillium lecanii* and neem based biopesticides are the green ecofriendly pest management options against a wide range of insect pests ofmany agri-horticultural crops (Eken *et al*., 2006; Luce´lia *et al*., 2011; Bajya *et al*., 2015; Halder *et al*., 2021). Both the biointensive and integrated modules comprised the combinations of entomopathogenic fungi and neem seed oil at half of their recommended doses at 1:1 ratio. Compatibility and synergistic activity of these duo biopesticides against insect pest management are well documented (Depieri *et al*., 2005; Subbulakshmi *et al*., 2012; Halder *et al*., 2018). The plant origin insecticide neem and its derivatives have diverse mode of action like antifeedant, insect growth regulation, oviposition deterrent as well as lethal activity (Chowdhary *et al*., 2001; Prakash *et al*., 2008). The integrated pest management module combining newer green chemistry molecules having different side of actions, entomopathogens and botanicals like neem successfully controlled the nefarious insect pest of tomato by conserving the associated beneficial fauna in tomato ecosystem.

From the table 2 it is shown that maximum CB ratio was obtained from integrated pest management module

followed by biointensive and chemical pest management modules. The module 3 *i.e*., integrated module had registered highest fruit yield which caused its highest CB ratio. Interestingly, the module 1 *i.e.,* biointensive pest management module had the second highest CB ratio of 1: 3.92. Lower cost of IPM inputs like Azadirachtin, neem oil, entomopathogenic fungi *viz*., *Beauveria bassiana*, *Lecanicillium* (=*Verticillium*) *lecanii* etc. compared to newer chemical insecticides could be the reason for higher cost benefit ratio of biointensive pest management module than the corresponding chemical module. In a similar vein, Kumari *et al*., 2021 documented that integrated module (seed treatment with Thiamethoxam 70% WS, removal of damaged cotyledonary leaves, spraying of Emamectin benzoate, spraying of neem oil, installation of cuelure traps, spraying of Spinosad) had recorded highest bitter gourd fruit yield (16 t ha^{-1}) and highest benefit cost ratio (2.61:1) along with lowest fruit fly damage in Hyderabad, India. In another study, in okra the integrated pest management module comprising sprayings of chlorantraniliprole, NSKE, Emamectin benzoate, *Bacillus thuringiensis* and nimbecidine their need based rotation was most effective in reducing the fruit borer damage (71.74 per cent) and yellow vein mosaic disease (17.75 per cent) with significant increase in the yield (177.7 q ha^{-1}) over control (Kodandaram *et al*., 2017).

CONCLUSION

Three different pest management modules were synthesized and evaluated against the major sucking insect pests of tomato. The integrated pest management module comprised spraying of imidacloprid, spiromesifen, indoxacarb, cyantraniliprole, chlorantraniliprole, *Beauveria bassiana* + Neem oil (1:1), *Lecanicillium lecanii* + Neem oil (1:1) and neem seed kernel extract (NSKE) starting from 30 DAT at 10 days intervals each harboured lowest fruit borer incidence accompanied with minimum whiteflies, leaf hoppers and aphid population with maximum PROC. Furthermore, the highest healthy fruit yields were recorded from the integrated pest management module accompanied with higher predatory mirid bugs and polyphagous spider populations. In terms of return, maximum net profit was obtained from this module with highest cost benefit ratio.

ACKNOWLEDGEMENT

The authors are thankful to the Director of ICAR-IIVR, Varanasi for providing the necessary research facilities during the experiments.

REFERENCES

Anonymous. 2018. Horticultural statistics at a glance. Published by Horticulture Statistics Division, Department of Agriculture, Cooperation & Farmers' Welfare, Ministry of Agriculture & Farmers' Welfare, Government of India, 10.

Bajya, D. R., Ranjith, M. and Raza, S. K. 2015. Evaluation of *Beauveria bassiana* against chickpea pod borer, *Helicoverpa armigera* and its safety to natural enemies. *Indian Journal of Agricultural Sciences,* **85** (3): 378–381.

Banik, S. and Halder, J. 2013. Protection of Crop Plants with Newer Chemical Molecules.*In*. Current Concepts in Crop Protection. Edited by Susanta Banik, ISBN: 978-93-80012-42-1, Published by Studium Press (India) Pvt. Ltd., New Delhi-110002, India, 307-349.

Bose, T. K. and Som, M. G. 1990.Vegetable Crops in India. Naya Prokash, 206 Bidhan Sarani, Calcutta, India. 249.

Chowdhary, H., Walia, S. and Dhingra, S. 2001. Bio-efficacy of azadirachtin, turmeric oil and their mixtures against bihar hairy caterpillar, *Spilosoma obliqua* (Walker). *Pesticide Research Journal,* **13** (2): 165–172.

Depieri, R. A., Martinez, S. S. and Menezes, A. O. Jr. 2005. Compatibility of the fungus, *Beauveria bassiana* (Bals.) Vuill. (Deuteromycetes) with extracts of neem seeds and leaves and the emulsible oil. *Neotropical Entomology,* **34** (4): 601–606.

Eken, C., Tozlu, G., Dane, E., Corhu, S. and Demirci, E. 2006. Pathogenicity of *Beauveria bassiana* (Deuteromycotina: Hypomycetes) to larvae of small poplar longhorn beetle, *Saperda populnea* (Coleoptera: Cerambycidae). *Mycopathologia,* **162**: 69–71.

Halder, J. and Rai, A. B. 2021. Emergence of new insect pests on vegetables during the last decade: a case study. *Current Horticulture*, **9** (1): 20-26.

Halder, J., Khushwaha, D., Rai, A. B., Nagendran, K. and Singh, B. 2018. Host plant mediated susceptibility of *Phenacoccus solenopsis* (Tinsley) to *Lecanicillium lecanii* (Zimmermann) Zare and Gams, neem oil and their combination. *Proceedings of National Academy of Sciences,*

India, Section- B: Biological Sciences, **88** (1): 241-248. (DOI 10.1007/s40011-016-0750-y).

Halder, J., Divekar, P. A. and Rani, A.T. 2021. Compatibility of entomopathogenic fungi and botanicals against sucking pests of okra: an ecofriendly approach. *Egyptian Journal of Biological Pest Control*, **31**:30. doi.org/10.1186/s41938-021-00378-6.

Halder, J., Kushwaha, D., Rai, A. B. and Singh, B. 2019. Biology and biorational management of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae): A global challenge to tomato production. *Proceedings of the Zoological Society*, **72** (2): 107-110. (DOI 10.1007/s12595-017- 0232-0).

Halder, J., Majumder, S. and Rai, A. B. 2021. Compatibility and combined efficacy of entomopathogenic fungi and neonicotinoid insecticides against *Myzus persicae* (Sulzer): An ecofriendly approach. *Entomologia Hellenica,* **30** (1): 24–32. https://doi.org/10.12681/eh.25417.

IRAC. 2022. Insecticide Resistance Action Committee (IRAC) mode of action classification scheme. Issued, March 2022; Version 10.2

Kodandaramm, M. H., Rai A. B. and Halder J. 2010. Novel insecticides for management of insect pests in vegetable crops: a Review. *Vegetable Science*, **37**(2): 109-123.

Kodandaram, M. H., Halder, J., Singh, N. and Rai, A. B. 2017. Pest management modules against major insect pests of okra. *Pesticide Research Journal*, **29** (2): 153-157.

Kumari, D. A., Suresh, V., Nayak, M. H., Lavanya, A. V. N. and Mamatha, A. 2021. Evaluation of different pest management modules in bitter gourd. *International Journal of Chemical Studies,* **9**(1): 587-590.

Luce´lia, S., Silva, L. A. D., da Silva, W.O.B., Correˆa, A. P. F., Rangel, D.E.N., Carlini, C.R., Schrank, A. and Vainstein, M. H. 2011.Virulence of the entomopathogenic fungus *Metarhizium anisopliae* using soybean oil formulation for control of the cotton stainer bug, *Dysdercus peruvianus*. *World Journal of Microbiology and Biotechnology,* **27** (10): 2297–2303.

Prakash, A., Rao, J. and Nandagopal, V. 2008. Future of botanical pesticide in rice, wheat, pulses and vegetable pest management. *Journal of Biopesticides,* **1**: 154-169.

Rai, A. B., Loganathan, M., Halder, J., Venkataravanappa, V. and Naik, P. S. 2014. Eco-friendly approaches for sustainable management of vegetable pests. Technical Bulletin, Indian Institute of Vegetable Research, Varanasi - 221305, Uttar Pradesh,104.

Roy, S., Halder, S., Singh, N., Rai, A.B., Prasad, R.N. and Singh, B. 2017. Do vegetable growers really follow the scientific plant protection measures? An empirical study from eastern Uttar Pradesh and Bihar. *Indian Journal of Agricultural Sciences*, **87** (12): 1668–1672.

Subbulakshmi, N., Ramarathinam, S., Anburaj, J., Sundaravadivelan, C., Kuberan, T., Kumar, P., Starlin, T. and Marimuthu, S. 2012. Compatibility of neem based oil nimbicidine with entomopathogenic fungi. *International Journal of Applied Biology,* **4**:12–15.

MS Received: 27 May 2022
MS Accepted : 02 August 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 27-31 (2022)

DOI Number: 10.5958/0974-4541.2022.00034.0

Evaluation of different integrated pest management modules against *Thrips parvispinus* Karny in chilli

Y. LALITHA PRIYA[1], K. SIREESHA[2], N. EMMANUEL[1], B. TANUJA PRIYA[2] and K. UMAKRISHNA[1]

[1,3,]Department of Entomology, [5]Department of Statistics, College of Horticulture, Dr.YSR Horticultural University, Venkataramannagudem-534101, West Godavari, Andhra Pradesh, India

[4]Horticulture Research Station, Dr.YSR Horticultural University, Lam-522034, Guntur, Andhra Pradesh, India

E-mail: lalithayalamati10@gmail.com

ABSTRACT: The present investigation was carried out to evaluate effectiveness of different integrated management strategies against *Thrips parvispinus* Karny. The experiment was conducted at, Horticultural Research Station, Dr. Y.S.R Horticultural University, Lam, Guntur during the year, 2021-22 with three replications in Randomized Block Design. Total seven modules including control were evaluated for management of *Thrips parvispinus*. Results revealed that Module-IV was most effective. Higher chilli yield of 32.22 q/ha with 87.08 % increase over untreated control was observed in M-IV (Mulch + blue sticky traps along with need based application of insecticides viz., fipronil 80WG @ 0.2 g/L, spinetoram 11.7SC @ 1 ml/L, spirotetramat 240SC @ 0.8ml/L, acetamapride 20%SP @ 0.2g/L, thiomethaxam 25%WG @ 0.2g/L, dimethoate 30%EC @ 2ml/L sprayed sequentially at 10 days interval) followed by Module-I and Module-III and they were at par with each other with recorded yield of 30.55 q/ha and 27.77 q/ha respectively with 86.37% and 85.01% increase over control.

Keywords: Chilli, integrated pest management modules, *Thrips parvispinus*

INTRODUCTION

Chilli is an important vegetable and spice crop grown throughout the world and it has immense commercial, dietary and therapeutic values. India is the largest producer, consumer and exporter of chilli in the world and cultivated in an area of 7.32 lakh hectares with a production of 19.88 lakh tonnes in 2020-21. In India, major chilli producing states are Andhra Pradesh, Telangana, Madhya Pradesh, Karnataka and West Bengal. In Andhra Pradesh chilli is cultivated in an area of 1.8 lakh hectares with a production of 8.36 lakh tonnes (2020-21) (www.dasd.gov.in) and contributes 75% to India's Chili exports. Guntur district in Andhra Pradesh alone contributes 30% of total chilli production in India, contributing major share in production and export of chilli (Mehta, 2017). The pest spectrum in chilli is over 293 insects and mite species debilitating the crop in the field as well as in storage (Anon, 1987). Butani (1976) reported more than 20 insect species in chilli from India of which thrips (*Scirtothrips dorsalis*), mites (*Polyphagotarsonemus latus*) and aphids (*Aphis gosypii* and *A. craccivora*) are the most damaging pests. Among the sucking pests, chilli thrips *Scirtothrips dorsalis* (Thripidae: Thysanoptera) was considered as the most important and serious pest as it attacks the crop from nursery till the harvest of the crop. But in the year 2021 a newly introduced invasive pest species *Thrips parvispinus* Karny was observed in chilli fields and subsequently this species has dominated and displaced the well-established chilli thrips, *Scirtothrips dorsalis* which was a regural pest on chilli (Sridhar *et al.,* 2021). *T. parvispinus*, is a member of "Thrips orientalis group" (Mound and Masumoto, 2005) and widespread pest species of quarantine importance. As chilli is being cultivated under high input pressure with huge investment and in larger areas (1.8lakh ha) in Andhra Pradesh most of the farmers worried about the crop loss and financial burden. Keeping this in view present investigation on evaluation of different integrated pest management modules against chilli flower thrips *T. parvispinus* was carried out.

MATERIALS AND METHODS

An experiment was conducted under open field conditions at Horticultural Research Station, Dr. Y.S.R Horticultural University, Lam, Guntur on evaluation of efficiency of different Integrated Pest Management modules against chilli flower thrips. For this experiment, variety LCA-620 was selected. The experiment was laid out in a randomized block design in an area of 500m^2 with seven modules including an untreated check. Experimental area was divided into three blocks and each module was replicated thrice for recording observations

Table 1. Evaluation of different integrated management modules against *Thrips parvispinus*

Module	November (30DAT)	December (60DAT)	January (90DAT)	February (120DAT)	March (150DAT)	Mean
	Thrips population per terminal leaves					
Module I	11.80 (3.58)	22.67 (4.85)	19.43 (4.51)	14.90 (3.98)	10.03 (3.32)	15.77
Module II	8.53 (3.08)	32.63 (5.79)	17.30 (4.27)	17.63 (4.31)	14.10 (3.88)	18.04
Module III	5.33 (2.51)	25.10 (5.10)	15.23 (4.02)	15.40 (4.04)	13.70 (3.83)	14.95
Module IV	11.27 (3.50)	20.07 (4.58)	17.33 (4.28)	13.00 (3.74)	11.93 (3.59)	14.72
Module V	14.20 (3.90)	27.33 (5.31)	21.87 (4.77)	18.90 (4.46)	15.37 (4.04)	19.53
Module VI	12.33 (3.65)	29.30 (5.50)	21.30 (4.72)	17.50 (4.29)	15.00 (3.99)	19.09
Module VII	14.93 (3.99)	34.73 (5.97)	28.97 (5.46)	20.90 (4.67)	16.17 (4.14)	23.14
CD	0.27	0.63	0.38	0.53	0.37	
CV	4.34	6.57	4.61	6.99	5.30	
SE (m)	0.09	0.2	0.12	0.17	0.12	

Table 2. Effect of IPM modules on yield and B:C

IPM module	Yield (q/ha)	increase in yield over control (%)	B:C Ratio
Module I	30.55	86.37	2.78
Module II	23.33	82.16	1.73
Module III	27.77	85.01	2.22
Module IV	32.22	87.08	2.80
Module v	4.72	11.74	0.59
Module VI	7.61	45.27	0.95
Module VII	4.16	0	0.56
C.D.	3.97		
SE(m)	1.28		
SE(d)	1.8		
C.V.	11.87		

and statistical analysis. Chilli seedlings of 45 days old were considered for transplanting. The population count of thrips (*Thrips parvispinus*) was taken at 30, 60, 90, 120 and 150 DAT (Days After Transplanting) and mean population was worked out. For counting thrips ten plants were selected randomly in each plot and tagged. The experiment was laid out with seven modules comprising of physical barriers (Mulch, Blue cover and white Non-oven 17 GSM plant cover made of polypropylene), biopesticides and botanical insecticides along with an untreated check. The IPM modules were randomized completely and each treatment was replicated thrice. For each treatment ten plants were labeled to record observations. The experimental details were as follows.

Treatment details

Module I: Mulch + need based application of insecticides *viz.,* Fipronil 80 WG @ 0.2g/L, Spinetoram 11.7SC @ 1ml/L - Spirotetramat 240SC @ 0.8ml/L - Acetamaprid 20%SP @ 0.2g/L-Thiomethaxam 25%WG @ 0.2g/L - Dimethoate 30%EC@ 2ml/L.

Module II: Mulch + Crop surrounded by plant cover (blue) + Need based application of Insecticides *viz.,* Fipronil 80 WG @ 0.2g/L - Spinetoram 11.7SC @ 1ml/L - Spirotetramat 240SC @ 0.8ml/L - Acetamaprid 20%SP @ 0.2g/L - Thiomethaxam 25%WG@ 0.2g/L - Dimethoate 30% EC @ 2ml/L sprayed sequentially with 10 days interval.

Module III: Mulch + Crop surrounded by plant cover (white) + Need based application of Insecticides *viz.,* Fipronil 80 WG @ 0.2g/L - Spinetoram 11.7SC @ 1ml/L - Spirotetramat 240SC @ 0.8ml/L - Acetamaprid 20%SP @ 0.2g/L - Thiomethaxam 25%WG @ 0.2g/L - Dimethoate 30%EC @ 2ml/L sprayed sequentially with 10 days interval.

Module IV: Mulch + Installation of blue sticky traps.+ Need based application of Insecticides *viz.,* Fipronil 80 WG @ 0.2g/L - Spinetoram 11.7SC @ 1ml/L - Spirotetramat 240SC @ 0.8ml/L -Acetamaprid 20%SP@ 0.2g/L - Thiomethaxam 25%WG @ 0.2g/L - Dimethoate 30%EC @ 2ml/L sprayed sequentially with 10 days interval.

Module V: Application of entomopathogens viz. *Beauveria bassiana*, *Lecanicillium lecani* and *Metarhizium anisopliae* @ 5g per litre applied alternatively at an interval of 10 days.

Module VI: Application of Neem oil @ 1 ml per litre and pongamia oil @ 1 ml per liter applied alone and in combination at an interval of 10days.

Module VII: Untreated check.

Chilli fruit yield

Harvesting of red chilli was done during 2022 Rabi season. The total fruit yield from each plot was taken and expressed in terms of dry chilli fruit yield per hectare basis and subjected for statistical analysis. Benefit cost ratio was calculated for all treatments.

Statistical analysis

Data obtained from management studies was subjected to suitable transformation and were analyzed by using ANOVA.

RESULTS AND DISCUSSION

Results (Table 1) indicated that during the vegetative stage (30DAT) Module III- {Mulch + Crop surrounded by plant cover (White) + Need based application of Insecticides *viz.,* Fipronil 80 WG @ 0.2g/L - Spinetoram 11.7SC @ 1ml/L - Spirotetramat 240 SC @ 0.8ml/L - Acetamaprid 20% SP @ 0.2g/L - Thiomethaxam 25% WG @ 0.2g/L - Dimethoate 30% EC @ 2ml/L } was effective in reducing the thrips population (5.33/terminal) followed by Module II- {Mulch + Crop surrounded by plant cover (Blue) + Need based application of Insecticides} (8.53/terminal). These two modules i.e., Module III and Module II were followed by Module IV {Mulch with blue sticky traps along need based application of Insecticides}, Module I - {Mulch+ Need based application of Insecticides}, Module VI {Application of Neem oil @ 1 ml per lit and pongamia oil @ 1 ml per liter applied alone and in combination} and these three modules were found to be at par with each other.

Similarly 60 DAT data indicated that that Module IV consisting mulch and blue sticky traps found to be effective over other treatments and which was at par with Module I - {Mulch+ Need based application of Insecticides} and Module III - {Mulch + Crop surrounded by plant cover (White) + Need based application of Insecticides}. Results after 90 DAT showed that the three modules Module III, Module IV, Module II were found to be superior over other treatments and were on par with each other. These three treatments were closely followed by Module I - {Mulch+ Need based application of Insecticides}.

After 120 DAT Module IV {Mulch + Installation of blue sticky traps.+ Need based application of Insecticides} was found to be effective in reducing the thrips population (13.00/terminal) which was at par with Module I (14.90/terminal) and Module III (15.40/terminal) all these three treatments Module IV, I, III were at par with each other.

At 150 DAT it was observed that the treatment Module I - {Mulch alone with need based application of Insecticides} 10.03/terminal was found to be effective which was at par with Module IV - {Mulch + blue sticky traps along with need based application of Insecticides} 11.93/terminal. However the thrips population was declined towards the month of March. Module II - {Mulch + Crop surrounded by blue cover (physical barrier) + Need based application of Insecticides} and Module III- {Mulch + Crop surrounded by plant cover (white) + Need based application of Insecticides} found to be effective upto vegetative stage of the crop growth. In case of Module II and Module III the plants nearer to the barrier showed change in height of plant due to difference in light intensity otherwise no difference was observed in plant growth compared to open field conditions. Once after reaching the flowering and fruiting stage no difference was observed between treatments with physical barriers and open field conditions. Salas *et al.* (2015) studied the effect of physical barrier and insect growth regulator on whitefly population and yield of pepper and observed that 50 mesh nylon net was effective in preventing the entry of whitefly and also observed that 27% decline in chilli yield. Scott *et al.* (1959) reported effectiveness of aluminium coloured mulch in *Frankiniella fusca, F.tritici* and *Scirtothrips dorsalis* in tomato. Greenough (1985) also reported significantly fewer thrips on tomato crop grown on aluminium coloured mulch. In the present study

Table 3. Effect of IPM modules on natural enemies

IPM module	Spiders / plant			Chrysopids / plant		
	60DAT	90DAT	Mean	60DAT	90DAT	Mean
Module I	0.4 (1.18)	0.30 (1.14)	0.35 (1.16)	0.13 (1.06)	0.03 (1.02)	0.08 (1.04)
Module II	0.43 (1.20)	0.23 (1.11)	0.33 (1.16)	0.27 (1.13)	0.13 (1.06)	0.20 (1.10)
Module III	0.53 (1.24)	0.33 (1.15)	0.43 (1.20)	0.20 (1.10)	0.10 (1.05)	0.15 (1.08)
Module IV	0.53 (1.24)	0.30 (1.14)	0.42 (1.19)	0.10 (1.05)	0.10 (1.05)	0.10 (1.05)
Module v	0.60 (1.26)	0.30 (1.14)	0.30 (1.20)	0.30 (1.14)	0.23 (1.11)	0.27 (1.13)
Module VI	0.73 (1.32)	0.37 (1.17)	0.55 (1.25)	0.17 (1.08)	0.17 (1.08)	0.17 (1.08)
Module VII	0.50 (1.22)	0.23 (1.11)	0.37 (1.17)	0.20 (1.10)	0.07 (1.03)	0.14 (1.07)
SE(m)	0.032	0.027	0.022	0.022	0.032	0.027
C.D.	NS	NS	NS	NS	NS	NS

Module IV {Mulch + blue sticky traps along with need based application of Insecticides) recorded significantly less thrips population which was at par with Module I – {Mulch+ Need based application of Insecticides} and Module II - {Mulch + Crop surrounded by plant cover (blue) + Need based application of Insecticides} compared to other Module V – {Application of entomopathogens *viz., Beauveria bassiana*, *Lecanicillium lecani* and *Metarhizium anisopliae*} was found to be less effective it may be due to lack of congenial conditions for their multiplication and due to severe pest build up. Present findings are in line with the observations made by Devi and Roy, (2017) stated that blue sticky traps can be use for mass trapping and monitoring of *Thrips tabaci* as a part of IPM component. Though the Module V could reduce the population but it was not as good as other modules, still this can be included in the management of *Thrips parvispinus* keeping in view of repellent action and safer to environment. In IPM module the treatments with entomopathogens or biopesticides like neem and pongamia oil can be used either in combination or can be alternated with pesticides to manage the resistance buildup in the pest population. Similar observations were made by Kardinam and Maris, (2021) on the ability of biopesticides to reduce the intensity of pest attack and also observed the inability of biological pesticides in reducing pest population of thrips. Kurbett *et al.* (2018) studied the effectiveness of different IPM modules and found that the chemi-intensive was most effective.

Yield

The results on fruit yield showed that Module IV - {Mulch + blue sticky traps along with need based application of Insecticides} has highest yield of 32.22 q/ha with 87.08 % increase over untreated control. Further, Module I - {Mulch+ Need based application of Insecticides} (30.55 q/ha) and Module III- {Mulch + Crop surrounded by plant cover (white) + Need based application of Insecticides} (27.77 q/ha) were equally effective with 86.37% and 85.01% increase over untreated control followed by Module II - {Mulch + Crop surrounded by blue cover (physical barrier) + Need based application of Insecticides} with 23.33 q/ha with 82.16% increase over untreated control. Module VI {Application of Neem oil @ 1 ml per lit and pongamia oil @ 1 ml per liter applied alone and in combination} (7.61 q/ha) with 45.27% and Module V – {Application of entomopathogens *viz., Beauveria bassiana*, *Lecanicillium lecani* and *Metarhizium anisopliae*} recorded lowest yield 4.72 q/ha with 11.74% increase over control but statistically on par with control (4.16) from (Table 2).

Natural enemies

Natural enemies spiders and chrysopids were observed in the crop ecosystem and recorded the data at 60DAT and 90DAT on number of spiders and chrysopids observed per module. Results stated that their was significant difference among the population of natural enemies with regard to

IPM modules and DAT since very fewer population was present. Stating that IPM modules has no effect on the natural enemies population (Table 3).

Economics

Among the modules, highest B: C ratio (2.80) was obtained with Module IV - {Mulch + blue sticky traps along with need based application of Insecticides} with a net return of Rs. 414440 which also obtained the highest yield (32.22 q/ha) when compared to other modules, followed by Module I - {Mulch+ Need based application of Insecticides} with B: C ratio of 2.78 with net returns of 391000. Based on the results with Module IV - {Mulch + blue sticky traps along with need based application of Insecticides *viz.,* Fipronil 80 WG @ 0.2g/L - Spinetoram 11.7SC @ 1ml/L - Spirotetramat 240 SC @ 0.8ml/L - Acetamaprid 20% SP @ 0.2g/L - Thiomethaxam 25% WG @ 0.2g/L - Dimethoate 30% EC @ 2ml/L} has been considered as effective for IPM of flower thrips in chilli (Table 3).

CONCLUSION

Based on experimental results it can be concluded that the treatments with Mulching and need based application of insecticides proved to be better compared to biopesticides and neem oil or pongamia oil alone or in combination. Hence, for the management of *Thrips parvispinus* Integrated Pest Management module consisting of mulch + Installation of blue sticky traps with need based application of insecticides proved to be effective instead of depending solely on spraying of insecticides.

REFERENCES

Anonymous, 1987. Progress Report 1987 for Asian Vegetable Research and Development centre, Taiwan. 77-99.

Butani, D. K. 1976. Pest and diseases of chillies and their control. Pesticides, **10**: 38-41.

Devi, M. S. and Roy, K. 2017. Comparable study on different coloured sticky traps for catching of onion thrips, *Thrips tabaci* Lindeman. *Journal of Entomology and Zoology Studies*, **5** (2): 669-671.

Greenough, D. R. 1985. Aluminum-surfaced mulch and disease resistance: Approaches to the control of tomato spotted wilt virus in solanaceous crops. MS Thesis, Louisiana State Univ., Baton Rouge.

Kardinan, A. and Maris, P. 2021. Effect of biopesticides against stem borer (*Lophobaris piperis*) and *Thrips sp*. on pepper (*Piper nigrum) E3S Web of Conferences*. 306, 01022.

Kurbett, A., Gopali, J. B., Allolli, T. B., Patil, S. and Kumar, V. and Kurubetta, K. 2018. Evaluation of different IPM modules against pest complex of Chilli (cv. *Byadgi dabbi*). Journal of entomology and zoology studies, **6**: 1991-1996.

Mehta, I. 2017. Chillies the Prime Spice a History. *IOSR Journal of Humanities and Social Science* (IOSR-JHSS). 22.**7**: 32-36.

Mound, L. A and Masumoto, M. 2005. The genus Thrips (Thysanoptera, Thripidae) in Australia, New Caledonia and New Zealand, *Zootaxa*,1020: 1-64.

Salas, R. A., Gonzaga, Z. C., Wu, D. L., Luther, G., Gniff ke, P. A. and Palada, M.C. 2015. Effects of physical barrier and insect growth regulator on whitefly control and yield of chili pepper (Capsicum annuum L.). *Journal of Food and Nutrition Sciences*, **3**(1-2): 13-19.

Scott, S. J., McLeod, P. J., Montgomery, F. W. and Handler. C. A. 1989. Influence of reflective mulch on incidence of thrips (Thysanoptera: Thripidae: Phlaeothripidae) in staked tomatoes. *Journal of Entomology Science*, **24**(4):422-427.

Sridhar, V., Rachana, R., Prasannakumar,N. R., Venkataravanappa, V., Sireesha, K.,Naik, O. S., Sreechandana, P. and Reddy, M. K. 2021. Dominance of invasive thrips, *Thrips parvispinus* (Karny) over existing chilli thrips, *Scirtothrips dorsalis* Hood on chilli in the southern states of India with a note on its host range: A likely case of species displacement. *Pest Management in Horticultural Ecosystems*, **27** (2): 132-136

MS Received: 08 August 2022
MS Accepted : 10 October 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 32-38 (2022)

DOI Number: 10.5958/0974-4541.2022.00035.2

Field efficacy of different biorationals and insecticides against brinjal shoot and fruit borer (*Leucinodes orbonalis* Guenee) under *terai* region of West Bengal

SURAJ SARKAR*[1], SUPRAKASH PAL[2], SHYAMAL SAHOO[3], NRIPENDRA LASKAR[3] and JOYDEB GHOSH[3]

[1]Cooch Behar Krishi Vigyan Kendra, [2]Regional Research Station (TZ), Directorate of Research [3]Department of Entomology, Faculty of Agriculture

UBKV, Pundibari, Cooch Behar, West Bengal, India

***E-mail**: surajskrento@gmail.com

ABSTRACT: Field efficacy of seven insecticides, including biorationals, was evaluated against *Leucinodes orbonalis* (Guenee) at the teaching farm, Uttar Banga Krishi Viswavidyalaya, during the two consecutive *rabi* seasons of 2018–19 and 2019–20. The results showed that chlorantraniliprole 18.5 SC, flubendiamide 39.35 SC, and novaluron 5.25% + emamectin benzoate 0.9% SC were the most successful treatments resulted in the lowest shoot infestation (2.24-6.05%) and fruit infestation (number basis: 11.01-13.29% and weight basis: 11.94-15.75%). *Bacillus thuringiensis* var. *kurstaki* and chlorantraniliprole 18.5 SC both produced the highest commercial fruit yields, ranging from 13.54 to 14.54 t/ha and 14.11 to 14.51 t/ha, respectively. Azadirachtin 50,000 ppm was the least effective among the tested insecticides against brinjal shoot and fruit borer.

Keywords: Brinjal, shoot and fruit borer*, Leucinodes,* IPM, biorational, bioefficacy

INTRODUCTION

Brinjal (*Solanum melongena* L.) is one of the most important vegetables cultivated worldwide and one of essential staple vegetables in Asian countries, particularly India. The insect infestation resulted in 70 to 92 percent yield loss in brinjal. There are about 140 different insect pests that attack brinjal, among which the shoot and fruit borer, *Leucinodes orbonalis* (Guenne) (Lepidoptera: Crambidae) (Ghosh and Senapati, 2009). Farmers typically rely on synthetic insecticides to treat pest problems because they produce quick results (Misra, 2008). These compounds have brought issues of pesticide resistance, persistent toxicity, optional nuisance outbreak, ecological devastation, and toxicity to beneficial insects. Additionally, it has been reported that *L. orbonalis* has developed resistance to the most commonly used insecticides due to irregular insecticide usage (Hegde *et al*., 2009). Therefore, it is necessary to look for safer and alternative methods (Gowda *et al*., 2017). The problems brought on by the misuse of chemical sprays can be reduced by incorporating biorationals. This served as the backdrop for the current study, which examined the field effectiveness of various biorational pesticides compared to chemical insecticides against brinjal shoot and fruit borer.

MATERIALS AND METHODS

The field study was conducted in the instructional farm in Uttar Banga Krishi Viswavidyalaya, Cooch Behar, West Bengal, during the *rabi* seasons of 2018–19 and 2019–20. The evaluation consists of seven insecticides and biorationals (azadirachtin 50,000 ppm, flubendiamide 39.35 SC, novaluron 5.25% + emamectin benzoate 0.9% SC, spinosad 45 SC, *Bacillus thuringiensis* var. *kurstaki*, and chlorantraniliprole 18.5 SC) and replications thrice in a randomized block design. 21-day-old seedlings of the 'lopcha' transplanted plots of 5 x 3 m dimension with a spacing of 60 x 50 cm. All the crop management practices were ensured for raising a healthy crop, except for plant protection measures. In each plot, five plants were randomly tagged and used to record the pest observations. Two sprays were carried out from 60 days following seeding at an interval of 15 days. The injured shoots were observed on the tagged plants one day before the application and three, seven, and ten days after spraying. The mean number of injured shoots per plot was calculated and expressed in percentage to determine the extent of the shoot damage. The fruits from brinjal plants were picked at an interval every two weeks, and the total number of injured fruits from each plot was counted and expressed in percentage. The fruit yield per plot was recorded and converted into per hectare. The data were subjected to appropriate transformation and analysed in OPSTAT statistical software.

RESULTS AND DISCUSSION

During 2018–19, the percent shoot damage ranged from 25.00 to 33.39% one day before insecticide

Table 1. Effect of different biorationals and insecticides on shoot damage due to *L. orbonalis* in brinjal (2018-19)

Tr. No.	Treatment	Dose	Shoot infestation (%) days after first spray				Shoot infestation (%) days after second spray			
			1 DBS	3 DAS	7 DAS	10 DAS	1 DBS	3 DAS	7 DAS	10 DAS
T1	*Bacillus thuringiensis* var. *kurstaki*	2g/l	25.49 (30.25) *	23.73 (29.02)	20.30 (26.70)	17.45 (24.62)	16.11 (23.65)	14.37 (22.24)	10.17 (18.56)	6.19 (14.35)
T2	Chlorantraniliprole 18.5SC	0.3 ml/l	28.41 (32.17)	21.11 (27.29)	17.08 (24.38)	13.22 (21.24)	12.53 (20.7)	10.11 (18.49)	3.84 (11.16)	2.24 (6.94)
T3	Spinosad 45 SC	1 ml/l	29.98 (33.12)	27.17 (31.34)	25.09 (30.04)	19.69 (26.33)	15.14 (22.86)	13.55 (21.56)	8.57 (16.96)	4.71 (12.22)
T4	Flubendiamide 39.35 SC	0.2 ml/l	28.08 (31.95)	22.04 (27.92)	18.52 (25.47)	11.06 (19.35)	16.56 (23.93)	11.88 (20.12)	7.46 (15.69)	5.79 (13.85)
T5	Novaluron 5.25% + Emamectin Benzoate 0.9% SC	1.5 ml/l	25.88 (30.54)	21.49 (27.56)	17.21 (24.48)	10.92 (19.27)	13.34 (21.38)	10.95 (19.11)	5.27 (13.23)	3.10 (8.30)
T6	Azadirachtin 50,000 ppm	3 ml/ litre	33.39 (35.26)	29.62 (32.95)	30.06 (33.21)	22.14 (28.04)	29.28 (32.72)	26.57 (30.98)	26.24 (30.74)	21.20 (27.36)
T7	Control (Water Spray)	-	25.00 (29.96)	31.54 (34.13)	23.05 (28.60)	29.21 (32.63)	30.55 (33.53)	28.35 (32.13)	29.95 (33.06)	29.61 (32.94)
	S.E. ±	-	1.34	1.55	1.09	1.09	1.09	1.35	1.44	2.29
	C.D. at 5%	-	NS	4.83	3.38	3.40	3.39	4.22	4.48	7.12

*Figures in parentheses are angular transformed values

Table 2. Bioefficacy of different biorationals and insecticides against shoot damage due to *L. orbonalis* in brinjal (First and second spraying-2019-20)

Tr. No.	Treatment	Dose	Shoot infestation (%) days after first spray				Shoot infestation (%) days after second spray			
			1 DBS	3 DAS	7 DAS	10 DAS	1 DBS	3 DAS	7 DAS	10 DAS
T1	*Bacillus thuringiensis* var. *kurstaki*	2g/l	28.22 (32.06)*	25.59 (30.30)	21.15 (27.32)	22.01 (27.91)	22.86 (28.54)*	17.37 (24.62)	13.28 (21.35)	11.63 (19.93)
T2	Chlorantraniliprole 18.5SC	0.3 ml/l	27.57 (31.6)	20.33 (26.78)	15.51 (23.17)	12.43 (20.61)	13.20 (21.27)	10.08 (18.48)	8.34 (16.65)	6.05 (14.18)
T3	Spinosad 45 SC	1 ml/l	31.02 (33.8)	28.21 (31.99)	23.32 (28.85)	17.92 (25.02)	18.90 (25.7)	15.24 (22.90)	12.43 (20.61)	10.85 (19.20)
T4	Flubendiamide 39.35 SC	0.2 ml/l	29.84 (33.1)	21.17 (27.29)	16.67 (24.08)	12.92 (20.78)	13.60 (21.6)	12.58 (20.75)	9.09 (17.50)	7.44 (15.82)
T5	Novaluron 5.25% + Emamectin Benzoate 0.9% SC	1.5 ml/l	27.96 (31.9)	24.29 (29.47)	17.70 (24.87)	16.38 (23.83)	17.20 (24.4)	14.32 (22.17)	11.32 (19.61)	9.20 (17.29)
T6	Azadirachtin 50,000 ppm	3 ml/l	33.39 (35.3)	30.90 (33.74)	29.18 (32.68)	28.33 (32.10)	29.21 (32.7)	23.90 (29.25)	24.57 (29.67)	24.66 (29.75)
T7	Control (Water Spray)	-	28.23 (32.00)	36.05 (36.87)	38.31 (38.21)	39.54 (38.93)	40.42 (39.4)	41.67 (40.18)	41.73 (40.22)	43.11 (41.01)
	S.E. ±	-	1.66	1.44	0.85	1.39	1.00	0.87	1.13	1.30
	C.D. at 5%	-	NS	4.49	2.64	4.32	3.20	2.70	3.51	4.06

*Figures in parentheses are angular transformed values

Table 3. Bioefficacy of different biorationals and insecticides against fruit damage (number basis) due to *L. orbonalis* in brinjal (2018-19 and 2019-20)

Tr. No.	Treatment	Dose	Mean per cent fruit infestation after each picking (Number Basis) during 2018-19			Mean per cent fruit infestation after each picking (Number Basis) during 2019-20		
			1st Picking	2nd picking	3rd picking	1st Picking	2nd picking	3rd picking
T1	*Bacillus thuringiensis* var. *kurstaki*	2g/l	19.60 (26.16) *	21.60 (27.24)	23.16 (28.54)	23.89 (29.09)*	25.42 (30.22)	28.69 (32.30)
T2	Chlorantraniliprole 18.5SC	0.3 ml/l	12.41 (20.24)	11.01 (19.24)	11.21 (19.34)	13.29 (20.77)	13.10 (21.14)	12.23 (20.04)
T3	Spinosad 45 SC	1 ml/l	18.45 (25.07)	29.48 (32.86)	33.26 (35.16)	18.28 (25.29)	27.08 (31.24)	26.70 (31.08)
T4	Flubendiamide 39.35 SC	0.2 ml/l	15.72 (23.23)	28.83 (32.35)	29.47 (32.85)	14.39 (18.40)	23.91 (29.23)	17.97 (25.04)
T5	Novaluron 5.25% + Emamectin Benzoate0.9% SC	1.5 ml/l	17.86 (20.76)	23.96 (29.28)	24.22 (29.46)	20.63 (22.51)	23.30 (28.66)	22.01 (27.92)
T6	Azadirachtin 50,000 ppm	3 ml/l	26.29 (30.47)	31.52 (34.02)	35.01 (36.25)	31.82 (34.11)	26.35 (30.83)	29.98 (33.19)
T7	Control (Water Spray)	-	35.84 (36.75)	39.12 (38.69)	44.59 (41.87)	42.93 (40.87)	37.36 (37.66)	41.33 (39.98)
	S.E. ±	-	3.94	2.24	1.79	6.01	1.86	1.79
	C.D. at 5%	-	NS	6.96	5.59	NS	5.79	5.57
	C.V. (%)	-	26.15	12.68	9.74	38.14	10.79	10.36

application (Table 1). Chlorantraniliprole 18.5 SC recorded the lowest shoot infestation at 3 days after spraying (DAS), followed by novaluron 5.25% + emamectin benzoate 0.9% SC, flubendiamide 39.35 SC, and *B. thuringiensis* var. *kurstaki*, however, the results are comparable with each other. The percent shoot damage at 7 DAS varied from 17.08 to 30.06 %, and application of novaluron 5.25% + emamectin benzoate 0.9% SC and chlorantraniliprole 18.5SC showed the lowest shoot damage compared to untreated control plots. During 10 DAS, flubendiamide 39.35 SC (11.06%), novaluron 5.25% + emamectin benzoate 0.9% SC (10.92%) showed the lowest shoot infestation rates.

Similarly, during the second spraying in 2018–19, the percent shoot damage ranged between 12.53– 30.55 (Table 1). The lowest shoot infestation was recorded at 3 DAS in chlorantraniliprole 18.5 SC (10.11%), closely followed by novaluron 5.25% + emamectin benzoate 0.9% SC (10.95%). The same trends were seen at 10 DAS, when plots treated with chlorantraniliprole 18.5 SC recorded the lowest shoot infestation (2.24%), followed by plots treated with novaluron 5.25% + emamectin benzoate 0.9% SC (3.10%), Spinosad 45 SC (4.71%), Flubendiamide 39.35 SC (5.79%), and *Bacillus thuringiensis* var. *kurstaki* (6.19%). All five of the study's treatments, except azadirachtin 50,000 ppm, offered more significant control over untreated plots (29.61 %) 10 days after the second spraying.

During 2019–20, the % shoot damage before the first spraying ranged from 27.57 to 33.39 (Table 2). The percent shoot damage ranged from 20.33 to 36.05 percent at 3 DAS. The application of flubendiamide 39.35 SC (21.17%), novaluron 5.25% + emamectin benzoate 0.9% SC (24.29%), *Bacillus thuringiensis* var. *kurstaki* (25.59%) and chlorantraniliprole 18.5 SC (20.33%) had comparable bioefficacy. At 7 DAS, flubendiamide 39.35 SC (16.67%) and chlorantraniliprole 18.5 SC (15.51%) recorded the lowest shoot infestation. The range of the shoot damage percentage at 10 DAS was 12.43 to 39.54. The lowest shoot infestation (12.43%) was recorded by chlorantraniliprole 18.5 SC, followed by flubendiamide 39.35 SC (12.92%).

Similarly, during the second spraying of 2019–20, the range of the percent shoot damage was 13.20–40.42. (Table 2). The chlorantraniliprole 18.5 SC reported the

Table 4. Bioefficacy of different biorationals and insecticides against fruit damage (weight basis) due to *L. orbonalis* in brinjal (2018-19 and 2019-20)

Tr. No.	Treatments	Dose	Mean per cent fruit infestation after each picking (Weight Basis) during 2018-19			Mean per cent fruit infestation after each picking (Weight Basis) during 2019-20		
			1st Picking	2nd picking	3rd picking	1st Picking	2nd picking	3rd picking
T1	*Bacillus thuringiensis* var. kurstaki	2g/l	23.02 (28.61) *	20.10 (26.61)	21.11 (27.33)	18.22 (25.24)	16.47 (23.90)	17.20 (24.49)
T2	Chlorantraniliprole 18.5SC	0.3 ml/l	15.78 (23.39)	14.04 (21.99)	13.98 (21.93)	12.20 (20.42)	11.94 (20.20)	12.38 (20.58)
T3	Spinosad 45 SC	1 ml/l	19.70 (26.31)	17.31 (24.58)	18.49 (25.45)	15.96 (23.51)	15.70 (23.33)	15.95 (23.52)
T4	Flubendiamide 39.35 SC	0.2 ml/l	17.29 (24.56)	16.54 (23.98)	17.77 (24.92)	14.93 (22.69)	14.51 (22.36)	14.33 (22.24)
T5	Novaluron 5.25% + Emamectin Benzoate 0.9% SC	1.5 ml/l	18.27 (25.27)	17.42 (24.63)	17.40 (24.64)	16.19 (23.71)	15.22 (22.94)	15.06 (22.81)
T6	Azadirachtin 50,000 ppm	3 ml/l	25.29 (30.17)	24.06 (29.35)	24.25 (29.45)	18.54 (25.49)	17.40 (24.64)	19.04 (25.85)
T7	Control (Water Spray)	-	35.18 (36.33)	37.62 (37.82)	37.95 (38.00)	28.19 (32.06)	28.98 (32.50)	30.91 (33.75)
	S.E. ±	-	1.08	0.67	0.83	0.62	0.87	0.45
	C.D. at 5%	-	3.39	2.08	2.58	1.94	2.72	1.41
	C.V. (%)	-	6.77	4.28	5.24	4.36	6.23	3.18

lowest shoot infestation at 3 and 7 DAS treatments (10.08% and 8.34%), followed by flubendiamide 39.35 SC (12.58% and 9.09%). Chlorantraniliprole 18.5 C (6.05%) had the lowest shoot infestation at 10 days after spraying, followed by flubendiamide 39.35 SC (7.44%). The findings are consistent with the results of Misra (2011), who reported that chlorantraniliprole at 40 and 50 g a.i./ha was the most effective against the brinjal shoot and fruit borer, reducing shoot damage by 95–97 %. Anil and Sharma (2011) also documented that the application of emamectin benzoate, novaluron, and spinosad resulted in 0.56, 0.96, and 1.25 percent shoot damage, respectively. Shirale *et al*. (2012) tested the effectiveness of new-generation insecticides against BFSB. They found that the plots sprayed with chlorantraniliprole 18.50% SC and flubendiamide 39.35% SC had the least percentage of fruit damage.

According to Swini Reddy and Kumar (2022), flubendiamide, emamectin benzoate, and chlorantraniliprole had the lowest rates of shoot infestation. Further, they also noted that Azadirachtin had shown the lowest effectiveness in suppressing BSFB, whereas spinosad offered a moderate level of control.

During 2019–20, the % shoot damage before the first spraying ranged from 27.57 to 33.39 (Table 2). The percent shoot damage ranged from 20.33 to 36.05 percent at 3 DAS. The application of flubendiamide 39.35 SC (21.17%), novaluron 5.25% + emamectin benzoate 0.9% SC (24.29%), *Bacillus thuringiensis* var. *kurstaki* (25.59%) and chlorantraniliprole 18.5 SC (20.33%) had comparable bioefficacy. At 7 DAS, flubendiamide 39.35 SC (16.67%) and chlorantraniliprole 18.5 SC (15.51%) recorded the lowest shoot infestation. The range of the shoot damage percentage at 10 DAS was 12.43 to 39.54. The lowest shoot infestation (12.43%) was recorded by chlorantraniliprole 18.5 SC, followed by flubendiamide 39.35 SC (12.92%).

Similarly, during the second spraying of 2019–20, the range of the percent shoot damage was 13.20–40.42. (Table 2). The chlorantraniliprole 18.5 SC reported the lowest shoot infestation at 3 and 7 DAS treatments (10.08% and 8.34%), followed by flubendiamide 39.35 SC (12.58% and 9.09%). Chlorantraniliprole 18.5 C

(6.05%) had the lowest shoot infestation at 10 days after spraying, followed by flubendiamide 39.35 SC (7.44%). The findings are consistent with the results of Misra (2011), who reported that chlorantraniliprole at 40 and 50 g a.i./ha was the most effective against the brinjal shoot and fruit borer, reducing shoot damage by 95–97 %. Anil and Sharma (2011) also documented that the application of emamectin benzoate, novaluron, and spinosad resulted in 0.56, 0.96, and 1.25 percent shoot damage, respectively. Shirale *et al*. (2012) tested the effectiveness of new-generation insecticides against BFSB. They found that the plots sprayed with chlorantraniliprole 18.50% SC and flubendiamide 39.35% SC had the least percentage of fruit damage. According to Swini Reddy and Kumar (2022), flubendiamide, emamectin benzoate, and chlorantraniliprole had the lowest rates of shoot infestation. Further, they also noted that Azadirachtin had shown the lowest effectiveness in suppressing BSFB, whereas spinosad offered a moderate level of control.

At first harvest/ picking during 2018–19, the percentage of fruits with infestation ranged from 12.41 to 35.84. (Table 3). All treatments outperformed the untreated control group, although there was no discernible difference between them. *Bacillus thuringiensis* var. *kurstaki* (21.60%) and chlorantraniliprole 18.5SC (11.01%) produced the best results at second pickings. Chlorantraniliprole 18.5SC, *Bacillus thuringiensis* var. *kurstaki*, and novaluron 5.25% + emamectin benzoate 0.9% SC substantially differed from the untreated control group. However, the outcomes from chlorantraniliprole 18.5SC were outstanding and far superior to those of all other treatments, including the untreated control. The percent fruit damage during third picking showed a similar pattern, with chlorantraniliprole 18.5SC recording the lowest mean percent fruit damage (11.21%), which was significantly better than all other treatments. Yousafi *et al*. (2015) recommended spinosad, flubendiamide, and emamectin benzoate to treat BFSB. Similarly, Vinayaka *et al.* (2019) also reported that the emamectin benzoate 5% SG and chlorantraniliprole 18.5% SC were most effective against BSFB. The insecticides *Bacillus thuringiensis* 5% WP and Azadirachtin 5% EC were shown to be the least effective against BFSB, whereas Spinosad 45% SC was found to be fairly effective. Saran *et al.* (2018) reported spinosad 45 SC @ 200 ml/ha, emamectin benzoate 5 SG @ 200 gm/ha, and chlorantraniliprole 20 SC @ 150 ml/ ha were found to be the most effective in lowering the incidence of the shoot and fruit borer.

After the first picking in 2019–20, the percentage of infested fruit (number of fruit basis) varied from 13.29 to 42.93 (Table 3). During second picking, the lowest percentage of fruit infection was found in chlorantraniliprole 18.5 SC treated plots (13.10%), followed by novaluron 5.25% + emamectin benzoate 0.9% SC (23.30%), flubendiamide 39.35 SC (23.91%), and *Bacillus thuringiensis* var. *kurstaki* (25.42%). Similar patterns emerged after the third picking, in which chlorantraniliprole 18.5 SC (12.23%), flubendiamide 39.35 SC (17.97%), and novaluron 5.25% + emamectin benzoate 0.9% SC (22.01 %) offered the best management in terms of lowest percent fruit infestation. After initial picking, the mean percent of fruit infection on a fruit weight basis ranged from 15.78 to 35.18 percent in the 2018–19 growing season (Table 4). On the fruit weight basis, chlorantraniliprole 18.5 SC treated plots showed the lowest percentage of fruit infestation (15.78 %), followed by flubendiamide 39.35 SC (17.29%), novaluron 5.25% + emamectin benzoate 0.9% SC (18.27%), and spinosad 45% SC (19.70%). After the second picking, spinosad 45% SC (17.31%), flubendiamide 39.35 SC (16.54%), and chlorantraniliprole 18.5 SC (14.04%) reported the lowest percentage of fruit infection based on fruit weight. After the third picking, a similar pattern was observed.

During 2019–20, after first picking, the mean percent of fruit infection on a fruit weight basis ranged from 12.20 to 28.19% (Table 4). On a fruit weight basis, chlorantraniliprole 18.5 SC treated plots had the lowest percentage of infested fruit (12.20%), followed by flubendiamide 39.35 SC (14.93%) and spinosad 45% SC (15.96%). The present study's findings show that based on the percent fruit damage (weight basis), chlorantraniliprole 18.5 SC had the most significant outcomes. Mainali *et al.* (2015) also recorded that plots treated with spinosad and chlorantraniliprole had the lowest mean fruit infection rates. Kameshwaran and Kumar (2015) reported that the plots treated with emamectin benzoate 25 WG @ 11 g a.i./ha and chlorantraniliprole 20 SC @ 40 g a.i./ha had the least amount of damage due to BSFB.

In both years, the yield of brinjal fruits differed significantly between different treatments at each of the three picking times. In 2018–19 and 2019–20, the yield varied between 11.48 and 14.11 t/ha and 10.50 and 14.67 t/ha, respectively. The chlorantraniliprole 18.5SC treated plots produced the highest overall yield in 2018–19 (14.11 t/ha), followed by *Bacillus thuringiensis* var. *kurstaki* (13.54 t/ha). The plots treated with flubendiamide 39.35 SC had the highest yield (14.67 t/ha) during 2019–20, followed by those treated with *Bacillus thuringiensis* var. *kurstaki* (14.54 t/ha). Similar findings were reported by Mainali *et al.* (2015), who claimed that the chlorantraniliprole treated plots had the highest marketable yield (32.03 mt/ha), followed by spinosad (30.93 mt/ha), with increases in marketable

Table 5. Yield of brinjal recorded in different biorational treatments in 2018-19 and 2019-20

Tr. No.	Treatments	Yield (t/ha) during 2018-19			Total Yield (t/ha)	Yield (t/ha) during 2019-20			Total Yield (t/ha)
		1st Picking	2nd picking	3rd picking		1st Picking	2nd picking	3rd picking	
T1	*Bacillus thuringiensis* var. kurstaki	4.22	4.32	5.00	13.54	4.30	5.00	5.24	14.54
T2	Chlorantraniliprole 18.5SC	4.28	4.49	5.34	14.11	4.17	5.02	5.32	14.51
T3	Spinosad 45 SC	3.57	4.30	5.08	12.95	4.40	4.58	5.21	14.19
T4	Flubendiamide 39.35 SC	4.00	4.16	4.15	12.31	4.53	5.06	5.08	14.67
T5	Novaluron 5.25% + Emamectin Benzoate 0.9% SC	3.83	4.03	4.79	12.65	4.02	4.58	5.07	13.67
T6	Azadirachtin 50,000 ppm	3.79	3.95	4.13	11.87	4.26	4.93	4.85	14.04
T7	Control (Water Spray)	3.62	3.84	4.02	11.48	3.19	3.64	3.67	10.50
	S.E. ±	0.14	0.08	0.12	-	0.12	0.16	0.08	-
	C.D. at 5%	0.44	0.24	0.37	-	0.37	0.49	0.26	-

fruit yield of 34.39 percent and 29.77 percent over the untreated check, respectively. Sarnabati and Ray (2017) noted that plots treated with chlorantraniliprole produced a maximum yield of 13.83 t/ha. Therefore, it was evident that in terms of brinjal yield, plots treated with chemical insecticides such as chlorantraniliprole 18.5 SC and flubendiamide 39.35 SC performed better than plots treated with biorationals.

CONCLUSION

The results of the present study show that chlorantraniliprole 18.5 SC treated plot had the lowest percentage of fruit and shoot infection during the year. Further, chlorantraniliprole 18.5 SC treated plots also had the highest marketable fruit output in terms of yield. Flubendiamide 39.35 SC, novaluron 5.25% + emamectin benzoate 0.9% SC, and *Bacillus thuringiensis* var *kurstaki* are the next best chemicals in terms of reducing pest damage and yield return. Azadirachtin 50,000 ppm was the least effective.

REFERENCES

Anil. and Sharma P. C. 2011. Evaluation of insecticides against brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee. *Indian Journal of Entomology*, **73**(4): 325-330.

Dwivedi, R. K., Tripathi, A., Pal, R. K. and Singh, D. K. 2014. Effect and ecofriendly management of BSFB (*Leucinodes orbonalis* Guenee) on brinjal. *International Journal of Plant Protection*, **7**: 287-291

Ghosh, S. K. and Senapati S. K. 2009. Seasonal fluctuation in the population of *Leucinodes orbonalis* Guen. in the sub-himalayan region of West Bengal, India and its control on eggplant (*Solanum melongena* L.). *Precision Agriculture*, **10** (5): 443-449.

Gowda, B.H., Ramesh, P.R. and Prashanth, J.M., 2017. Biointensive management of brinjal shoot and fruit borer, *Leucinodes orbonalis* Guen.: Technology demonstration and impact. Pest Management in Horticultural Ecosystems, **23**(1): 60-63.

Hedge, JNR, Girish. and Chakravarthy, A. K. 2009. Integrated management of brinjal shoot and fruit borer, *Leucinodes orbonalis*. Proceedings "International conference on Horticulture for livelihood security and economic growth", November 9-12, 2009, University of Agriculture Science, Banglore, 1103-1107.

Jha, S. K., Jayakrishnan, S. and Madhuban, G. 2006. Persistence of chlorpyrifos on eggplant fruits for the management of shoot and fruit borer, *Leucinodes Orbonalis* Guen. *Annals of Plant Protection Sciences*, **14**(1): 116-119.

Kameshwaran, C. and Kumar, K. 2015. Efficacy of newer insecticides against the brinjal, *Solanum melongena* (L.) shoot and fruit borer, *Leucinodes orbonalis* (Guen.) in Karaikal district, U.T. of Puducherry. *Asian Journal of Bio Science*, **10**(2): 119-128.

Mainali, R. P., Paneru, R. M., Pokhrel, P. and Giri, Y. P. 2015. Field bio-efficacy of newer insecticides against eggplant fruit and shoot borer, *Leucinodes orbonalis* guenee. *International Journal Applied Sciences Biotechnology*, **3**(4): 727-730

Misra, H.P., 2008. New promising insecticides for the management of brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee. *Pest Management in Horticultural Ecosystems*, **14**(2): 140-147.

Misra, H. P. 2011. Bio-efficacy of chlorantraniliprole against shoot and fruit borer of brinjal, *Leucinodes orbonalis* guenee, *Journal of Insect Science*, **24** (1): 60-64.

Saran, S., Singh, D.V., Singh, A., Kumar, S. and Kumar, U. 2018. Bio-efficacy of selective eco-friendly insecticides and bio pesticides against shoot and fruit borer, *Leucinodes orbonalis* (Guenee) in brinjal. *Journal of Pharmacognosy and Phytochemistry*, **7**(6): 1690-1694

Sarnabati, L. and Ray, D. C. 2017. Efficacy of newer insecticides against brinjal shoot and fruit borer *Leucinodes orbonalis* Guen. in Manipur. *Indian Journal of Entomology*, **79** (1): 55-58.

Shirale, D., Patil, M., Zehr, U. B. and Parimi, S. 2012. Evaluation of newer insecticides for the management of brinjal fruit and shoot borer *Leucinodes orbonalis* (Guenee). *Indian Journal of Plant Protection*, **40** (4): 273-275

Swini Reddy, CST and Kumar, A. 2022. Efficacy of selected insecticides against brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guenee). *The Pharma Innovation Journal*, **11**(4): 1327-1330

Vinayaka, K. S., Singh, B., Yadav, S. S. and Nayak, S. B. 2019. Efficacy of Different Insecticides against brinjal fruit borer (*Leucinodes orbonalis* Guenee) and their impact on fruit yield. *Journal of Entomology and Zoology Studies*, **7**(3): 66-66

Yousafi, Q., Afzal, M. and Aslam, M. 2015. Management of Brinjal Shoot and Fruit Borer, *Leucinodes orbonalis* Guenee, with Selected Insecticides. *Pakistan Journal of Zoology*, **47**(5): 1413-1420

MS Received: 31 August 2022
MS Accepted : 15 November 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 39-47 (2022)
DOI Number: 10.5958/0974-4541.2022.00036.4

In-vitro compatibility of entomopathogenic fungus, *Lecanicillium lecanii* (Zimm.) Zare and Gams with insecticides and fungicides

TEJASWI. G. GOWDA* and O. P. REJI RANI

Department of Agricultural Entomology, College of Agriculture Kerala Agricultural University, Vellayani, Thriruvananthapuram, Kerala, India

***E-mail:** tejaswigowda93@gmail.com

ABSTRACT: The compatibility of *Lecanicillium lecanii* with insecticides and fungicides was tested to identify and incorporate the compatible chemicals in the IPM package for sucking pest management. Among the new generation insecticides tested, we found flubendiamide 39.35% SC, chlorantraniliprole 18.5% SC, imidacloprid 17.8% SL and thiamethoxam 25% WG were found to be compatible with *L. lecanii* at all the three doses. Thiamethoxam, 25 % WG, was the least inhibitive at recommended dose with the highest Biological index (BI) of 80. Of the old-generation insecticides, dimethoate 30 % EC was compatible, while malathion 50 % EC, quinalphos 25% EC and chlorpyrifos 20% EC were toxic to *L. lecanii.* Quinalphos, 25 % EC, was the highly inhibitive insecticide with a BI of 14-18, exhibiting a significant reduction in growth, sporulation, and germination of the fungus. Among the fungicides tested, copper oxychloride 50% WP and azoxystrobin 23% SC were moderately toxic. Other fungicide *viz*., tebuconazole 25% EC, hexaconazole 5% EC, carbendazim 50% WP and mancozeb 75% WP were found to be toxic to *L. lecanii.*

Keywords- *Lecanicillium lecanii*, compatibility, biological index, fungicides

INTRODUCTION

The development of entomopathogenic fungi as environmentally acceptable substitutes for chemical pesticides has advanced significantly, and some species have been commercialised. The most commonly used entomopathogenic fungi are *Beauveria bassiana* (Bals.) Vuill., *Lecanicillium lecanii*, *Metarhizium anisopliae* (Metschn.) Sorokin, *Metarhizium acridum* (Driver & Milner), and *Isaria fumosorosea* Wize. *Lecanicillium lecanii* is widely used for managing the sucking pests of several crops. In the greenhouse condition, it is an efficient biocontrol agent for managing *Trialeurodes vaporariorum* Westwood (Kim *et al.,* 2002). It is the most widely distributed species, capable of causing epizootics in tropical and subtropical regions with prevalent warm, humid conditions (Nunez *et al.,* 2008).

The compatibility of the biological control agents with other crop protection chemicals will be critical to their successful implementation. Their compatibility with chemical fungicides used to combat plant fungal infections is particularly significant to growers. It is crucial to understand how commercial fungicides and insecticides affect entomopathogenic fungi since they could affect the pathogen's effectiveness and persistence on plant surfaces and in agricultural soils. The use of selective insecticides and fungicides in combination with entomopathogens can improve pest control efficiency by reducing the quantity of pesticides used, the risk of environmental contamination and the expression of insecticide resistance in pests (Shah *et al.,* 2009). In light of this, the present study was conducted to determine the compatibility of *L. lecanii* with the insecticides and fungicides commonly used for pest management.

MATERIALS AND METHODS

Eight insecticides and six fungicides of different chemical groups, including new and old generations, commonly used in the agroecosystem, were tested for their compatibility using the poisoned food technique suggested by Moorhouse *et al.,* (1992). The assays were carried out under *in vitro* conditions. *Lecanicillium lecanii* (isolate no. V1 8) was originally sourced from the National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru and was revived by passing through eggplant mealybug, *Coccidohystrix insolitus* (Green). Compatibility was tested at three doses, the recommended, half the recommended, and double the recommended doses for insecticide (Table 1). For fungicides, the compatibility was tested only at the recommended dose (Table 3). Compatibility was assessed based on radial growth, sporulation and germination of the fungus in the poisoned medium.

The effect of insecticides and fungicides on colony growth was assessed in a poisoned medium prepared using double strength PDA. The required quantity of chemicals was dissolved in sterile double distilled water and added to an equal amount of molten double strength PDA. The poisoned PDA was immediately poured into Petri plates for solidification. The plates were then

Table 1. Radial growth of *L. lecanii* on PDA poisoned with insecticides

Insecticide	Concentrations (%)	Dose	Mean colony diameter (cm)		Growth inhibition (%) on 21 DAI
			10 DAI	21 DAI	
Flubendiamide 39.35 % SC	0.0025	0.5x	2.23 (1.49)^{bc}	3.90 (1.98)^{d}	12.75
	0.005	x	2.13 (1.46)^{c}	3.70 (1.92)^{e}	17.23
	0.01	2x	2.10 (1.45)^{c}	3.40 (1.84)^{f}	23.94
Chlorantraniliprole 18.5 % SC	0.003	0.5x	2.10 (1.45)^{c}	4.20(2.05)^{cd}	6.04
	0.006	x	2.07 (1.44)^{c}	4.10(2.03)^{bc}	8.28
	0.012	2x	1.87 (1.37)^{d}	3.90 (1.98)^{d}	12.75
Imidacloprid 17.8 % SL	0.003	0.5x	2.33 (1.53)^{ab}	4.40(2.10)^{ab}	1.57
	0.006	x	2.17 (1.47)^{bc}	4.30(2.07)^{abc}	3.80
	0.012	2x	2.13 (1.46)^{c}	4.10 (2.03)^{cd}	8.28
Thiamethoxam 25 % WG	0.0025	0.5x	2.13 (1.46)^{c}	4.40 (2.10)^{ab}	1.57
	0.005	x	2.2 (1.48)^{bc}	4.20 (2.05)^{bc}	6.04
	0.01	2x	2.13 (1.46)^{c}	4.10 (2.02)^{cd}	8.28
Malathion 50 % EC	0.05	0.5x	1.4 (1.18)^{gh}	2.47 (1.57)^{i}	44.74
	0.1	x	1.47 (1.21)^{g}	2.30 (1.52)^{j}	48.55
	0.2	2x	1.30 (1.14)^{h}	2.20 (1.48)^{j}	50.78
Quinalphos 25 % EC	0.025	0.5x	0.80 (0.89)^{i}	1.10 (1.05)^{k}	75.39
	0.05	x	0.80 (0.89)^{i}	1.10 (1.05)^{k}	75.39
	0.1	2x	0.77 (0.87)^{i}	1.00 (1.00)^{k}	77.63
Dimethoate 30 % EC	0.02	0.5x	1.73 (1.32)^{de}	3.63 (1.91)^{e}	18.79
	0.04	x	1.67 (1.29)^{ef}	3.60 (1.90)^{e}	19.46
	0.08	2x	1.50 (1.22)^{g}	2.90 (1.7)^{g}	35.12
Chlorpyrifos 20 % EC	0.03	0.5x	1.53 (1.24)^{fg}	2.70 (1.64)^{h}	39.60
	0.06	x	1.47 (1.21)^{g}	2.50 (1.58)^{i}	44.07
	0.12	2x	1.27 (1.13)^{h}	2.27 (1.51)^{j}	49.22
Control			2.47 (1.57)^{a}	4.47 (2.11)^{a}	-
CD (0.05)			(0.063)	(0.051)	
S.E(m) ±			0.069	0.107	

DAI-Days after inoculation, x- recommended dose, Figures in the parentheses are square root transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA

inoculated with a 5 mm disc of the seven-day-old actively growing culture of *L. lecanii*, using a flame-sterilized cork borer and incubated at room temperature. PDA without pesticides served as control. The experiment was laid out in a Completely Randomized Design (CRD), where each treatment was replicated thrice. Observations were recorded on the radial growth of fungus on the 10th and 21st Day after Inoculation (DAI).

Spore count was enumerated from 21-day-old cultures. Conidia of the fungus were dispersed in sterile water (10 mL) with 0.02 % tween 20 by scraping off the mycelia with a sterilized L rod. Ten µL each of the suspensions was transferred into a haemocytometer using a micropipette for counting the spores. For germination studies, spore suspension of the fungus was prepared from 21-day-old culture, and the spore count was adjusted to 105 conidia mL-1 by serial dilution method. A sterile glass slide was evenly coated with a drop of molten poisoned PDA and was allowed to dry in a laminar airflow chamber. After drying, 100 µL of the spore suspension was dropped onto a glass slide and spread uniformly. The slide was incubated in a Petri dish lined with moistened filter paper for 24 h at room temperature. After 24 h, the slides were observed under 40 × magnifications in a compound microscope to count 100 spores randomly, and the numbers of germinated spores were noted. Spores with germ tubes more than the diameter of the spores were considered germinated.

Inhibition of the fungal growth parameter in the poisoned medium was calculated using the following formula

$$\text{Per cent inhibition} = [(C - T) / C] \times 100$$

C= colony growth or germination per cent or

Table 2. Spore count and germination of *L. lecanii* on PDA poisoned with insecticides

Insecticide	Concentration (%)	Dose	Spore count (10^7 spores mL^{-1})	Sporulation Inhibition (%)*	Germination (%)	Germination Inhibition (%)**
Flubendiamide 39.35 % SC	0.0025	0.5x	2.96 (1.72)^{cd}	27.98	93.00 (74.74)^{b}	7.00
	0.005	x	2.48 (1.57)^{cde}	39.66	92.67 (74.34)^{bc}	7.33
	0.01	2x	2.36 (1.54)^{def}	42.58	87.33(69.36) ^{defghi}	12.67
Chlorantraniliprole 18.5 % SC	0.003	0.5x	3.09 (1.76)^{bc}	24.82	89.67(71.25)^{cdefg}	10.33
	0.006	x	2.27 (1.51)^{ef}	44.77	87.00 (68.99) ^{efghi}	13.00
	0.012	2x	1.98 (1.41)^{ef}	51.82	85.33 (67.59)^{hi}	14.67
Imidacloprid 17.8 % SL	0.003	0.5x	3.01 (1.72)^{bcd}	26.76	90.33 (71.92)^{bcde}	9.67
	0.006	x	2.21 (1.49)^{ef}	46.23	86.33 (68.36)^{fghi}	13.67
	0.012	2x	1.80 (1.34)^{fg}	56.20	85.00 (67.32)^{i}	15.00
Thiamethoxam 25 % WG	0.0025	0.5x	3.73 (1.93)^{ab}	9.25	91.00 (72.56)^{bcd}	9.00
	0.005	x	2.46 (1.55)^{cde}	40.15	89.67 (71.38)^{cdef}	10.33
	0.01	2x	2.45 (1.56)^{cde}	40.39	86.00 (68.05)^{ghi}	14.00
Malathion 50 % EC	0.05	0.5x	0.38 (0.59)^{hi}	90.75	74.67 (59.82)^{j}	25.33
	0.1	x	0.31 (0.53)^{i}	92.46	71.67 (57.89)^{jk}	28.33
	0.2	2x	0.05 (0.22)^{j}	98.78	70.33 (57.02)^{jk}	29.67
Quinalphos 25 % EC	0.025	0.5x	0.06 (0.25)^{j}	98.54	52.33 (46.34)^{mn}	47.67
	0.05	x	0.04 (0.18)^{j}	99.03	47.00 (43.28)^{n}	53.00
	0.01	2x	0.03 (0.16)^{j}	99.27	32.67 (34.85)^{op}	67.33
Dimethoate 30 % EC	0.02	0.5x	2.18 (1.48)^{ef}	46.96	90.33 (71.92)^{bcde}	9.67
	0.04	x	2.00 (1.41)^{ef}	51.34	89.00(70.64)^{defgh}	11.00
	0.08	2x	1.34 (1.16)^{g}	67.40	87.67(69.46) ^{defghi}	12.33
Chlorpyrifos 20 % EC	0.03	0.5x	0.58 (0.76)^{h}	85.89	68.33 (55.76)^{kl}	31.67
	0.06	x	0.48 (0.69)^{hi}	88.32	63.33 (52.74)^{l}	36.67
	0.12	2x	0.36 (0.6)^{hi}	91.24	53.33 (46.91)^{m}	46.67
Control			4.11 (2.03)^{a}		100 (89.71)^{a}	
CD (0.05)			(0.207)		(3.244)	
S.E(m) ±			0.251		3.411	

DAI-Days after inoculation, x- recommended dose
* Values in parentheses are square root transformed ** Values in parentheses are arc sine transformed.

spore count in control

T= colony growth or germination per cent or spore count in treatment

Compatibility status was finally confirmed by calculating Biological Index (BI) as proposed by Rossie-Zalaf (2008)

BI = [47 x VG + 43 x SP + 10 x GER] / 100 where,

VG - Vegetative growth of the fungal colony (%) in relation to control; SP - Sporulation (%) in relation to control; GER - Conidial germination (%) in relation to control

BI values were grouped into three categories of toxicological classification *viz.,* 0 to 41 = toxic;

42 to 66 = moderately toxic; >66 = compatible.

The data were subjected to analysis of variance (ANOVA) using WASP 2 software and treatment variations were related.

RESULTS AND DISCUSSION

Compatibility with insecticides

In general, the new generation insecticides chlorantraniliprole 18.5 % SC, imidacloprid 17.8 % SL, flubendiamide 39.35 % SC and thiamethoxam 25 %

Table 3. Compatibility status of *L. lecanii* with insecticides based on biological index

Insecticide	Dose	VR	SP	GER	BI	Compatibility status
Flubendiamide 39.35% SC	0.0025	88.64	71.90	93	82	COMPATIBLE
	0.005	84.09	60.22	92.667	75	COMPATIBLE
	0.01	77.27	57.49	87.333	70	COMPATIBLE
Chlorantraniliprole 18.5% SC	0.003	93.18	75.18	89.667	85	COMPATIBLE
	0.006	95.45	55.18	87	77	COMPATIBLE
	0.012	88.64	48.15	85.333	71	COMPATIBLE
Imidacloprid 17.8% SL	0.003	100.00	73.24	90.333	88	COMPATIBLE
	0.006	97.73	53.84	86.333	78	COMPATIBLE
	0.012	93.18	43.77	85	71	COMPATIBLE
Thiamethoxam |25% WG	0.0025	100.00	90.85	91	95	COMPATIBLE
	0.005	95.45	59.95	89.667	80	COMPATIBLE
	0.01	93.18	59.49	86	78	COMPATIBLE
Malathion 50% EC	0.05	56.06	9.34	74.667	38	TOXIC
	0.1	52.27	7.64	71.667	35	TOXIC
	0.2	50.00	1.12	70.333	31	TOXIC
Quinalphos 25% EC	0.025	25.00	1.48	52.333	18	TOXIC
	0.05	25.00	0.80	47	17	TOXIC
	0.01	22.73	0.63	32.667	14	TOXIC
Dimethoate 30% EC	0.02	81.82	52.97	90.333	70	COMPATIBLE
	0.04	82.58	48.71	89	69	COMPATIBLE
	0.08	65.91	32.60	87.667	54	MODERATLY TOXIC
Chlorpyrifos 20% EC	0.03	61.36	14.18	68.333	42	TOXIC
	0.06	56.82	11.65	63.333	38	TOXIC
	0.12	51.52	8.76	53.333	33	TOXIC

BI values were grouped into three categories of toxicological classification *viz.,* 0 to 41 = toxic; 42 to 66 = moderately toxic; >66 = compatible.

WG, were the least inhibitory on growth, sporulation and germination of *L. lecanii* at all the three test doses (Table 1). In terms of growth, the recommended dose inhibition was below 17.23 per cent. Among these, imidacloprid 17.8% SL caused the least growth inhibition (3.80 per cent), followed by thiamethoxam 25 % WG (6.40 per cent). At half the recommended dose, these insecticides caused less than 12 per cent growth inhibition. At their double dose, less than 23.94 per cent inhibition was recorded. These insecticides significantly inhibited the sporulation of *L. lecanii* (table 4). All the new-generation insecticides caused nearly 40 to 46 per cent inhibition at the field dose. Germination was inhibited by less than 11 per cent at half doses and below 15 per cent at their recommended and double the recommended doses, which is minimal compared to old-generation insecticides. Non-inhibitive effect of chlorantraniliprole 18.5% SC in this experiment is in corroboration with the findings of Sitta *et al*. (2009) in *Metarhizium anisopliae* and Vijayasree (2013) in *L. lecanii.*

Imidacloprid and thiamethoxam were reported to exhibit less growth inhibition in *L. lecanii* and *Beauveria bassiana*, as substantiated in the experiments by Filho *et al.* (2001) and Kakati *et al.* (2018). Both thiamethoxam and imidacloprid had no adverse impact on the germination of *L. lecanii*, as reported by Gurulingappa *et al.* (2011). Ummer and Kurien (2021) reported that imidacloprid 17.8% SL caused only 4.19 per cent inhibition in *L. lecanii*. The sporulation inhibition by these new-generation insecticides is supported by the findings of Oliveira *et al.* (2003), where thiamethoxam showed 21.39 per cent sporulation inhibition in *B. bassiana*, while Akbar *et al.* (2012) observed 49.48 per cent inhibition in sporulation of *M. anisopliae*.

Among the old-generation insecticides tested, dimethoate 30% EC was comparatively less inhibitive, with an 18 to 19 per cent reduction in growth at its recommended and half the recommended doses. Growth inhibition was maximum with quinalphos 25 %

Table 4. Effect of fungicides on growth, sporulation and germination of *L. lecanii*

Fungicide	Concentrations (%)	Mean colony diameter (cm)*		Growth inhibition (%)
		10 DAI	21 DAI	
Copper oxychloride 50% WP	0.2	1.5 (1.41)[b]	3.27 (1.94)[b]	35.88
Azoxystrobin 23% SC	0.1	1.33 (1.35)[c]	2.97 (1.86)[c]	41.76
Carbendazim 50%WP	0.2	0.00 (0.71)[d]	0.00 (0.71)[e]	100
Mancozeb 75% WP	0.3	1.27 (1.33)[c]	2.40 (1.7)[d]	52.94
Hexaconazole 5% EC	0.15	0.00 (0.71)[d]	0.00 (0.71)[e]	100
Tebuconazole 25%EC	0.2	0.00 (0.71)[d]	0.00 (0.71)[e]	100
Control		2.67 (1.78)[a]	5.10 (2.37)[a]	
CD (0.05)		(0.054)	(0.063)	
S.Em ±		0.384	0.761	

DAI- Days after inoculation. * Values in parentheses are square root transformed values

EC (75.39 per cent), followed by malathion 50 % EC (48.55%) and chlorpyrifos 20 % EC (44.07 per cent). The same inhibitive trend was observed in sporulation, where all these insecticides caused more than 80 per cent inhibition. Quinalphos, 25 % EC, severely deterred the sporulation (>98 per cent) at all three test doses. Sporulation inhibition was high in malathion at 50 % EC (> 90 per cent) and in chlorpyrifos 20 % EC (> 80 %).

Germination inhibition was highest in quinalphos 25 % EC (47.67 to 67.33 per cent), followed by malathion 50 % EC and chlorpyrifos 20 % EC (25.33 to 29.67 per cent and 31.67 to 46.67 per cent, respectively). Dimethoate 30 % EC was comparatively less inhibitory (9.67 to 12.33 per cent).

The non-inhibitory nature of dimethoate 30 % EC in this study is supported by findings of Armarkar and Chikte (2008) and Kakati *et al.* (2018), where it was found to be compatible with *L. lecanii* causing only 19.63 and 21.25 per cent inhibition in growth. However, it was reported to have an adverse effect on the growth of *B. bassiana*, with 59.25 per cent inhibition (Dhanya *et al.,* 2019). The inhibitory nature of chlorpyrifos 20% EC and malathion 20% EC to entomopathogens such as *B. bassiana* was confirmed in the studies conducted by Rachappa *et al.* (2007), where there was a 58 per cent reduction in growth by malathion 50% EC and 69 per cent reduction by chlorpyrifos 20% EC.

Quinalphos 25% EC was highly inhibitive to the growth (75 to 77 per cent), sporulation (99 per cent) and germination (47 to 67 per cent). The inhibitory effect of quinalphos noted in this study is supported by the findings of various researchers. Rajanikanth *et al.* (2010) and Faraji *et al.* (2016) reported the non-compatibility of quinalphos 25% EC where there was a total inhibition in the conidial germination of *B. bassiana.*

The reason for inhibition noted with many of the old-generation insecticides, as opined in the study carried out by Rani, (2000) in the entomopathogenic fungus *Fusarium pallidoroseum* (Cooke) Sacc, might be due to the alteration in C: N ratio in the poisoned medium to non-ideal proportions. This may be why fungi respond differently in different media into which other chemicals are added. Only if the carbon and nitrogen source is available to the fungi to metabolise can it grow and sporulate well. The compatibility status calculated based on the Biological index (Table 5) reveals that malathion 50% EC, quinalphos 25% EC and chlorpyrifos 20% EC were "toxic' with BI ranging from 14 to 38. Only dimethoate 30% EC was found to be 'compatible' with the BI index of 69. The incompatibility of *L. lecanii* with chlorpyrifos is in agreement with studies conducted by Abidin *et al.,* (2017), where it was found to be toxic to *B. bassiana* and *M. anisopliae* based on the BI index (39.32 and 24.40 respectively). All four new-generation insecticides tested were highly compatible at all three test doses, with BI Indices ranging from 71 in chlorantraniliprole 18.5% SC to 95 in thiamethoxam 25% WG.

Table 5. Effect of fungicides on sporulation and germination of *L. lecanii*

Fungicide	Concentrations (%)	Spore count (10 spores mL)*	Sporulation Inhibition (%)	Germination (%)**	Germination Inhibition (%)
		21 DAI		24 h	
Copper oxychloride 50% WP	0.2	0.37 (0.93)[c]	91.16	98.33 (83.87)[ab]	0.67
Azoxystrobin 23% SC	0.1	0.60 (1.05)[b]	85.68	95.67 (78.49)[b]	3.36
Carbendazim 50%WP	0.2	0.00 (0.71)	100	0 (0.29)[c]	100
Mancozeb 75% WP	0.3	0.04 (0.73)[d]	99.045	0 (0.29)[c]	100
Hexaconazole 5% EC	0.15	0.00 (0.71)	100	0 (0.29)[c]	100
Tebuconazole 25%EC	0.2	0.00 (0.71)	100	0 (0.29)[c]	100
Control		4.19 (2.17)[a]	-	99.00 (86.48)[a]	-
CD (0.05)		(0.066)		(5.79)	
S.Em ±		0.581		19.735	

DAI- Days after inoculation. * Values in parentheses are square root transformed values
** Values in parentheses are arc sine transformed.

Compatibility with fungicides

Among the fungicides studied for compatibility, azoxystrobin 23% SC was comparatively less inhibitive (Tables 4 and 5). The inhibition was 41.76 per cent and 95 per cent, respectively, in growth and sporulation. However, the reduction in germination was only 3.33 per cent. Silva *et al.* (2013) reported the least inhibition in the growth and sporulation of *M. anisopliae*. On the contrary, Zumaeta (2014) pointed out that azoxystrobin 300 ppm reduced germination by 81 per cent and growth by 51 per cent.

Copper oxychloride 50% WP caused 31 and 97.87 per cent reduction in growth and sporulation. In terms of germination, its inhibition was negligible (1 per cent). The inhibitory effect of copper oxychloride 50% WP on the growth and sporulation of entomopathogenic fungi noted in this study follows the report of Olan and Cortez (2003), who found that there was 79.24 per inhibition in *L. lecanii. B. bassiana* caused 45-55 per cent inhibition in sporulation (Rachappa *et al.,* 2007; Usha *et al.,* 2014). Mancozeb 75% WP was found to be highly poisonous. It caused 51 per cent growth inhibition, 99 per cent sporulation inhibition and 100 per cent germination inhibition. Mancozeb is known to affect cellular respiration, interrupting the Krebs cycle in multiple stages, thus it inhibitory the growth and as well as germination of fungi (Liñan, 1997). Gonzalez *et al. (*2012) found that mancozeb 75% WP @ 2000 mg kg-1 exhibited a spore load of 2.3 x 107 mg kg-1 in *L. lecanii*, while there was 34.3 per cent growth inhibition. It was also reported to cause complete inhibition in the germination of *Lecanicillium muscarium* by Ali *et al. (*2013) and *Isaria fumosorosea* Wize, by Bernal *et al.* (2014).

Carbendazim 50% WP, hexaconazole 5% EC and tebuconazole 25% EC caused 100% inhibition in all the growth parameters. This complete inhibition of *L. lecanii* by carbendazim is in concordance with the observation of Krishnamoorthy and Visalakshi (2007) and Ummer and Kurien (2021). Inhibitory properties of hexaconazole 5% EC were reported by Raj *et al.* (2011) in *B. bassiana*, Lavanya and Matti (2020) and Johnson *et al. (*2020) in *M. anisopliae*, where there was a total arrest.

Compatibility status, when assessed for fungicides, showed that azoxystrobin 23% SC and copper oxychloride

Table 6. Compatibility status of *L. lecanii* with fungicides based on biological index

Fungicides	Dose	VR	SP	GER	BI	Compatibility status
Copper oxychloride 50% WP	1x	64.05	8.78	99.32	44	Moderately toxic
Azoxystrobin 23% SC	1x	64.05	14.35	96.63	46	Moderately toxic
Mancozeb 75% WP	1x	47.05	0.93	0	23	Toxic
Carbendazim 50%WP	1x	0	0	0	0	Toxic
Hexaconazole 5% EC	1x	0	0	0	0	Toxic
Tebuconazole 25%EC	1x	0	0	0	0	Toxic

50% WP were moderately toxic to the fungus (Table 8). Other fungicides *viz*., carbendazim 50% WP, mancozeb 75% WP, hexaconazole 5% EC and tebuconazole 25% EC was "toxic" to the fungus.

Bartlett *et al.* (2002) suggested that the toxicity of tebuconazole and hexaconazole, the triazole fungicides, is due to ergosterol biosynthesis inhibition, consequently preventing the formation of the fungal cell membrane. Mancozeb which was found to be toxic in this study, was earlier reported to be moderately toxic to *L. lecanii* by Gonzalez *et al.* (2012). This variation may be attributed to the fact that in their study, only growth and sporulation were considered for computing compatibility index based on the scale of classification put forth by the International Organisation of Biological Control (IOBC).

It can be concluded that the new generation insecticides are very well compatible with *L lecanii*, and the old generation insecticide is toxic. Among fungicides, copper oxychloride 50% WP and azoxystrobin 23 % SC are moderately toxic, and other fungicides were classified as toxic to *L. lecanii*. Therefore, compatible insecticides can be used along with *L. lecanii* to suppress the non-targeted pests, while moderately toxic fungicides can be used sequentially to manage diseases.

ACKNOWLEDGEMENT

The authors are thankful to Kerala Agricultural University, Thrissur, Kerala, India for providing infrastructural facilities and research fund to carry out research work.

REFERENCES

Abidin, A. F., Ekowati, N. and Ratnaningtyas, N. I. 2017. Compatibility of insecticides with entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*. *Scripta Biologica,* **4**(4): 273-279.

Akbar, S., Shoaib, F., Asifa, H., Hafiza, T. G., Muhammad, A., Muhammad, N. M., Muhammad, N. and Muhammad, B. K. 2012. Compatibility of *Metarhizium anisopliae* with different insecticides and fungicides. *African Journal of Microbiology Research,* **6**(17): 3956-3962.

Ali, S., Huang, Z. and Ren, S. 2013. Effect of fungicides on growth, germination and cuticle degrading enzyme production by *Lecanicillium muscarium*. *Biocontrol Science and Technology,* **23**(6): 711-723.

Armarkar, S. V. and Chikte, P. B. 2008. Compatibility of *Verticillium lecanii* with different chemical pesticides. *Journal of Plant Diseases and Protection,* **3**(1): 43-45.

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M. and Parr-Dobrzanski, B. 2002. The strobilurin fungicides. *Pest Management Science,* **58**: 649–662.

Bernal, E.P.G, Gomez Alvarez, M. I. and Zuluaga Mogollon, M. V. 2014. *In vitro* compatibility *Isaria fumosorosea* (Wize.) Brown y Smith (Hypocreales: Clavicipitaceae) with commercial plaguicides. *Acta Agronomica*, **63**(1): 48-54.

Dhanya, M. K., Sathyan, T., Murugan, M., Ashokkumar, K. and Surya, R. 2019. *In-vitro* compatibility of entomopathogenic fungi with synthetic insecticides and neem oil. *Pesticide Research Journal,* **31**(2): 275-281.

Faraji, S., Shadmehri, A. and Mehrvar, A. 2016. Compatibility of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* with some pesticides. *Journal of Entomological Society of Iran,* **36** (2): 137-146.

Filho, A. B., Almeida, J. E. and Lamas, C. 2001. Effect of thiamethoxam on entomopathogenic microorganisms. *Neotropical Entomology,* **30** (3): 437-447.

Gonzalez, L. C., Nicao, M. E. L., Muino, B. L., Perez, R. H., Sanchez, D. G. and Martínez, V.L. 2012. Effect of six fungicides on *Lecanicillium (Verticillium) lecanii* (Zimm.) Zare and Gams. *Journal of Food, Agriculture and Environment,* **10** (2): 1142-1145.

Gurulingappa, P., Mc Gee, P. and Sword, G. A. 2011. *In vitro* and *in planta* compatibility of insecticides and the endophytic entomopathogen, *Lecanicillium lecanii*. *Mycopathologia,* **172** (2): 161-168.

Johnson, J.M., Deepthy, K.B. and Chellappan, M. 2020. Tolerance of *Metarhizium anisopliae* Sorokin isolates to selected insecticides and fungicides. *Entomon,* **45** (2): 143-148.

Kakati, N., Dutta, P., Das, P. and Nath, P.D., 2018. Compatibility of entomopathogenous fungi with commonly used insecticides for management of banana aphid transmitting banana bunchy top virus (BBTV) in Assam banana production system. *International Journal of Current Microbiology and Applied Sciences,* **7**(11): 2507-2513.

Kim, H. K., Hoe, H. S., Suh, D. S., Kang, S. C., Hwang, C. and Kwon, S. T. 2002. Gene structure and expression of the gene from *Beauveria bassiana* encoding bassiasin I, an insect cuticle-degrading serine protease, *Biotechnology Letters,* **21**: 777-783.

Krishnamoorthy, A. and Visalakshi, P. G. 2007. Influence of some pesticides on entomopathogenic fungus *Lecanicillium (Verticillium) lecanii* (Zimm.) Zare and Gams. *Journal of Horticultural Sciences,* **2**(1): 53-57.

Lavanya, D.S. and Matti, P., 2020. Compatibility of Entomopathogenic Fungi, *Metarhizium anisopliae* with Pesticides. *International Journal of Current Microbiology and Applied Sciences,* **9**(2): 714-721.

Liñan, C. 1997. Farmacología vegetal. España. p. 108.

Moorhouse, E. R., Gillsepie, A.T., Sellers, E.K and Charnley, A.K., 1992. Influence of fungicides and insecticides on the entomogenous fungus, *Metarhizium anisopliae*, a pathogen of the vine weevil, *Otiorhynchus sulcatus*. *Biocontrol Science and Technology*, **82**: 404 – 407.

Nunez. E. J., Iannacone and Omez H. G. 2008. Effect of two entomopathogenic fungi in controlling *Aleurodicus cocois* (Hemiptera: Aleyrodidae). *Chilean Journal of Agricultural Research,* **68**: 21-30.

Olan, H. J. F. and Cortez, M. H. 2003. Effect of three fungicides on seven strains of the entomopathogen *Lecanicillium* (*Verticillium lecanii*). *Manejo Integrado de Plagas Y Agroecologia*, **69**: 21-26.

Oliveira, C. N. D., Neves, P. M. O. J. and Kawazoe, L. S. 2003. Compatibility between the entomopathogenic fungus *Beauveria bassiana* and insecticides used in coffee plantations. *Scientia Agricola,* **60**(4): 663-667.

Pillai, K.G. and Visalakshy, P.N. 2017. Entomopathogenic fungi for the management of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) on gerbera (*Gerbera jamesonii*) in polyhouses. *Pest Management in Horticultural Ecosystems*, **23**(1): 31-36.

Rachappa, V., Lingappa, S. and Patil, R. K. 2007. Effect of agrochemicals on growth and sporulation of *Metarhizium anisopliae* (Metschnikoff) Sorokin. *Karnataka Journal of Agricultural Sciences,* **20**(2): 410-413.

Raj, G. A., Janarthanan, S., Samuel, S. D., Baskar, K. and Vincent, S. 2011. Compatibility of entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin isolated from Pulney hills, Western Ghats of Tamil Nadu with insecticides and fungicides. *Elixir Agriculture,* **40**: 5563-5567.

Rajanikanth, P., Subbaratnam, G. V. and Rahaman, S. J. 2010. Compatibility of insecticides with *Beauveria bassiana* (Balsamo) Vuillemin for use against *Spodoptera litura* Fabricius. *Journal of Biological Control,* **24**(3): 238-243.

Rani, R. O. P. 2000. Production and evaluation of the fungus *Fusarium pallidoroseum* (Cooke) Sacc. as a biopesticide against pea aphid *Aphis craccivora* Koch. Ph. D thesis, Kerala Agricultural University, Thrissur, 137p.

Rossie-Zalaf, L. S., Alves, S. B., Lopes, R. B., Silveira, N. S. and Tanzini, M. R. 2008. Interaction of microorganisms with other pest and disease control agents. In: Alves, S. B. and Lopes, R. B., (eds), *Microbial Control of Pest in Latin America: Advances and Challenges*. Fealq, Piracicaba, Brazil. 270-302.

Shah, F. A., Ansari, M. A., Watkins, J., Phelps, Z., Cross, J. and Butt, T. M. 2009. Influence of commercial fungicides on the germination, growth and virulence of four species of entomopathogenic fungi. *Biocontrol Science and Technology,* **19**(7): 743-753.

Silva, R. A. D., Quintela, E. D., Mascarin, G. M., Barrigossi, J. A. F. and Liao, L. M. 2013. Compatibility of conventional agrochemicals used in rice crops with the entomopathogenic fungus *Metarhizium anisopliae*. *Scientia Agricola,* **70**(3): 152-160.

Sitta, R. B., Gouveia, L. and Sosa-Gomez, D. R. 2009. Compatibilidade de inseticidas com fungos entomopatogênicos. Jornada Acadêmica da Embrapa Soja, 4 (Embrapa Soja. Documento, 312). Londrina: Editado por Odilon Ferreira Saraiva, Pula Geron Saiz Melo. Retrieved from https://ainfo.cnptia.embrapa.br/digital/bitstream/item/72358/1/ID-29937pdf

Ummer, N. and Kurien, S., 2021. *In vitro* evaluation on compatibility of *Lecanicillium lecanii* with selected pesticides. *Pesticide Research Journal,* 33(1): 105-108.

Usha, J., Babu, N. M. and Padmaja, V. 2014. Detection of compatibilty of entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. with pesticides, fungicides and botanicals. *International Journal of Plant, Animal and Environmental Sciences,* **4**(2):613-624.

Vijayasree, V. 2013. Efficacy and biosafety of new generation insecticides for the management of fruit borers of cowpea, brinjal and okra. Ph. D thesis, Kerala Agricultural University, Thrissur, 294 p.

Zumaeta, J. 2014. Efecto de diferentes concentraciones de Azoxystrobin sobre la germinación y crecimiento de Lecanicilium Jecanii y *Metarhizium anisopliae* en condiciones de laboratorio. Universidad Nacional de Trujillo. 65p.

MS Received: 22 August 2022
MS Accepted : 08 October 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 48-52 (2022)

DOI Number: 10.5958/0974-4541.2022.00037.6

Preferential response of melon fruit fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) to major tomato hybrids

R. VIJAY[1], G. KESHAVAREDDY[1*] and K. R. VASUDEVA[2]

[1]Department of Agricultural Entomology, University of Agricultural Sciences, GKVK, Bengaluru-560065, Karnataka, India

[2] Department of Post Harvest Technology, College of Horticulture, Bengaluru

*** E-mail:** keshavaa_reddy@rediffmail.com

Abstract: The five major tomato hybrids were evaluated to check the preferential response of melon fruit fly, *Zeugodacus cucurbitae*. All the five tomato hybrids were selected based on highest number of seedlings sold in a year by different nurseries in Kolar and Chikkaballapur districts during 2019. The field studies indicated that Abhinav was the most susceptible hybrid for melon fly infestation followed by Saaho, US-448 and Alankar. Meghdoot was relatively resistant hybrid among five major hybrids. The correlation studies indicated that large pedicel diameter, high total soluble sugar (TSS) content and low phenol contents are the major factors responsible for highest fruit infestation by melon fruit fly on tomato.

Key words: Hybrid, infestation, tomato, *Zeugodacus cucurbitae*

Introduction

Tomato (*Solanum lycopersicum* L.) is an important edible and widely grown vegetable in the world (Ganeshan and Chethana, 2009). It belongs to the botanical family *Solanaceae*. It is an herbaceous plant with woody stem which can grow up to 1–3 m. As a true fruit it is botanically classified as berry and its soft tissue consists of pericarp walls with a thin layer of epicarp. Tomato is the third most preferred vegetable after potato and onion in India and ranks second after potato in the world. It is a commonly used vegetable in the Indian culinary. It is a healthy vegetable and minimizes the risk of heart diseases, diabetes as well as cognitive function disorders due to its antioxidant, anti-inflammatory and anti-carcinogenic property. Lycopene and bioflavonoids in tomatoes are considered as cancer fighting agents (Wu *et al*., 2011). Ripened red tomato contains powerful antioxidant. Lycopene and vitamin-E prevent low density lipoprotien oxidation effectively. This vegetable helps in rapid skin cell replacement because of its unique vitamin-C content and used for sunburn treatment (Bhowmik *et al*., 2012).

Tomato is grown across the world covering the area of 4.76 mha with a production of 182.25 mt. India is the second largest tomato growing country in the world after China and being grown in area of 0.79 mha with the production of 19.37 mt and productivity of 24.65 t/ha (Anonymous, 2018). Karnataka is the third major producer after Andhra Pradesh and Madhya Pradesh with an area of 64,250 ha, production of 20.81 lakh tonnes and average productivity of 34.3 t/ha which is much higher than national average (Anonymous, 2018). Kolar and Chikkaballapur are the major tomato growing districts of Karnataka state (Anonymous, 2017).

The insect pests have been potential threat in tomato production, particularly due to emerging and invasive pests. The incidence of insect pests varies from season to season and crop growth stages. The fluctuation of insect pests are largely governed by different weather factors during crop growing period and season. Many insect pests feed on tomato starting from germination to harvesting resulting in reduction of yield and fruit quality. But the incidence of fruit flies was unheard of. Melon fruit fly, *Zeugodacus cucurbitae* is an emerging pest in tomato ecosystem and is causing serious economic threat to tomato production as well as quality. The tomato fruits become increasingly susceptible to fruit fly attack at close to harvest and even more when the rainy season starts. The melon fruit fly cause peculiar mode of infestation in tomato, where adult female fly starts laying eggs under the pedicel of tomato making use of visual and olfactory cues. Immediately after egg hatching, larvae of fruit fly penetrate into the fruit and feed on the internal content of the fruit without any external symptoms of damage. Infested fruits start to rot, liquid oozes out from the fruits, as the larvae completely feeds on the fruit, the infested fruits drop off and pupation takes place in soil (Brevault and Quilici, 2007). In this view, the current study on the preferential response of melon fruit fly to major tomato hybrids was carried to bring out the relatively resistant hybrid which prevents/ resist the attack of *Z. cucurbitae* on tomato.

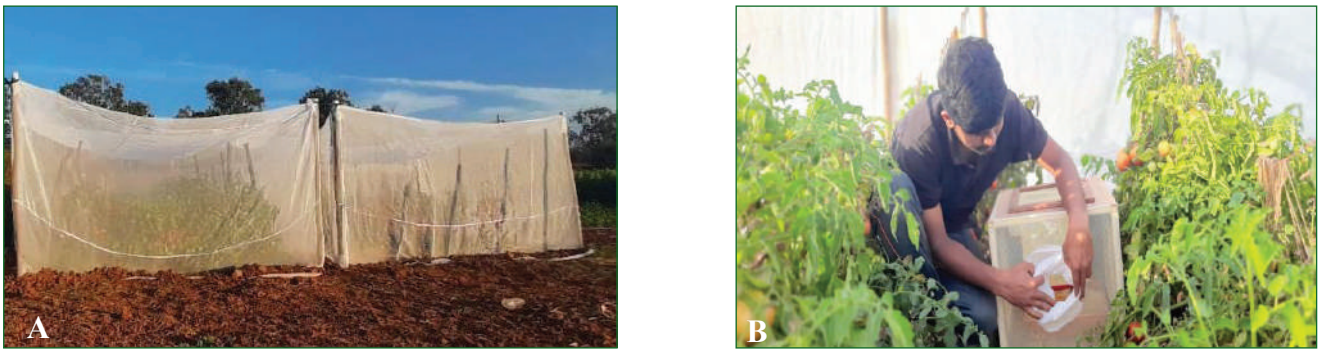


Fig. 1: **A**: Experimental plots covered with nylon mesh before the release of fruit flies (*Z. cucurbitae*);
B: Flies released into experimental plot covered by nylon mesh

Materials and methods

Study area

The experiment was conducted in the research fields of All India Network Project on Agricultural Acarology, Zonal Agricultural Research Station (ZARS), UAS, GKVK, Bengaluru. The study was conducted during *rabi* season of 2019-20. The research farm is located in between 77°34′17″ E long. 13°04′55″ N lat. at an average elevation of 920 m above mean sea level.

Climate details

The climate of UAS, GKVK, Benagluru comes under eastern dry zone of Karnataka. It receives mean average rainfall of 86.32 mm rainfall during *rabi* season. The mean minimum and maximum temperature is 27.58^{0}C and 17.2^{0}C respectively. The mean maximum and minimum relative humidity is 91% and 57.2% respectively during the cropping period. The meteorological data for the study period was collected from Department of Meteorology, UAS, GKVK, Bengaluru.

Visit to various nurseries to record on sale of tomato seedlings

The survey was conducted on the sale of varieties/ hybrids wise tomato seedlings to farmers in one year by making visits to the selected nurseries in both the districts during 2019. Later based on maximum number of seedlings sold to farmers in a year, a total of five major tomato hybrids that are widely cultivated by farmers in both the districts have been selected for further studies like per cent fruit infestation and fruit fly preferential studies.

Field experiment

Field study on preferential response of fruit fly to five different tomato hybrids was conducted in a Randomized Block Design (RBD) method. Each hybrid with four replications was maintained. Each replication consists of three plants of each hybrid and a total of fifteen plants. The plot size of each replication was 3×4 meter and was completely covered with nylon mesh to prevent the damage to tomato crop by other insect pests (Figure 1A). At the fruit maturity and ripening stage (75 DAT), seven days old hundred adult melon fruit flies with sex ratio of 1:1 were released into each nylon mesh (Figure 1B). Later, the observations on per cent fruit infestation was recorded at 7 days time intervals, up to 21 days. At different time intervals, per cent fruit infestation in each plant was recorded by dividing number of infested fruits with total number of fruits observed. Later, total per cent fruit fly infestation was calculated for each treatment.

Insect culture

Melon fruit fly, *Z. cucurbitae* infested tomato fruits were collected from tomato fields of Kolar and Chikkaballapur districts of Karnataka state and were kept on plastic trays with 5 cm thick sterilized fine sand and kept inside acrylic rearing cages in laboratory for pupation. Later, pupae were separated from sand carefully and kept in petri plates for adult emergence. The emerged adults were allowed to mate and used to maintain culture in the laboratory.

Lab experiment

The per cent fruit infestation by fruit fly was correlated with different physical and biochemical fruit parameters like fruit diameter, pedicel diameter, fruit shape, pericarp and epicarp thickness, pH and total soluble sugars (TSS) of fruit juice, total phenols and lycopene content of the fruits of different tomato hybrids. The physical and biochemical parameters of tomato fruits of different hybrids were derived at Department of Post Harvest Technology, College of Horticulture, Bengaluru. The following different procedures were followed to calculate the above parameters

Table1. Hybrid/variety wise seedlings sold by different nurseries in Kolar and Chikkaballapur Districts during 2019

Sl. No.	Hybrid/Variety	Total no. of seedlings sold in a year
1.	Abhinav	51.5 lakhs
2.	US- 448	34.2 lakhs
3.	Alankar	15 lakhs
4.	Saaho	14.25 lakhs
5.	Meghdoot	12.5 lakhs
6.	Rishika	4.5 lakhs
7.	Ansal	4 lakhs
8.	Samarth	2.5lakhs
9.	Siri plus	2 lakhs
10.	TO-6242	1.5 lakhs
11.	Prabha	1.5 lakhs
12.	Madan	1 lakh
13.	Virang	1 lakh
14.	TO-1057	0.3 lakh
15.	US-440	0.3 lakh

a) Assessment of fruit shape of different tomato hybrids

Fruit shape of different tomato hybrids were determined using descriptors for tomato developed by International Plant Genetic Resource Institute.

b) Assessment of fruit diameter, pedicel diameter, pericarp and epicarp thickness

Three randomly selected tomato fruits from each hybrid were collected in paper covers and taken to the Department of Post Harvest Technology, College of Horticulture. Different fruit parameters like fruit diameter, pedicel diameter, pericarp and epicarp thickness were noted using electronic digital caliper (Gadget hero's digital LCD vernier caliper 0–150 mm). Later, the mean values of three fruits was calculated.

c) pH of fruit juice

pH of fruit juices of different hybrids were measured using digital pH meter of Contech Instruments Ltd.

d) Total soluble sugars of fruit juice

Total Soluble Sugars (TSS) of fruit juice was recorded with digital pocket refractometer (Atago - 0 to 53 ^{0}B) and expressed in Brix (^{0}B).

e) Estimation of total phenol content of different tomato hybrids

Total phenol content of different tomato hybrids were estimated using the following steps and procedure. 5.0 g of tomato sample was crushed with 20 ml of 80% ethanol in a pestle and mortar and the volume was made up to 50 ml. From the extracted volume, 0.5 ml of aliquot was taken in a test tube and 0.2ml of Folin-Ciocalteau reagent was added to aliquot, 3.3 ml of distilled water was added and mixed well. After 2 min, 1ml of 2.0% Na_2CO_3 solution was added into each tube and allowed to stand at room temperature for 30 minutes, the absorbance was recorded in a spectrophotometer at 700 nm. Using absorbance value total phenol content was estimated with following formula (Singleton and Rossi, 1965).

Total phenol content

$$(\text{mg gallic acid equivalents/100 gm}) = \frac{\text{Absorbance} \times \text{Std. value} \times \text{Total volume of extract (ml)} \times 100}{\text{Assay volume (ml)} \times \text{Weight of tissue (gm)} \times 1000}$$

f) Estimation of lycopene in different tomato hybrids

Fruits were randomly sampled from the experimental plots with minimum sample size of 10 gm, the pulp was repeatedly extracted with acetone using pestle and mortar until the residue become colourless. Acetone extracts were pooled and transferred into a separating funnel containing 30 ml petroleum ether. 20 ml of 5% sodium sulphate solution was added, mixed gently and left the solution to settle for few minutes. The above layer with lycopene mixed petroleum ether was extracted and absorbance was recorded in a spectrophotometer at 503nm. The total lycopene content was calculated using following formula (Lichtenthaler, 1987).

$$\text{Lycopene (mg in 100g sample)} = \frac{31.206 \times \text{Absorbance}}{\text{Wt of sample (g)}}$$

Statistical Analysis

The data was subjected to single factor Analysis of Variance (ANOVA). The critical difference (CD) at 5% probability level was used as the test criterion.

Results and discussion

Visit to various nurseries on sale of tomato seedlings

A preliminary survey was conducted on the sale of tomato seedlings to farmers for the year 2019 by making visits to the selected nurseries in both Kolar and Chikkaballapur Districts. Among fifteen different tomato hybrids/ varieties sold by various nurseries, five major hybrids were selected for further studies. These hybrids were selected exclusively based on maximum number of seedlings sold by the nurseries to the farmers in a year. Among them Abhinav (51.5 lakh seedlings) was the

Table 2. **Physical, biochemical parameters and % fruit infestation of five tomato hybrids**

Sl. No.	Tomato hybrid	Fruit shape	Fruit diameter (mm)	Pedicel diameter (mm)	Stalk length (mm)	Epicarp thickness (mm)	Pericarp thickness (mm)	TSS (°B)	pH	Phenols (mg of gallic acid in 100gm)	Lycopene (mg in 100gm)	Per cent fruit infestation
1	Saaho	Slightly flattened	53.59	8.30	10.30	1.51	7.03	4.37	4.48	54.94	7.48	20.60 (44.29)[c]
2	US-448	Slightly flattened	55.59	9.93	10.77	1.00	5.42	4.50	4.19	56.69	7.50	20.13 (43.57)[c]
3	Alankar	Slightly flattened	52.52	9.20	11.07	1.29	6.40	4.00	4.43	60.38	5.80	14.34 (37.00)[b]
4	Meghdoot	Rounded	51.01	6.97	11.03	1.23	6.20	3.37	4.49	66.59	7.70	9.04 (29.43)[a]
5	Abhinav	High rounded	50.52	10.67	11.67	0.67	5.46	4.57	4.53	42.03	7.21	26.61 (51.01)[d]
											Sem±	**1.84**
											CD	**5.68**
											CV	**8.98**

*Values in the parentheses are Arc sine transformed.

major tomato hybrid that is being grown predominantly by farmers followed by US-448 (34.2 lakh seedlings), Alankar (15 lakh seedlings), Saaho (14.25 lakh seedlings) and Meghdoot (12.5 lakh seedlings) (Table 1).

Field experiment

The results of the preferential studies of fruit fly, *Z. cucurbitae* under open field conditions revealed that the per cent fruit infestation vary among different hybrids of tomato. Abhinav was the most susceptible hybrid among five hybrids with 26.61 per cent infestation followed by Saaho (20.60%), US-448 (20.13%), Alankar (14.34%). Meghdoot was found resistant among five hybrids with only 9.04 per cent fruit infestation (Table 9). The variation in per cent fruit infestation of five hybrids were subjected to correlation analysis with biochemical and physical parameters and are presented in Table 2.

The correlation studies revealed that the per cent fruit infestation by fruit fly had significant positive correlation with total soluble sugars (+0.94) and pedicel diameter (+0.83). The per cent fruit infestation showed significant negative correlation with total phenol content of fruits (-0.96, P=0.05) (Table 3). Abhinav fruits have more total soluble sugars (TSS) 4.57 ^{0}B. This may be the one reason for fruit fly preference to infest Abhinav fruits when compared to other major hybrid fruits which have relatively less total soluble sugars.

Present preferential investigation results also supported by Nehra *et al.* (2019) who reported that total soluble sugars had significant positive correlation with fruit fly infestation in round gourd varieties, whereas total phenol content had negative correlation with fruit fly infestation.

Haldhar *et al.* (2013) found significant positive correlation (r = 0.97) between larval density per fruit and per cent fruit infestation during the evaluation of eleven genotypes of musk melon against melon fruit fly. Further, they also reported that total sugars, reducing sugars, non-reducing sugars and pH were consistently high in susceptible genotypes and were low in resistant ones. On the other hand, total phenol content of fruit was highest in resistant lines but was low in susceptible genotypes. These results are corroborated with our present investigation on preferential studies of fruit fly on tomato hybrids.

The per cent fruit infestation showed non-significant negative correlation with epicarp thickness (-0.56). There was no significant correlation between per cent fruit infestation and pH of fruit juice, pericarp thickness, lycopene content, fruit diameter and stalk length. The fruit shape of all the five major hybrids were determined using descriptors for tomato developed by International Plant Genetic Resource Institute and found that Abhinav fruit was high rounded, Alankar, Saaho, US-448 fruits were slightly flattened whereas of Meghdoot fruit was round shape. It was found that the fruit shape was also have an impact on fruit fly infestation. Abhinav with high rounded fruit shape was found highly susceptible to fruit fly infestation when compared with other hybrids. Dhillon *et al.* (2005) also reported that per cent fruit infestation and larval density per fruit vary with fruit shape, size and toughness of the fruits in bitter gourd genotypes. Bitter gourd fruits with deep ribs were highly susceptible to melon fruit fly infestation.

Table 3. Correlation co-efficient (r) between fruit fly infestation and different physical-biochemical parameters of five tomato

	pH	TSS	Epicarp thickness	Pericarp thickness	Lycopene	Total phenol	Pedicel diameter	Fruit diameter	Stalk length	% infestation
pH	1.00									
TSS	-0.29	1.00								
Epicarp thickness	0.06	-0.41	1.00							
Pericarp thickness	0.41	-0.32	0.92	1.00						
Lycopene	-0.07	-0.01	-0.11	-0.15	1.00					
Total phenol	-0.20	-0.82	0.67	0.40	-0.04	1.00				
Pedicel diameter	-0.27	0.85	-0.72	-0.64	-0.27	-0.82	1.00			
Fruit diameter	-0.87	0.41	0.29	0.01	0.08	0.17	0.16	1.00		
Stalk length	0.33	0.01	-0.83	-0.67	-0.23	-0.45	0.46	-0.69	1.00	
% infestation	-0.01	0.94*	-0.56	-0.36	0.10	-0.96*	0.83*	0.10	0.22	1.00

* Significant at P= 0.05

Conclusion

Use of resistant cultivars is one of the most important component in managing insect pest in agriculture ecosystem as the other methods incur huge burden on farmers. Among five major tomato hybrids examined for preferential response of melon fruit fly, Abhinav was the most susceptible hybrid with highest per cent fruit infestation and Meghdoot was relatively resistant. These results were supported by correlation analysis with various physical and biochemical parameters of tomato fruits. With this, farmers can be suggested to go for Meghdoot rather than Abhinav hybrid in order to resist/ reduce the attack of fruit fly on tomato.

References

Anonymous. 2017. Annual Report (2017-18). *Indian Horticulture database.* National

Horticulture Board, Ministry of Agriculture, Govt. of India, 256 pp.

Anonymous. 2018. Annual Report (2018-19). *Indian Horticulture database.* National Horticulture Board, Ministry of Agriculture, Govt. of India, 203 pp.

Anonymous. 2018. *FAO STAT Statistical Database.* Food and Agriculture Organization of the United Nations, USDA, Washington, D. C.

Bhowmik, D., Kumar, S. K. P., Paswan, S. and Srivastava, S. 2012. Tomato- A natural medicine and its health benefits. *Journal of Pharmacognosy and Phytochemistry,* **1**(1): 24–36.

Brevault, T. and Quilici, S. 2007. Visual response of the tomato fruit fly, *Neoceratitis cyanescens* to colored fruit models. *Entomologia Experimentalis et Applicata,* **125**: 45–54.

Dhillon, M. K., Singh, R., Naresh, J. S. and Sharma, N. K. 2005. Influence of physico-chemical traits of bitter gourd, *Momordica charantia* L. on larval density and resistance to melon fruit fly, *Bactrocera cucurbitae* (Coquillett). *Journal of Applied Entomology,* **129**: 393–399.

Haldhar, S. M., Bhargava, R., Choudhary, B. R., Pal, G. and Kumar, S. 2013. Allelochemical resistance traits of muskmelon (*Cucumis melo*) against the fruit fly (*Bactrocera cucurbitae*) in a hot arid region of India. *Phytoparasitica*, **41**: 473–481.

Lichtenthaler, H. K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymology,* **148:** 350–382.

Nehra, S., Singh, S., Samota, R. G., Choudhary, S. K. and Choudhary, A. L. 2019. Screening of round gourd varieties for resistance against fruit fly, *Bactrocera cucurbitae* (Coquillett). *Journal of Pharmacognosy and Phytochemistry*, **8**(4): 1101–1107.

Singleton, V. L. and Rossi, J. A. 1965. A colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture,* **16:** 144–158.

Wu, Z., Sun, S., Wang, F. and Guo, D. 2011. Establishment of regeneration and transformation system of *Lycopresicon esculentum* Microtom. *British Biotechnology Journal,* **3**: 53–60.

MS Received: 12 October 2022
MS Accepted : 08 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 53-57 (2022)

DOI Number: 10.5958/0974-4541.2022.00038.8

Occurrence of *Aphis odinae* van der Goot and its natural enemies in cashew

K. VANITHA, T. N. RAVIPRASAD, H. RAJASHEKARA and BABLI MOG

ICAR-Directorate of Cashew Research, Puttur- 574 202, Karnataka, India

E-mail: vanitha.k@icar.gov.in

ABSTRACT: *Aphis odinae*, a polyphagous aphid, was found infesting cashew trees in Puttur, Karnataka. The damage symptoms caused by these aphids, their seasonality and their natural enemies were recorded in this study. The incidence of aphids was observed from August and April every year. The aphids infest emerging flushes, inflorescences and developing nuts. Severe damage to inflorescence resulted in the drying of flowers and the premature fall of tiny nuts. However, medium-sized cashew nuts usually mature or tolerate the aphid infestation without any damage. The size and germination percentage of aphid infested matured nuts, and healthy nuts indicated not much variation. Under field conditions, four species of coccinellids and three species of syrphids were found predating on different stages of aphids and brought their population under control within a short period. The species of ants associated with aphids were also documented. Considering the incidence and severity of the damage, it is a minor pest of cashew in the study area.

Keywords: Cashew, aphids, coccinellids, syrphids, damage symptoms

INTRODUCTION

Cashew (*Anacardium occidentale* L.) is an important edible tree nut crop grown in parts of tropical and subtropical regions, including India. Cashew kernels are rich in nutrients and used in several confectioneries; cashew apples are also nutritious. Cashew trees are infested by several insect pests, among which tea mosquito bugs and stem and root borers are important. Besides, several minor insect pests damage cashew sporadically; a few are secondary pests of regional importance. *Aphis* (*Toxoptera*) *odinae* van der Goot (Aphididae), also known as mango aphid, is considered a minor pest of cashew, which occurs on new flushes, flowers as well as developing nuts. It is a polyphagous pest, infesting at least 45 plant families (Blackman and Eastop, 2000). The common plants include citrus, tea, coffee, mango, *Aralia* sp., *Rhododendron* sp. etc. (Lokeshwari *et al*., 2014; Vidya and Rajanna, 2014; Singh and Singh, 2017).

The *A. odinae* is widely distributed in eastern and southeastern Asia and Africa (Barbagallo and Santos, 1989; Martin, 1989, Dwomoh *et al*., 2008), almost all the cashew growing regions of India (Pillai *et al*., 1976). It has been associated with the transmission of at least two plant viruses: peanut green mosaic virus and peanut stripe virus (Plantwise, 2022). Generally, aphids are reported as minor pests in cashew and natural enemies play a major role in regulating the pest population. The information on the seasonality of pests, their natural enemies and the associated insects like ants is to be documented in the changing climatic scenario. Hence, the present study was taken to understand the pest status of aphids and the influence of their natural enemies.

MATERIALS AND METHODS

Random surveys were conducted in 60 ha of cashew plantations of ICAR-Directorate of Cashew Research, Puttur, Karnataka, during 2019-2021 for the occurrence of aphids and their natural enemies. The population of aphids and its predators were recorded monthly on randomly selected trees. The larvae of different predators were collected, brought to the laboratory and reared on *A. odinae* for the emergence of adults. The nature of the damage on shoots, flowers and developing apples and nuts upon aphid infestation was also recorded. To assess the damage status of aphids, net caged trees of cashew variety, VRI-3 having aphid infestation were observed periodically for the pest population buildup and subsequent damage on cashew flowers, apples and nuts. The severely infested nuts were harvested upon maturity and compared with un-infested nuts for the variations in size, weight and germination percentage.

RESULTS AND DISCUSSION

Surveys in the cashew plantations during 2020-22 indicated that the incidence of aphids was noticed in cashew from August till April in Puttur, Karnataka, in a sporadic manner. Infestation occurred initially on new shoots and was subsequently observed on flowers and nuts. In Ghana, its incidence is reported during February-March on mature trees (Dowmoh *et al*., 2008). The number of aphids, including adults and nymphs, varied between 10 and 303 per flush or nut during the study period. At Goa, the population varied from 84.44 to 203.07nymphs and adults per leaf (Maruthadurai and Singh, 2018). Ants played a major role in disseminating aphids to the new plant parts. This pest infests the young cashew shoots, inflorescences and developing nuts.

Flower drying due to aphid damage | Tending of aphids by red ants

Fig 1. *Aphis odinae* infestation on cashew inflorescences

Nature of damage

Large numbers of aphids occur on young shoots and leaves. But no apparent damage on the shoots was observed due to feeding by aphids, except slightly discoloured streaks on the severely infested shoots without any shoot drying. This could be due to the influence of natural enemies, especially syrphids and coccinellids, which occurred on the aphid infested shoots within 10 to 15 days of aphid infestation and brought down its population subsequently. *Aphis odinae* also sucks the sap on the buds and the cashew flowers, and the nut set was observed generally in most infested inflorescences. However, during heavy infestation by aphids and natural enemy free conditions (caged inflorescences), total drying of flowers was noticed because of intensive feeding damage under the high population pressure of aphids. According to Barzman *et al*. (1996), although pseudococcids, coccids and aphids are sap-sucking feeders, they have never been identified as significant pests of cashew.

Later, aphids also occurred on developing cashew nuts but not on cashew apples. Nuts of very early stages dried prematurely and fell off. However, medium-sized cashew nuts matured usually without any significant damage. But the severely infested nuts expressed black discolouration. There was no significant reduction in the size of aphid infested nuts compared to un-infested nuts. However, drying and curling of leaves, inflorescences and malformation of nuts and apples were reported at Goa due to aphid infestation (Marudhadurai and Singh, 2018), which might be due to higher population pressure of aphids under reduced population of natural enemies or variation in varietal reaction to aphid infestation. The mean length, breadth and width of un-infested nuts of VRI-3 cashew variety ones are 3.10, 2.36 and 1.62 cm, respectively (N=100 Nos), while the values of severely infested nuts were 3.01, 2.40 and 1.60 cm, respectively (N=30 Nos). The mean nut weight of un-infested and infested nuts was 5.68 and 5.56 g, respectively. Further, good germination was also observed in those aphid infested nuts (92 %), similar to un-infested nuts (94 %). Studies on biochemical changes in mango plants upon *T. odinae* infestation indicated a significant reduction in the amount of total soluble sugars and free amino acid content in infested shoots in mango (Lokeshwari *et al*., 2014) and other crops (Singh and Sinha, 2011).

Fig 2. *A. odinae* with exuviae on developing nuts

Table 1. Occurrence and damage symptoms of *A. odinae* in cashew plant parts

Plant part infested	Season of occurrence	Aphid population (No.)	Damage symptom
Leaves and young flushes	August-December	10-99 / flush	No clear symptoms. Mild brown streaks develop at heavy infestation (but natural enemies (NE) reduces the aphid population subsequently).
Inflorescences	November-March	15-106 / inflorescence	Flower drying without nut set under NE free condition.
Cashew nuts	March-April	86-303 / developing nut	Early-stage nuts - drying and premature drop. Medium sized developing nuts- black discolorations, small dots on nut surface in severely infested nuts.

Table 2. Influence of *A. odinae* on cashew nut parameters

Parameters	Severely infested nuts (Mean±SD)	Healthy nuts (Mean±SD)
Nut Length (cm)	3.02±0.14	3.11±0.08
Breadth (cm)	2.40±0.13	2.36±0.21
Width (cm)	1.61±0.08	1.69±0.16
Weight (g)	5.57±0.07	5.68±0.12
Germination of nuts (%)	92	94

Natural enemies

Under field conditions, the aphid population is controlled by several predatory insects, mainly coccinellids and syrphids. During our field collections, though few mummified bloated dark aphids were encountered, the emergence of parasitoids was not found in them under laboratory conditions. Hence parasitoids could not be documented. However, intensive surveys may yield information on parasitoids of *A. odinae* in cashew plantations. Among coccinellids, the common species observed in cashew that predate the aphids are *Scymnus castaneus* Sicard, *Pseudaspidimerus flaviceps* (W.), *Cheilomenes sexmaculata* (F.) and *Coccinella transversalis* F. The other coccinellid species recorded in the cashew plantations of Puttur include *Anegleis cardoni* (Weise), *Illeis cincta* (F.), *Brumoides suturalis* (F.) etc. Still, their predation on *A. odinae* could not be noticed during the surveys.

The syrphid species associated with *A. odinae* in cashew at Puttur include *Paragus serratus* (F.), *Ischiodon scutellarins (*F.) and *Dideopsis aegrota* (F). Vidya & Rajanna (2014) reported three species of coccinellids, four species of syrphids and a species each of hemerobid and chrysopid as aphidophagous predators on *A. odinae*. In Goa, three species of coccinellid and syrphids were reported on *A. odinae*. Among the coccinellids, *P. flaviceps* was dominant, followed by *C. sexmaculata*, which was in accordance with Vidya and Rajanna (2014). While in Goa, *S. castaneus* was the dominant species (Maruthadurai and Singh, 2018). Among syrphids, *D. aegrota* was abundant compared to the other two species. In Goa, *P. serratus* was the dominant coccinellid, followed by *D. aegrota*. The maggots of *P. serratus* and *I. scutellaris* were found predating on *A. craccivora* and *T. odinae* (Satapathy 1993; Joshi *et al*., 1999).

It was observed that under field conditions, these natural enemies play a significant role in rapidly bringing down the aphid population within 10-15 days, so that aphids remain a minor pest in cashew. A maximum of three grubs of predators were found on a single shoot or nut. Sometimes, two or more natural enemies co-exist in the same niche. A study at ICAR-DCR, Puttur revealed that when the cashew trees are covered with fine mesh nylon net cages during the flowering season for pollination studies, the population of aphids flared up as there were no natural enemies of the aphids. As a result, flower drying and reduced nut set were noticed in those caged trees, which proved the role of natural enemies in managing this aphid species in field conditions.

Association of ants

Cashew plants attract a lot of ant species towards their extra floral nectarines. The common ant species attended aphids in cashew plants are *Oecophylla smaragdina* (F.), *Anaplolepis gracilipes* (Smith), *Crematogaster* sp., *Camponotu scompressus* (F.), *Camponotus* sp., *Myrmicaria brunnea* Saunders, *Prenolepis naoroji* Forel *etc*. In Ghana, aphids were closely associated with *Oecophylla*

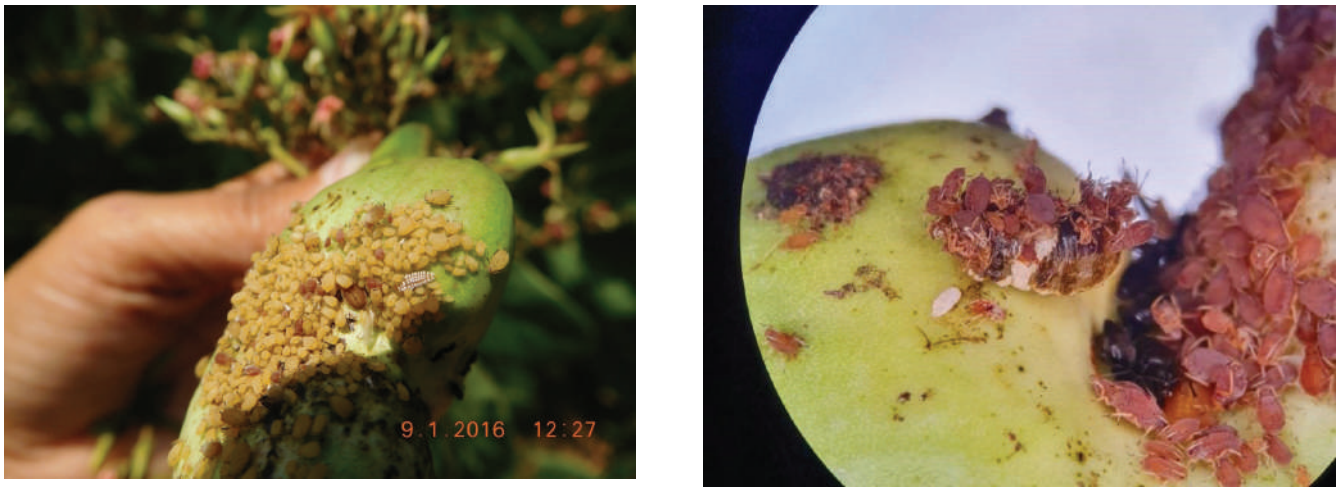


Fig 3. Predators of aphids, Left: Coccinellid grubs (*P. flaviceps* and *S. castaneus*), right: syrphid larva (*D. aegrota*)

longinoda, *Crematogastar striatula* and *Camponotus olivieri* on cashew fruits (Dowmoh *et al*., 2008). The foraging ants, syrphids and coccinellids all were seen on the same aphid infested shoots or nuts. As aphids are minor pests in cashew and natural enemies significantly reduce their population, management measures are not required. Furthermore, Peng *et al*. (1999) found that *O. smaragdina* do not affect homopteran natural enemies during flowering and fruiting periods, resulting in very little damage to the cashew crop. *O. longinoda* might also not affect homopteran natural enemies on cashew (Sreekumar *et al*., 2019).

Fig 4. Tending of *A. odinae* by *Prenolepis* sp. ants and a grub of *P. flaviceps* (brown) seen among aphids

REFERENCES

Barbagallo, S. and L. A. Santos. 1989. *Toxopteraodinae* (V.D.G.) (Homoptera: Aphididae), infesting (*Anacardium occidentale* L.) in Mozambique. (*Toxopteraodinae* (V.D.G.) (Homopter: Aphididae), uma nova praga do cajueiro (*Anacardium occidentale* L.) em Mocambique.), *Phytophaga,* **3**: 163–171.

Barzman, M. S., Mills, N. J. and Nguyen, T. T. C. 1996. Traditional knowledge and rational for weaver ant husbandry in the Mekong delta of Vietnam. *Agriculture and Human Values,* **13**: 2-9.

Blackman, R. L. and Eastop, V. F. 2000. Aphids on the World's Crops-An Identification and Information Guide. The Natural History Museum. John Wiley and Sons, Ltd., New York.

Dwomoh, A., J. B. Ackonor and J. V. K. Afun. 2008. Survey of insect species associated with cashew (*Anacardium occidentale* Linn.) and their distribution in Ghana E. *African Journal of Agricultural Research*, **3** (3): 205-214.

Lokeshwari, D., Verghese, A., Shivashankar, S., Krishna Kumar, N. K., Manjunatha, H. and Venugopalan, R. 2018. Effect of *Aphis odinae* (Hemiptera: Aphididae) infestation on sugars and amino acid content in mango. *African Entomology,* **22** (4): 823–827.

Martin, J. H. 1989. Identification, occurrence and pest status of *Toxopteraodinae* (Van der Goot) (Hemiptera: Aphididae) in Africa. *Bulletin of Entomological Res*earch, **79**: 611.

Maruthadurai, R. and N. P. Singh. 2017. A report on occurrence of aphidophagous predators of *Aphis odinae* (van der Goot) (Hemiptera: Aphididae) in cashew ecosystem from Goa, India. *Journal of Threatened Taxa*, **9** (2): 9858–9861. http:// doi. org/10.11609/jott.2435.9.2.9858-9861.

Peng, R. K., Christian, K. and Gibb, K. 1999. Utilization of green ants, *Oecophyllas maragdina*, to control cashew insect pests, Rural Industries Research and Development Corporation Canberra, Australia, p. 88.

Pillai, G. B., Dubey, O. P. and Singh, V. 1976. Pests of cashew and their control in India. A review of current status. *Journal of Plantation Crops,* **4**: 37-50.

Plantwise, 2022 . https://www.plantwise.org/knowledgebank/datasheet/54272#Impact Section. Accessed on 5th October 2022.

Satapathy, C. R. 1993. Bio-ecology of major insect pests of cashew (*Anacardium occidentale* L.) and evaluation of certain pest management practices. PhD Thesis. University of Agricultural Sciences, Bangalore, India.

Singh, G. and Singh, R. 2017. Food plant records of Aphidini (Aphidinae: Aphididae: Hemiptera) in India. *Journal of Entomology and Zoology Studies*, **5** (2): 1280-1302.

Singh, P. and Sinhal, V. K. 2011. Effect of aphid infestation on the biochemical constituents of mustard (*Brassica juncea*) plant. *Journal of Phytology,* **3** (8): 28–33.

Sreekumar, K.M., Dhanya, C. and Gavas, R., 2019. Population dynamics of red ant, *Oecophylla smaragdina* in cashew orchards and its augmentation for biological control. *Pest Management in Horticultural Ecosystems*, **25**(2): 211-215.

Sunil, J., Ballal, C.R. and Rao, N.S. 1999. Evaluation of biotic potential of syrphid predators, *Ischiodon scutellaris* (Fabr.) and *Paragus serratus* (Fab.) (Diptera: Syrphidae). *Journal of Aphidology,* **13**: 9–16.

Vidya, M. and Rajanna, K. M. 2014. Role of Insect Predators in the Control of *Toxoptera odinae* (Hemiptera: Aphididae) in Cashew Plantation. *Biopesticides International,* **10** (1): 112–115.

MS Received: 21 October 2022
MS Accepted : 17 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 58-63 (2022)
DOI Number: 10.5958/0974-4541.2022.00039.X

Preference of pumpkin beetles, *Aulacophora* spp. (Coleoptera: Chrysomelidae) to different cucurbitaceous hosts

P. MANIKANDAN[1,2], M. SARAVANARAMAN[1], K. SUGUNA[1] and V. SELVANARAYANAN[1]

[1]Department of Entomology, Faculty of Agriculture, Annamalai University, Tamil Nadu, India
[2]Division of Eco technology, M.S. Swaminathan Research Foundation, Tamil Nadu, India
E-mail: rudhran323@gmail.com

ABSTRACT: Preference for two species of pumpkin beetles, *i.e. Aulacophora foveicollis* (Lucas) and *Aulacophora intermedia* (Jacoby) (Galerucidae: Coleoptera) towards six cucurbitaceous hosts were evaluated at the Department of Entomology, Faculty of Agriculture, Annamalai University, Tamil Nadu, India. In the field evaluation, the population of *A. foveicollis* was the highest in pumpkin (12.83 adults/ plant) and least in snake gourd (0.61 adults/ plant). However, the population of *A. intermedia* was the maximum in bottle gourd (11.89 adults/ plant) and was least in bitter gourd (5.17 adults/ plant). In the laboratory free choice test, *A. foveicollis* preferred pumpkin, while *A. intermedia* preferred ridge gourd. Bitter gourd was the least preferred by both species. A similar preference pattern was observed in the confinement test also. Orientation assay using an olfactometer revealed that preference towards different hosts by *A. foveicollis* was bottle gourd> pumpkin >ridge gourd, cucumber >snake gourd > bitter gourd, and that of *A. intermedia* was ridge gourd > bottle gourd > pumpkin > cucumber > snake gourd > bitter gourd. The maximum number of trichomes recorded on the bottle gourd was followed by the snake gourd, whereas less trichome density was recorded on the cucumber. Trichome density had a significant positive correlation with *the A. intermedia* population and a non-significant negative correlation with *A. foveicollis*.

Keywords: *Aulacophora foveicollis*, *A. intermedia*, Host preference, cucurbits

INTRODUCTION

Red pumpkin beetles are predominant pests of cucurbitaceous vegetables (Raman and Annadurai, 1985). Three species of red pumpkin beetles, *viz*., *Aulacophora foveicollis*, *A. cincta*, and *A. intermedia* (Galerucidae: Coleoptera), inflict severe damage from the seedling to maturity stage. The adults of *A. foveicollis* are red-coloured, while *A. cincta* are grey with black having a glistening yellow-red border, and *A. intermedia* are blue in colour. The female can lay 150-300 numbers of eggs. The grubs feed on the roots portion, and adults feed on leaves and flowers, and the resultant damage range extends from 35% to 75% (Saljoqi and Khan, 2007). The response of *Aulacophora* spp on the cucurbitaceous hosts differs, and it can be exploited for the behavioural management of the beetle. Hence, the preference of two species of red pumpkin beetles *viz., Aulacophora foveicollis* (Lucas) and *Aulacophora intermedia* (Jacoby) towards six cucurbitaceous hosts were evaluated under laboratory and field conditions.

MATERIALS AND METHODS

In situ preference of pumpkin beetles

The field evaluation was carried out at the Department of Entomology, Faculty of Agriculture, Annamalai University, Tamil Nadu, India. The seeds of cucurbitaceous vegetables *viz*., pumpkin (*Cucurbita maxima* Linn.), bottle gourd (*Lagenaria siceraria* (Molina) Standl.), ridge gourd (*Luffa acutangula* Linn.), snake gourd (*Trichosanthes cucumerina* Linn.), bitter gourd (*Momordica charantia* Linn.) and cucumber (*Cucumis sativus* Linn.) were procured from commercial seed stores and local farmers. The field experiments were laid out in a Randomized Block Design in a plot size of 2.5m×2.5m, and three such replicated plots were maintained. Regular agronomic practices were followed. To evaluate the preference of red pumpkin beetles towards different cucurbitaceous crops, the population of beetles was recorded between 6 am and 8 am when the feeding activity is more. The number of adult beetles on the leaves, flowers, and stems of three plants were counted at 15, 30, 45, 60, 75, and 90 days after sowing, and the data were pooled together.

Mass culturing of pumpkin beetles

For the laboratory experiments, a homogenous population of red pumpkin beetle cultured on the pumpkin was used. Plants were raised in cement pots (50 cm × 30 cm × 30 cm) and caged using a 1.5m × 1.5m × 2m sized net cage. Two species of red pumpkin beetles, *A. foveicollis* and *A. intermedia* were collected from the experiment field and released into separate cages. Periodically fresh plants were placed inside the

Table 1. Field incidence of *Aulacophora foveicollis* on different cucurbits

Host	Number of adult / plant						Mean
	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	
Pumpkin	1.33 (1.52)^{b}	6.00 (2.64)^{c}	17.67 (4.31)^{d}	22.67 (4.86)^{f}	16.33 (4.16)^{e}	13.00 (3.73)^{c}	**12.83**
Bitter gourd	0.00 (1.00)^{a}	1.33 (1.52)^{a}	2.67 (1.91)^{a}	5.33 (2.51)^{c}	1.67 (1.62)^{b}	1.33 (1.52)^{b}	**2.06**
Bottle gourd	1.00 (1.41)^{b}	4.33 (2.30)^{b}	5.33 (2.51)^{b}	11.33 (3.51)^{d}	13.67 (3.82)^{d}	0.00 (1.00)^{a}	**5.94**
Ridge gourd	1.67 (1.62)^{bc}	4.67 (2.37)^{bc}	5.33 (2.51)^{b}	3.00 (1.97)^{b}	1.33 (1.52)^{b}	0.33 (1.13)^{ab}	**2.72**
Snake gourd	0.00 (1.00)^{a}	1.33 (1.52)^{a}	2.33 (1.82)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	**0.61**
Cucumber	2.33 (1.82)^{c}	6.00 (2.64)^{c}	13.33 (3.78)^{c}	15.67 (4.08)^{e}	7.00 (2.82)^{c}	0.33 (1.13)^{ab}	**7.44**
S.Ed.	**0.10**	**0.14**	**0.11**	**0.12**	**0.09**	**0.18**	
C.D. (p=0.05)	**0.24**	**0.32**	**0.25**	**0.27**	**0.21**	**0.42**	

*****Mean of three replications. Values in parenthesis are square root transformed. Value with different alphabets differs significantly.

Table 2. Field incidence of *Aulacophora intermedia* on different cucurbits

Host	Number of adult / plant						Mean
	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	
Pumpkin	1.33 (1.52)^{a}	4.67 (2.37)^{bcd}	13.67 (3.82)^{c}	16.33 (4.16)^{de}	7.67 (2.94)^{b}	0.67 (1.27)^{a}	**7.39**
Bitter gourd	1.33 (1.52)^{a}	4.33 (2.30)^{bc}	5.33 (2.51)^{a}	7.67 (2.94)^{a}	12.33 (3.64)^{c}	0 (1.00)^{a}	**5.17**
Bottle gourd	3.33 (2.07)^{b}	5.33 (2.50)^{cd}	8.33 (3.05)^{b}	17.67 (4.31)^{e}	21.67 (4.76)^{d}	15 (3.99)^{d}	**11.89**
Ridge gourd	6.67 (2.76)^{c}	12.33 (3.65)^{d}	7.67 (2.94)^{b}	11.67 (3.55)^{c}	5.67 (2.57)^{a}	0.33 (1.13)^{a}	**7.39**
Snake gourd	2.33 (1.82)^{b}	1.33 (1.52)^{a}	7.33 (2.88)^{b}	15.67 (4.08)^{d}	19.67 (4.54)^{d}	8.33 (3.05)^{c}	**9.11**
Cucumber	2.67 (1.91)^{b}	3.67 (2.15)^{b}	7.67 (2.94)^{b}	10.33 (3.36)^{b}	8.33 (3.05)^{b}	2.33 (1.82)^{b}	**5.83**
S.Ed.	**0.11**	**0.15**	**0.11**	**0.07**	**0.10**	**0.15**	
C.D. (p=0.05)	**0.25**	**0.33**	**0.24**	**0.16**	**0.22**	**0.33**	

Mean of three replications. Values in parenthesis are square root transformed. Value with different alphabets differs significantly

cages, and the test insects were allowed to multiply within the cage and used for laboratory experiments.

In vivo feeding preference of pumpkin beetles towards selected hosts by free choice

The preference of pumpkin beetles towards various hosts was evaluated under a completely randomized design under laboratory conditions of 28±20C and 90 per cent relative humidity. Fresh and insect damage-free leaves of the six cucurbit hosts were collected from the experimental field. Leaf discs (3.7 cm2) were excised from the respective hosts and were placed at equidistance on a moist filter paper inside plastic Petri plates (20 cm dia). Overnight pre-starved adult beetle was released inside @ one per Petri plate. Three replications were maintained for both species of red pumpkin beetles. Leaf area fed was calculated using graph sheets at 12, 24, and 48 hrs after the adult release.

***In vivo* feeding preference of pumpkin beetles towards selected hosts under confinement**

Leaf discs of 3.7 cm^2 excised from the six cucurbit hosts were individually placed on filter paper inside plastic Petri plates (9 cm diameter). One overnight pre-starved adult beetle was confined to feed on the leaf discs. Three replications were maintained for each host and both species of red pumpkin beetle. Leaf area fed was calculated using graph sheets at 12, 24, and 48 hrs after the adult release.

***In vivo* evaluation of orientation of pumpkin beetles towards selected hosts**

The olfactory preference of *A. foveicollis* and *A. intermedia* towards different hosts was evaluated using an olfactometer (Mascot Enterprises, Coimbatore, India). Finely chopped leaves of selected host plants were kept in different hands of an olfactometer @ three hosts at a time. The evaluation was done for all six hosts by replacing the hosts. Twenty-five beetles pre-starved overnight were released at the centre of the olfactometer per set. The central chamber was vacuumed, and mild air was sent through the opening of the hands. Beetles were also replaced when the hosts were replaced in the hands. The number of insects oriented towards the different hosts was recorded. Each experiment was repeated thrice. The orientation of red pumpkin beetles towards other hosts was calculated in percentage.

Estimation of diversity and density of trichomes on selected cucurbit hosts

To identify the reasons for the preference for red pumpkin beetle, the density and types of trichomes present in the abaxial and adaxial leaf surfaces and petioles of the selected hosts were estimated based on micromorphology description for cucurbits given by Ali and Al-Hemaid (2011). One square centimetre section was cut from the leaves of specified hosts. The sectioned samples were observed under a compound microscope (10 X magnification). The number of trichomes and types of trichomes was counted and expressed as trichome density per square centimetre area.

Statistical analysis

The data obtained from the field and laboratory evaluation were analyzed statistically as per the methods described by Panse and Sukhatme (1978).

RESULTS AND DISCUSSION

Incidence of *A. foveicollis* and *A. intermedia* on selected hosts

The field incidence of red pumpkin beetles was noticed in all the test hosts. At 15 DAS, the maximum population of *A. foveicollis* was recorded on cucumber, followed by ridge gourd, pumpkin, and bottle gourd, as against no population on the bitter gourd and snake gourd. Similarly, a higher preference for red pumpkin beetles towards cucumber was observed by Mahmood *et al*. (2005), Khan (2015), and Laila *et al.* (2015). The mean data shows that the maximum population of beetles was observed on pumpkins, followed by cucumber. Significant variation in the beetle population was observed during various days of observation. The hierarchy of preference towards other hosts was cucumber > bottle gourd>ridge gourd >bitter gourd (Table 1). Hassan *et al*. (2012) reported that bitter gourd was less preferred in all the stages.

Host preference of *A. intermedia* varied significantly among the different days of observation. In the early stages of crop growth, ridge gourd was highly preferred, whereas, in the later stages, bottle gourd was the most preferred. The highest mean population of *A. intermedia* was recorded on bottle gourd, and the lowest was on bitter gourd. The order of preference of *A. intermedia* towards other hosts was snake gourd > pumpkin >ridge gourd > cucumber. Similar observations were recorded by Vandana *et al*. (2001), Roy and Pande, 1990 and Mehta and Sandhu (1992).

***In vivo* feeding preference of pumpkin beetles to selected hosts**

In a free choice test, cucumber was the most preferred host by *A. foveicollis*, followed by pumpkin, bottle gourd, snake gourd, and ridge gourd and no leaf area consumption was recorded on bitter gourd. Similarly, in earlier studies,

Table 3. Leaf area consumption of red pumpkin beetles

Host	Leaf area consumption (Sq.cm) free-choice test						Leaf area consumption (Sq.cm) no-choice test					
	A. foveicollis			*A. intermedia*			*A. foveicollis*			*A. intermedia*		
	12 HRS	24 HRS	48 HRS	12 HRS	24 HRS	48 HRS	12 HRS	24 HRS	48 HRS	12 HRS	24 HRS	48 HRS
Pumpkin	0.27 (1.12)^{a}	0.51 (1.22)^{b}	0.97 (1.39)^{b}	0.06 (1.03)^{bc}	0.1 (1.04)^{b}	0.17 (1.08)^{b}	0.06 (1.03)^{b}	0.11 (1.05)^{a}	0.23 (1.10)^{b}	0.39 (1.18)^{c}	0.77 (1.33)^{d}	1.18 (1.47)^{b}
Bitter gourd	0 (1.00)^{a}	0 (1.00)^{a}	0 (1.00)^{a}	0 (1.00)^{a}	0 (1.00)^{a}	0 (1.00)^{a}	0.01 (1.00)^{a}	0.08 (1.04)^{a}	0.17 (1.08)^{ab}	0.01 (1.00)^{a}	0.03 (1.01)^{a}	0.06 (1.03)^{a}
Bottle gourd	0.09 (1.04)^{a}	0.16 (1.07)^{ab}	0.31 (1.14)^{a}	0 (1.00)^{a}	0 (1.00)^{a}	0 (1.00)^{a}	0.03 (1.01)^{a}	0.16 (1.07)^{a}	0.21 (1.09)^{ab}	0.02 (1.01)^{a}	0.07 (1.03)^{a}	0.14 (1.06)^{a}
Ridge gourd	0.02 (1.01)^{a}	0.06 (1.02)^{a}	0.13 (1.06)^{a}	0.3 (1.14)^{a}	0.51 (1.22)^{d}	0.98 (1.40)^{c}	0.28 (1.13)^{c}	0.55 (1.24)^{b}	0.88 (1.37)^{c}	0.26 (1.12)^{b}	0.39 (1.18)^{b}	0.98 (1.40)^{b}
Snake gourd	0 (1.00)^{b}	0.03 (1.01)^{a}	0.25 (1.11)^{a}	0.08 (1.04)^{c}	0.18 (1.08)^{c}	0.26 (1.12)^{b}	0.05 (1.02)^{a}	0.11 (1.05)^{a}	0.14 (1.06)^{a}	0.02 (1.01)^{a}	0.05 (1.02)^{a}	0.07 (1.03)^{a}
Cucumber	0.5 (1.44)	1.67 (1.62)^{c}	2.8 (1.94)^{c}	0.02 (1.01)^{ab}	0.03 (1.01)^{ab}	0.07 (1.03)^{a}	0.73 (1.31)^{d}	1.46 (1.50)^{c}	1.84 (1.68)^{d}	1.1 (1.45)^{d}	2.03 (1.74)^{d}	2.4 (1.84)^{c}
SE(d)	**0.08**	**0.08**	**0.08**	**0.01**	**0.02**	**0.02**	**0.01**	**0.03**	**0.02**	**0.02**	**0.04**	**0.04**
C.D.	**0.19**	**0.18**	**0.18**	**0.02**	**0.03**	**0.05**	**0.02**	**0.05**	**0.03**	**0.04**	**0.08**	**0.08**

*Mean of three replications. Values in parenthesis are square root transformed. Value with different alphabets differs significantly.

Table 6. Correlation between Red Pumpkin beetles and trichome density of leaves (Pearson correlation)

	Trichome density
A.fovicollis	
Population	-0.002^{NS}
Feeding preference	-0.563^{NS}
A.intermedia	
Population	0.943**
Feeding preference	0.076^{NS}

NS- Non-significant
*- Significant at 0.05 % level of probability
**-Significant at 0.05 % level of probability

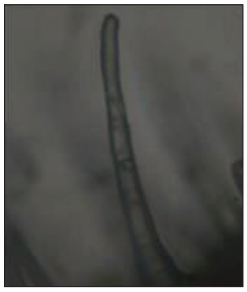


Plate. A. Trichomes of Bottle gourd

Plate. B. Trichomes of Snake gourd

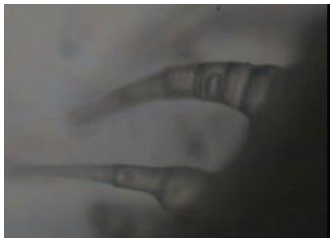


Plate. C. Trichomes of Pumpkin Plate.

D. Trichomes of Ridge gourd

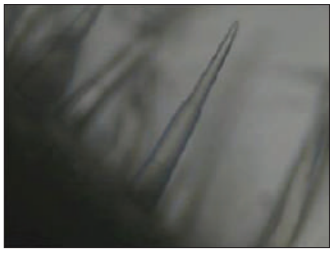


Plate. E. Trichomes of Bitter gourd

Plate. F. Trichomes of Cucumber

cucumber was the most preferred and bitter gourd was the least preferred host of *A. foveicollis* (Khan *et al.,* 2011 and Khan *et al.,*2012). *A. intermedia* registered the maximum consumption of ridge gourd followed by snake gourd, pumpkin, and cucumber; there was no feeding on the bitter gourd and bottle gourd (Table 3). In the confinement test, the maximum leaf consumption by *A. foveicollis* was on cucumber *A. intermedia* fed the maximum on pumpkins which is a deviation from the free choice test (Table 3).

Orientation of pumpkin beetles towards selected hosts

In the orientation assay, the maximum number of *A. foveicollis* oriented towards the bottle gourd, followed by pumpkin, ridge gourd, cucumber, and snake gourd. In contrast, the orientation of *A. intermedia* was the maximum towards the ridge gourd. Orientation of both species was the minimum towards snake gourd and little gourd (Table 4).

Density and diversity of trichomes on the selected host plants and its impact on the preference of *A. foveicollis* and *A. intermedia*

Three types of trichomes, *viz.,* Type-I (thin-walled, irregular shape and flattened disc at base), Type-II (Curved pointed apical cell) and Type-III (short, thick-walled, swollen at the base and pointed tip) as described by Ali and Al-Hemaid, (2011) were observed on the selected hosts. Various types of trichomes were observed in test hosts *viz.,* bottle gourd (Types-I) (Plate A), snake gourd (Type-I, II & III) (Plate B), pumpkin (2 types) (Plate C), ridge gourd (1 type) (Plate D), bitter gourd (3 types) (Plate E) and cucumber (1 type) (Plate F) (Table.5). The highest number of trichomes, irrespective of types was observed in bottle gourd leaves followed by snake gourd and the lowest number of trichomes was observed on cucumber (Table 5). There is no significant effect of trichome density present in the host plants against *A. foveicollis* on population and feeding preference but a significant positive effect on *the A. intermedia* population (Table 6). Dalin *et al*. (2008) reported that trichome density in various plants was suggested as the probable source of resistance against many soft-bodied insects. In the case of red pumpkin beetles, hard-bodied, coleopteran pests were not affected by trichomes in the selected test hosts.

CONCLUSION

Among the host plants, Pumpkin was the most preferred host of *A. foveicollis,* whereas bottle gourd was the most preferred host of *A. intermedia* in both field and laboratory conditions. No significant effect was observed between red pumpkin beetle preference and trichomes density.

ACKNOWLEDGEMENT

The authors thank the authorities of Annamalai University for providing necessary facilities to conduct the experiment.

REFERENCES

Ali, M. A. and Al-Hemaid, F. M. A. 2011. Taxonomic significance of trichomes micromorphologyin cucurbits. *Saudi Journal of Biological Sciences*, **18**: 87–92

Dalin, P., Agren, J., Bjorkman, C., Huttumen, P. and Karkkainen K. 2008. Leaf trichome formation

and plant resistance to herbivory. In: Schaller A, ed. Induced plant resistance to herbivory. Springer Science + Business Media, pp 89-105.

Hassan, K., Uddin, M. M. and Haque, M. A. 2012. Host suitability of red pumpkin beetle, *Aulacophora foveicollis* (Lucas) among different cucurbitaceous hosts. *International Research Journal of Applied Life Sciences,* **1** (4): 91-100.

Khan, M. M. H., Alam, M. Z. and Rahman, M. M. 2011. Host preference of red pumpkin beetle in a choice test under net case condition. *Bangladesh Journal of Zoology,* **39** (2): 231-234.

Khan, M. M. H., Alam, M. Z., Rahman, M. M. and Miah, M. I. and Hossain, M.M. 2012. Influence of weather factors on the incidence and distribution of red pumpkin beetles infesting cucurbits. *Bangladesh Journal of Agricultural Research,* **37** (2): 361-367.

Khan, S. A. 2015. Relative infestation of red pumpkin beetle on different cucurbit vegetables. *Pakistan Entomology*, **37** (1): 45-47

Laila, K., Maqsood, S. and Amjad, U. 2015. Host Preference of Red Pumpkin Beetle (*Aulacophorafaveicollis*) Lucas (Chrysomelidae: Coleoptera) among different Cucurbits. *Journal of Entomology and Zoology Studies*, **3** (2): 100-104.

Mahmood, T., Khokhar, K. M., Hussain, S. I. and Laghari, M. H. 2005. Host preference of red pumpkin beetle, *Aulacophora (Raphidopalpa) foveicollis* (Lucas) among cucurbit crops. *Sarhad Journal of Agriculture,* **21** (3): 473-475.

Mehta, P. K. and Sandhu, G. S. 1992. Cucurbitacins and other biochemicals in relation to cucurbits susceptibility to red pumpkin beetles. *Proceedings of the Indian National Science Academy*, **58** (6): 371-376.

Panse, V. G. and Sukhatme, P. V. 1984. Statistical methods for agricultural workers. Third Edition, Indian Council of Agricultural Research, New Delhi, 2 - 378.

Raman, K. and Annadurai, S. 1985. Host selection and food utilization of red pumpkin beetle, *Raphidopalpa foveicollis* (Lucas) (Chrysomeliade: Coleoptera). *Indian Academy of Science. (Animal Science)*, **94** (5): 547-556.

Rathodi, S.T., Borad, P.K. and Bhat, N.A., 2009. Bio-efficacy of neem based and synthetic insecticides against red pumpkin beetle, *Aulacophora foveicollis* (Lucas) on bottle gourd. *Pest Management in Horticultural Ecosystems*, **15**(2): 150-154.

Roy, D. C. and Pande, Y. D. 1991. Seasonal incidence, host preference and feeding rate of red pumpkin beetle, *Raphidopalpa foveicollis* (Lucas) in Tripura. *Indian Journal of Agricultural Science,* **61** (8): 603-607.

Saljoqi, A. U. R. and Khan, S. 2007. Relative abundance of the red pumpkin beetle, *Aulacophora foveicollis* (Lucas), on different cucurbitaceous vegetables. *Sarhad Journal of Agricultural Science,* **23** (1): 110-114.

Vandana, R. M., Prashad, P. R. and Rao, N. V. 2001. Host preference of *Raphidopalpa foveicollis* (Lucas). *Vegetable Science*, **28** (1): 95-97.

MS Received: 05 December 2022
MS Accepted : 29 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 64-71 (2022)
DOI Number: 10.5958/0974-4541.2022.00040.6

Bioefficacy of insecticides and plant based oils against red spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) in okra

D. NIRUBA[1]*, M. CHANDRASEKARAN[1], C. GAILCE LEO JUSTIN[2] and A. KALYANASUNDARAM[1]

[1]Department of Plant Protection, Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli – 620 027

[2] Horticulture Research Station, Pechiparai.

***E-mail**: nirubatcbm@gmail.com

ABSTRACT: Field trials were conducted at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli district, Tamil Nadu, to evaluate the bioefficacy of insecticides and plant-based oils against red spider mite, *Tetranychus urticae* (Koch) in okra during *rabi* 2021 and *summer* 2022. The results revealed that the highest per cent reduction over control of the red spider mite population was recorded in spiromesifen 25 EC @ 0.8 ml/l (73.28% and 67.78%), followed by propargite 57 EC @ 3ml/l (70.82% and 65.51%) in both the seasons, respectively. Among the plant-based oils, karanj oil was most effective against red spider mites, with a per cent reduction over control of 58.7% and 47.98% in *rabi,* 2021 and *summer* 2022, respectively. Mahua oil (54.02% and 41.72%) and camphor oil (51.62% and 38.74%) moderately control the red spider mite in okra. Based on moderate to high efficacy and safer natural enemies and environment, spiromesifen 25 EC @ 0.8 ml/l, propargite 57 EC @ 3ml/l and karanj oil 2ml/l would be used as an effective component in the IPM module for okra red spider mite.

Keywords: Bioefficacy, red spider mite, okra, insecticides, plant-based oils, karanj oil

INTRODUCTION

Okra, commonly known as lady's finger, is one of the predominant vegetables cultivated throughout India. Okra is a rich source of protein, carbohydrates, fat, iron, iodine and vitamins like A, B, and C, essential components of the human diet (Halder *et al.,* 2005). As a nutritious vegetable, okra is the best food to address doubling farmers' income as well as the problem of malnutrition. The production of okra is impacted by several biotic and abiotic factors, including insect pests and diseases (Gulati *et al.,* 2004). The crop is susceptible to various insect and mite pests, of which red spider mite, *Tetranychus urticae* Koch is most predominant In India (Gupta, 1985; Singh *et al.,* 1987). The first sight of infestation by red spiders mite resulted in a chlorotic, stippled appearance on the leaves. Heavily infested leaves turn pale, dry up, and fall off from the plants, which appear weak, and the photosynthetic activity is seriously hampered.

Using conventional insecticides based on the crop stage has proved to be okra's most effective pest control practice (Krishnakumar and Srinivasan, 1987). The time lag between pesticide application and harvesting is critical in vegetable crops like okra. However, the farmers need to be made aware of the use of pesticides at the fruiting stage and non-adoption of a safe waiting period leads to pesticide residues above Maximum Residual Limit (MRL). The residues of non-approved pesticides were detected in 1180 vegetable samples, and okra was found to have a higher level of pesticides above MRL among those vegetables as reported by Monitoring of Pesticide Residues at National Level (MPRNL) (Anon 2015). Considering the limitations of using insecticides alone and pesticide residue accumulation, the present study was conducted to determine the efficacy of new insecticides, plant-based oils, and safer insecticides in managing red spider mites.

MATERIALS AND METHODS

The present field trials were conducted at the experimental farm of Anbil Dharmalingam Agricultural College and Research Institute, TNAU, Tiruchirappalli, Tamil Nadu, to find out the efficacy of insecticides and plant-based oils against red spider mite, *T. urticae*, during the season of *rabi,* 2021 and *summer,* 2022. The experiments are laid out in a Randomized Block Design (RBD) with eight treatments, including untreated control and replicated thrice. The treatments namely neem oil 3%, karanj oil 2ml/l, mahua oil 3%, camphor oil 1ml/l, azadirachtin 0.03WSP 5.0g/10l, spiromesifen 25EC 0.8ml/l, propargite 57EC 3ml/l were evaluated. All the treatments had two sprays except the untreated control. Okra (Summer gold hybrid) seeds were sown

Table 1. Efficacy of insecticides and Plant based oils against red spider mite, *Tetranychus urticae* in okra (Rabi 2021)- First spray

Treatments	Mite (1 cm²/ 3 leaves/plant) *					
	Pre-treatment	1 DAT	3 DAT	7 DAT	14 DAT	Mean
T_1- Neem oil @ 3%	31.40	15.80	14.07	11.80	15.87	
	(5.65)	(4.04)^{b}	(3.82)^{c}	(3.51)^{d}	(4.05)^{b}	14.38
T_2- Karanj oil @ 2ml/l	33.00	15.07	13.00	10.00	16.00	
	(5.79)	(3.95)^{b}	(3.67)^{b}	(3.24)^{c}	(4.06)^{b}	13.52
T_3- Mahuaoil @ 3%	34.67	16.20	14.13	12.67	17.00	
	(5.93)	(4.09)^{c}	(3.83)^{d}	(3.63)^{e}	(4.18)^{d}	15.00
T_4- Camphor oil @ 1ml/l	31.80	16.87	15.67	13.40	17.33	
	(5.68)	(4.17)^{d}	(4.02)^{e}	(3.73)^{f}	(4.22)^{d}	15.82
T_5- Azadirachtin 0.03 WSP @ 2.0 g/l	31.20	10.43	12.07	14.13	16.53	
	(5.63)	(3.31)^{b}	(3.54)^{b}	(3.83)^{b}	(4.13)^{bc}	13.29
T_6- Spiromesifen 25 EC @ 0.8 ml/l	31.80	5.13	6.13	8.80	15.20	
	(5.68)	(2.37)^{a}	(2.58)^{a}	(3.05)^{a}	(3.96)^{a}	8.82
T_7- Propargite 57 EC @ 3 ml/l	31.93	5.80	6.80	10.00	15.80	
	(5.70)	(2.51)^{a}	(2.70)^{a}	(3.24)^{a}	(4.04)^{a}	9.60
T_8- Untreated control	31.33	33.13	32.47	31.13	32.60	
	(5.64)	(5.80)^{e}	(5.74)^{f}	(5.62)^{g}	(5.75)^{e}	32.33
SEd	NS	0.07	0.09	0.04	0.06	-
CD (p=0.05)	NS	0.15	0.19	0.09	0.12	-

*Mean of three replications
DAT- Days After Treatment
Figures in the Parentheses are √x+0.5 transformed values
Values in the column followed by same letters are not different statistically, (p=0.05) by LSD
NS – Non-significant

Table 2. Efficacy of insecticides and plant-based oils against red spider mite, *Tetranychus urticae* in okra (Rabi 2021)- Second spray

Treatments	Mite (1cm^2 /3 leaves/plant) *						Reduction over control (%)	Yield(t/ha)
	1 DAT	3 DAT	7 DAT	14 DAT	Mean	Cumulative mean#		
T_1- Neem oil @ 3%	15.20 (3.96)^b	13.53 (3.75)^c	11.27 (3.43)^c	15.60 (4.01)^b	13.90	14.14	55.99	3.5
T_2- Karanj oil @ 2ml/l	14.67 (3.89)^b	12.53 (3.61)^b	9.47 (3.16)^{bc}	15.40 (3.99)^b	13.02	13.27	58.70	4.00
T_3- Mahuaoil @ 3%	15.73 (4.03)^d	13.73 (3.77)^e	12.20 (3.56)^d	16.53 (4.13)^{cd}	14.55	14.78	54.02	3.2
T_4- Camphor oil @ 1ml/l	16.40 (4.11)^d	15.07 (3.95)^e	12.80 (3.65)^e	16.80 (4.16)^d	15.27	15.54	51.62	3.04
T_5- Azadirachtin 0.03 WSP @ 2.0 g/l	9.73 (3.20)^c	11.53 (3.47)^d	13.67 (3.76)^b	16.00 (4.06)^{bc}	12.73	13.01	59.51	4.48
T_6- Spiromesifen 25 EC @ 0.8 ml/l	4.73 (2.29)^a	5.60 (2.47)^a	8.33 (2.97)^a	14.73 (3.90)^a	8.35	8.58	73.28	5.60
T_7- Propargite 57 EC @ 3 ml/l	5.47 (2.44)^a	6.33 (2.61)^a	9.60 (3.18)^{ab}	15.20 (3.96)^a	9.15	9.38	70.82	5.12
T_8- Untreated control	31.60 (5.67)^e	31.93 (5.70)^f	32.40 (5.74)^f	31.80 (5.68)^e	31.93	32.13	55.99	2.08
SEd	0.08	0.07	0.06	0.05	-	-	-	-
CD (p=0.05)	0.17	0.16	0.12	0.11	-	-	-	-

*Mean of threereplications
#Mean of first and second spraying
DAT- Days After Treatment
Figures in the Parantheses are √x+0.5 transformed values
Values in the column followed by same letters are not different statistically, (p=0.05) by LSD
NS – Nonsignificant

with a spacing of 60×45cm. All the agronomic packages and practices Tamil Nadu Agricultural University recommended were followed to raise the crop, except the plant protection practices. The treatments were imposed when the red spider mite population crossed ETL. The observations on the incidence of the red spider mite were recorded before treatment and on 1, 3, 7 and 14 days after insecticide application. Mite populations were assessed in one cm^2 area on the top, middle and bottom leaves in each of the five randomly selected and tagged plants from each replication. The reduction of the red spider mite population in respective treatments over control was computed. using the formula (Susheelkumar *et al.,* 2020).

STATISTICAL ANALYSIS

The data was statistically analysed after square root transformation using AGRES software. The treatment mean values were compared by Latin Square Design (LSD).

RESULTS AND DISCUSSION

Field efficacy of insecticides and plant-based oils against red spider mite in okra presented in Table 1, 2, 3 and 4.

Rabi 2021

The pre-treatment population count ranged from 31.2 to 34.67/cm2/3 leaves/plant. Significant differences among the treatments were noted on 1, 3, 7 and 14 DAS; all treatments were superior to untreated control. After the first spraying, the minimum mean population of 8.82/cm^2/3 leaves/plant was recorded in spiromesifen 25EC, followed by propargite 57EC (9.60/cm^2/3 leaves/plant), Azadirachtin 0.03 WSP (13.29/cm^2/3leaves/plant), karanj oil (13.52/cm2/3 leaves/plant), Neem oil @ 3% (14.38/cm^2/3leaves/plant), Mahua oil @ 3% (15.00/cm^2/3leaves/plant), and Camphor oil (15.82/cm^2/3leaves/plant) as against untreated control (32.33/cm^2/3leaves/plant) (Table 1). After imposed second spraying, the mean mite population varied from 8.35 to 31.93/cm^2/3 leaves/plant, and a similar trend in population reduction was observed (Table 2).

The cumulative mean data of two sprayings revealed that spiromesifen 25 EC recorded the minimum population of red spider mite (8.58/cm^2/3 leaves/plant) followed by propargite 57 EC (9.38/cm^2/3 leaves/plant) and azadirachtin 0.03 WSP (13.01/cm^2/3leaves/plant) with per cent reduction over control of 73.28, 70.82 and 59.51, respectively and among the plant-based oils karanj oil recorded the minimum mite population of 13.27/cm^2/3leaves/plant with 58.70 per cent reduction over control. The maximum yield was recorded in the effective treatment spiromesifen 25 EC (5.6 t/ha), followed by propargite 57 EC (5.12 t/ha), azadirachtin 0.03 WSP (4.48 t/ha), karanj oil (4.00 t/ha), neem oil (3.5 t/ha), mahua oil (3.2 t/ha), camphor oil (3.04 t/ha) as against untreated control (2.08 t/ha).

Summer 2022

Before spraying of treatments, the mite population ranged from 49.4 to 50.47/cm^2/3 leaves. After the first spraying was imposed, the mean mite population varied from 16.33 to 31.03/cm^2/3 leaves/plant. The minimum mean mite population was recorded in spiromesifen 25 EC (16.33/cm^2/3 leaves/plant), followed by propargite 57 EC (17.52/cm^2/3 leaves/plant), azadirachtin 0.03 WSP (20.93/cm^2/ 3 leaves/plant), karanj oil (26.43/cm^2/3 leaves/plant), neem oil (28.05/cm^2/3 leaves/plant), mahua oil (29.50/cm^2/3 leaves/plant), camphor oil (31.03/cm^2/3 leaves/plant), as against untreated control (50.25/cm^2/3 leaves/plant) (Table 3). After the second spraying, the mean mite population varied from 15.77 to 49.38/cm2/3 leaves/plant, and a similar trend in population reduction was observed (Table 4). The cumulative mean data of two spraying indicated that spiromesifen 25 EC recorded the minimum population of red spider mite (16.05/cm^2/3 leaves/plant), followed by propargite 57 EC (17.18/cm^2/3 leaves/plant), azadirachtin 0.03 WSP (20.57/cm^2/3 leaves/plant) with per cent reduction over control of 67.78, 65.51 and 58.72 respectively. Among the plant-based oils, karanj oil @ 2ml/l recorded the minimum population of 25.92/cm^2/3 leaves/plant with a 47.98 per cent reduction over control.

The maximum yield was recorded in the effective treatment spiromesifen 25 EC (4.96 t/ha), followed by propargite 57 EC (4.64 t/ha), azadirachtin 0.03 WSP (4.16 t/ha), karanj oil (3.68 t/ha), as against 1.76 t/ha in the untreated control. The results from the present field trial against red spider mites in okra showed that among the treatments, spiromesifen25 EC was the most effective against red spider mites, followed by propargite 57 EC and azadirachtin 0.03 WSP. From the plant oils used, the karanj oil effectively controlled the mite population, followed by neem, mahua, and camphor.

Spiromesifen is a systemic insecticide/acaricides belonging to the class of spirocyclic tetronic acid derivatives, which act as inhibitors of acetyl-coenzyme-A carboxylase and causes a reduction in total lipid biosynthesis. Propargite is a systemic and contact insecticide. It interferes with the key mite enzyme systems, which causes interruption of normal metabolism, respiration, and electron transport functions in the nervous system of mites. Plant-based oils act

Table 3. Efficacy of insecticides and Plant based oils against red spider mite, *Tetranychus urticae* in okra (Summer 2022) - First spray

Treatments	Mite (1 cm²/ 3 leaves/plant) *					
	Pre-treatment	1 DAT	3 DAT	7 DAT	14 DAT	Mean
T_1- Neem oil @ 3%	49.40	31.60	27.40	23.33	29.87	
	(7.06)	(5.67)^{b}	(5.28)^{c}	(4.88)^{c}	(5.51)^{c}	28.05
T_2- Karanj oil @ 2ml/l	50.00	30.00	25.87	21.73	28.13	
	(7.11)	(5.52)^{b}	(5.13)^{b}	(4.72)^{b}	(5.35)^{b}	26.43
T_3- Mahua oil @ 3%	50.47	34.07	28.60	24.73	30.60	
	(7.14)	(5.88)^{d}	(5.39)^{e}	(5.02)^{e}	(5.58)^{d}	29.50
T_4- Camphor oil @ 1ml/l	50.07	34.80	30.33	26.13	32.87	
	(7.11)	(5.94)^{e}	(5.55)^{f}	(5.16)^{f}	(5.78)^{e}	31.03
T_5- Azadirachtin 0.03 WSP @ 2.0 g/l	51.07	18.00	18.13	20.53	27.07	
	(7.18)	(4.30)^{c}	(4.32)^{d}	(4.59)^{d}	(5.25)^{bc}	20.93
T_6- Spiromesifen 25 EC @ 0.8 ml/l	50.00	9.93	11.53	18.47	25.40	
	(7.11)	(3.23)^{a}	(3.47)^{a}	(4.36)^{a}	(5.09)^{a}	16.33
T_7- Propargite 57 EC @ 3 ml/l	50.33	11.27	12.33	19.67	26.80	
	(7.13)	(3.43)^{a}	(3.58)^{b}	(4.49)^{ab}	(5.22)^{a}	17.52
T_8- Untreated control	49.93	49.73	50.60	50.20	50.47	
	(7.10)	(7.09)^{f}	(7.15)^{g}	(7.12)^{g}	(7.14)^{f}	50.25
SEd	NS	0.04	0.05	0.04	0.04	-
CD (p=0.05)	NS	0.09	0.11	0.08	0.08	-

*Mean of three replications
DAT- Days After Treatment
Figures in the Parentheses are √x+0.5 transformed values
Values in the column followed by same letters are not different statistically, (p=0.05) by LSD
NS – Non-significant

Table 4. Efficacy of insecticides and plant-based oils against red spider mite, *Tetranychusurticae* in okra (Summer 2022)- Second spray

Treatments	Mite (1cm² /3 leaves/plant) *						Reduction over control (%)	Yield(t/ha)
	1 DAT	3 DAT	7 DAT	14 DAT	Mean	Cumulative mean#		
T_1- Neem oil @ 3%	30.40 (5.56)[b]	26.73 (5.22)[c]	22.27 (4.77)[c]	28.67 (5.40)[c]	27.02	27.53	44.73	3.36
T_2- Karanj oil @ 2ml/l	28.67 (5.40)[b]	24.73 (5.02)[b]	20.93 (4.63)[b]	27.27 (5.27)[b]	25.40	25.92	47.98	3.68
T_3- Mahua oil @ 3%	33.27 (5.81)[d]	27.27 (5.27)[e]	23.87 (4.94)[d]	29.87 (5.51)[d]	28.57	29.03	41.72	2.88
T_4- Camphor oil @ 1ml/l	33.93 (5.87)[e]	29.07 (5.44)[f]	25.07 (5.06)[e]	31.93 (5.70)[e]	30.00	30.52	38.74	2.40
T_5- Azadirachtin 0.03 WSP @ 2.0 g/l	17.20 (4.21)[c]	17.67 (4.26)[d]	19.60 (4.48)[c]	26.33 (5.18)[bc]	20.20	20.57	58.72	4.16
T_6- Spiromesifen 25 EC @ 0.8 ml/l	9.93 (3.23)[a]	10.73 (3.35)[a]	17.80 (4.28)[a]	24.60 (5.01)[a]	15.77	16.05	67.78	4.96
T_7- Propargite 57 EC @ 3 ml/l	10.67 (3.34)[a]	11.67 (3.49)[bc]	18.93 (4.41)[a]	26.13 (5.16)[ab]	16.85	17.18	65.51	4.64
T_8- Untreated control	49.27 (7.05)[f]	49.27 (7.05)[g]	49.80 (7.09)[f]	49.20 (7.05)[f]	49.38	49.82	44.73	1.76
SEd	0.04	0.05	0.05	0.04	-	-	-	-
CD (p=0.05)	0.09	0.12	0.11	0.08	-	-	-	-

*Mean of three réplications
#Mean of first and second spraying
DAT- Days After Treatment
Figures in the Parentheses are √x+0.5 transformed values
Values in the column followed by same letters are not different statistically, (p=0.05) by LSD
NS – Non-significant

as feeding deterrents against the mite. Bathani *et al*. (2019) reported similar results that diafenthiuron 50 WP was most effective for controlling mites, followed by abamectin 1.9 EC and propargite 57 EC in sesame. Biradar and Nadaf (2014) reported that bifenazate 240 SC and propargite 57 EC significantly reduced grapes' mite population (0.30 mites/3 leaves). Patel *et al.* (2017) evaluated the bioefficacy of different acaricides against brinjal mite *and Tetranychus urticae* and found that spiromesifen 0.02% and fenazaquin 0.01% were most effective against the mite. The maximum fruit yield was recorded in spiromesifen 0.02% treated plot (37.91 quintal/ha) followed by fenazaquin 0.01% (36.95 quintal /ha).

The effectiveness of plant-based oils against mites, as recorded in the present study, was closely related to Patel *et al.* (2020), who reported that neem oil 0.5% was found to be most effective, followed by NSKE 5% against mites in brinjal. Further, Raghavendra *et al.,* (2017) also proved that tulsi leaf extract @ 10%, neem oil @ 3% and nochi leaf extract @ 5% were found to be the best with per cent reduction over control of 81.15, 80.58 and 79.98 respectively, which can be recommended as an alternative to synthetic chemical acaricides for the management of *Tetranychus urticae.* Baskaran and Sathyaseelan (2019) recorded that Azadirachtin 1 %, neem oil + mahua oil 3% was effective against mites in okra. Bullar *et al.,* (2021) reported that pongamia (karanj) extract was effective against mites in brinjal.

CONCLUSION

The present study showed that spiromesifen 25EC was most effective against red spider mites in okra, followed by propargite 57EC and azadirachtin. Among the plant oils, karanj oil effectively reduced the mite population, which can be used as an effective component in the IPM module for okra red spider mites.

ACKNOWLEDGEMENT

The authors are grateful to all the faculty members of Department of Plant Protection, Anbil Dharmalingam Agricultural College, and Research Institute Tiruchirappalli, for providing field, necessary facilities for carrying out this study.

REFERENCES

Abrol, D. P. and Singh, J. B. 2003. Effect of insecticides on the resurgence of the red spider mite, *Tetranychus cinnabarinus* Boisdual on brinjal in Jammu, India. *Journal of Asia-Pacific Entomology*, **6**(2): 213-219.

Anon, R. and Pallavi, M. S. 2021. Determination, Dissipation and Decontamination of Thiamethoxam Using LC-MS/MS in Okra Fruits. *Indian Journal of Entomology*, 1-6.

Baskaran, V. and V, Sathyaseelan. 2019. Evaluation of eco-friendly agents against, red spider mite, *Tetranychus urticae* koch on okra. *Innovative Farming,* **4** (2):115-117.

Bathani, S. B., M. L. Patel. and N. J. Hadiya. 2019. Bio-efficacy of different acaricides against broad mite, *Polyphagotarsonemus latus (*Banks) in Kharif sesame. *Journal of Pharmacognosy and Phytochemistry,* **8** (3):4758 - 4762.

Bhullar, M., Heikal, H., Kaur, P. and Kaur. R. 2021. Efficacy of natural products and biorationals against two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) infesting brinjal (*Solanum melongena* L.) under protected cultivation. *International Journal of Acarology,* **47** (8): 677-683.

Biradar, A. P. and A. M. Nadaf. 2014. Bio efficacy of Bifenazate 240 SC (Floramite) against mites on Grape, *Vitis vinifera*." *Annals of Plant Protection Sciences,* **22** (2): 317-319.

Chakrabotry, G., Roy, D. and Sarkar, P. K. 2015. Effect of temperature on tea red spider mite (*Oligonychuscoffeae*Niether) and its management using cyflumetofen 20SC. *The Bioscan*, **10**(1): 1093-1098.

Chandra Sekhar, D., Jagdishwar Reddy, D., Rahman, S. J., Ranga Reddy, A. and Narendranath, V. V. 2006. February. Ecology and management of red spider mite, *Tetranychus urticae* Koch on grape. In *International Symposium on Grape Production and Processing* 785 (335-342).

Ghosh, S. K. 2013. Incidence of red spider mite (*Tetranychusurticae* Koch) on okra (*Abelmoschus esculentus* (L.) Moench) and their sustainable management. *Current Biotica*, **7**(1&2): 40-50.

Ghosh, S. K. and Chakraborty, K. 2014. Bio-efficacy of plant extracts against red spider mite (*Tetranychus spp*.) infesting brinjal (*Solanum melongena* L.). *Research journal of Agricultural and Environmental Sciences*, **1**(1): 26-31.

Gulati, R. 2004. Incidence of *Tetranychus cinnabarinus* (Boisd.) infestation in different varieties of *Abelmoschus esculentu*s L. *Annals of Plant Protection Sciences,* **12** (1):45-47.

Gupta, A. 1985. Effect of environment on the life processes of Earias fabia Stoll, a pest of bhindi, *Hibiscus esculentus* (Okra) in an arid climate in and around Jodhpur (Raj.). *Transactions of Indian Society of Desert Technology and University Centre of Desert Studies*.

Halder, J., A. B. Rai and M. H. Kodandaram.2013. Compatibility of neem oil and different entomopathogens for the management of major vegetable sucking pests. *National Academy Science Letters,* **36** (1):19-25.

Karabhantanal, S. S., Udikeri, S. S., Vastrad, S. M. and Wali, S. Y. 2012. Bio efficacy of different acaricides against red spider mite, *Tetranychus urticae* on grapes. *Pest Management in Horticultural Ecosystems*, **18**(1): 94-97.

Krishnakumar, N. K. and Srinivasan. K. 1987. Efficacy and economics of pest control of okra with conventional and synthetic pyrethroid insecticides. *Indian Journal of Plant Protection,* **15**: 81-83.

Kumar, S., Sachan, S. K., Singh, R. and Singh, D. V. 2020. Bio-efficacy of some newer insecticides and bio-pesticides against whitefly (*Bemisia tabaci* Gennadius) in Brinjal ecosystem. International Journal of Conservation Science, **8**(5): 1883-1888.

Patel, N. B. and C. C. Patel. 2017. Relative bio-efficacy of different acaricides against brinjal mite, *Tetranychus urticae Koch*. *International Journal of Current Microbiology and Applied Sciences,* **6** (7): 4353 - 4363.

Patel, N. B., R. K. Thumar and C. C. Patel. 2020. Efficacy of different bio-pesticides against brinjal mite, *Tetranychus urticae* Koch. *Journal of Entomology and Zoology Studies*, **8** (3):1049-1053.

Peter, C. and David, B. V.1988. Evaluation of some insecticides for the control of major insect pests of okra and their side effects. *International Journal of Pest Management*, **34**(1): 76-80.

Raghavendra, K.V., C. Chinniah., G.T. Jayasimha and R. Gowthami. 2017. Bio-efficacy of plant derivatives and natural oils against two-spotted spider mite, *Tetranychus urticae Koch*." *Journal of Entomology and Zoology Studies,* **5**:1456-1461.

Roobakkumar, A., Subramaniam, M. S., Babu, A., and Muraleedharan, N. 2010. Bioefficacy of certain plant extracts against the red spider mite, *Oligonychus coffeae* (Nietner) (Acarina: Tetranychidae) infesting tea in Tamil Nadu, India. *International Journal of Acarology*, **36**(3): 255-258.

Sangeetha, S. and Ramaraju, K. 2013. Relative toxicity of fenazaquin against two-spotted spider mite on okra. *International journal of vegetable science*, **19**(3): 282-293.

Shukla, A., G. G. Radadia and G. D. Hadiya. 2017. Estimation of loss due to two spotted spider mite, *Tetranychus urticae Koch* (Acari: Tetranychidae) infesting brinjal. *International Journal of Current Microbiology and Applied Science,* **6** (9): 2145-2150.

Singh, A. K, Koul, K, Shankar, U, Singh, S. K, Mondal, A, and Singh, M. 2018. Seasonal incidence and management of red spider mite, *Tetranychus urticae* Koch on Okra, *Abelmoschus esculentus* (L.) Moench. *Journal of Entomology and Zoology Studies*, **6**: 650-656.

Singh, R. 1987. Effect of okra fruit blocks, seeds and pericarp on post-embryonic development of *Earias vittella (*Fab.) in relation to some phytochemicals of selected okra genotypes. *Proceedings: Animal Sciences,* **96** (4): 361-367.

Yadav, S. K, Kumawat, K. C., Deshwal, H. L. and Kumar, S. 2020. Evaluation of sequences of insecticides, biopesticides and bioagents against major insect pests of okra. *Journal of Entomology and Zoology Studies*, **8**(5): 1870-1875.

MS Received: 27 July 2022
MS Accepted : 29 September 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 72-77 (2022)

DOI Number.: 10.5958/0974-4541.2022.00041.8

Development and evaluation of formulations of *Lecanicillium lecanii* against *Myzus persicae*

Y. B. VARUN[1], S. H. RAMANAGOUDA[2], J. B. GOPALI[3], SHASHIKANT EVOOR[2], T. N. SACHINKUMAR[2] and S. H. RASHMI[2]

[1]Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bengaluru 560089, India

[2]College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka

[3]Regional Horticultural Research and Extension Center, Kumbapur farm Dharwad, Karnataka

***E-mail**: goudaento@gmail.com

ABSTRACT: Evaluation of oil and talc-based formulations of an indigenous isolate of *Lecanicillium lecanii* (Zimm.) Zare and Games was performed at Kittur Rani Chanamma College of Horticulture (KRCCH), Arabhavi. Different formulations of *Lecanicillium lecanii* were developed and tested against cabbage aphid, *Myzus persicae* (Sulzer) under laboratory and field conditions. Among the various formulations screened, ground nut and sesamum oil-based formulations recorded the highest mean mortality of 96.66 per cent, followed by sunflower oil (95%), coconut oil (94.15%) and mustard oil (93.33%). However, most formulations of *L. lecanii* developed recorded more than 81.50% mean mortality of *M. persicae* under laboratory conditions. Further, based on the efficacy under laboratory conditions, the four best formulations of *L. lecanii* were evaluated against *M. persicae* under field conditions. Among the selected formulations, the maximum per cent reduction *of M. persicae* was recorded in sesamum oil, resulted in 15.14 and 18.60 per cent reductions over control after the first and second sprays.

Keywords: *Lecanicillium lecanii*, *Myzus persicae,* Sesamum oil, Ground nut oil

INTRODUCTION

In agroecosystems, indiscriminate usage of chemical insecticides disturbed the ecosystem's sustainability due to the mortality of predators, parasitoids and pollinators. Therefore, the farmers are inclined towards organic insect pest management (personal communication, while survey), which is valid for the other parts of the country and the world (Parvatha Reddy, 2008). There is a need for ecologically, economically and socially acceptable technology for insect pest management. The biological control of insect pests is a viable partial alternative to insecticides in Integrated Pests Management (IPM). Among the biological control agents, microbial bioagents are considered the best, of which entomopathogenic fungi is the first organism to cause disease in insect and are most important for insect pest management (Fan *et al.,* 2007).

Lecanicillium lecanii is an important entomopathogenic fungus that suppresses sucking insect pests like aphids, leafhoppers, thrips, whitefly, scales, mealy bugs and mites (Goettel *et al.,* 2008; Shinde *et al.*, 2010; Pillai and Visalakshy, 2017; Mani *et al.,* 2016). Cabbage is one of the important crops among the cruciferous vegetables in India. Several insect pests attack this crop, *viz.,* cutworm, diamondback moth, cabbage looper, cabbage leaf webber, tobacco leaf eating caterpillar and green peach aphid. Among the insect pests of cabbage, the cabbage aphid, *Myzus persicae* (Sulzer) is an important sucking pest, which not only reduces the marketable yield but also reduces consumer preference by producing honeydew (Akbar *et al.*, 2010). To keep the menace of the cabbage aphids under control during the seedling and head development stage, the farmers are employing insecticides that result in pesticide residue, pollution to the environment and negatively affecting the non-target organism. Therefore, an attempt was initiated to manage the cabbage aphid with bio-control agents by developing and evaluating formulations of the native isolate of *L. Lecanii*.

MATERIAL AND METHODS

Development of formulations of *Lecanicillium lecanii*

Rearing of cabbage aphid

The wild population of the test insect, the cabbage aphid, *Myzus persicae*, was collected from Kittur Rani Chanamma College of Horticulture (KRCCH) vegetable fields, Arabhavi, during which year. The culture of the test insect was maintained on cabbage seedlings under net house conditions in pots. When plants were 15-20 days old, the field-collected aphids were released,

and the culture of aphids was maintained until all the experiments were completed (Gokak *et al.*, 2017). All the laboratory experiments were conducted in the Biocontrol laboratory, Department of Entomology, K. R.C. College of Horticulture Arabhavi.

Preparation of oil formulations of *Lecanicillium lecanii*

The oil formulations were prepared by following the method of the previously reported procedure with slight modifications (Bhanu Prakash *et al.*, 2015). The different edible oils, such as groundnut, sunflower, coconut, mustard, sesamum and soybean, were sterilized at 121 oC for 15 minutes. They were later cooled under room temperature and utilized for the preparation of oil formulation of *L. lecanii*. Six oil formulations of *L. lecanii* were prepared separately by mixing 20 ml spore suspension of *L. lecanii* with 10 ml of autoclaved cooled oils with tween-20 (0.05 %) and 1 ml of a sticker in one liter of sterilized distilled water and finally filtered through a muslin cloth.

Preparation of powder formulations of *Lecanicillium lecanii*

Different powder formulations of *L. lecanii* were developed by mixing the spore powder of *L. lecanii* obtained from different grains (rice, bajra, wheat and ragi) with talc powder in the ratio of 1:1. Later combination of both spore and talc powder were grinded for 30 seconds to made it as fine dust and finally packed in a sterilized polythene bag.

Preparation combination of oil and powder formulations of *Lecanicillium lecanii*

Sixteen combinations of oil and spore powder of *L. lecanii* were prepared by mixing different oils, spore dust and spore suspension in a proportion of 10:10:20 with tween 20 (0.05 %) and one ml of sticker in 1 liter of sterilized distilled water and finally filtered through muslin cloth.

Evaluation of developed formulations of *Lecanicillium lecanii* against *Myzus persicae* under laboratory condition

A total of 26 different formulations of *L. lecanii* were developed which includes six oil, four powder based and 16 combination of both oil and powder. All the developed formulations of *L. lecanii* and control (distilled water) were evaluated against *M. persicae* under laboratory conditions for their efficacy.

Bioassay study

About 2.0 ml of each formulations of *L. lecanii* ($2x10^8$ cfu/ml) dissolved in one liter of water and the solution was smeared on the detached fresh cabbage leaf disc (9 cm dia.) and later it was shade dried and placed on Petri dish containing a thin layer of one per cent water agar. A batch of uniform size of 20 net house reared *M. persicae* nymphs were released to each Petri dish. The Petri dish were maintained at room temperature 27.0 ± 1.0 ºC and the relative humidity of 70.0 ± 5.0 per cent. Another group of 20 uniform size of *M. persicae* was released on the leaf smeared with double distilled sterilized water, served as control. Each treatment was replicated twice with a 20 *M. persicae* per replication.

Observations recorded

The mortality of *M. persicae* was recorded after three, five and seven days after treatment (DAT). The moribund aphids were also counted as dead. The per cent aphid mortality was worked out by using following formula.

$$\text{Per cent aphid mortality} = \frac{\text{Number of dead/ moribund aphids}}{\text{Number of live aphids}} \times 100$$

Evaluation of selected formulations of *Lecanicillium lecanii* against cabbage aphid under field condition

Based on laboratory observation, we selected four best formulation of *L. lecanii* to evaluate against *M. persicae* the under field condition. The experiment was conducted in a randomized block design with three replication and eight treatments at College of Horticulture (COH), Bagalkot. The imposition of treatments were made once the infestation of *M. persicae* reached the economic threshold level in field condition at fifteen days interval. The observations were recorded on number of aphids per leaf on one day before and three, five, seven, ten and 15 days after each spray.

$$\text{Reduction over control (\%)} = \frac{\text{(Control – Treatments)}}{\text{Control}} \text{ x } 100$$

RESULTS AND DISCUSSION

A total of 26 different formulations of *L. lecanii* (oil, talc-based and a combination of both) were screened against *M. persicae* under laboratory conditions. The result revealed that the per cent mortality increased with the advancement of the exposure period. The highest mean mortality of *M. persicae* was recorded in groundnut and sesamum oil formulations (96.66 %), followed by sunflower oil (95%), coconut oil (94.16%), and a combination of groundnut oil + rice grains (93.33%)**.** However, most formulations developed

Table 1. Details of the selected formulations of *Lecanicillium lecanii* tested under field condition

Treatments	Formulations	Dosage
T_1	Ground nut oil formulation (GNO) @ 2×10^8cfu/ml	2 ml/l
T_2	Sesamum oil formulation (SEO) @ 2×10^8 cfu/ml	2 ml/l
T_3	Rice grain formulation (RCG) @ 2×10^8 cfu/ml	2 g/l
T_4	Combination of GNO+RCG@ 2×10^8 cfu/ml	2 ml/l
T_5	NSKE	4%
T_6	Commercial formulation of *L. lecanii*@ 2×10^8 cfu/ml	2 g/l
T_7	Imidacloprid 17.8 SL	0.25 ml/l
T_8	Untreated Control	-

Statistical analysis: Data on aphid counts were subjected to square root transformation for reliable analysis and treatments means were compared by Duncan's Multiple Range Test (DMRT) by using ICAR- Web Agri Stat Package (WASP) software.

recorded more than 81.66 per cent mean morality of *M. persicae,* which was significantly superior over untreated control (Table 2). The reasons for more efficacies of all developed formulations might be due to indigenous isolate of *L. lecanii* and their more virulence, the synergist effect of vegetable oils and grain media and talc powder, and they supply good nutrients, mainly carbohydrates which favour better multiplication of spores. In addition, there was a controlled climatic condition with low temperature and high humidity.

The effectiveness of entomopathogenic fungi after three days of treatment reached a maximum of 90.00 per cent, and after five days of treatment, more than 90.00 per cent, and still it was more after seven days of treatment and reached the peak of a cent per cent mortality of *M. persicae* in all the developed formulations of *L. lecanii*. The efficacy of the entomopathogenic fungi began within 48 h after inoculation, and the hyphae penetrated the integument, epithelial and epidermal cells. After 72h, the fat tissues were damaged, and mortality reached 100 per cent after 96 hrs, as explained by Ei-Sinary (2006) and Quesada- Moraga *et al.* (2006). Among different grains tested, the spores multiplied on rice and sorghum grains recorded the maximum mortality of mealy bugs (96 per cent) after nine days of treatment, as reported by Banu (2013). Similarly, Karthikeyan and Selvanarayanan (2013) reported about a cent per cent morality of *Aphis gossypii and Bemisia tabaci* in a liquid formulation of *L. lecanii* while 93.33 per cent mortality of *A. devastans* was recorded. Three oil and one crude formulation of *Nomuraea (Metarhizium) rileyi* were evaluated against third-instar larvae of *Spodoptera litura* by Sharmila *et al.* (2015). The mean larval mortalities of *S. litura* of 96.67, 93.33, 86.67 and 76.67 per cent were recorded in groundnut, sunflower and coconut oil and crude formulations of *N. rileyi* (1x108 cfu/ml), respectively, at 10 days after treatment. Among the different formulations of *L. lecanii* that were developed and evaluated against *M. persicae,* the oil-based formulations recorded the highest mortality of *M. persicae* compared to using different grains alone and in combination with edible oils. The reason was that oils form a cover over the conidial surface, prevent drying before actual germination and help in more prolonged survival, assist by disrupting the waxy layer of insect cuticle, and better penetration of peg into integument.

Similarly, a field experiment was conducted to evaluate the effect of selected formulations of *L. lecanii* against *M. persicae*. The data on the bio efficacy of various treatments in reducing the aphid population after the first and second sprays are furnished in Table 3. A day before the spray, the population of aphids were uniform across the experimental plot, and it differed significantly after the first spray on different days of treatments. The mean number of aphids per leaf was significantly lowest (3.11 aphids/ leaf) in the imidacloprid treatment, followed by NSKE (5.97 aphids /leaf). Among the selected formulations, sesamum oil recorded the significantly lowest mean aphids (7.45 aphids/ leaf), which was on par with groundnut oil (7.47 aphids/ leaf) and groundnut oil + rice grain (7.67 aphids/ leaf).

Table 2. *In-vitro* evaluation of formulations of *Lecanicillium lecanii* against *Myzus persicae*

Treatments	Per cent mortality of *Myzus persicae*			
	3 DAT	5 DAT	7 DAT	Mean
T_1-Ground nut oil formulation (GNO)	90.00 (71.56) [a]	100 (89.71) [a]	100 (89.71) [a]	96.66 (79.46) [a]
T_2 -Sun flower oil formulation (SFO)	87.50 (69.29) [ab]	97.50 (80.90) [b]	100 (89.71) [a]	95.00 (78.26) [a]
T_3 -Coconut oil formulation (CNO)	85.00 (67.21) [bc]	97.50 (80.90) [b]	100 (89.71) [a]	94.16 (76.01) [b]
T_4 - Soybeanoil formulation (SYO)	82.50 (65.27) [cd]	95.00 (77.07) [c]	100 (89.71) [a]	92.50 (74.10) [bc]
T_5-Mustard oil formulation (MSO)	82.50 (65.27) [cd]	97.50 (80.90) [b]	100 (89.71) [a]	93.33 (75.03) [bc]
T_6 - Sesamum oil formulation (SEO)	90.00 (71.56) [a]	100 (89.71) [a]	100 (89.71) [a]	96.66 (79.46) [a]
T_7 -Rice grain formulation (RCG)	77.50 (61.68) [e]	97.50 (80.90) [b]	100 (89.71) [a]	91.66 (73.21) [c]
T_8 -Ragi grain formulation (RGG)	72.50 (58.37) [f]	92.50 (74.10) [d]	100 (89.71) [a]	88.33 (70.02) [d]
T_9 -wheat grain formulation (WHG)	70.00 (56.78) [fg]	90.00 (71.56) [e]	100 (89.71) [a]	86.66 (68.57) [de]
T_{10} -Bajra grain formulation (BJG)	72.50 (58.37) [f]	92.50 (74.10) [d]	100 (89.71) [a]	88.33 (70.02) [d]
T_{11} -Combination of MSO+RCG	57.50 (49.31) [j]	95.00 (77.07) [c]	100 (89.71) [a]	84.16 (66.54) [e-h]
T_{12} -Combination of MSO+RGG	65.00 (53.72) [ghi]	92.50 (74.56) [d]	100 (89.71) [a]	85.83 (67.88) [def]
T_{13} -Combination of MSO+WHG	65.00 (53.72) [ghi]	90.00 (71.56) [e]	100 (89.71) [a]	85.00 (67.30) [efg]
T_{14} - Combination of MSO+BJG	65.00 (53.72) [ghi]	87.50 (69.29) [f]	100 (89.71) [a]	84.16 (66.54) [e-h]
T_{15} -Combination of SEO+RCG	62.50 (52.23) [hij]	97.50 (80.90) [b]	100 (89.71) [a]	86.66 (68.57) [de]
T_{16} -Combination of SEO+RGG	65.00 (53.72) [ghi]	92.50 (74.10) [d]	100 (89.71) [a]	85.83 (67.88) [def]
T_{17}- Combination of SEO+WHG	62.50 (52.23) [hij]	85.00 (67.21) [g]	95.00 (77.07) [b]	81.66 (64.64) [h]
T_{18} -Combination of SEO+BJG	67.50 (55.24) [fgh]	92.50 (74.10) [d]	100 (89.71) [a]	86.66 (68.57) [de]
T_{19} -Combination of GNO+RCG	80.00 (63.43) [de]	100 (89.71) [a]	100 (89.71) [a]	93.33 (75.03) [bc]
T_{20} -Combination of GNO+RGG	77.50 (61.68) [e]	97.50 (80.90) [b]	100 (89.71) [a]	91.66 (73.21) [c]
T_{21} -Combination of GNO+WHG	70.00 (56.78) [fg]	87.50 (69.29) [f]	100 (89.71) [a]	85.83 (67.88) [def]
T_{22} -Combination of GNO+BJG	57.50 (49.31) [j]	90.00 (71.56) [e]	100 (89.71) [a]	82.50 (65.27) [gh]
T_{23} -Combination of CNO+RCG	70.00 (56.78) [fg]	95.00 (77.07) [c]	100 (89.71) [a]	88.33 (70.02) [d]
T_{24} -Combination of CNO+RGG	60.00 (50.76) [ij]	97.50 (80.90) [b]	100 (89.71) [a]	85.83 (67.88) [def]
T_{25} -Combination of CNO+WHG	70.00 (56.78) [fg]	90.00 (71.56) [e]	100 (89.71) [a]	86.66 (68.57) [de]
T_{26} -Combination of CNO+BJG	57.50 (49.31) [j]	92.50 (74.10) [d]	100 (89.71) [a]	83.33 (65.90) [fgh]
T_{27} -Distilled water as control	0.00 (0.28) [k]	12.50 (20.61) [h]	35.00 (36.22) [c]	15.83 (23.44) [i]
S. Em ±	0.73	0.42	0.57	0.55
C. D. at 1%	2.87	1.64	2.27	1.87
CV (%)	1.85	0.78	0.93	1.83

DAT-Days after treatment; Figures in the parenthesis are Arc sin transformed values
In a column, means followed by same alphabet(s) do not differ significantly by DMRT (P=0.01)

In contrast, the commercial formulation of *L. lecanii* and rice grain formulation recorded 8.03 and 8.30 aphids per leaf, respectively. After the first spray, the untreated control recorded the maximum mean number of aphids per leaf (8.78 aphids/ leaf). However, after different days of treatments, there was a significant difference recorded with respect to the number of aphids per leaf. The mean number of aphids was significantly lowest in the imidacloprid (3.82 aphids /leaf) treatment, followed by NSKE (12.75 aphids /leaf). Among the selected formulations, the significant lowest mean number of aphids per leaf was recorded in sesamum (18.68 aphids /leaf), groundnut oil (19.15 aphids /leaf) and rice grain in combination with groundnut oil (19.75 aphids/leaf), and they were on par with each other. The untreated control recorded the highest 22.95 mean number of aphids per leaf in the cabbage crop.

The results under field conditions revealed that all the treatments significantly reduced the *M. persicae* over the untreated control. The maximum per cent reduction *in M. persicae* was recorded in imidacloprid followed by NSKE, sesamum oil, groundnut oil, a combination of groundnut oil with rice grain, rice grain formulation and commercial formulations *of L. lecanii* after the first and second spray. The results of the present findings are compared with the earlier workers. Sahayaraj and Namachivayam (2011) reported *L. lecanii* reduced about 62 per cent of *A. craccivora* at 39 days after seedling emergence (DASE). Further, Shivakumar *et al*. (2022) reported that *Verticillium lecani* is effective in reducing the aphid *Aphis nerii* population under laboratory (73.3%) and field conditions (43.84 %). The repeated application of an oil-based formulation of *L. lecanii* at 15 days intervals showed 70 per cent mortality of *P. marginatus* under field conditions by Gulsar and Gopalakrishnan (2012). The effect of all vegetable oils and grain media showed a positive response on the growth and development of *L. lecanii* and increased mortality of *M. persicae.* All the formulations of *L. lecanii* significantly recorded increased mortality with the advancement of the exposure period and reached a cent per cent mortality of *M. persicae* compared to untreated control.

ACKNOWLEDGMENT

Authors are grateful to Head, Division of Entomology, COH Bagalkot, for supporting and giving valuable suggestion throughout the study.

REFERENCES

Akbar, M. F., Haq, M. A., Parveen, F., Yasmin, N. and Khan M., F. U. 2010. Comparative management of cabbage aphid *Myzus persicae* (Sulzer) (Aphididae: Hemiptera) through bio- and synthetic - insecticides. *Pakistan Entomology,* **32**(1): 13-17.

Banu, J. G. 2013. Effect of different storage conditions on spore viability of *Lecanicillium lecanii* formulations and infectivity to mealy bug, *Paracoccus marginatus. International Journal of Plant Protection*, **6**: 334-337.

Bhanu Prakash, G. V. S., Sankar, U. V. R. and Padmaja, V. 2015. Development and testing of mycopesticide formulations of *Metarhizium anisopliae* (Metschnikoff) for shelf life and field application against *Spodoptera litura* (Feb.) larvae. *International Journal of Bioassays*, **4** (9): 4284-4289.

El-Sinary, G. H. 2006. Influence of the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) on the mature larvae of the potato tuber moth, *Phthorimaea operculella* (Zeller) under different degrees of temperature and relatively humidity. *Journal of Agricultural Science,* **27**: 4151 - 4161.

Fan, Y., Fang, W., Guo, S., Pei, X., Zhang, Y. and Xiao, Y. 2007. Increased insect virulance in *Beauveria bassiana* strains over expressing an engineered chitinase. *Applied and Environmental Microbiology*, **73**: 295-302.

Goettel, M. S., Koike, M., Kim, J. J., Aiuchi, D., Shinya, R. and Brodeur, J. 2008. Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. *Journal of Invertebrate Pathology*, **98**: 256-261.

Gokak, R., Ramanagouda, S. H. and Jayappa, J. 2017. Collection, isolation and bioassay studies of indigenous isolates of *Lecanicillium lecanii* (Zimm.) Zare and Games against *Myzus persicae* (Sulzer). *Journal of Entomology Zoology Studies*, **5** (2): 686-691.

Gulsar, B. J. and Gopalakrishnan, N. 2012. Development of formulations of a native entomopathogenic fungus, *Lecanicillium lecanii* and testing virulence against mealy bug, *Paracoccus marginatus* infesting cotton. *Indian Journal of Plant Protection,* **40** (3): 182-186.

Mani, M., Smitha, M.S. and Najitha, U., 2016. Root mealybugs and their management in horticultural crops in India. *Pest Management in Horticultural Ecosystems*, **22**(2): 103-113.

Parvatha Reddy, P. 2008. Organic farming for sustainable agriculture. Scientific publishers.

Pillai, K.G. and Visalakshy, P.N., 2017. Entomopathogenic fungi for the management of whitefly, Bemisia tabaci (Hemiptera: Aleyrodidae) on gerbera (Ge5bera jamesonii) in polyhouses. *Pest Management in Horticultural Ecosystems*, **23**(1): 31-36.

Quesada-Moraga, E., J. Carrasco-Diaz, A. and C. Santiago-Alvarez. 2006. Insecticidal and antifeedant activities of proteins secreted by entomopathogenic fungi against *Spodoptera littoralis* (Lep: Noctuidae). *Journal of Applied Entomology,* **130**: 442-452.

Sahayaraj, K. and Namachivayam, S. K. R. 2011. Field evaluation of three entomopathogenic fungi on groundnut pests. *Tropicultura*, **29** (3): 143-147.

Sharmila, T., Manjula, K. and Murali Krishna, T. 2015. Evaluation of oil formulations of *Nomuraearileyi* (Farlow) Samson against *Spodoptera litura* under laboratory conditions. *International Journal of Plant Sciences*, **10** (1): 29-32.

Shinde, S. V., Patel, K. G., Purohit, M. S., Pandya, J. R. and Sabalpara, A. N. 2010. *Lecanicilliumlecanii* (Zimm.) Zare and Games an important Biocontrol agent for the management of insect pests -a review. *Agricultural Research Communication Centre,* **31** (4): 235 - 252.

Shivakumara, K.T., Keerthi, M.C. and Polaiah, A.C., 2022. Efficacy of different biorational insecticides against *Aphis nerii* Boyer de Fonscolombe (Hemiptera. Aphididae) on Gymnema sylvestre (R. Br) under laboratory and field conditions. *Journal of Applied Research on Medicinal and Aromatic Plants*, **28**, p.100358.

MS Received: 30 November 2022
MS Accepted : 19 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 78-80 (2022)
DOI Number: 10.5958/0974-4541.2022.00042.X

Studies on abundance and visitation rate of pollinators in radish (*Raphanus sativus* L.)

M. MAHESH[1]*, K. PADMINI[2], G. K. RAMEGOWDA[1], R. VENUGOPALAN[2], M. ANJANAPPA[1]

*[1]College of Horticulture, Bengaluru, University of Horticultural Sciences, Bagalkot,
[2] ICAR-Indian Institute of Horticultural Research, Hesaraghatta lake post, Bengaluru – 560089, India

***E-mail:** mahesh11orange@gmail.com

ABSTRACT: Radish, *(Raphanus sativus* L.,) a popular edible root vegetable (Family: Brassicaceae), is a highly cross-pollinated crop completely dependent on pollinators for seed set and yield. The present study was carried out at the experimental field of ICAR- Indian Institute of Horticultural Research, Bengaluru, during the *rabi*, 2020- 2021, to study the foraging behaviour of insect pollinators of *R. sativus*. Results indicated that four insect species from Hymenoptera viz., *Apis florea, Apis dorsata, Tetragonula iridipennis,* and *Apis cerana* and a butterfly*, Delias eucharis* (common jezebel larva), were observed visiting radish flowers. Peak insect visitor activity was observed between 09.00 A.M. to10.00 A.M. and 12.00 P.M. to 01.00 P.M. during peak flowering time in January. Among the four species of honey bees, *T. iridipennis* was the most abundant (29%), followed by *A. cerana* (28%), *A. florea* (25 %) and *Apis dorsata* (18%).

Keywords: Radish, *Raphanus sativus* L., pollinator visitation rate, honey bee

INTRODUCTION

Radish, *Raphanus sativus* L. (Family: *Brassicaceae*), is a popular edible root vegetable native to the Mediterranean region. It is a cross-pollinated crop with a sporophytic system of self-incompatibility; hence, it completely dependant on pollinators. Pollinators of major field and horticultural crops include honey bees, bumblebees, and many species of solitary bees. The extent of natural cross-pollination in radish is 65% (Stewart *et al.,* 2002). However, the low seed yield problem in radish prevails ranging from 2 to 20g/plant in open pollination. The reason for less seed setting could be due to lesser pollinator availability and visitation and only a few reports are available in radish. Hence, in the present study, an experiment was carried out to determine the abundance of pollinators and their visitation rate in radish in open pollination.

MATERIALS AND METHODS

The field experiment was conducted during the *rabi* season from 2020 to 2021 at ICAR-Indian Institute of Horticultural Research, Bengaluru. The radish (Cv. Arka Nishant) was planted on October 25, 2020, in a plot of 6.6 × 3.6 m, and the experimental design followed was factorial RBD with three replications. The number of plants per plot was 40, with a spacing of 60×60cm. An abundance of major insect pollinators on radish flowers was recorded during the peak flowering period of the crop. The number of pollinators visiting per plant/ minute was noted in five randomly selected plants. These observations were recorded from 07:00 A.M. to 05:00 P.M. at half an hour at ten-minute intervals for seven days each at peak flowering. Insecticides were not used during the blooming period to encourage pollinator activity.

RESULTS AND DISCUSSION

During the study period, it was observed that only honey bee species visited radish flowers regularly and abundantly, whose observations are documented in this study. Apart from these bees, few butterflies were observed in a scattered and negligible proportion, of which only the identity of common jezebel (*Delias eucharis*) could be established. Data on visitation rates by different honey bee species on radish (Cv. Arka Nishant) flowers at different hours during February, 2021 are represented in Table 1. Irrespective of the day hours, the significantly highest number of bees visited in a minute (4.85) was by *T. iridipennis,* followed by *A. cerana* (4.55) and *A. florea* (4.13). It was almost half of the bees visited by *A. dorsata* (2.90 bees/plant/minute) (Table 1). The study was in agreement with Divija *et al.* (2022) and Sharma *et al.* (2016) on radish blooms Cv. Pusa Himani and Pritish *et al.* (2012) on Radish Cv. Punjab Safeda. The work by Nandini *et al.* (2018) on okra flower also supported that the abundance of Hymenopterans was maximum than other insects.

Irrespective of the bee species, significantly the highest number of bees visited (6.50 bees/plant/min) between 11:00 to A.M. to12:00 P.M. of a day, followed by 10:00 to 11:00 AM, 9:00 AM to 10:00 AM and 12:00 AM to13:00 P.M., which were on par with each other (11-14% bees/plant/min) (Fig.1). Similarly, Mahfouz *et al.* (2012) observed a maximum number of honeybees from 9:00 AM to 11:00 AM. The number of bees was

Table 1. Visitation rate of pollinators in radish Cv. Arka Nishant (number per plant per minute) during daytime

Bee Species	0700 - 0800	0800 - 0900	0900 - 1000	1000 - 1100	1100 – 1200	1200 - 1300	1300 - 1400	1400 - 1500	1500 - 1600	1600 - 1700	Mean A
Apis florea	3.44	4.14	4.44	5.86	6.00	5.16	3.71	3.13	2.71	2.71	4.13
Apis cerana	0.29	5.43	6.86	5.43	6.43	4.44	5.00	5.02	4.43	2.15	4.55
Tetragonula iridipennis	2.72	2.43	7.44	8.14	9.43	7.14	2.86	2.72	3.29	2.29	4.85
Apis dorsata	0.71	4.00	2.30	1.71	4.14	3.29	2.71	2.85	4.14	3.15	2.90

Factors	C.D.	SE(d)	SE(m)
Bee Species	0.030	0.015	0.010
Day hours	0.047	0.023	0.017
Bee Species X Day hours	0.094	0.047	0.033

Fig 1. Insect foragers on radish flowers

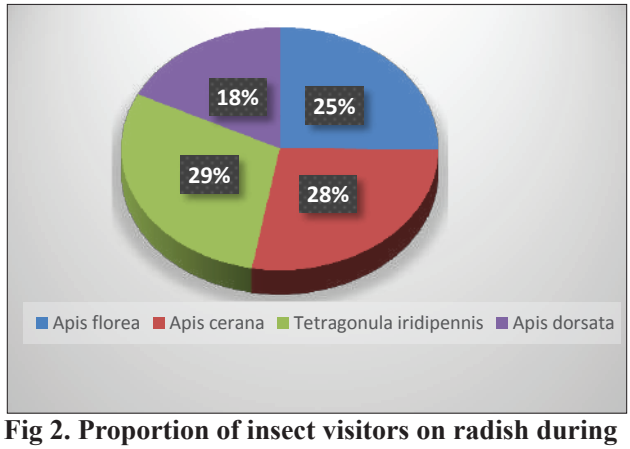


Fig 2. Proportion of insect visitors on radish during the peak flowering period

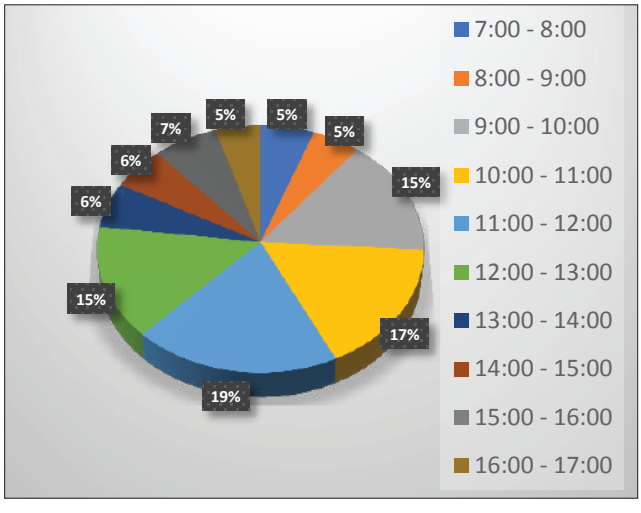


Fig 3. The abundance of insect visitors on radish flowers from morning 7 AM to evening 5 PM

drastically reduced from 3:00 PM to 4:00 PM. Neeraj and Ramashrit (2005) also found that the maximum foragers (*Apis* spp.) were at 11:00 AM, while the least number was observed at 3:00 PM.

When both the bee species and day hours were considered, significantly highest flowers were visited by *T. iridipennis* between 11:00 AM to 12:00 noon, followed by *A. cerana* and *A. florea,* which were on par with each other and less number of visitations by *A. dorsata*. Among the four species studied, *A. cerana* was the early visitor in huge numbers (5.43 flowers at 8:00 to 9:00 AM and 10:00 to 11:00AM which was statistically third highest for that species after 11:00AM to 12:00 noon and 9:00 to 10:00 AM to 13:00 hr of a day and during the rest of the day, it was half of the mean flower visited. Flower visitation by *A. florea* was almost consistent for most of the day, with peak hours from 11:00 AM to 12:00 noon. The *A. dorsata* visitation rate appeared in

a tri-modal distribution with peaks from 11:00 to 12:00 noon, 15:00 to 16:00, and 9:00 to 10:00AM (Table 1). *T. iridipennis* had the highest abundance (29 %) of the four honey bee species, followed by *A. cerana* (28 %) and *A. florae* (25 %), with *A. dorsata* being the lowest (Fig. 2). They play an important role in flower pollination. The total abundance of all visitor bees was most significant in the middle of the day, *i.e.,* at 10 AM and 01.00 PM. Murphy and Robertson (2000) also reported that the abundance and diversity of insect pollinators varied considerably between observational periods. Other studies also illustrated that the abundance of insect pollinators differed across the time of the day but increased around midday (10:00 AM to 12:00 noon) (Semida and Elbanna 2006; Andrej and Anton 2006). Considering all these parameters for establishing the effective visitors of the crops, *T. iridipennis, A. cerana,* and *A. florea* were the most effective visitors of the radish crop in this study.

CONCLUSION

Based on the current findings, radish flowers were found to be highly attractive to a wide range of insect species, particularly those in the order Hymenoptera. *Tetragonula iridipennis, Apis cerana, Apis florea,* and *Apis dorsata* were the most abundant and frequent visitors to the radish flower, according to abundance and visitation rate.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Director, ICAR- IIHR, Bengaluru for providing the necessary facilities for the experiment.

REFERENCES

Andrej, S. and Anton, I. 2006. Pollinators of *Helleborus niger* in Slovenian naturally occurring populations. *Acta Agriculturae Slovenica,* **87** (2): 205 – 211.

Chandrashekhar, G.S. and Sattigi, H. N. 2006. Pollinator fauna and foraging activity of bees and other pollinators in radish. *Karnataka Journal of Agricultural Sciences*, **19** (3): 719–721.

Divija, S. D., Sravan Kumar, P., Krishnarao, G. and Nisarga, G. S. 2022. Diversity, abundance and foraging behaviour of insect pollinators in radish (*Raphanus raphanistrum* subsp. *sativus* L.). *Journal of Asia-Pacific Entomology,* **25:**101909

Mahfouz, H. M., Kamel., S. M., Belal., A. H. and Said, M. 2012. Pollinators visiting sesame *Sesamum indicum* l.) Seed crop with reference to foraging activity of some bee species. *Cercetari agronomice in Moldova,* **15**(2):49-55.

Murphy, C. and Robertson, A. 2000. Preliminary study of the effects of honey bees (*Apis mellifera*) in Tongariro National Park. *Science Conservation.* **139**: 1-18.

Nandhini, E., Padmini, K., Venugopalan, R., Anjanappa, M. and Lingaiah, H. B. 2018. Flower-visiting insect pollinators of okra (*Abelmoschus esculentus*) (L.) Moench) in Bengaluru region, *Journal of Pharmacognosy and Phytochemistry,* **7**(2): 1406-1408.

Neeraj, K. and Ramashrit, S. 2005. Relative abundance of *Apis* spp. on rabi season sunflower (*Helianthus annus* L.). *Journal of the Entomological Research*, **29** (1): 65-69.

Pritish, J. 2012. Studies on insect pollinators on seed crop of radish. M.Sc. thesis submitted to CCS Haryana Agricultural University. 1-60.

Semida, F. and Elbanna, S. 2006. Impact of introduced honeybees on native bees at St. Katherine Protectorate, South Sinai, Egypt. *International Journal of Agriculture and Biology,* **8**(2): 191-194.

Sharma, H., Sapna, L. and Kiran, R. 2016. *Apis cerana* F. as an important natural pollinator of radish (Raphanus sativus L.) under mid-hill conditions of Himachal Pradesh. *Inv bio-resources. Stress Management,* **7**(5):1156-1160.

Sattigi, H. N., Lingappa, S. and Kulkarni, K. A. 1996. Seasonal variation of foraging behaviour of Indian bee*, Apis cerana* Fab. *Karnataka Journal of Agricultural Sciences*, **1:** 43-48.

Uma, Pratap, Verma, L. R. 1994. Pollination of radish by *Apis cerana*. *Journal of Apicultural Research,* **33**(4): 237-241.

Verma, S. K. and Phogat, K. P. S. 1994. Impact of pollination by honeybees, *Apis cerana* on yield of radish under caged condition of Himalayan hills. *Indian Bee Journal,* **45**: 183-186.

MS Received: 06 November 2022
MS Accepted : 28 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 81-86 (2022)

DOI Number: 10.5958/0974-4541.2022.00043.1

Effect of medicinal plants on cocoon parameters of PM×CSR2 inoculated with *Bm*NPV and *Staphylococcus sciuri*

K. V. ANITHARANI[1]*, R. N. BHASKAR[1], MANJUNATH GOWDA[1], S. CHANDRASHEKHAR[1], and ANITHA PETER[2]

[1]Department of Sericulture, [2]Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India

***E-mail:** anitharani.kv710@gmail.com

ABSTRACT: The silkworm *Bombyx mori* L. is an economically important insect and is prone to many diseases, including flacherie. To manage this disease, chemical based bed disinfectants are being used. These bed disinfectants leave residual toxicity in the rearing bed and inside the rearing house. Given these constraints, using plant extracts and their biomolecules was found to be an alternative to manage the grasserie and flacherie diseases. The leaves of *Adhatoda vasica* and *Phyllanthus niruri* contain many secondary metabolites that possess antimicrobial properties against a wide range of pathogens. The aqueous and methanolic leaf extracts of *A. vasica* and *P. niruri* administered to fourth and fifth instar batches of silkworm (PM×CSR2) recorded the maximum cocoon weight (1.94 and 1.81 g) shell weight (0.326 and 0.298 g), pupal weight (1.58 and 1.46 g) and shell ratio (17.65 and 16.69 %) in methanolic extracts of *P. niruri,* followed by *A. vasica,* aqueous extract of *P. niruri* and *A. vasica.* Whereas the inoculated (*Bm*NPV and *S. sciuri*) batches also recorded the same trend of observations (1.56 and 1.64., 0.255 and 0.271., 1.24 and 1.32 g., 16.21 and 16.05 %) in both fourth and fifth instar silkworms compared to their controls.

Keywords: Silkworms, PM×CSR2, *Adhatoda vasica, Phyllanthus niruri,* cocoon parameters

INTRODUCTION

The silkworm *Bombyx mori* L. is affected by many diseases, among which, flacherie is one of the severe diseases of silkworms caused by bacteria and viruses, with disease incidence of 57.22 per cent of all other diseases of the silkworm in Karnataka (Chopade *et al*., 2021). The flacherie refers to flaccidity in the larva, and the affected silkworm become feeble, lethargic and possess transparent cephalo-thoracic regions. As a result, the larvae vomit gut juice and extrude soft faeces with higher water content (Shah, 2016). The incidence of flacherie was higher during summer, followed by the rainy season and low during winter. The bacterial flacherie of silkworms is caused by different groups of bacteria: *Streptococcus faecalis, Staphylococcus aureus, Serratia* sp. and *Bacillus* sp. (Sugun, 2000). To manage these diseases, chemical based bed disinfectants are being used. Applying chemical disinfectants and their formulations for controlling flacherie leaves residual toxicity in rearing beds and houses. Given these constraints, biomolecules derived from botanical sources could be found as an alternative to treat the grasserie and flacherie diseases. Several botanicals revealed they are effective in silkworm rearing and disease management (Manimegalai and Chandramohan, 2006).

Recent interest has shifted to use of safer and natural botanicals to manage pathogens in sericulture. In this regard, medicinal plants with active constituents that show antimicrobial activity against microorganisms should be exploited to develop disinfectants against a wide range of pathogens (Madhusudhan *et al.,* 2018). The leaves of *Adhatoda vasica* and *Phyllanthus niruri* contain many secondary metabolites. They have been reported to have antiviral (Serkedjieva, 2004; Yasuhara- Bell *et al*. 2010) and antibacterial properties (Choudhary *et al.* 2017).

MATERIALS AND METHODS

Collection and sample preparation

The leaves of medicinal plants *Adhatoda vasica* (Adusoge) and *Phyllanthus niruri* (Kirunelli) were collected from 'Sanjeevini Vatika' (Herbal Garden), Department of Horticulture, UAS, GKVK, Bengaluru. The required quantity of fresh leaves of each plant was harvested and surface sterilized with 70 per cent ethyl alcohol, then washed with sterile distilled water and shade dried. The shade-dried plant samples were then slowly powdered in an electric blender, sieved, and stored in desiccators (Krishnaprasad *et al.*, 1979).

Preparation of plant extract

The aqueous and methanolic extracts of *A. vasica* and *P. niruri* were prepared by the soxhlet extraction method (Ajanal *et al*., 2012) using distilled water and methanol as solvents. Ten grams of leaf powder from both medicinal plants were taken in 250 ml of solvents (Distilled water and methanol), and extracts were prepared (10:250 g/ml). Later, by using a rotary evaporator, these extracts were reduced to 100 ml. The extracts were evaluated on

fourth and fifth instar larvae of PM×CSR2 (Kolar Gold) against *Bm*NPV and *Staphylococcus sciuri*.

Inoculation of silkworms with bacterial isolate

Inoculation of silkworms was done on the fourth and fifth instar first day, *i.e*., immediately after the third and fourth moult. The bacterial stock was prepared, from which 10^{-7} dilution (2.33×10^{9} CFU/ml) was prepared using 9 ml distilled water. The newly moulted fourth and fifth instar larvae were starved for 6 hours and distributed in trays. Each treatment contained three replications (50 larvae/replication). The suspension of haemolymph bacteria was smeared on mulberry leaves and fed to silkworms at 1 ml/50 larvae.

Inoculation of silkworms with *Bm*NPV

The serial dilution (10^{-9}) of *Bm*NPV suspension (6.75×10^{4}) was prepared using distilled water. After the complete feeding of mulberry leaves smeared with bacteria, the larvae were fed with a mulberry leaf smeared with 1.00 ml of diluted PIBs suspension. Three batches were kept as a control in which larvae were fed with leaves smeared with distilled water, the second batch was a leaf with methanol, and the third batch was only mulberry leaves without any application. All the larvae of each treatment and control were fed on fresh mulberry leaves till spinning.

Application of botanical extracts

After 30 minutes of inoculation with PIBs, each botanical extract (1:3) was smeared on leaves and fed to silkworms at 1 ml/50 larvae (Fig. 1). The control batches were fed with distilled water and methanol-sprayed leaves.

Fig 1. Mulberry leaves smeared with leaf extracts before administration

RESULTS AND DISCUSSION

A significant difference was observed between the healthy and inoculated batches, whereas the results were on par with the three controls. The interaction effect between plant extracts and the health of silkworms in both the instars administered showed non-significant results (Table 1). The aqueous and methanolic leaf extracts of *A. vasica* and *P. niruri* were administered to the fourth and fifth instar batches of silkworm (PM×CSR2). The data on cocoon weight registered maximum in methanolic extract of both *P. niruri* (1.94 and 1.81 g/cocoon*)* and *A. vasica* (1.89 and 1.78 g) followed by aqueous extracts (1.81 and 1.72., 1.78 and 1.69 g), respectively. Further, the *Bm*NPV and *S. sciuri* inoculation to silkworms followed by botanical extract administration have recorded the cocoon weight of 1.47, 1.54, 1.48 and 1.56 g in the fourth and 1.52, 1.60, 1.53 and 1.64 g in fifth instar silkworms administered with aqueous and methanolic extracts of *A. vasica* and *P. niruri.* Furthermore, the control batches *viz*., distilled water, methanol and absolute control recorded higher cocoon weight in healthy (1.67, 1.58 and 1.57g in the fourth instar; 1.67, 1.62 and 1.62 g in the fifth instar) compared to inoculated silkworms (1.44, 1.46 and 1.37 g in fourth instar; 1.46, 1.50 and 1.45 g in fifth instar).

Significant results were recorded for shell weight over the control in plant extracts administered in batches of fourth and fifth instars (Table 2). The methanolic extract of *P. niruri* recorded the highest shell weight in healthy and inoculated batches of the fourth (0.326 and 0.255 g) and fifth instar (0.298 and 0.271 g) compared to the methanolic extract of *A. vasica* (0.301 and 0.251., 0.289 and 0.259 g), aqueous extract of *P. niruri* (0.293 and 0.234., 0.278 and 0.247 g) and *A. vasica* (0.291and 0.229., 0.273 and 0.239 g). The fourth instar treated silkworm batch recorded significantly higher shell weight (0.260, 0.276, 0.264 and 0.291 g) than the fifth instar batches (0.256, 0.274, 0.263 and 0.284 g). The control batches *viz.*, distilled water control (0.259 and 0.217, 0.257 and 0.209 g), methanolic control (0.251 and 0.209., 0.250 and 0.210 g), and absolute control (0.249 and 0.206., 0.257 and 0.209 g) recorded significantly lesser shell weight compared to botanical treated batches of both healthy and infected silkworms (Table 2). The interaction effect between plant extracts and the health of silkworms (healthy and infected) was found non-significant.

The effect of administering medicinal plant extracts to healthy and inoculated batches of fourth and fifth instar PM×CSR2 on pupal weight was assessed, and recorded significant results. The pupal weight of 1.58, 1.51, 1.45 and 1.45 g was recorded and found non-significant in the

Table 1. Effect of administration of plant extracts of *Adhatoda vasica* and *Phyllanthus niruri* on cocoon weight (g) of fourth and fifth instar treated batches of *B. mori*

Treatments	IV instar			V instar		
	Healthy	Inoculated	Mean	Healthy	Inoculated	Mean
***A. vasica* -Aqueous**	1.78	1.47	1.63	1.69	1.52	1.61
A. vasica-Methanol	1.89	1.54	1.71	1.78	1.60	1.69
P. niruri-Aqueous	1.81	1.48	1.64	1.72	1.53	1.63
P. niruri-Methanol	1.94	1.56	1.75	1.81	1.64	1.72
Distilled water control	1.67	1.44	1.55	1.67	1.46	1.56
Methanol control	1.58	1.46	1.52	1.62	1.50	1.56
Absolute control	1.57	1.37	1.47	1.62	1.45	1.54
Mean	1.75	1.47	1.61	1.70	1.53	1.62
Results	**A**	**B**	**AB**	**A**	**B**	**AB**
F-test	*	*	NS	*	*	NS
S.Em ±	0.05	0.02	0.06	0.04	0.02	0.06
CD at 5 % level	0.13	0.07	0.19	0.12	0.06	0.17

*Significant at 5 % level, NS: Non-significant; A: Plant extracts, B: Health of silkworm

Table 2. Effect of administration of plant extracts of *Adhatoda vasica* and *Phyllanthus niruri* on shell weight (g) of fourth and fifth instar treated batches of *B. mori*

Treatments	IV instar			V instar		
	Healthy	Inoculated	Mean	Healthy	Inoculated	Mean
A. vasica - Aqueous	0.291	0.229	0.260	0.273	0.239	0.256
A. vasica- Methanol	0.301	0.251	0.276	0.289	0.259	0.274
P. niruri-Aqueous	0.293	0.234	0.264	0.278	0.247	0.263
P. niruri-Methanol	0.326	0.255	0.291	0.298	0.271	0.284
Distilled water control	0.259	0.217	0.238	0.257	0.209	0.233
Methanol control	0.251	0.209	0.230	0.250	0.210	0.230
Absolute control	0.249	0.206	0.228	0.257	0.209	0.233
Mean	0.282	0.229	0.255	0.272	0.235	0.253
Results	**A**	**B**	**AB**	**A**	**B**	**AB**
F-test	*	*	NS	*	*	NS
S.Em ±	0.005	0.003	0.007	0.009	0.005	0.012
CD at 5 % level	0.015	0.008	0.021	0.025	0.013	0.035

*Significant at 5 % level, NS: Non-significant; A: Plant extracts, B: Health of silkworm

fourth instar healthy silkworm batch administered with methanolic extract of *P. niruri, A. vasica,* aqueous extract of *P. niruri* and *A. vasica,* respectively. The trend was the same in the fifth instar healthy batch, which recorded 1.46, 1.44, 1.39 and 1.37 g of pupal weight compared to their controls.

Further, in the pathogen (*Bm*NPV and *S. sciuri*) inoculated batches, significant results were found for the maximum pupal weight of 1.24, 1.22, 1.19 and 1.17 g in methanolic extract of *P. niruri, A. vasica,* aqueous extract of *P. niruri* and *A. vasica* of fourth instar. The trend was same in the fifth instar inoculated batch (1.32, 1.30, 1.24 and 1.22 g). Among the three control batches maintained for healthy and inoculated batches of both the instars, the healthy silkworms recorded maximum pupal weight (1.37, 1.34 and 1.33., 1.35, 1.32and 1.31 g) compared to inoculated batches (1.14, 1.18 and 1.11., 1.16, 1.17 and1.17 g) in distilled water, methanol and absolute control, respectively (Table 3).

The *in-vivo* effect of botanical extracts and their additive effect on *Bm*NPV and *S. sciuri* inoculation to the fourth and fifth instar of PM×CSR2 registered non-significant results for shell ratio (Table 4). The highest shell ratio of 17.65 and 16.21., 16.69 and 16.05 per cent

Table 3. Effect of administration of plant extracts of *Adhatoda vasica* and *Phyllanthus niruri* on pupal weight (g) of fourth and fifth instar treated batches of *B. mori*

Treatments	IV instar			V instar		
	Healthy	Inoculated	Mean	Healthy	Inoculated	Mean
A. vasica -Aqueous	1.45	1.17	1.31	1.37	1.22	1.30
A. vasica-Methanol	1.51	1.22	1.36	1.44	1.30	1.37
P. niruri-Aqueous	1.45	1.19	1.32	1.39	1.24	1.31
P. niruri-Methanol	1.58	1.24	1.41	1.46	1.32	1.39
Distilled water control	1.37	1.14	1.25	1.35	1.16	1.26
Methanol control	1.34	1.18	1.28	1.32	1.17	1.24
Absolute control	1.33	1.11	1.22	1.31	1.17	1.24
Mean	1.44	1.18	1.31	1.38	1.23	1.30
Results	**A**	**B**	**AB**	**A**	**B**	**AB**
F-test	NS	*	NS	NS	*	NS
S.Em ±	0.04	0.02	0.06	0.03	0.02	0.05
CD at 5 % level	0.12	0.06	0.17	0.10	0.05	0.14

*Significant at 5 % level, NS: Non-significant; A: Plant extracts, B: Health of silkworm.

in the methanolic extract of *P. niruri*, followed by *A. vasica* (17.47 and 16.11., 16.64 and 16.00 %). However, the aqueous extract of *P. niruri* (17.18 and 16.09., 16.63 and 15.98 %) and *A. vasica* (16.57 and 16.01., 16.43 and 15.96 %) recorded comparatively less shell percentage in healthy and inoculated batches of both fourth and fifth instars. Between the instars, the fourth instar treated batches found a maximum shell ratio (16.93, 16.64, 16.79 and 16.29 %) compared to the fifth instar (16.37, 16.30, 16.32 and 16.19 %).

Similar results were observed by Rudroju *et al*. (2017), who studied the effect of leaf extracts of *Trichosanthes cucumerina* L. on the cocoon parameters of the flacherie-infected silkworm. The methanolic extract of the leaf showed the highest cocoon characteristics *viz*., cocoon weight (1.94±0.11g), shell weight (0.39± 0.01g) and pupal weight (1.54±0.08g) over the control (1.63±0.02 g). The study on the efficacy of nine different medicinal plant extracts for managing late larval flacherie of silkworm (PM×CSR2) and cocoon parameters was carried out by Manjunatha *et al.* (2020). Among nine medicinal plant extracts administered, *Phyllanthus niruri* was found effective by enhancing the cocoon parameters *viz.,* cocoon weight (10.51 g/10 cocoons), shell weight (1.610 g/10 cocoon shells), pupal weight (8.90 g/10 pupae) and shell ratio (16.46 %) as reflected in the present study.

The aqueous extract of *Ziziphus jujuba* L. was fortified to fifth instar PM×CSR2 larvae (Sunil and Chandrashekhar, 2016) and recorded maximum cocoon weight (1.766, 1.531 and 1.723 g/cocoon), shell weight (0.309, 0.264 and 0.33 g/shell), pupal weight (1.459, 1.267 and 1.393 g/pupa) and shell ratio (17.65, 17.29 and 19.37 %) at 1:2, 1:4 and 1:8 concentrations compared to control (1.322, 0.221, 1.101 g and 16.80 %). Further, the ethanolic extract of *Ocimum sanctum* (2 %) was administered to fifth instar silkworms (PM×CSR2) and recorded cocoon parameters *viz*., cocoon weight, shell weight, pupal weight and cocoon shell ratio (Devi and Bai, 2015), which was found similar with the present study where the cocoon weight, shell weight, pupal weight and cocoon shell ratio were found more in botanical administered silkworm batches compared to healthy and infected controls because of the presence of biomolecules which acts as antimicrobial agents against virus and bacteria in infected batches. In contrast, the biomolecules exhibited an additive effect in healthy batches by supplying extra protein molecules for silk synthesis.

Sisodia and Gaherwal (2019) recorded the effect of amla plant extract on *Bacillus subtilis* infected silkworm and found increased cocoon shell weight (0.178±1.56 g/shell) compared to control (0.16 ±1.40 g/shell). Further, Chavan and Bhawane (2016) also studied the effect of ethanolic plant extract on *Bm*NPV infection and cocoon parameters of pure Mysore and CSR2 silkworm breeds. *Curcuma longa* recorded maximum cocoon weight (995.20 mg/cocoon) and shell weight (147.50 mg/shell) in CSR2. In contrast, in PM, the maximum cocoon weight (931.3 mg/cocoon) was recorded in *Bougainvillea spectabilis,* with shell weight (222.0 mg/shell) in *A. Mexicana,* which is in line with the present findings. Kuntamalla *et al.* (2015) recorded

Table 4. Effect of administration of plant extracts of *Adhatoda vasica* and *Phyllanthus niruri* on shell ratio (%) of fourth and fifth instar treated batches of *B. mori*

Treatments	IV instar			V instar		
	Healthy	Inoculated	Mean	Healthy	Inoculated	Mean
A. vasica -Aqueous	16.57	16.01	16.29	16.43	15.96	16.19
A. vasica-Methanol	17.47	16.11	16.79	16.64	16.00	16.32
P. niruri-Aqueous	17.18	16.09	16.64	16.63	15.98	16.30
P. niruri-Methanol	17.65	16.21	16.93	16.69	16.05	16.37
Distilled water control	16.86	15.62	16.24	16.14	15.92	16.03
Methanol control	16.94	15.61	16.28	16.30	15.84	16.07
Absolute control	16.74	14.99	15.87	16.08	15.82	15.95
Mean	17.06	15.81	16.43	16.42	15.94	16.18
Results	**A**	**B**	**AB**	**A**	**B**	**AB**
F-test	NS	NS	NS	NS	NS	NS
S.Em ±	0.63	0.34	0.89	0.48	0.26	0.68
CD at 5 % level	1.83	0.98	2.59	1.40	0.75	1.98

NS: Non-significant; A: Medicinal plants, B: Health of silkworm

higher single cocoon weight (1.571 g), shell weight (0.258 g), pupal weight (1.316 g) and silk ratio (15.97 %) in 3 per cent concentration of aqueous leaf extract of *O. sanctum* compared to other concentrations (1, 2 and 4 %).

The effect of botanical extract of turmeric, amla, asparagus, bael, berhavia, garlic and basil on the shell ratio of silkworms infected with *Bacillus* sp. revealed that the silkworms treated with Boerhavia leaf extract recorded a maximum shell ratio (18.12 %) compared to amla leaf extract (17.04 %) (Priyadharshini *et al*. 2009). The increase in cocoon parameters may be due to phytochemical constituents such as steroids, alkaloids and flavonoids that inhibit the gut microorganisms which compete with the host for nutrients. The ingredients have stimulated the synthesis of silk proteins and nucleic acids, which there by increase silk content.

CONCLUSION

The plant extracts administered to silkworms were inhibited the pathogen multiplication and recorded maximum cocoon weight, shell weight, pupal weight and shell ratio in methanolic extracts of *P. niruri* followed by *A. vasica*, aqueous extract of *P. niruri* and *A. vasica* administered healthy and pathogen inoculated (*Bm*NPV and *S. sciuri*) silkworms. The reduced pathogenicity and increased cocoon parameters were due to the presence of many secondary metabolites which possess antimicrobial property against wide range of bacteria and virus.

REFERENCES

Ajanal, M., Gundkalle, M. B. and Shradda, U. N. 2012. Estimation of total alkaloid in *Chitrakadivati* by UV-Spectrophotometer. *Ancient Science Life,* **31**(4): 198-201.

Chaudhary, E., Chauhan, R. and Chauhan, N. 2017. Assessment of bactericidal and phytochemical properties of *Adhatoda vasica*various extracts against gram positive and gram negative bacteria. *International Journal of Current Microbiology and Applied Science,* **6**(7): 2660-2669.

Chavan, J. A. and Bhawane, G. P. 2016. Effect of *Bm* NPV infection and subsequent oral treatment of ethanolic plant extracts on cocoon and post cocoon characters in PM and CSR2 *B. mori* L. *Biolife*, **4**(3): 475-481.

Chopade, P., Raghavendra, C. G., Mohana Kumar, S. and Bhaskar, R. N. 2021.Assessment of diseases in *Bombyxmori* silkworm – A survey. Global Transitions Proceedings, **2:** 133–136.

Devi, P. S. V. and BAI, M. R. 2015. Effect of *Ocimum sanctum* L. plant extract on the economic parameters of silkworm, *Bombyxmori* L. *Journal of Entomology and Studies*, **3**(2): 62-64.

Krishnaprasad, K. S., Siddaramaiah, A. L. and Lingaraju, S. 1979. Possible control of muscardine disease of silkworms by using a few plant extracts. *Current Research*, **8**(5): 79-80.

Kuntamalla, S., Sathish, J. and Anitha, J. 2015. Effect of medicinal botanical (*Ocimum sanctum*), family, Labiateae on commercial parameters of the silkworm, *Bombyxmori,* L. *International Journal of Multidisciplinary Current Research*, 76-78.

Madhusudhan, K. N., Poojanag, N., Moorthy, S. M., Mary Josepha, A. V. and Sivaprasad, V. 2018. Evaluation of antibacterial potential of botanicals against silkworm bacterial pathogen (*Bacillus thuringiensis*). *International Journal of Pharmaceutics and Drug Analysis,* **6** (2): 339-341.

Manimegalai, S. and Chandramohan, N. 2006. Efficacy of botanicals against grasserie disease of silkworm, *Bombyx mori* L. *Sericologia,* **46**: 15-23.

Manjunath, G. C., Doreswamy, C., Vasundhara, M. and Sanathkumar, V. B. 2020. *Invitro* evaluation of antibacterial efficacy of certain medicinal plants against bacterial isolates associated with late larval flacherie disease of silkworm, *Bombyx mori* L. *International Journal of Current Microbiology and Applied Science,* **10**: 533-542.

Priyadharshini, P., Aruchamy, M. C. and Ramanna, S. K. 2009. Evaluation of antibacterial efficacy of certain botanicals against bacterial pathogen *Bacillus* sp. of Silkworm, *Bombyx mori* L. *International Journal of Industrial Entomology,* **18** (1): 49-52.

Rudroju, S. S., Akula, S., Janga, S. and Kuntamalla, S. 2017. Effect of leaf and seed extracts of *T. cucumerina* L on antibacterial activity and economic traits of mulberry silkworm, *Bombyx mori* L. *Journal of Entomology and Zoology Studies*, **5** (2): 889-894.

Serkedjieva, J. 2004. Antiviral activity of red marine algae *Ceramium rubrum*. *Phytotherapy Research*, **18:** 480-483.

Shah, A. I. 2016. Flacherie – A threat to the socio economic fabric of India: A review. International Journal of Advanced Research, **4** (9): 692-695

Sisodia, N. S. and Gaherwal, S. 2019. Effects of amla extract (*Phyllanthus emblica*) on biological and economical characteristics of the silkworm, *Bombyx mori. Pramana Research Journal,* **9** (6): 1022-1028.

Sugun, R. 2000. Status and management of Thatte disease of silkworm. *Advances in Tropical Sericulture Proc. Natl. Sem. Tropic Seric*.UAS GKVK Bengaluru, 84-86.

Sunil, B. A. and Chandrashekhar, J. H. 2016. Studies on rearing performance of *Bombyx mori* L. (race: PM ×CSR2) with fortification of mulberry leaves using plant extracts. *Kaav International Journal of Science, Engineering and Technology,* **3**(4): 118-143.

Yasuhara-Bell, J., Yang, Y., Barlow, R. and Trapido-Rosenthal, H. 2010. *In vitro* evaluation of marine-microorganism extracts for anti-viral activity. *Virology Journal,* **7**:18.

MS Received: 30 November 2022
MS Accepted : 29 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 87-97 (2022)

DOI Number: 10.5958/0974-4541.2022.00044.3

Biology and morphometrics of peach fruit fly, *Bactrocera zonata* (Saunders) on mango

M. K. JENA* and S. R. PATEL

Department of Entomology, N. M. College of Agriculture, Navsari Agricultural University, Navsari-396 450, Gujarat, India

***E-mail id**- jenamanoj401@gmail.com

ABSTRACT: The investigation on biology and morphometrics of *Bactrocera zonata* (Saunders) was undertaken at the Department of Entomology, Navsari Agricultural University, Navsari, Gujarat, India during 2021-22. The eggs were smooth, white, shiny, translucent, rice grain shaped, slightly curved, elongated and tapered at the anterior end but broadly rounded at the posterior end. The hatching percentage was 82.63±6.94%. The incubation, maggot, pre-puparial, puparial, pre-oviposition, oviposition and post-oviposition periods were 2.40±0.48, 7.20±1.51, 0.77±0.11, 8.10±1.02, 12.3±1.13, 13.30±2.45 and 5.10±0.72 days, respectively. The morphometric measurements of egg, maggot, pre-puparia, puparia, adult male and female were also studied along with duration of different life stages. The pupationtook place at depth of 0.50 to 6.00 cm in moist soil. The sex ratio of male: female was 1:1.20. The fecundity of gravid female was 176±31.8 eggs/female. The total life cycle was completed in 22.00 to 36.00 days on mango.

Keywords: Biology, morphometrics, *Bactrocera zonata*, mango

INTRODUCTION

The peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the most destructive pests of horticultural crops causing huge crop loss in various regions of the world. It is native to South and Southeast Asia. It was first recorded in Iraq in 1972 (El-Haidari *et al*., 1972). It is, a close relative and resource competitor of *B. dorsalis* (Hendle), which is currently distributed in more than 20 countries including India, Pakistan, Mauritius, Reunion, Arabian Peninsula and North Africa. Just as *B. dorsalis*, *B. zonata* adults are highly invasive, strong fliers and have high reproductive potential with females laying up to 564 eggs. It is a polyphagous pest and has been reported to infect fruits of peach, mango, citrus, papaya, watermelon and Alfalfa (Al-Ali, 1977; Alzubaidy, 2000; Stonehouse *et al*., 2002; Abdulrazak *et al*., 2016). The pest caused 25 to 50% losses in guava fruits (Syed *et al*., 1970). The damages caused by peach fruit fly may be reached 100% of fruit without control (Hardy, 1997; Jena *et al*., 2022a). These are regarded as quarantine pests (Joomaye *et al*., 2000; Jena *et al*., 2022b).The knowledge about different life stages of insect pest, morphometrics of various life stages and their developmental time duration is highly helpful to increase awareness about pest. The monitoring of pre-oviposition, oviposition and post-oviposition periods, fecundity and the adult longevity is helpful for developing management strategies that will keep mangoes and environment away from detrimental effect of insecticides. This investigation will be also help in the identification of pest. Therefore, the present investigation was carried out to study the biology and morphometrics of *B. zonata* under laboratory condition.

MATERIALS AND METHODS

Studies on the selected aspects of biology of *B. zonata* infesting mango was carried out at the Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari during 2021-22. The data on temperature and relative humidity were recorded daily in the laboratory during the entire investigation period.

Rearing technique: The initial culture of *B. zonata* was raised by collecting infested fruits of mango from the College farm, N.M. College of Agriculture, Navsari and Regional Horticultural Research Station Farm, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat. Infested fruits were kept in rearing jar having 15cm diameter and 20cm height on a 5cm thick layer of sieved moist sand to obtain the puparia. The top of each jar was covered with white

Table 1. Morphometrics of different life stages of *B. zonata* on mango

Stages	Length (mm)			Breadth (mm)		
	Min.	Max.	Mean±S.D.	Min.	Max.	Mean ± S.D.
Egg	0.72	1.04	0.90±0.10	0.14	0.27	0.20±0.04
Maggot						
First instar	1.34	1.48	1.43±0.05	0.18	0.28	0.23±0.03
Second instar	5.20	6.40	5.90±0.39	0.60	1.20	0.97±0.22
Third instar	7.68	8.80	8.05±0.45	1.40	1.52	1.47±0.04
Pre-puparium	7.10	8.40	7.83±0.40	1.90	2.12	2.01±0.08
Puparium	4.10	8.40	6.75±1.49	2.10	2.12	2.11±0.01
Adult						
Male	4.20	5.10	4.72±0.24	8.42	11.40	10.25±0.93
Female	5.32	6.21	5.87±0.31	10.40	12.60	11.68±0.87

n=20

muslin cloth to prevent the maggots from escaping. When all the full grown maggots entered in to the sand for pupation, rotten fruits were removed from the jars. Sand in the jar was sieved after every 4 to 5 days to collect the puparia. Thereafter puparia were transferred in clean plastic bottle having 1.50cm diameter and 7.50cm height, individually. These bottles were covered with lid to prevent the escaping of flies. The flies emerged were utilized for further studies on biology.

Freshly emerged adults were paired and confined in glass jars having 15cm diameter and 20cm height covered with white muslin cloth bag. One end of bag was held in position on the top of the jar with the help of rubber band, while the other end of the bag was kept open for introducing the adults in to the jar. The open end of the bag was tightened with rubber band to prevent the adults from escaping. Such jars were put in wooden cages (45.50×46.50×76.50cm) to prevent damage of rats and ants. A cotton swab having five per cent sugar solution was suspended inside the jar as food to the adult flies. One physiologically mature fruit of mango was placed inside the glass jar for oviposition by female. The fruit was replaced after observing the oviposition puncture. The fruit punctured due to egg laying was cut open with a fine razor blade and eggs laid if any were confirmed using magnifying lens. About (2×1×1cm) size piece of fruit having eggs was smoothly cut and transferred in a separate Petri dish and it was observed twice a day for their hatching. Eggs were carefully transferred with a fine hair brush on a glass slide and observed under microscope to study their morphometric characters.

When eggs hatched out, the neonate maggots were gently transferred on a fresh fruit slice (2×2×1cm); later on, they were kept in a Petri dish for further rearing. The food (fruit slices) as well as Petri dishes were changed every day to avoid microbial development on fruit slice. The maggots were reared following this method until they were full grown and transferred along with Petri dish in small glass jar having 15cm diameter and 20cm height filled with a layer of 5cm moist sand. The jars were covered with muslin cloth duly tightened with rubber bands to prevent the escape of maggots.

Egg: Eggs were examined under the microscope for studying their colour, shape and size, while for measurement it was gently transferred under compound microscope with the help of moist hair brush. The Trinocular microscope (SZ-61; Make: Olympus) attached with software scope Plate (Version 3.1) was used for measuring the eggs. To study the incubation period, twenty freshly laid eggs on fruit (slice) were observed daily in the morning and evening till hatching. The eggs were considered as hatched when tiny maggots came out from it, whereas hatching percentage was calculated from the number of eggs hatched out of total numbers of eggs kept under observation.

Maggot: About 2cm thick fruit slices of mango were kept individually in Petri dish. A slice was slightly ruptured with the help of scalpel for easy entry of the maggot. The newly hatched maggots were transferred individually on that slice. Maggots were reared till they underwent pupation. The food was changed every morning to maintain sanitation in the Petri dish. Newly hatched,

Fecundity: To determine the fecundity, the number of eggs laid in fruit by females were counted and the average fecundity was calculated.

Longevity: Longevity of male and female were calculated separately from the date of emergence to the death of adult.

Total life cycle: The period from eggs laid to the death of adult was considered as the total life cycle.

RESULTS AND DISCUSSION

Egg: The female *B. zonata* laid eggs in clusters of 2 to 14 eggs underneath the rind of the fruit with the help of a sharp ovipositor at a depth of about 1 to 4mm. The eggs were embedded in the pulp of fruit vertically or slightly angled and twisting with each other. Similar observations were also made by Narayanan and Batra (1960), Butani (1979) and Amur *et al*. (2017). The eggs were smooth, white, shiny, translucent, rice grain shaped, slightly curved, elongated and tapered at the anterior end but broadly rounded at the posterior end. They turned dark brown colour as they were nearer to the hatching. Similar findings were also found by Amur *et al*.(2017) as well as Naik *et al*.(2017).The length and breadth of eggs varied from 0.72 to 1.04mm with an average of 0.90±0.10mm and 0.14 to 0.27mm with an average of 0.20±0.04mm, respectively (Table 1). The present findings are similar to those made by Dale (2002) who recorded that the length and breadth of eggs of *B. zonata* varied from 0.75 to 1.01mm and 0.16 to 0.25mm, respectively in size but it is less accordance with Leghari (2013) who reported that the size of eggs varied from 0.50 to 0.60mm of both fruit flies, *B. dorsalis* and *B. zonata*.

The incubation period of eggs of *B. zonata* varied from 1.60 to 3.40 days with an average of 2.40±0.48 days on mango (Table 2). Almost similar observations on incubation period was also reported by Dale (2002) who reported the incubation period as 1.00 to 2.50 days on mango for *B. zonata*; 10.16, 3.46, 2.04, 1.42 and 1.54 days at 15, 20, 25, 30 and 35°C temperature, respectively for *B. zonata* (Duyck *et al*., 2004); 4.50±0.50, 3.18±0.18, 2.40±0.40 and 2.00±0.50 days at 20, 25, 30 and 35°C

Table 2. Duration of different life stages of *B. zonata* on mango

Stage	Periods		
	Min.	Max.	Mean±S.D.
Incubation period (Days)	1.60	3.40	2.40±0.48
Hatching percentage (%)	65.00	95.00	82.63±6.94
Total maggot period (Days)	4.00	10.00	7.20±1.51
Pre-puparial period (Days)	0.58	0.96	0.77±0.11
Puparial period (Days)	6.00	9.00	8.10±1.02
Sex ratio (Male: female)	1:1.00	1: 1.70	1:1.20
Adult period (Days)			
Pre-oviposition	10.00	14.00	12.30±1.13
Oviposition	9.00	18.00	13.30±2.45
Post-oviposition	4.00	6.00	5.10±0.72
Fecundity (eggs/female)	120	240	176.00±31.81
Longevity (Days)			
Male	10.00	18.00	13.95±2.06
Female	14.00	26.00	21.50±3.14
Total life cycle (Days)			
Male	22.00	29.00	25.58±1.88
Female	28.00	36.00	30.94±2.80

n=20

Plate 1: Eggs of *B. zonata*

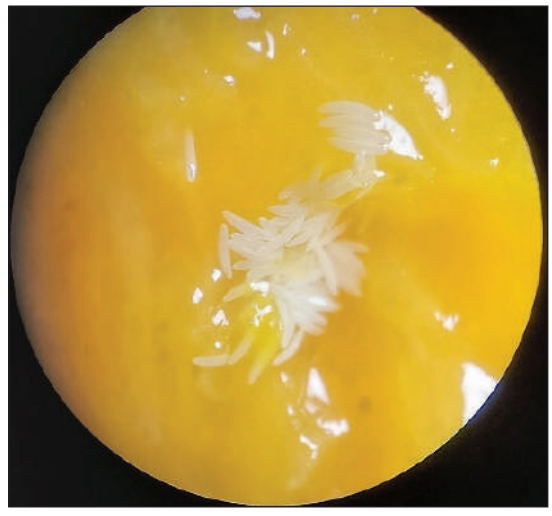


Plate 2: Microscopic view of eggs

Plate 3: First instar maggot of B. *zonata*

Plate 4: Second instar maggot of B. *zonata*

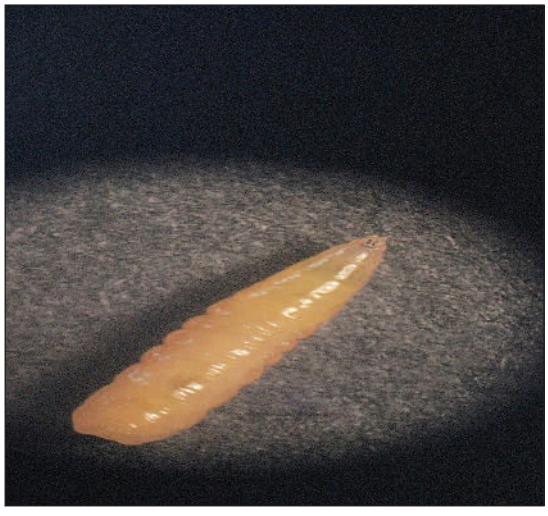


Plate 5: Full grown maggot of B. *zonata*

Plate 6: Pre-puparium of B. *zonata*

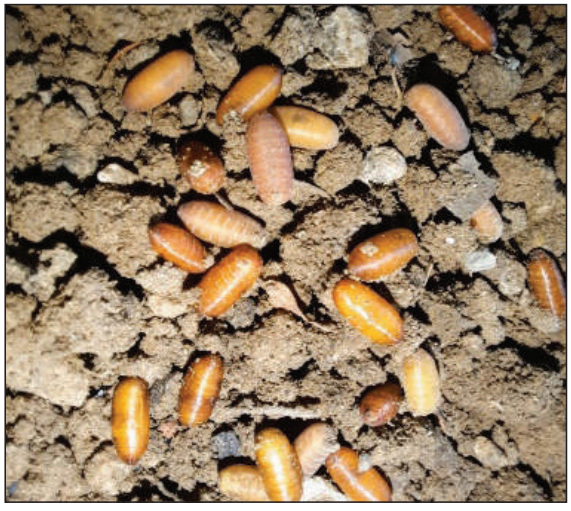


Plate 7: Puparia of *B. zonata*

Plate 8: Newly emerged adults of *B. zonata*

Plate 9: Adult male of *B. zonata*

Plate 10: Adult female of *B. zonata*

second instar and fully grown maggots were observed under microscope to study the colour, shape and size.

Pre-puparium: A stage, when full grown maggot ceased feeding and became inactive was considered as pre-puparial stage. Such maggots were transferred with food to glass jar having 15cm diameter and 20cm height and have 5cm layer of moist sand at the bottom to facilitate pupation. Observations on colour, shape and size of pre-puparial stage were also be recorded. The length and breadth of pre-puparial stage were measured under microscope. The pre-puparial period was recorded for individual maggot reared on fruit.

Puparium: The puparia were collected by sieving moist sand in the jar and their shape, size, colour and period were studied. The length and breadth were also measured. Puparial period was calculated from the date of formation of puparium to the date of emergence of the adult from the puparium.

Adult: The newly emerged adults were observed critically for their shape, colour and sex differences while ten adults each of male and female were taken from the stock culture and killed by placing in KCN bottle for studying their size under microscope critically.

Sex ratio: To study the sex ratio, puparia were kept in the glass jar on the layer of 5cm moist sand and jar was covered with white muslin cloth fixed with a rubber band to prevent the escape of adults. Sex ratio was calculated by separating males and females, based on their morphological characters.

Pre-oviposition, oviposition and post-oviposition period: Pre-oviposition period was calculated from the date of emergence of female to the date of starting of egg laying. Oviposition period was calculated from the date of starting egg laying to the date of ceasing of egg laying. Post-oviposition period was calculated from the date of ceasing of egg laying to the death of female.

temperature, respectively for *B*. *zonata* (Younes and Akel, 2010); 11.30±1.50, 6.30±2.10 and 1.80±0.30 days at 20, 25 and 40°C for *B*. *zonata* (Fetoh *et al*., 2012); 1.00 to 2.00 days for *B*. *zonata* (Leghari, 2013); 8.00 to 11.00, 5.00 to 6.00, 1.00 to 4.00 and 1.00 to 2.00 days at 15, 20, 25 and 30°C temperatures, respectively for *B*. *zonata* (Ali, 2016).

The hatching percentage of eggs of *B*. *zonata* varied between 65.00 to 95.00 per cent with an average of 82.63±6.94 per cent on mango (Table 2). The present findings are akin with Dale (2002) who recorded that the hatching percentage of *B*. *zonata* was 66.67 to 91.67 per cent for *B*. *zonata*; 30.24, 83.06, 90.33 and 71.18 per cent at 20, 25, 30 and 35°C temperature, respectively for *B*. *zonata* (Younes and Akel, 2010); 66.00 to 97.00 per cent at 40°C and 25°C, respectively for *B*. *zonata* (Fetoh *et al*., 2012); 86.00, 86.00, 68.00 per cent at 30, 25, 20±2°C temperature and 63.00 per cent at field conditions for *B*. *zonata* (Abu-Ragheef and Al-Jassany, 2018).

Maggot: The damaging stage of *B*. *zonata i.e*., maggot underwent metamorphosis and passed through three instars with different size and morphology which is supported by Christenson and Foote (1960), Weems *et al*. (2015) and Amur *et al*., (2017).

The first instar maggots were translucent and white with slightly yellowish colour except its mouth parts, which were black in colour. The head was pointed and slightly bent downward with a pair of mandibular hooks. The maggot was apodous with three thoracic and nine abdominal segments. The cuticle of maggot was so translucent that the internal organs were visible through it. They were generally less active as compared to other two instars. Similar morphological characters were also observed by Narayanan and Batra (1960) who observed that young maggots of *D*. *dorsalis* were white, translucent as well as Amur *et al*. (2017) who noticed that the 1st instar was inactive and small in size as compared to two other instars. Additionally, similar findings were found by Naik *et al*. (2017) who observed that the freshly hatched maggot of *B*. *dorsalis* was pale white in colour with translucent body. The length of newly emerged maggot varied from 1.34 to 1.48mm, while the breadth varied from 0.18 to 0.28mm on mango (Table 1). The average length of newly emerged maggot (first instar) was 1.43±0.05 mm whereas, the breadth measured was 0.23±0.03 mm. A similar finding was reported by Narayanan and Batra (1960) who observed that young maggot of *D*. *dorsalis* was measured about 1.50×0.30mm. However, the present findings are slightly deviated from the work of Kalia and Yadav (2015) who recorded that the mean body length and width of *B*. *dorsalis* of the freshly hatched maggot were 2.87±0.74 and 2.25±0.70mm, respectively. Further, Amur *et al*. (2017) reported that the first instars measured 2.60±0.75mm in length and 0.27±0.82mm in width; the length and width of maggots of *B*. *dorsalis* on custard apple were 6.07±1.97 and 1.75±0.89mm, respectively (Naik *et al*., 2017) which deviate from the present findings.

The second instar maggot of *B*. *zonata* was slightly larger and more yellowish than first instar maggot possessing the externally visible alimentary canal. These findings are agreed with those of Amur *et al*. (2017) who noticed that the second instar of *B*. *dorsalis* had a distinguishing characteristic *i.e*., presence of externally visible alimentary canal. The length of second instar maggot varied from 5.20 to 6.40mm with an average of 5.90±0.39mm, while the breadth varied from 0.60 to 1.20mm with an average of 0.97±0.22mm on mango (Table 1). These findings are slightly different from those of Amur *et al*. (2017) who reported that the second instars of *B*. *dorsalis* measured 5.88±0.55mm in length and 2.34±0.70mm in width.

The third instarmaggotof *B*. *zonata* was apodous, longer and broader with cephalic end and blunt at posterior end. The colour of full grown maggot was yellowish and more opaque than both newly emerged or young maggot and second instar maggot. A black mole was present on anterior and caudal side. It fed rapidly in the pulp of mango, formed the tunnels and holes in the fruit pulp and peel, came outside the fruit by holes of peel, moved fast and jumped. The black-coloured mouth hooks were retractile and extended outside the body at the time of feeding. The morphological characters and behaviours observed during the present investigation are similar to those described by Narayanan and Batra (1960) who observed that full grown maggot had a habit of jumping short distance for finding a suitable place for pupation. The present findings are also in agreement with those of Butani (1979) who reported that the maggot of *D*. *dorsalis* was yellow at later stage and head of cephalic segment was pointed anteriorly and trapezoidal in outline; Amur *et al*. (2017) who noted that the third instar maggot of *D*. *dorsalis* fed rapidly in the pulp of mango, formed the tunnels and holes in the fruit pulp and peel, came outside the fruit by holes of peel, moved fast and jumped. Black mole was present on anterior and caudal side; Naik *et al*. (2017) who revealed that the matured maggots of *D*. *dorsalis* were brownish yellow, cylindrical, apodous, frugivorous with an elongated body, pointed anteriorly or cephalic end and blunt posteriorly. The black-coloured mouth hooks were retractile and extended outside the body at the time of feeding. The length and breadth of full grown maggot varied between 7.40 to 8.80mm with an average of 8.05±0.45mm and 1.40 to 1.52mm

with an average of 1.47±0.04mm, respectively (Table 1). In past, Narayanan and Batra (1960) who observed that the fullgrown maggot of *B*. *dorsalis* was 8.00 to 9.00mm long and 1.50mm broad across the posterior end which is in favour of the present findings. However, the present findings are slightly deviated from those of Kalia and Yadav (2015) who recorded that the mean body length and width of *B*. *dorsalis* of full grown maggot was 8.18±0.84 and 2.25±0.70mm, respectively; Amur *et al*. (2017) who reported that the third instar maggot of *B*. *dorsalis* measured 7.69±0.72mm in length and 3.58±0.25mm in width.

Initially the young maggots of *B*. *zonata* emerged from eggs were found sluggish but after feeding for a few hours it became active and bored in to the pulp of fruit and fed there on. The maggots inhabited in liquefied pulp and hanged head downward with their posterior spiracles at the liquid surface. The maggots after full feeding moved from the centre of the fruit where they had been feeding on the soft and fermented skin of fruit. On completion of full development, the maggots bored holes; exited out through hole and fell to the sand or soil for pupation by their jumping movement. It was further noted that the exit holes made for pupation by full grown maggot on damaged fruit was very clearly visible. After leaving the fruit, matured maggot wandered on soil haphazardly and looked for suitable site for pupation.

The total maggot period of *B*. *zonata* ranged from 4.00 to 10.00 days with an average of 7.20±1.51 days (Table 1). These findings are more or less in confirmity with Dale (2002) who recorded the maggot period varied from 6.30 to 9.20 days on mango for *B*. *zonata*; 30.00, 10.00, 5.00, 4.00 and 4.00 days at 15, 20, 25, 30 and 35°C, respectively for *B*. *zonata* (Duyck *et al*., 2004); 14.20±0.20, 10.30±0.33, 7.75±0.05 and 7.01±0.01 days at 20, 25, 30 and 35°C temperature, respectively for *B*. *zonata* (Younes and Akel, 2010); 33.70±3.20, 24.30±4.00, 16.70±2.10, 10.30±1.50 and 6.70±0.60 days from 20°C to 40°C for *B*. *zonata* (Fetoh *et al*., 2012); 26.00 to 29.00, 12.00 to 14.00, 7.00 to 9.00 and 4.00 to 5.00 days at 15, 20, 25 and 30°C temperatures, respectively for *B*. *zonata* (Ali, 2016); 8.20, 19.30 and 13.60 days at 30±2°C, 20±2°C (when the maggots fed on artificial diet) and field conditions (When the maggots fed on mandarin fruit), respectively for *B*. *zonata* (Abu-Ragheef and Al-Jassany, 2018). Rashmi *et al*. (2020) who opined that the duration of the maggot stage of *B*. *zonata* significantly shortened with increase in the range of 16 to 36°C from 53.40 to 6.60 days which is again in tally with the present findings.

Pre-puparium: After having full maggot development, the maggot came out of fruit through the exit hole. Thereafter, it fell on the soil by jumping movement (coiling the body), wandered for some time on soil and finally entered in to soil for pupation. The length and breadth of pre-puparium ranged between 7.10 to 8.40mm with an average of 7.83±0.40mm and 1.90 to 2.12mm with an average of 2.01±0.08mm, respectively (Table 1).The pre-puparial period was 0.58 to 0.96 days with an average of 0.77±0.11days (Table 2). Dale (2002) who recorded that the pre-puparial period of *B*. *zonata* was 16.00 to 23.00 hrs on mango which is agree with the present investigation. However, Amur *et al*. (2017) who found that the pre-puparial period was 2.07±0.86 days which is slightly deviated from the present findings.

Prominent. Additionally, there was the presence of black dot on the posterior portion of puparium. These findings are agreed with those of Narayanan and Batra (1960); Kalia (1992); Kalia and Yadav (2015); Naik *et al*. (2017). The length and breadth of puparium of *B*. *zonata* varied between 4.10 to 8.40 and 2.10 to 2.12mm, respectively. The average length and breadth of puparium measured as 6.75±1.49 and 2.11±0.01mm (Table 1).The length and breadth of puparium measured during present studies were more or less similar to the reports of Kalia (1992) who reported that puparium of *B*. *dorsalis* measured 5.15×2.10, 4.10×1.40, 5.30×2.23 and 4.40×2.30mm in average length and breadth on mango varieties, Dashehari, Amrapali, Mallika and Bangalora, respectively; 4.47±0.64mm in length and 2.69±0.16mm in width on mango (Amur *et al*., 2017); 4.08±0.50mm in length and 1.82±0.69mm in width on custard apple (Naik *et al*., 2017).

The puparial period of *B*. *zonata* varied between 6.00 to 9.00 days with an average of 8.10±1.02 days (Table 2). The more or less similar findings was recorded by Rana *et al*. (1992) who observed that the puparial period of *B*. *zonata* ranged between 7.00 to 10.00 days; 7.03 to 40.90 days on guava for *B*. *zonata* (Mohamed, 2000); 7.40 to 8.80 days on mango for *B*. *zonata* (Dale, 2002); 53.00, 20.00, 10.00, 8.00 and 8.00 days at 15, 20, 25, 30 and 35°C temperatures, respectively for *B*. *zonata* (Duyck *et al*., 2004); 14.01±0.01, 9.50±0.25, 7.01±0.01 and 5.81±0.11 days at 20, 25, 30 and 35°C temperatures, respectively for *B*. *zonata* (Younes and Akel, 2010); 47.67±2.50, 14.33±0.60, 7.80±0.30 and 6.67±0.60 days at 20, 25, 30 and 35°C, respectively for *B*. *zonata* (Fetoh *et al*., 2012); 34.00 to 39.00, 15.00 to 17.00, 10.00 to 12.00 and 7.00 to 9.00 days at 15, 20, 25 and 30°C temperature, respectively for *B*. *zonata* (Ali, 2016); 32.40 days (at 16°C), 19 days (20°C), 13.80 days (24°C) and 8.60 days (28°C), 7.40 days (32°C) and 6.20 days (36°C) for *B*. *zonata* (Rashmi *et al*., 2020). However, the present findings are less similar with those of Abu-Ragheef and

Al-Jassany (2018) who noted that the pupation period of *B. zonata* were 10.80, 23.90 and 22.30 days at 30±2°C, 20±2°C temperature and field conditions, respectively.

Adult: When *B. zonata* fly was ready to emerge from the puparium, it pushed the upper end of puparium and came out by bursting the puparial case. Thereafter, it slowly crawled through soil and reached to the soil surface. The newly emerged adult looked faint and sluggish; however, after sometime the wing was found fully opened by fluttering movement. The adults were stout, reddish brown in colour with hyaline wing, yellow legs and the thorax is reddish brown in colour. The adult flies possessed two prominent compound eyes on the dorso-lateral region of the head and aristate type of antennae. The abdominal tergites were free. In scutellar and thoracic region, a pair of yellow coloured lateral vittae were present. A pair of dark marks on tergum III and no medial dark line except tergum was observed on abdomen. The wings of adult consisted of discontinuous band expanding in to a spoton costal margin, which is a typical character of identifying the *B. zonata*. The hind wings were modified in to a short tubular structure with rounded end. In male, the abdominal end was rounded with pecten while, it was developed in to pointed ovipositor in case of female. Moreover, the male *B. zonata* were slightly smaller than female flies. This finding is concurrence with Narayanan and Batra (1960) who reported that the male of *D. dorsalis* was smaller than the female. Naik *et al*. (2017) reported that the abdomen of *B. dorsalis* was blunt in adult male and smaller in size than that of the female, whereas adult females were easily distinguishable by the presence of tapering abdomen extending in to an ovipositor and comparatively larger than the males which is again in favour of the present findings.

The length of adult of *B. zonata* male with wing expansion ranged from 4.20 to 5.10mm and that of breadth varied from 8.42 to 11.40mm. While, the length of adult female with wing expansion ranged from 5.32 to 6.21mm and breadth from 10.40 to 12.60mm, respectively (Table 1). The average length and breadth of male from wing expansion was (4.72±0.24) × (10.25±0.93) mm. The average length and breadth of female from wing expansion was (5.87±0.31) × (11.68 ± 0.87) mm.The present findings are deviated from the findings of Kalia (1992) who reported that the size of females (across the wing × length) of *B. dorsalis* were 14.90×8.65, 12.30×6.25, 14.40×8.20, 14.00×8.20mm and that of the males were 13.80×8.15, 11.50×5.20, 13.70×7.40, 13.70×7.65mm on mango varieties, Dashehari, Amrapali, Mallika and Bangalora, respectively. Naik *et al*. (2017) recorded that the length and breadth of the male adult varied from 4.91 to 7.23mm and 10.10 to 12.65mm, respectively while these were found to vary from 6.70 to 8.98mm and 12.20 to 16.50mm for female adult, respectively which is not in favour of the present findings due to different species.

Sex ratio: The *B. zonata* adults were differentiated in to their sexes based on their morphological characters and the sex ratio was worked out by separating and counting the males and females emerged. The sex ratio of male: female was 1:1.20 (Table 2). A more or less similar trend was seen by Rana *et al*. (1992) who observed that the sex ratio of male and female *B. zonata* was 1:1.10 when reared on guava; 1:1.11 for *B. zonata* on mango (Dale, 2002); 1:1.33, 1:1.12, 1:0.79 and 1:1.04 for *B. zonata* at 20, 25, 30 and 35°C temperature, respectively (Younes and Akel, 2010); 1:1.22, 1:1.10, 1:1.06, 1:1.00 and 1:3.00 on Banana, guava, papaya, sapota and mango, respectively (Kalia, 2015); 1:3.00 for *B. dorsalis* (Amur *et al*., 2017); 1:1.00, 1:1.70, 1:1.10 and 1:1.09 and 1:0.92, respectively when *B. zonata* was reared on papaya, mango, guava and Robusta and Ekaki varieties of banana (Jayanthi and Verghese, 2002). However, Abu-Ragheef and Al-Jassany (2018) reported that the sex ratio female:male were 0.54:1, 0.79:1, 0.79:1 for *B. zonata* at 20, 25, 30±2°C temperature (when the maggots fed on artificial diet), respectively and it was 0.58:1 in field conditions (when the maggots fed on mandarin fruit) which does not support the present findings. Some discrepancy among the results pertaining to sex ratio was observed which is might be due to change in host fruits as well as the prevailing environmental conditions.

Pre-oviposition period: The pre-oviposition period of *B. zonata* ranged between 10.00 to 14.00 days with an average of 12.30±1.13 days on mango (Table 2). These findings are more or less tally with those of Dale (2002) who noted that the pre-oviposition period of *B. zonata* was found to be 12.00 to 14.00 days with an average of 13.20 days on mango; 27.67±2.50, 18.00±2.00, 12.33±0.60, 8.00±2.00 and 0.00±0.00 days at 20, 25, 30, 35 and 40°C temperature, respectively for *B. zonata* (Fetoh *et al*., 2012); 14.00 to 17.00 days on guava for *B. zonata* (Rana *et al*., 1992); 18.00 to 22.00 days for *B. dorsalis* (Qureshi *et al*., 1993); 18.00 to 22.00 days for *B. zonata* (Shehata, 2008); 29.40±2.88, 22.80±2.86, 20.40±1.82 and 17.80±1.10 days at 20, 25, 30 and 35°C temperature, respectively for *B. zonata* (Younes and Akel, 2010); 20.67, 21.71, and 20.71 days of females copulated repeatedly, delayed copulation and copulated once, respectively for *B. zonata* (Younes and Akel, 2010); 26.40, 43.60 and 29.00 days at 30±2°C, 20±2°C (when the maggots fed on artificial diet) and field conditions (when the maggots fed on mandarin fruit), respectively for *B. zonata* (Abu-Ragheef and Al-Jassany, 2018). El-

Minshawy *et al*. (1999) revealed that the pre-oviposition period of *B*. *zonata* was found to be 45.00 to 60.00 days for *B*. *zonata* at 25°C which is deviated from the present findings. The variation in pre-oviposition period was observed due to change in host fruits as well as environmental conditions.

Oviposition period: The oviposition period of *B*. *zonata* varied from 9.00 to 18.00 days with an average of 13.30±2.45 days (Table 2). A similar trend was found by Rana *et al*. (1992) who observed that the oviposition period of *B*. *zonata* on guava was 12.00 to 17.00 days; 13.00 to 18.00 days with an average of 15.00 days for *B*. *zonata* on mango (Dale, 2002); 9.80±2.59, 13.00±2.92, 29.80±3.03 and 28.40±5.41days at 20, 25, 30 and 35°C temperature, respectively for *B*. *zonata* (Younes and Akel, 2010); 29.00 to 38.00 days with an average of 33.14 days in case of repeated copulation and 9.00 and 14.00 days with an average of 11.14 days in *B*. *zonata* females copulated once whereas in females copulated after 14.00 days post-emergence, it was 12.00 and 23.00 days with an average of 16.43 days (Younes and Akel, 2010); 67.33±3.60, 55.00±5.00, 34.33±1.10 and 18.00±2.00 days from 20 to 35°C, respectively for *B*. *zonata* (Fetoh *et al*., 2012).However, the present findings are deviated from those of El-Minshawy *et al*. (1999) who registered that the average oviposition period of *B*. *zonata* was 70.00 to 90.00 days at 25°C; 117.50 days for *B*. *zonata* adult females reared on artificial diet at 25°C (El-Gendy, 2002); 3.00 to 7.00 days for *B*. *zonata* adult females reared on artificial diet at 25°C (Mohamed, 2003). The differences in oviposition period observed due to change in the diet type, copulation type as well as prevailing environmental conditions.

Post-oviposition period: The post-oviposition period of *B*. *zonata* varied from 4.00 to 6.00 days with an average of 5.10±0.72 days (Table 2). These findings are concurrence with those of Fetoh *et al*. (2012) who found that post-oviposition period of *B*. *zonata* was 12.33±2.50, 8.00±1.00, 6.00±1.70 and 4.67±1.50 days at 20, 25, 30 and 35°C, respectively; 4.20, 9.40 and 5.30 days at 30±2°C, 20±2°C (when the maggots fed on artificial diet) and field conditions (when the maggots fed on mandarin fruit), respectively for *B*. *zonata* (Abu-Ragheef and Al-Jassany, 2018). The present findings show a less similar trend with those of Rana *et al*. (1992) who noticed that the post-oviposition period of *B*. *zonata* was found to be 16.00 to 43.00 days on guava; 21.00 to 26.00 days with an average of 22.80 days on mango (Dale, 2002). The variation in the post-oviposition period was due to the change in the hosts as well as environmental conditions.

Fecundity: The egg laying capacity of gravid female varied from 120 to 240 eggs/female with an average of 176.00±31.81 eggs/female (Table 2).In past, Rana *et al*. (1992) recorded that single female *B*. *zonata* laid 191 to 259 eggs when reared on guava; 121 to 146 eggs were laid by single female *B*. *zonata* on mango (Dale, 2002); 62.80±14.24, 224.64±31.79, 241.00±12.96 and 160.20±12.96 eggs were laid by single female *B*. *zonata* at 20, 25, 30 and 35°C temperature, respectively (Younes and Akel, 2010) which are in close proximity with the present findings. Abu-Ragheef and Al-Jassany (2018) revealed that the highest number of eggs were laid by *B*. *zonata* in virgin reproduction was 83.20 egg/female at 30±2°C temperature and the lowest was 65.40 egg/female at 20±2°C and hatching rate was zero which is slightly deviated from the present findings. The deviation is seen due to the change in the type of copulation and environmental conditions.

Longevity: The longevity varied from 10.00 to 18.00 days with an average of 13.95±2.06 days whereas the female longevity ranged from 14.00 to 26.00 days with an average of 21.50±3.14 days (Table 2). Thus, the study indicated that the *B*. *zonata* females lived longer than male. These findings are similar to those of Fetoh *et al*. (2012) who revealed that the male durations of *B*. *zonata* reared on different temperature degrees from 20 to 40°C were decreased in an ascending manner with raising the temperature degrees, as: 87.67±2.50, 65.67±7.40, 37.67±17.60, 16.67±2.90 and 0.00±0.00 days.The female longevity of *B*. *zonata* showed decreasing in the longevity period (107.33±3.80, 81.00±7.00, 52.67±1.50 and 30.67±4.00 days) with increasing temperature from 20 to 35°C.

Total life cycle: The total life cycle starting from egg to death of adult of male and female varied from 22.00 to 29.00 days and 28.00 to 36.00 days, respectively. The average duration of life cycle of *B*. *zonata* male and female were 25.58±1.88 and 30.94±2.80 days, respectively (Table 2). The life cycle (from egg to death of adult) of male is shorter than the female.A more or less similar trend was observed by Dale (2002) who revealed that the total life cycle was 37.00 to 42.00 days for male and 51.00 days for female when *B*. *zonata* reared on mango; male duration of 39.55 days and 43.20 days for females when adults of *B*. *zonata* reared on artificial diet at 25°C (Mohamed, 2003); 87.67±2.50, 65.67±7.40, 37.67±17.60 and 16.67±2.90 days for males and 107.33±3.80, 81.00±7.00, 52.67±1.50 and 30.67±4.00 days for females when *B*. *zonata* reared on different temperature degrees from 20 to 35°C (Fetoh *et al*., 2012); 16.90, 37.10 and 32.60 days at 30±2°C, 20±2°C (when the maggots fed on artificial diet) and field conditions (when the maggots fed on mandarin fruit), respectively for *B*. *zonata* (Abu-Ragheef and Al-

Jassany, 2018); 96.00 days to 14.00 days at 16 to 36°C temperature, respectively for *B. zonata* (Rashmi *et al*., 2020). The slight variation in the longevity of *B. zonata* was noticed due to variation in temperature, humidity, host fruits and other environmental factors.

CONCLUSION

The adult female of *B. zonata* searched for a physiologically mature fruits just after mating and laid eggs just below the rind of the fruit. The maggots emerged from the eggs feed on the pulp of the fruits after sometime and pass through three different instars. The fullgrown maggot enters into the soil for pupation. The adults emerged from the puparia feed on nectar and other liquefied juice, mate and again oviposit in mature fruit. Thus, *B. zonata* complete the life cycle in 22 to 36 days. The life cycle (from egg to death of adult) of male *B. zonata* is shorter than the female. The various descriptions on biology and morphometrics of *B. zonata* would help in identification of the species and distinguish it from other species of fruit flies. It also helps to differentiate the male and female species. Understanding the biology would be helpful for finding out the weak link of the insect and selection of appropriate management practices.

ACKNOWLEDGEMENT

The authors are highly thankful to the Principal and Head, Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat for providing necessary technical support throughout the research.

REFERENCES

Abdulrazak, A., Hameed, A., Samir, A., Naema, I., Ali, K., Khalid, M. and Saad, A. 2016. New record of peach fruit fly *Bactrocera zonata* (Saunders) (Tephritidae: Diptera) in Iraq. *Arab and near East Plant Protection Newsletter*, **69**: 3p.

Abu-Ragheef, A. H. and Al-Jassany, R. F. 2018. Study of some biological aspects of peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae): In laboratory and field Study. *Journal of Agriculture, Biology and Healthcare*, **8**(12): 67-74.

Al-Ali, A. 1977. Phytophagous and entomophagous insects and mites of Iraq. Natural history research center Publication No. 33. Al -Zahra Press Baghdad, 142.

Ali, M. A. 2016. Effect of temperature on the development and survival of immature stages of the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *African Journal of Agricultural Research*, **11**(36): 3375-3381.

Alzubaidy, M. 2000. Economic Importance and Control/ Eradication of peach fruit fly, *Bactrocera zonata*. *Arab Journal of plant protection*, **18**: 139-142.

Amur, A., Memon, N., Shah, M. A., Ansari, A., Solangi, D. A. and Mal, B. 2017. Biology and morphometric of different life stages of the oriental fruit fly (*Bactrocera dorsalis* Hendle) on three varieties of mango of Sindh, Pakistan. *Journal of Animal and Plant Sciences*, **27**(5): 1711-1718.

Atwal, A. S. 1976. Agricultural Pest of India and South-East Asia. Kalyani Publishers, Ludhiana, pp. 189-190.

Butani, D. K. 1979. Insects and Fruits. Periodical Expert Agency, Delhi, pp. 210-211.

Dale, N. S. 2002. Studies on the biology of *Bactrocera zonata* (Saunders) and management of fruit flies in mango orchards. M.Sc. (Agri.) Thesis submitted to Gujarat Agriculture University, S. K. Nagar. (Unpublished).

Duyck, P. F., David, P. and Quilici, S. 2004. A review of relationships between interspecific competition and invasions in fruit flies (Diptera: Tephritidae). *Ecological Entomology*, **29**: 511-520.

El-Gendy, I. R. 2002. Studies on peach fruit fly, *Bactrocera zonata* at El-Beherira governorate. M. Sc. Thesis, Faculty of Agriculture, Alexandria University, Egypt.

El-Haidari, H., Fattah, Y. and Sultan, J. 1972. Contribution to the Insect fauna of Iraq. Part 4. Direct. Gen., 17.

El-Minshawy, A. M., El-Eryan, M. A. and Awad, A. I. 1999. Biological and morphological studies on the guava fruit fly, *Bactrocera zonata* (Saunders) found recently in Egypt. *8th National Conference on Pests & Diseases of Vegetable & Fruit in Ismailia*, Egypt, 71-82.

Fetoh, B. E. A., Gawad, A. A. A., Shalaby, F. A. and Elyme, M. F. 2012. Temperature-Dependent Development and Degree Days Models of the Peach Fruit Fly *Bactrocera zonata* (Saunders) and the Cucurbit Fly, *Dacus ciliatus* (Loew). *International Journal of Environmental Science and Engineering*, **3**: 85-96.

Hardy, D. E. 1997. Taxonomy and distribution of the oriental fruit flies and related species (Tephritidae: Diptera). In: *Proceedings of the Hawaiian Entomological Society*, **20**: 395-428.

Jayanthi, K. P. D. and Verghese, A. 2002. A simple and cost-effective mass rearing technique for the tephritid fruit fly, *Bactrocera dorsalis* (Hendle). *Current Science*, **82**(3): 266-268.

Jena, M. K., Patel, S. R. and Sahoo, S. 2022a. Fruit Fly: A Destructive Pest of Horticultural Crops and Its Management Strategies. Applied Entomology and Zoology (Volume-8). Akinik Publishers, New Delhi, India, 149-176.

Jena, M. K., Patel, S. R., Bairwa, K. L. and Sahoo, S. 2022b. The Melon Fruit Fly, *Bactroceracucurbitae*: A Menace to Horticultural Crops. Current Research in Agricultural Entomology (Volume-2). Bright Sky Publishers, New Delhi, India, 49-74.

Kalia, V. and Srivastava, M. L. 1992. Ovipositional behaviour and development of the oriental fruit fly, *Dacus* (*Strumeta*) *dorsalis* on development stages of mango fruit. *Bulletin of Ent*omology, **33** (12): 88-93.

Kalia, V. K. and Yadav, B. 2015. Cost effective mass rearing of the oriental fruit fly, *Bactrocera dorsalis* (Hendle) round the year. *International Research Journal of Natural and Applied Sciences*, **2**: 6.

Leghari, H. B. 2013. Mango fruit fly is hitting hard to the mango growers in Sindh. *Pakistan Standard Time Article*, 1-22.

Mohamed, A. M. 2000. Effect of constant temperatures on the development of the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *Assiut Journal of Agricultural Science*, **31**: 329-337.

Mohammed, A. A. 2003. Studies on the peach fruit fly, *Bactrocerazonata* (Saunders) and its control in Fayoum governorate, Egypt. M. Sc. Thesis, Faculty of Agriculture, Fayoum University, Egypt.

Naik, H. S., Jagadeesh, K. S. and Basavaraju, B. S. 2017. Biology and biometrics of oriental fruit fly, *Bactrocera dorsalis* (Hendle) (Diptera: Tephritidae) on custard apple, *Annona squamosa* L. *International Journal of Current Microbiology and Applied Sciences*, **6**(12): 3859-3864.

Narayanan, E. S. and Batra, H. N. 1960. Fruit Flies and Their Control. Indian Council of Agricultural Research, New Delhi, India, 1-68.

Quershi, Z. A., Hussian, T., Cary, J. R. and Dowell, R. V. 1993. Effect of temperature on development of *Bactrocera zonata* (Saund.) (Diptera: Tephritidae). *Pan- Pacific Entomologist*, **69**(1): 71-76.

Rana, J. S., Prakash, O. and Verma, S. K. 1992. Biology of guava fruit fly infesting guava fruits in Haryana and influence of temperature and relative humidity on its incidence. *Crop Research*, **5**(3): 525-529.

Rashmi, M. A., Verghese, A., Rami Reddy, P. V., Kandakoor, S. and Chakravarthy, A. K. 2020. Effect of climate change on biology of oriental fruit fly, *Bactrocera dorsalis* Hendle (Diptera: Tephritidae). *Journal of Entomology and Zoology Studies*, **8**(3): 935-940.

Shehata, N. F., Younes, M. W. F. and Mahmoud, Y. A. 2008.Biological Studies on the Peach Fruit Fly, *Bactrocera zonata* (Saunders). *Egypt Journal of Applied Sciences Research*, **4**(9): 1103-1106.

Stonehouse, M., Mahmood, A., Poswal, K., Mumforda, N., Baloch, M., Chaudhary, H., Makhdum, G. and Huggett, D. 2002. Farm field assessments of fruit flies (Diptera: Tephritidae) in Pakistan: distribution, damage and control. *Cropprotection*, **21**(1): 661-669.

Syed, R. A., Ghani, M. A. and Murtaza, M. 1970. Studies on the tephritids and their natural enemies in West Pakistan. III. *Dacus zonatus* (Saunders). *Technical Bulletin*, pp.1-16.

Weems, H. V., Heppner, J. B., Nation, J. L. and Steck, G. J. 2015. Oriental Fruit Fly, *Bactrocera dorsalis* (Hendle) (Insecta: Diptera: Tiphiritidea); University of Florida/IFAS Extension.

Younes, M. W. F. and Akel, F. A. 2010. Effect of temperature on development and reproduction of peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: tephritidae). *Egypt Journal of Experimental Biology* (*Zoology*), **6**(2): 255-261.

MS Received: 12 November 2022
MS Accepted : 27 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 98-102 (2022)

DOI Number: 10.5958/0974-4541.2022.00045.5

Dissipation, persistence and risk assessment of imidacloprid 17.8 SL on tomato

SANJAY KUMAR SAHOO* and VINOD KUMAR DUBEY

Department of Entomology, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur) 848125 Bihar, India

***E-mail :** sksahoo@rpcau.ac.in

ABSTRACT: Studies were conducted to evaluate the persistence, dissipation, and risk assessment of Imidacloprid 17.8 SL in tomato. After the third spray, fruit samples were separately collected from treated and untreated control plots at various intervals. The tomato samples were processed following modified QuEChERS technique and analyzed through UHPLC. When tomato was treated at 35and 70 g a.i. ha^{-1}after the third spray, the average initial deposits of imidacloprid on tomato were reported to be 0.34 and 0.65 mg kg^{-1}, respectively. After five and seven days following the last application at the single dose and double dose, imidacloprid residue in tomato was dissipated at below the LOQ of 0.05 mg kg^{-1}. For risk assessment of imidacloprid on tomato, the TMRC were calculated and compared with MPI. The results showed that, the TMRC values were below MPI in all the sampling days for both the dosages. Marketable size fruits should be plucked before the application of any insecticide so that 2-3 days will required for next picking. Hence, the study suggested aminimum waiting period of three days for safe consumption of tomato when applied with imidacloprid at a single dose.

Keywords: Imidacloprid, waiting periods, half-life, tomato, UHPLC

INTRODUCTION

Tomato (*Solanum Iycopersicum* Linn.) is one of the important major vegetables farmed at a global level (Engindeniz, 2006). It is widely grown in subtropical regions of north India, with 845 thousand ha area, under tomato cultivation and a production of 21181 thousand tonnes with a productivity of 25.07 MT/ha. In Bihar, tomato cultivation covered 62.70 thousand ha area, production 1161.79 thousand tonnes with a productivity of 18.53 MT/ha (Anonymous, 2021). The crop is affected by weeds, pathogens, and insect pest infestation. The fruit borers (*Helicoverpa armigera* (Hübner), aphids (*Aphis gossypii* Glover), and whitefly (*Bemisia tabaci* Gennadius) are the main insect pests that attack tomatoes and cause considerable economic harm to tomato growers. Pesticides are used on tomato crops to prevent pest infestation. The most common method of control of these pests is by the application of insecticides. When we compared from the food categories of plant origin like bread and others, the level of pesticide residue in vegetables and fruits that are mostly consumed as raw or semi-processed would be higher (WHO, 2003). Even though it has been suggested to use conventional insecticides, such as organochlorines, organophosphates, carbamates, and synthetic pyrethroids against a different species of insect pests of tomato (Claeys *et al.,* 2011). While the usage of these insecticides has helped the tomato growers to control the insect pests, it is frequently associated with environmental damage, chemical residues, and the emergence of pesticide resistance. Moreover, imidacloprid has been found to be highly efficient against many insect pests in tomato.

Imidacloprid[1-(6-chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2-ylideneamine] is a broad-spectrum systemic insecticide newly introduced in the Indian subcontinent by Bayer India Ltd.as Confidor 200 g $litre^{-1}$ SL and Gaucho 700 g kg^{-1} WS. It has an ovel mode of action, acting as an agonist of the nicotinyl acetylcholine receptor (Bai *et al.,* 1991; Businelli *et al.,* 1992). This new chloro-nicotinyl compound is effective against various insect pests and is used for seed dressing, soil treatment and foliar treatment in different crops (Mullins, 1993).

For the analysis of pesticide residues is one of the crucial for tackling growing consumer concerns about contamination issues. The two main origins of pesticide residues in food and crops are when pesticide that persist in the soil and pesticide that is directly applied to crops growing in the field (Businelli *et al.,* 1992). Pesticide residue contamination of food sources, particularly vegetables, is one of the biggest hazards to public health. Since there seems to be much information on the residues of imidacloprid on tomato is not available, the current investigation was to focus on the quantification of imidacloprid residues on tomato fruits that is required to fixed consumer and environmental safety. Therefore, the present study was carried out to know the dissipation, persistence, and risk assessment of imidacloprid on tomato.

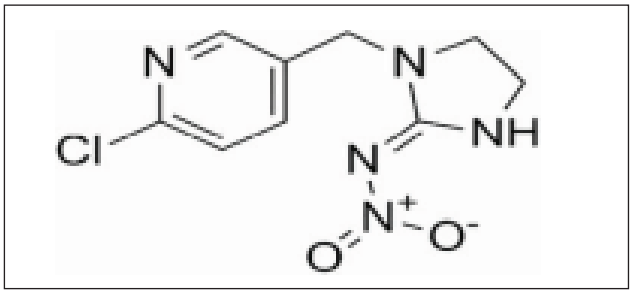
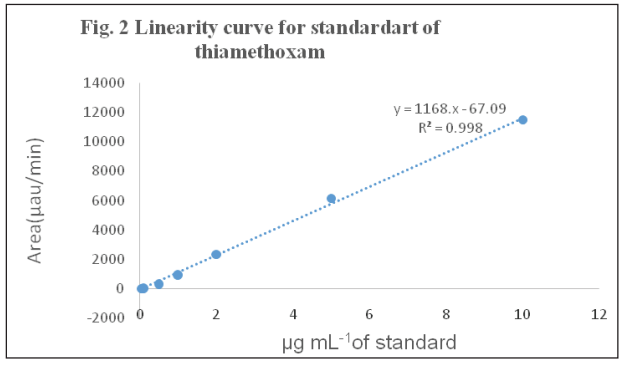


Fig. 1 Depicting chemical structure of imidacloprid



MATERIALS AND METHODS

Chemicals and reagents

Imidacloprid certified reference materials with a purity rating of 99.80% were provided by Dr Ehrenostrofer, Germany. Analytical grade solvents were used to prepare the standard solution and to extract the sample. Imidacloprid stock solutions were made in a concentration of 400.00 µg mL^{-1} in acetonitrile. To obtain concentrations of 100, 10, 2, 1, 0.5, 0.1, and 0.05 µg mL^{-1}, these solutions were serially diluted. Seven different concentrations of the standard solution (10, 5, 2, 1, 0.5, 0.1, and 0.05 µg mL^{-1}) were injected during the linearity study (Fig. 2).

Experimental sites

The field experiment was carried out in 2021 at the Dr. Rajendra Prasad Central Agricultural University in Pusa (Samastipur), Bihar, India, as a supervised field trial using standard agronomic practices for production of tomato (var. Kashi Vishesh), were planted under a Randomized Block Design (RBD) with three treatments, including untreated control. In control plots, insecticides were not used, and in another two treatment, the approved dose 35 and double of the approved dose 70 g a.i. $ha^{-1}$of imidacloprid insecticides were applied. Three times at ten-day intervals, imidacloprid was sprayed on the plants, the first spray starting at the fruit initiation stage. Each and every plot included a 50 m^2 area and three replications of each treatment. 500 g of tomato fruits were taken at random after the last application at the following times *viz.,*0 (2 hours), 1, 3, 5, 7, 10, and 15 days. Samples were brought to the lab for the analysis of pesticide residues for further extraction, cleaning, and quantification.

Extraction and cleanup

The tomato fruit samples were processed following modified Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) technique and analyzed through Ultra High Performance Liquid Chromatography (UHPLC). The sample brought from the field was cut and crushed using a mixer, and 15 g of brinjal and tomato fruits were weighed separately from the representative crushed sample into a 50 mL centrifuge tube and added with 30 mL of acetonitrile followed by addition of 10 ± 0.1 g of sodium chloride (NaCl) and centrifuged. An aliquot of 15 mL was added to a tube containing 10 ± 0.1g of anhydrous sodium sulphate (Na_2SO_4). Dispersive solid phase extraction was used to clean up the acetonitrile extract.The contents of a test tube containing 6 mL of acetonitrile, 0.15 ± 0.01 g of primary secondary amines

Table 1. Residue of imidacloprid in tomato after 3rd spray @ 35 and 70 g a.i. ha^{-1}

Days After Spraying (DAS)	Amount of Residue (mg kg^{-1})			
	35 g a.i. ha^{-1}		70 g a.i. ha^{-1}	
	Mean ± SD	Per cent Dissipation	Mean ± SD	Per cent Dissipation
Before application	<LOQ*	-	<LOQ	-
0 (2hrs after spray)	0.34 ± 0.02	-	0.65±0.05	-
1	0.23 ± 0.04	32.35	0.31± 0.03	52.30
3	0.09 ±0.006	73.53	0.18±0.02	72.30
5	<LOQ	-	0.08±0.02	87.69
7	<LOQ	-	<LOQ	-
10	<LOQ	-	<LOQ	-
15	<LOQ	-	<LOQ	-

*(LOQ = Limit of Quantification 0.05 mg kg^{-1})

Table 3. Theoretical maximum residue contributions (TMRC) on tomato fruits for Imidacloprid 17.8 S

Interval (days)	*MPI (µg/ person^{-1} day $^{-1}$)	Tomato 35 g a.i. ha^{-1} Average residues (µg g^{-1})	#TMRC (µg/ person^{-1} day $^{-1}$) Rural	#TMRC (µg/ person^{-1} day $^{-1}$) Urban	70 g a.i. ha^{-1} Average residues (µg g^{-1})	TMRC (µg/ person^{-1} day^{-1}) Rural	TMRC (µg/ person^{-1} day^{-1}) Urban
0	3300	0.34	9.52	9.86	0.65	18.52	18.85
1	3300	0.23	6.44	6.67	0.31	8.68	8.99
3	3300	0.09	2.52	2.61	0.18	5.04	5.22
5	3300	≤0.05*	-	-	0.08	2.24	2.32
7	3300	≤0.05	-	-	≤0.05	-	-
10	3300	≤0.05	-	-	≤0.05	-	-
15	3300	≤0.05	-	-	≤0.05	-	-

*(LOQ = Limit of Quantification 0.05 mg kg^{-1})

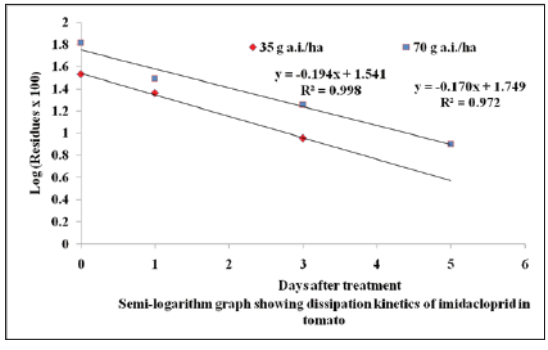


Fig 3. Semi-logarithm depicting dissipation kinetics of imidacloprid in tomato

(PSA) sorbent, and 0.90 ± 0.01 g of anhydrous $MgSO_4$ was extensively vortexed and centrifuged for three minutes. This acetonitrile extract was separated into three milliliters and used for analysis.

Instrumentation and estimation

Imidacloprid residues was analyzed throughUHPLC equipped with PDA (Photo Diode Array)detector with C18 column.The residues were calculated by comparing the peak area of the standards to samples performed under equilibrium conditions.The solvent system used was acetonitrile: HPLC water at 70:30 with a flow rate of 0.3 ml min^{-1}. The retention time was observed 3.097 min.

RESULTS AND DISCUSSION

Effectiveness of the developed techniques

Fortification studies were performed at various concentration prior to the field trial to evaluate the effectiveness of the method. To achieve this, untreated control samples of tomato fruits were fortified with imidacloprid concentrations of 0.05, 0.25, and 0.5 mg kg^{-1}, and the samples were evaluated using the aforementioned techniques.The plots of the untreated control samples and the reagent blanks were both evaluated in the same way in order to look for interferences caused by the substrate and reagents utilized, respectively. The residues data were presented as such as the recovery rate was higher than 80% in all the replicates.

Dissipation and persistence of imidacloprid on tomato

The residue's data are presented in mg kg^{-1} and dosein g a.i. ha^{-1}. After thirddays of treatment (DAT) on tomato, the average preliminary deposits of imidacloprid were found to be 0.34 and 0.65, respectively. On the first day following the last treatment, these residues decreased in tomatoes (0.23 and 0.31) showing per cent dissipation of 32.35 and 52.30, respectively.On 3rd day, in tomato the residues dissipated at approved dosage 73.53% and 72.30% at the double of the approved dose. The residues at both dosages reached below the LOQ of 0.05 mg kg^{-1} within a week of the last application, as demonstrated in (table1 and fig. 3). According to the aforementioned results, imidacloprid residues increased with larger application rates. These findings are very similar to those of (Manal *et al.,* 2022), who investigated the imidacloprid residue on tomato. The average preliminary deposits of imidacloprid were found to be 0.921 and 0.641 mg/kg in the leaves and fruit.The dissipation of imidacloprid in cucumber fruits after application also studied by (Hassanzadeh *et al.*, 2012), who reported that, The average initial deposits of imidacloprid on the cucumber fruits were found to be 1.93 and 3.65 mg kg^{-1} at the single and double dosages,

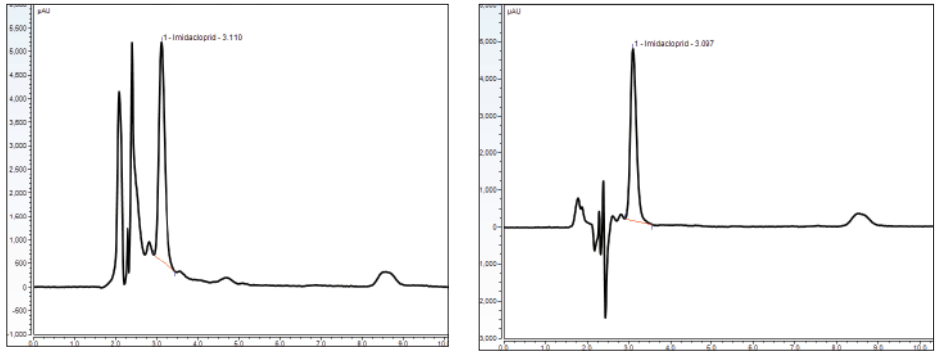


Fig. 3 UHPLC chromatograms for imidacloprid standard 0.05 µg/ml andtomato samples for single dose after 3rd application of imidacloprid

Table 2. Dissipation parameters of imidacloprid residues in tomato

Dissipation parameters	3rd spray (MRL = 0.5 mg kg-1)	
	Single dose (35g a.i. ha-1)	Double dose (70 g a.i. ha-1)
K_1 (b)	-0.194	-0.170
K_2 (a)	1.541	1.749
$T_{1/2}$	1.55	1.77
T_{tol}	-	0.29
R^2	0.998	0.972
Y	-0.194x + 1.541	-0.170x + 1.749

K_1 = "Slope of the regression line",K_2 = "Initial deposit obtained as in the regression equation, $T_{1/2}$ = "Residual half-life (in days)", T_{Tol}= "Time (in days) required for the pesticide residue to reach below the maximum residue limit (MRL) of 0.6 mg kg^{-1} ", R^2 = "Coefficient of determination".

respectively. The average preliminary deposits of imidacloprid were found to be 1.33 and 2.38 mg kg^{-1}, respectively, following the application of imidacloprid in tomato (Dharumarajan *et al.,* 2009).

Waiting period for imidacloprid in tomato

The data depicted regarding dose of the imidacloprid in (table 2) in g a.i.ha^{-1}. Half-life value ($T_{1/2}$) is often described simply and broadly as the time required to dissipate initial residues to half (Gunther and Blinn, 1955). According to Hoskins formula (1961),time was taken for residue to reach below Maximum Residue Limit (MRL) (T_{tol}) and half-life value ($T_{1/2}$) was calculated in days.

"Maximum residue limit (MRL)" of thiamethoxam in tomato was approved at 0.5 mg/kg (Anonymous, 2022). Determination of dissipation kinetics regarding residues of imidacloprid in tomato after third application is expressed in the form of semi-logarithm graphs (fig. 4 for imidacloprid, a linear relationship was obtained by plotting log concentration of residue multiplied by 100) against time. It confirms that, declination in residues of imidacloprid shows first order kinetic reaction.

After the third application of imidacloprid the half-life values in tomato were found to be 1.55 and 1.77 day resulting from the dose of single and double accordingly. When applied at the approved dose, initial deposit of imidacloprid in tomato was below the MRL. However, three days waiting period is suggested as picking interval for tomato is 2-3 days (table 2).

Risk assessment of imidacloprid in tomato fruit samples

The data demonstrated in (table 3)regarding MPI(µg/ $person^{-1}$ day^{-1}), TMRC (µg/ $person^{-1}$ day^{-1}) and dose of imidacloprid in (g a.i. ha^{-1}).A moderately toxic pesticide is more likely to cause risk assessment to occur from high exposure than from low exposure to a very

toxic pesticide. The Theoretical Maximum Residue Contributions (TMRC) of imidacloprid residues in tomato fruits were estimated at different time intervals and compared to the Maximum Permissible Intake (MPI) in order to determine the risk (table 3). The Acceptable Daily Intake (ADI) values of imidacloprid were 0.06 mg kg^{-1} body weight (Anonymous, 2022).Taking the average daily consumption of tomato fruit as 28 g for rural diet and 29 g for urban diet (Anonymous, 2022), the average residues of imidacloprid on tomato were used to calculate TMRC value. MPI was calculated by multiplying ADI with the weight of an average person (55 kg), the value of MPI which came out to be 3300, respectively. TMRC values of tomato for imidacloprid applied at single and double doses were determined to be 9.52 and 18.52for a rural diet, and 9.86 and 18.85for an urban diet. After treating samples of tomato fruit with imidacloprid at both applied dosages, it was reported that the TMRC values were lower than MPI for all the sampling days. But, considering the picking interval, minimum three day waiting period may be suggested before consumption of tomato.

CONCLUSION

Imidacloprid half-life (t½) is values on tomato were found to be 1.55 and 1.77 days, respectively, after three applications of imidacloprid 17.8SL. Theoretical maximum residue contributions for imidacloprid were reported to be much lower than MPI on tomato fruits at 0 (2 hours DAT) for both dosages, according to residue data. Therefore, a minimum three days waiting period is advised before tomato can be consumed safely when used at the recommended dose in order to avoid any harm to consumers' health.

ACKNOWLEDGEMENT

The trial was conducted under the university funded project. The authors are thankful to the university authority for providing the research project. The professor and Head, Entomology Department, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur) - 848125, Bihar, India, is greatly appreciated by the authors for providing all necessary research facilities.

REFERENCES

Anonymous.2021. www.indiastat.com (Accesed on 30.12.2022)

Anonymous.2022. https://fssai.gov.in (Accesed on 30.12.2022)

Anonymous.2022.https://www.fao.org/fao-who-codex alimentarius/codex texts/dbs/pestres/pesticides/en/ (Accesed on 30.12.2022)

Businelli, A., Vischetti, C. and Coletti, A. 1992. Validation of the k-oc approach for modeling the fate of some herbicides in Italian soils. *Fresenius Environmental Bulletin*, **1:** 583-87.

Bai, D., Lummis, S. C. R., Leicht, W., Beer, H. and Sattelle, B. D. 1991. Actionof imidacloprid and a related nitromethylene on cholinergic receptors of an identified insect motor neurone. *Pesticides Science,* **33** (2): 197-204.

Claeys, W. L., Jean-Francois, S., Bragard, C., Maghuin-Rogister G., Luc, P. and Schiffers, B. 2011. Exposure of several Belgian consumer groups to pesticide residues through fresh fruit and vegetable consumption. *Food Contamination*, **22**: 508-16.

Dharumarajan, S., Dikshit, A. K. and Bala, S. K. 2009. Persistence of combination-mix (Beta cyfluthrin +imidacloprid) on tomato (*Lycopersicon esculentum*). *Pesticides Research Journal*, **21**(1): 83-85.

Engindeniz, S. 2006. Economic analysis of pesticide use on processing tomato growing: A case study for Turkey. *Crop Protection,* **25**: 534-41.

Gunther, F. A. and Btinn R. C.1955. Analysis of insecticides and acaricides Interscience publishers Inc. New York.

Hoskins, W. M. 1961. Mathematical treatment of the loss of pesticide residues. *Plant Protection Bulletin* (FAO), **9**:163-168.

Hassanzadeh, N., Esmaili S. A. and Bahramifar, N. 2012. Dissipation of Imidacloprid in Greenhouse Cucumbers at Single and Double Dosages Spraying. *Journal of Agricultural Science and Technology,* **14**: 557-564.

Manal, A. A., Abdel Razik., Zamzam, M., Al Dhafar., Aisha, M., Alqahtani., Mohamed .A. Osman and Mohamed. E. Sweelam. 2022. Dissipation and Residues of Imidacloprid and Its Efficacy against Whitefly, Bemisia tabaci, in Tomato Plants under Field Conditions. Molecules, **27**:7607. https://doi.org/10.3390/molecules27217607

Mullins, J. W. 1993. Imidacloprid: a new nitroguanidine insecticide, in Pest control with enhanced environmental safety, ACS Symposium Series ł24, American Chemical Society, Washington, DC, 183-198.

World Health Organization. 2003. GEMS/Food Regional Diets (Regional per Capita Consumption of Raw and Semi-Processed Agricultural Commodities.

MS Received: 06 December 2022
MS Accepted : 28 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 103-109 (2022)

DOI Number: 10.5958/0974-4541.2022.00046.7

Host dynamics and molecular characterization of neo tropical invasive Bondar's Nesting Whitefly (BNW), *Paraleyrodes bondari* Peracchi (Hemiptera: Aleyrodidae) in Andhra Pradesh

P. V. RAGHUTEJA[1*], N. B. V. CHALAPATHI RAO[1], E. PADMA[2], B. NEERAJA[1], A. KIREETI[1], V. GOVARDHANA RAO[1], G. KOTESWARA RAO[1] and **B. V. K. BHAGAVAN[3]**

[1]Dr. YSRHU - Horticultural Research Station (HRS), Ambajipeta- 533214, Dr. B. R. Ambedkar Konaseema District, Andhra Pradesh, India

[2]Dr. YSRHU - College of Horticulture (COH),Venkataramannagudem- 534101, West Godavari District, Andhra Pradesh

[3]Dr. YSRHU - Horticultural Research Station (HRS), Peddapeta- 532421, Srikakulam District, Andhra Pradesh

[*]**E-mail:v**iswanadharaghuteja@gmail.com

ABSTRACT: The Bondar's Nesting Whitefly (BNW), *Paraleyrodes bondari* Peracchi is a new exotic pest of coconut since December, 2018 in India. It has spread across the nation since its first report and occurring in several horticultural crops especially in the East Godavari District of Andhra Pradesh. The host dynamics of BNW were studied and its incidence, intensity and severity on different host plants were also recorded. The host plants observed were Coconut, Oilpalm, Banana, Cinnamom, False rubber, Mango, Jackfruit, Guava, Temple pod and Hibiscus etc. The incidence and intensity were observed to be highest in Coconut and Oilpalm, while least was recorded in Hibiscus plant. The identity of BNWwas further confirmed through amplification of mt. COI gene obtained for the isolate which was sequenced and submitted to NCBI- GenBank (Acc. No. ON739183).

Keywords: *Paraleyrodes bondari*, BNW, Andhra Pradesh, host dynamics, neotropical, invasive, molecular characterization.

INTRODUCTION

Whiteflies (Hemiptera: Aleyrodidae) are plant-feeding, polyphagous sucking pests of global significance because of their function as vectors of plant diseases as well as inducing secondary deposits of sooty moulds on leaf surfaces by honeydew production, thus disturbing photosynthetic activity (Dickey *et al*. 2015). They superficially resemble tiny flies, with more than 1550 species described worldwide (Ouvrard and Martin, 2018). Among the insect pests, globally over the past 25 years, exotic whiteflies invaded several countries causing direct losses in agriculture, horticulture and forestry. Such reported invasive whiteflies in India are the spiralling whitefly, *Aleurodicus dispersus* Russell, which is known to breed on 320 host plants belonging to 225 genera and 73 families (Sundararaj and Pushpa, 2012); solanum whitefly, *Aleurothrixus trachoides* (Back) reported to breed on 24 plant species including medicinal plants (Sundararaj *et al*. 2018) and rugose spiraling whitefly (RSW), *Aleurodicus rugioperculatus* Martin that invaded in 2016 (Shanas*et al*. 2016; Sundararaj and Selvaraj, 2017; Chalapathi Rao *et al.* 2018 and Sushmitha *et al.* 2020) and found breeding on more than 20 host plants including coconut. In December 2018, Central Plantation Crops Research Institute (CPCRI) recorded two exotic whitefly species, *P. bondari* Peracchi and *P. minei* Iaccarino on coconut palms of Kerala and issued a pest alert (CPCRI, 2019).First incidence of the neotropical invasive Bondar's Nesting Whitefly (BNW), *P. bondari* Peracchi (Hemiptera: Aleyrodidae) in India was reported on coconut palms from Kerala (Chandrika *et al*. 2018). Very recently, it was observed that BNW has been invaded into different host plants of Andhra Pradesh. The occurrence of this pest on different host plants was confirmed during the survey in East Godavari District of Andhra Pradesh. The host range of BNW was discussed in this article.

MATERIALS AND METHODS

Systemic and continuous surveys were conducted to identify different host plants of BNW and percentage of BNW incidence and intensity on different host plants were worked out. The severity of BNW was assessed based on the following criteria, i.e. low: 0-10 live adult nests /leaf; moderate: 11-20 live adult nests/leaf; severe: >20 live adult nests /leaf.

Occurrence of Bondar's Nesting Whitefly in Andhra Pradesh state

In Andhra Pradesh, Bondar's Nesting Whitefly (BNW)

Table 1. New host plants of *P.bondari* Peracchi in Andhra Pradesh

Common Name	Scientific Name	Order	Family	Distribution	Whiteflies intermingled on the infested leaf	Incidence (%)	Intensity per leaf (%)	Severity
Coconut	*Cocos nucifera*	Arecales	Arecaceae	A.P, Kerala and Tamil Nadu	*Aleurodicus rugioperculetus* Martin, *P.minei* Iaccarino	59	32	Medium to high
Oilpalm*	*Elaeis guineensis*	Arecales	Arecaceae	A.P	*Aleurodicus rugioperculetus* Martin	45	27	Low to medium
Banana*	*Musa paradisiaca*	Zingiberales	Musaceae	A.P, Kerala	*Aleurodicus rugioperculetus* Martin	27	16	Low to medium
Cinnamom*	*Cinnamomum verum*	Laurales	Lauraceae	A.P	*Aleurodicus rugioperculetus* Martin	23	14	Low to medium
False Rubber	*Ficus elastic*	Rosales	Moraceae	A.P	-	26	18	Low to medium
Mango*	*Mangifera indica*	Sapnidales	Anacardiaceae	A.P	*Aleurodicus rugioperculetus* Martin	24	12	Low to medium
Jackfruit	*Artocarpus heterophyllus*	Rosales	Moraceae	A.P	*Aleurodicus rugioperculetus* Martin	18	14	Low to medium
Guava	*Psidium guajava*	Myrtales	Myrtaceae	A.P	*Aleurodicus disperses* Russell, *Aleurodicus rugioperculetus* Martin	19	28	Low to medium
Temple pod*	*Cassia fistula*	Fabales	Fabaceae	A.P	-	12	15	Low
Hibiscus*	*Hibiscus rosa-sinensis*	Malvales	Malvaceae	A.P	*Aleurodicus rugioperculetus* Martin	16	9	Low to Medium

*Hosts on which *P. bondari* has occurred for the first time in India

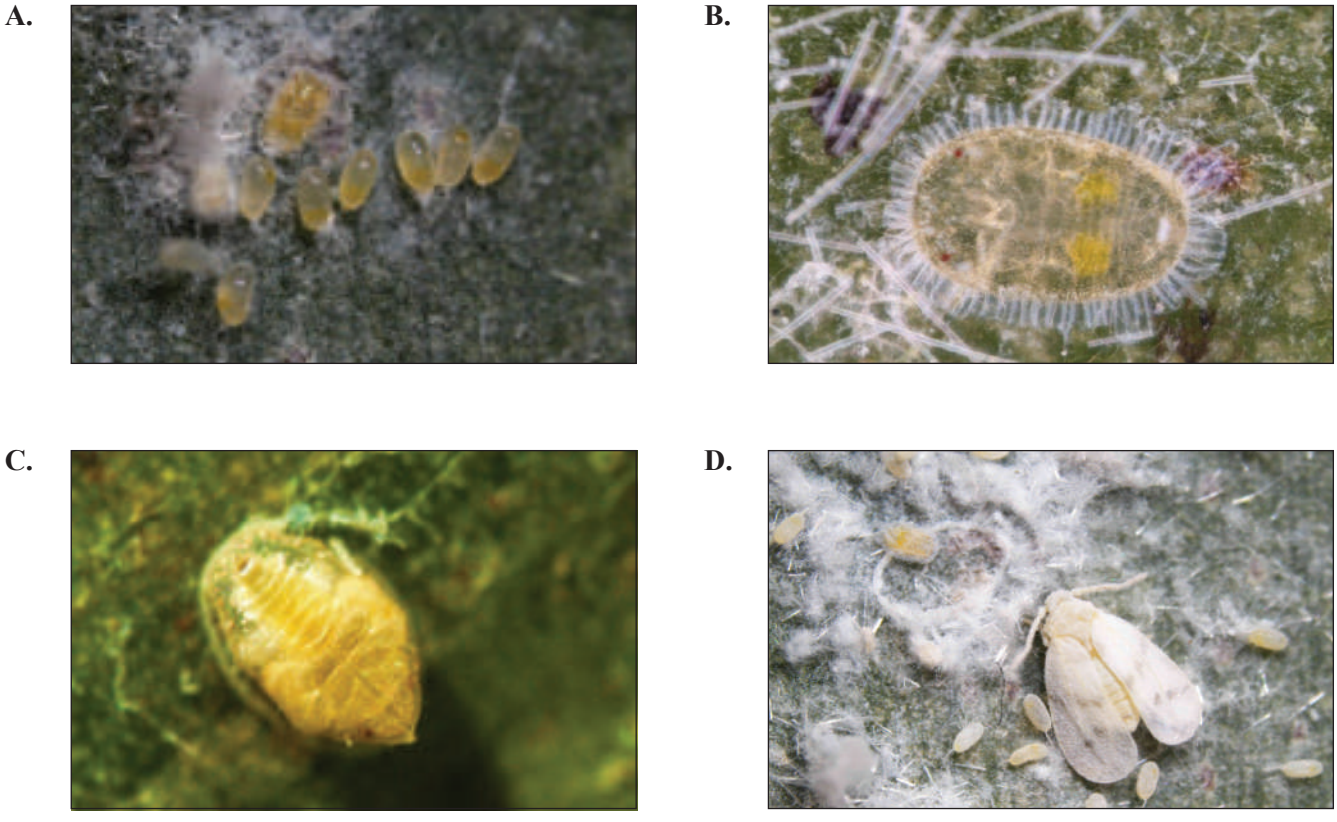


Fig 1. Life stages of BNW: A) Stalked Eggs and CrawlersB) Nymphs C) Pupae D) Adults

was observed on coconut plantations firstly in villages of East Godavari district. Based on typical characteristics of BNW, samples were collected from different locations and brought to the Biocontrol Research Laboratory, Coconut Research Station, Ambajipeta for morphological identification which was confirmed by Principal Scientist Dr. N. B. V. Chalapathi Rao as Bondar's Nesting Whitefly based on characteristics described by Perrachi. The incidence of BNW was confirmed after the survey in East Godavari District of Andhra Pradesh state. The pest was detected in all the coconut growing villages present in the vicinity of Konaseema region of East Godavari District. During the survey, this pest was also observed on different host plants and their samples were collected for further identification.

A total of 10 different hosts (Table 1) were identified during survey. Among them, coconut is already identified as host plant of BNW in Andhra Pradesh, while host plants like oilpalm, Guava, Banana and some ornamental plants etc. are firstly recorded as preferred host of BNW in India. As higher economic importance of plantation crops mainly in Konaseema region of Andhra Pradesh state, the concern about occurrence of BNW has raised the risk of becoming major pest.

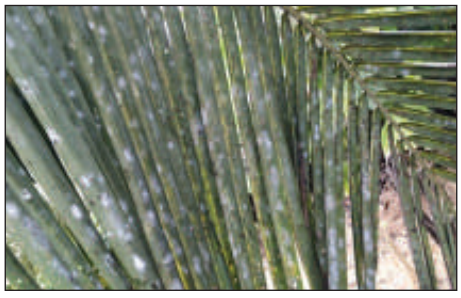
However, this perilous neotropical invasive Bondar's Nesting Whitefly (BNW) pest was firstly reported from coconut (*Cocos nucifera* L.) palms at Kayamkulam, Kerala, Peninsular India during December, 2018 (Joseph rajkumar *et al.,* 2019). Vidya *et al.* (2019) reported the incidence of BNW on Custard Apple, Jackruit, Capsicum, Cinnamom, Mango, Banana, Guava, Teak, *Macaranga peltata, Brideliaretusa*, *Morinda citrifolia, Leucanea leucocephala* in Andaman and Nicobar Islands.

Biology

Adults lay stalked eggs in clusters around the nest and mobile crawlers are seen active in the colony. The crawlers settle down on finding a suitable feeding point.

Nymphs are creamy yellow, transparent, oval shaped, absolutely flat up to 0.9 mm. The margin of the nymph is covered with a band of white wax and short setae are observed all around. On the dorsal side of the final-instar nymph, there are two shiny fibre glass like strands on the anterior side, eight on the posterior side and four setae (two longer and two shorter) located on the middle region. As the nymphs advance to the fourth-instar, there is excessive production of shiny fibreglass-like strands (Josephraj kumar *et al.* 2019).

Adults are powdery white, about 0.95 mm long, with two conspicuous oblique grey bands across the forewings together forming a **typical "X" pattern**.



A.

B.

C.

D.

E.

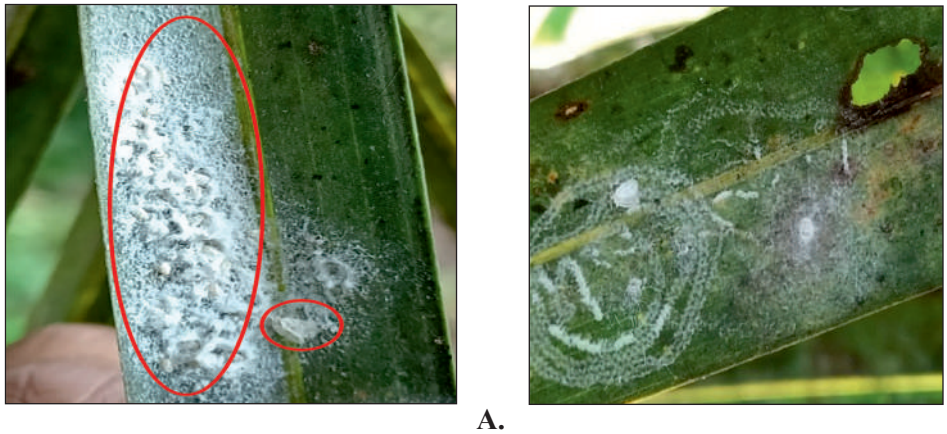
F.

G.

H.

I. **J.**

Fig 2: Occurence of Bondar's Nesting Whitefly, *P. bondari*in different hosts A. Coconut, B. Oilpalm, C. Banana, D. Cinnamom, E. False Rubber, F. Mango, G. Jackfruit, H. Guava, I. Templepod and J. Hibiscus



A.

B. **C.**

Fig 3: Co-existence of Rugose Spiralling Whitefly (RSW) and Bondar's Nesting Whitefly (BNW) on A. Coconut, B. Guava and C. Jackfruit

Severity of Bondar's Nesting Whitefly

The invasive pest *P.bondari* Perrachi (Hemiptera: Aleyrodidae), commonly known as the Bondar's Nesting Whitefly (BNW) was firstly observed in the 10 different host plants after reported in coconut from Konaseema region of Andhra Pradesh. The characteristics of BNW were shown same as observed in the coconut palm which is usually confined to the abaxial surface of palm leaflets shying away from the sunlight on account of its photo-sensitiveness. Adults and nymphs produce honeydew leading to deposits of sooty mould which affects the photosynthetic efficiency. The feeding damage by BNW has not become as intense as that of RSW with minimum honey dew and sooty mould deposits recorded so far on palm leaflets.

The following stages (Fig. 2 and 3) were observed during the survey for the confirmed report of this invasive pest in all these hosts.

Molecular Characterization of exotic Bondar's Nesting Whitefly (BNW)

Genomic DNA was isolated from individual adults

Table 2. List of nucleotide sequences of BNW retrieved from GenBank

Accession Number	Species	Location	Per cent Identity (%)	Reference
MW488198.1	*P. bondari*	Karnataka, India	100.00	Shivaji *et al*. 2020
MW041899.1	*P. bondari*	Karnataka, India	100.00	Shivaji *et al*. 2020
MK343480.1	*P. bondari*	Kerala, India	100.00	Josephrajkumar *et al*. 2019
KP032215.1	*P. bondari*	Florida	100.00	Dickey *et al*. 2015
MZ026894.1	*P. bondari*	Tamil Nadu, India	100.00	Ramasubramanian *et al.* 2021

of *P. bondari* collected from Horticultural Research Station (HRS), Ambajipeta using HiPurA™. Insect DNA Purification Kit (Hi-Media, India) following the manufacturer's instruction. The extracted DNA was eluted in 200 µl elution buffer. The quality of extracted DNA was tested using the spectrophotometer and 1.2 per cent agarose gel electrophoresis and stored at -20°C for further use. The DNA samples of RSW were subjected to PCR analysis for confirmation using mitochondrial COI gene specific primers, LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HC02198 (5′- TAAACTTCAGGGTGACCAAAAAAT CA-3′) (Dickey *et al.* 2015 and Josephraj Kumar *et al.* 2020).

Amplification was carried out in a 20 µl reaction volume which contained 2 µl of 10X Buffer [10 mMTris–HCl (pH 9.0), 50 mMKCl, 1.5 mM $MgCl_2$, 0.01% gelatin], 0.8 µl dNTPs (0.25 mM each), 3 µl of the forward and reverse primer's (2µM each), 0.3 µl of 3U *Taq* DNA polymerase (Genei Laboratories Pvt. Ltd., India) and 35 ng DNA. The PCR cycling condition consisting of an initial denaturation at 94ºC for 2 min followed at 35 cycles of denaturation at 94ºC for 30 sec, annealing at 50 ºC for 1 min and extension at 72 ºC for 1 min, with a final extension at 72 ºC for 10 min. After amplification, the PCR products were fractioned on 1.2 per cent agarose gel stained with ethidium bromide in 1X TBE buffer by electrophoresis. The expected amplicon size was found to be 700 bp in all the samples. The DNA was eluted from gel and sequenced by Sanger's di-deoxy chain termination method (Barcode Biosciences Pvt. Ltd.).

The nucleotides were aligned and edited using Bio edit software. The sequences were deposited in NCBI and accession number was obtained (Acc. No. ON739183).

Based on the nucleotide sequence analysis of *P. bondari* isolates shared 100 per cent similarity with previouslyreported BNW isolate coconut cytochrome c oxidase subunit I (COX 1) gene, partial cds m (Acc. No. MW488198.1), isolate BNWKa1 (Acc. No. MW041899.1), isolate WFM 2 (Acc. No. MK343480.1), isolate BNW_856-1 (Acc. No. KP032215.1), isolate SBITR-Pb01 (Acc. No. MZ026894.1) (Table 2).`

Omongo *et al.* (2018) confirmed the identity of BNW, *P. bondari* adults by sampling from the affected Cassava field of Uganda and subjecting partial mt. COI gene sequences (632 bp) to NCBI BLAST. The sequences matched 100 per cent nucleotide identity (haplotype Acc. No. MH 178372) and 100 per cent nucleotide identity with bondar's nesting whitefly, *P. bondari,* Peracchi (Acc. No. KP032215). Josephraj Kumar *et al.* (2019) determined the identity of bondar's nesting whitefly (BNW) by confirming the partial mt. COI gene sequences (675 bp) (Acc. No. MK343480) shared 100 per cent nucleotide identity with bondar's nesting whitefly (Acc. No. KP 032215.1 *P. bondari* isolate BNW 856–1).

As the invasive pest, *P. bondari* has been already reported in coconut with causing significant damage in Konaseema region of Andhra Pradesh state. But recently it has been seen that this pest invaded into the different ten host plants. After the morphological and molecular identification of this pest, this is the first confirmed report of *P. bondari* on Oilpalm, False rubber, Temple pod, Hibiscus in India and first time observed in Mango, Banana, Cinnamom and Guava in Andhra Pradesh state. This evident the alarming situation to the growers of these crops as this pest may spread to other growing areas of Andhra Pradesh state.

ACKNOWLEDGEMENT

The author is highly thankful to Dr. A. Joseph Rajkumar, Principal Scientist (Agri. Entomology) ICAR-Central Plantation Crops Research Institute (CPCRI) Kasargod, Kerala for identifying the invasive pest upto genus level.

REFERENCES

Chalapathi Rao, N. B. V., Rakshith, R. D., Krishna, R.G. and Ramanandam, G. 2018. A review on rugose spiralling whitefly, *Aleurodicus rugioperculatus* martin (Hemiptera: Aleyrodidae) in India. *Journal of Pharmacognosy and Phytochemistry,* **7**(5): 948-953.

Chandrika, M., Josephrajkumar, A., Singh, L. S. and Das, A. 2018.New distributional record of rugose spiralling whitefly on coconut in Kamrup and Nalbari districts of Assam. *Indian Coconut Journal,* **61**(4): 19-21.

CPCRI 2019. Pest alert - A new invasive Bondar's nesting whitefly reported on coconut from Kerala, India and First record of invasive nesting whitefly, *Paraleyrodesminei* Iaccarino (Homoptera: Aleyrodidae). http://www.cpcri.gov.in/images/images/ news/ICAR-CPCRI/PestAlert/Paraleyrodes bondari1.pdf and http://www.cpcri.gov.in/images/images/news/Pest alert/ whitefly.pdf (Accessed on 14 January 2019).

Dickey, A. M., Stocks, I. C., Smith, T., Osborne, L. and McKenzie, C. L. 2015. DNA barcode development for three recent exotic whitefly (Hemiptera: Aleyrodidae) invaders in Florida. *Florida Entomologist*.**9**: 473-478.

Josephrajkumar, A., Chandrika, M., Merin, B., Arya, K., Krishna, K. V., Vinayaka, H. and Chowdappa, P. 2019. First record of the invasive Bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi on coconut from India. *Phytoparasitica,* **47**: 333-339.

Josephrajkumar, K. A., Chandrika, M., Merin, B., Prathibha, P. S., Vinayaka, H. and Krishna, V.K. 2020. Diagnosis of invasive whitefly species co-occurring on coconut. *Current Science,* **119** (7): 1101-1105.

Omongo, C. A., Namuddu, A., Okao, O. G., Alicai, T., Van, B. S., Ouvrard, D. and Colvin, J. 2018. Occurrence of Bondar's nesting whitefly, *Paraleyrodesbondari* (Hemiptera: Aleyrodidae), on cassava in Uganda. *Revista Brasileira de Entomologia*, **62**: 257- 259.

Ouvrard, D. and Martin, J. H. 2018. The Whiteflies – Taxonomic checklist of the world's whiteflies (Insecta: Hemiptera: Aleyrodidae). https://doi.org/10.5519/0095728, http://www.hemiptera-databases.org/whiteflies/ – searched on 13 December 2018.

Peracchi, A. L. 1971. Doisaleirodideospragas de *Citrus* no Brasil (Homoptera, Aleyrodidae).Arquivos do Museo Nacional, *Rio de Janeiro*, **54**: 145-151.

Ramasubramanian, T., Janani, S., Yogambal, C., Mohan, K.S. and Geetha, N. 2021.DNA Barcode of Bondar's Nesting Whitefly on Sugarcane. https://www.ncbi.nlm.nih.gov/nucleotide/MW047062.1, accessed 1 May 2021.

Shanas, S., Job, J., Joseph, T. and Anju, K. G. 2016. First report of the invasive rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) from the Old World. *Entomon,* **41**(4), 365-368.

Shivaji, T. H., Thava, P. P. R., Josephraj, K. A., Santhosh, K. P., Merin, B. and Nirmal, K . B . J . 2020. Characterization of whiteflies infesting arecanut. https://www.ncbi.nlm.nih.gov/nucleotide/MW047062.1, accessed 14 January 2021.

Shivaji, T. H., Thava, P. P. R., Josephraj, K. A., Santhosh, K. P., Merin, B. and Nirmal, K.B.J. 2020. *Paraleyrodes bondari* isolate BNWKa1 cytochrome C oxidase subunit I (COX1) gene, partial cds; mitochondrial.https://www.ncbi.nlm.nih.gov/nuccore/MW041899.1, accessed 14 January 2021.

Sundararaj, R. and Pushpa, R. 2012. In B. V. David (Ed.). The whitefly or Mealywing bugs: Bioecology, Host specificity and management (Vol. 2011, pp. 20–57). Germany: Lambert Academic Publishing.

Sundararaj, R. and Selvaraj, K. 2017. Invasion of rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae): A potential threat to coconut in India. *Phytoparasitica*, **45**: 71-74.

Sundararaj, R., Amuthavalli, T. and Vimala, D. 2018. Invasion and establishment of the solanum whitefly, *Aleurothrixus trachoides* (Back) (Hemiptera: Aleyrodidae) in South India. *Current Science*, **115** (1): 29-31.

Sushmitha, S., Sujatha, A., Emmanuel, N., Umakrishna, K. and Salomi, S. D. R. 2020. Incidence, intensity and host dynamics of rugose spiralling whitefly, *Aleurodicus rugioperculetus* Martin (Hemiptera: Aleyrodidae) in Andhra Pradesh. *Pest management in Horticultural Ecosystem,* **26**(1): 109-120.

Vidya, C. V., Sundararaj, R., Dubey, A. K., Bhaskar, H., Mani, C. and Henna, M. K. 2019. Invasion and establishment of Bondar's nesting whitefly,*Paraleyrodesbondari* Peracchi (Hemiptera: Aleyrodidae) in Indian mainland and Andaman and Nicobar Islands.*Entomon,* **44**(2): 149-154.

MS Received: 06 December 2022
MS Accepted : 27 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 110-114 (2022)

DOI Number: 10.5958/0974-4541.2022.00047.9

Identification of efficient attractant for the management of shot hole borer, *Xylosandrus compactus* infesting Coffee

A. ROOBAKKUMAR*, P. KRISHNA REDDY, M. S. UMA, TINTUMOL, K., H.G. SEETHARAMA and N. SURYA PRAKASH RAO

Entomology Division, Central Coffee Research Institute, Coffee Research Station – 577 117, Chikkamagaluru Karnataka, India

***E-mail**- roobakkumar@gmail.com

ABSTRACT: The shot hole borer (SHB), *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae: Scolytinae), is an emerging pest of Robusta coffee in India. Traps with attractants are commonly used to capture the ambrosia beetles for purposes of monitoring, studying population dynamics, predicting outbreaks, and mass trapping to reduce damage. The relative attractiveness of several common attractants and the influence of their concentration were evaluated in the field against *X. compactus* using Broca traps in this study. The tested attractants exhibited clear differences in attractiveness and the preliminary field studies at coffee plantations revealed that absolute ethanol attracted more shot hole borer adults than coffee twig extracts, distillery ethanol, and ethanol methanol combinations. The number of beetles trapped varied in different places during multi-location field trials, but in general, 50% of absolute ethanol-baited traps captured the highest number of beetles in all the locations. This study demonstrates 50% absolute ethanol can be used as an effective attractant for coffee shot hole borer and enables a cost-effective option for the control of *X. compactus* in coffee.

Keywords: Coffee, ambrosia beetle, *Xylosandrus compactus*, ethanol, mass trapping

INTRODUCTION

The Ambrosia beetle, *Xylosandrus compactus* (Coleoptera: Curculionidae) is a polyphagous pest that affects over 200 species, including coffee, forestry, and ornamental plants (Venkataramaiah and Sekhar,1964; Matsumoto, 2002,;Wu *et al.,* 2007; Chong *et al*., 2009; Gao *et al.,* 2017). It is native to Asia and is mostly found in subtropical and tropical locations (Hayato, 2007). In many countries, this beetle is predominantly reported as a Robusta coffee pest (Chacko, 1978; Hara and Beardsley, 1979; Vasquez *et al.,* 1996; Egonyu *et al.,* 2009). Until recently, it was considered as one of the minor pests and natural pruners in India. However, the incidence of this pest has grown significantly in recent years, particularly in Robusta coffee, and it has caused substantial damage, particularly in young plants (Roobakkumar, 2019). Though the exact reasons for the pest flare-ups in field situations are not clear, it is assumed that changes in climatic conditions and innovative grower practices such as drip irrigation coupled with fertigation, which contributes to luxurious vegetative growth with more succulent secondary and tertiary branches, are favoring pest build-up. The insect prefers green and succulent coffee shoots, but it will also infest the main stem if the plant is relatively young. The adult female beetles bore the coffee branches and makes a tunnel inside the branch for inoculating the symbiotic fungus *Ambrosiella xylebori.* Once the fungus developed inside the tunnel, adult beetle started to lay eggs and the emerged larvae grow inside by feeding on the fungus. Adult beetles and larvae do not feed on the tissues of the coffee tree; however, they survive by feeding the fungus inoculated by female adult beetle (Roobakkumar, 2019). This cryptic nature of the lifestyle inside the coffee plants makes it exceedingly difficult to manage this pest using conventional control methods. The available management methods for the pest are of limited success as the beetles are within the branches and do not feed directly on the vascular tissues. Pruning followed by the burning of infested twigs and application of fungicide, Propiconazole is the management measure adopted by coffee growers in India. When infestation levels become high, sanitation is a major concern to growers, as the removal of the copious amount of bearing branches from coffee plants significantly reduces the yield for the current and coming years (Greco and Wright, 2015). Though the recommended measures are effective, it is not economically feasible for most of the small coffee growers as it involves a large workforce. Further, the indiscriminate use of pesticides could contribute to undesirable problems in coffee plantations. At this juncture, switching to large-scale non-chemical management strategies is necessary to compete on the international market for a reasonable price for Indian coffee. The enormous crop loss caused by this pest and its life cycle warranted the development of easy, dependable, and environmentally sustainable management strategies. Traps baited with a chemical attractant are gaining importance nowadays for monitoring, analyzing the population dynamics, forecasting outbreaks, and mass

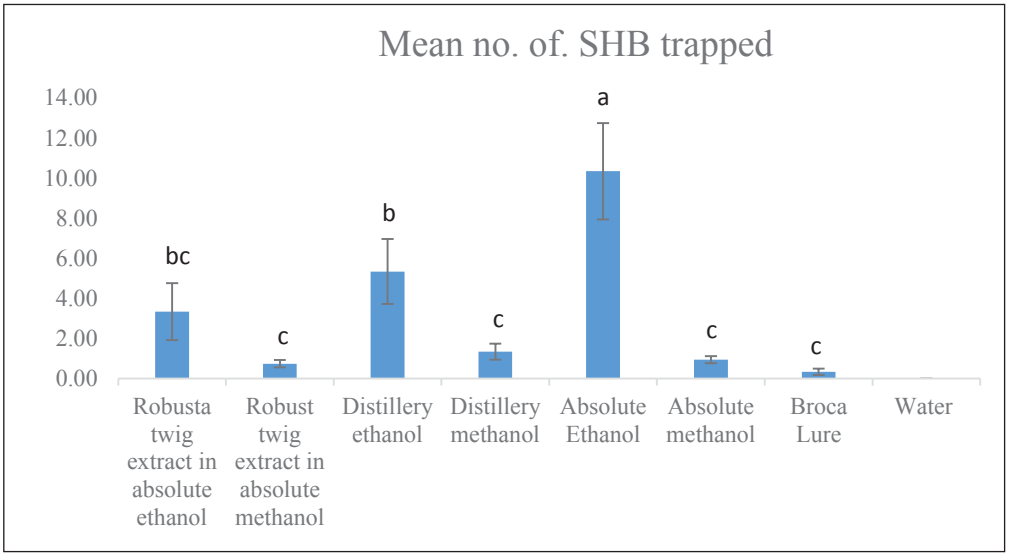


Fig. 1. Mean number of *X. compactus* captured per week in traps baited with different lures on robusta coffee plants (Means followed by the same letter do not differ significantly at *P* = 0.05 according to DMRT)

trapping. So far, the mass trapping strategy is not studied well for coffee shot hole borer in India. As mass trapping is one of the best IPM strategies if a strong attractant is available, the current study was initiated to find out an effective attractant for the management of coffee shot hole borer.

MATERIALS AND METHODS

Study Location

All the experiments except multilocation field trials were conducted in 40-year-old Robusta plants (Variety, Old Robusta) at the research farm of Central Coffee Research Institute (CCRI) located at 850 MSL (13°22'N75°28'E) in the district of Chikkamagaluru, Karnataka, India. This study site had enough shot hole borer infestation (around 80% of the trees had one or more infested twigs at the time of the survey). For multi-location field trials apart from CCRI, three private estates were selected viz., Nithin estate (Palya, Sakaleshpura), Hadhige estate (Mudigere), and Narendra estate (Munnurpal, Kalasa) from three different coffee growing zones of Karnataka, India.

The Trap

The trap used for all our experiments is the BROCA trap which is originally used for the management of Coffee Berry Borer. The trap consists of two units made up of polypropylene which can be threaded together, a small vial of 14 ml capacity with 10 ml lure is kept inside the trap and the whole trap is hung on the branches of the coffee plants approximately at the height of 1.5 m from the ground. Water is filled in the bottom half of the trap, which acts as the trap since the beetles that fall in the water are unable to fly again. The traps were recharged at weekly intervals by changing the water and checking for the level of the lure in the vials.

The attractants

Three field trials were conducted to evaluate the attractants to trap *X. compacts* in coffee plantations in south India. Trial 1 was designed to evaluate seven different attractants *viz.,* twig extracts of Robusta obtained using ethanol and methanol which is normally used for the attraction of insects. The other treatments like absolute ethanol (99.9% purity) and methanol (99.9% purity) of analytical grade were purchased from SD fine chemicals; distillery ethanol (95% purity) and methanol (95% purity) were purchased from the local distillery units. Solvent extracts were prepared following the methodology proposed by Karunaratne *et al*., 2008 by shaking the dried ground coffee twigs (500 g) of cultivar old Robusta with the distilled solvent, respectively ethanol and methanol (1.5 L) at 27° C for 24 hours followed by filtering and concentrating below 40° C on a rotary evaporator to obtain the extract, which was dried in a vacuum oven for 24 h before use.

Trial 2 was designed to compare the attractiveness of the most attractive lure from trial 1. Hence different concentrations of distillery ethanol and absolute ethanol were evaluated in the second trial. In the third trial, selected attractants were evaluated at multilocation to confirm the results.

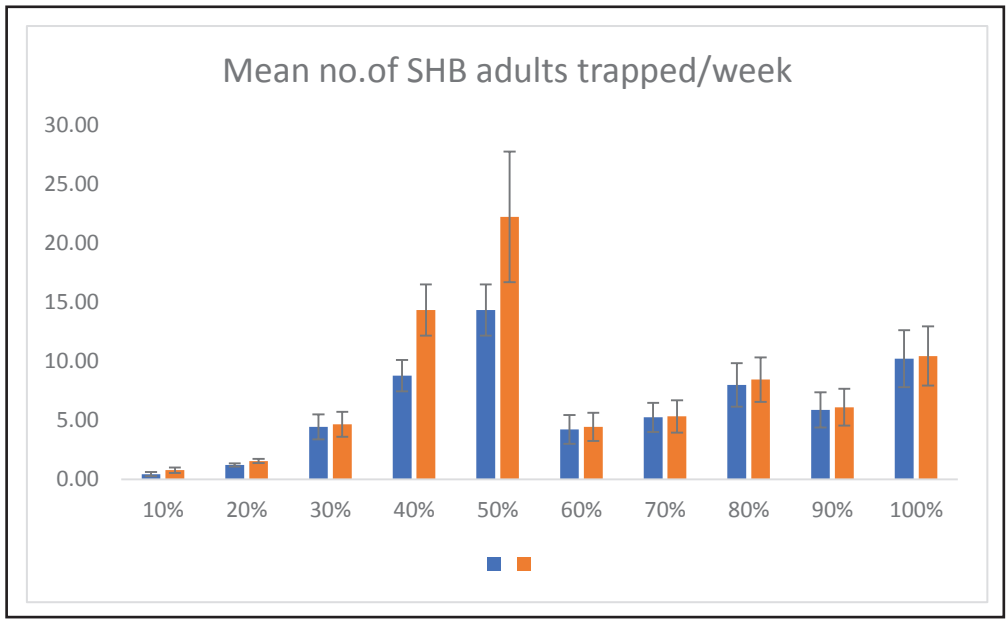


Fig. 2. Mean number of *X. compactus* captured per week in traps baited with different concentrations of Distillery ethanol (DE) and Absolute ethanol (AE) on robusta coffee plants (Means followed by the same letter do not differ significantly at *P* = 0.05 according to DMRT).

Experimental Design and Statistics

Each treatment was replicated 5 times. Broca Traps were deployed for all the studies. Traps were placed in a grid at 15 m intervals, 1.5m height above the ground on coffee plants and used a completely randomized design for the placement of treatments in the field. Experiments were conducted from September 2019 to February of 2020 and November 2020 to January 2021, trapped beetles were collected at weekly intervals. Every week, all the replications were rotated clockwise to avoid the experimental site error. 10 ml of each of the tested lures are placed in a small plastic vial inside the Broca traps which ran for the entire study period of 2 months. Insects were collected weekly by pouring the fluid through a fine (200 µ mesh) sieve. The insects retained on the sieve were transferred to the Entomology Laboratory (CCRI) for identification and counting. Differences between treatments on the attraction of shot hole borer adults were studied using analysis of variance (ANOVA) and the means were separated by Duncan's multiple range test (DMRT) (SPSS 10.0 1999).

RESULTS

Trial 1

The preliminary trapping studies conducted from September to November 2019 revealed that significant differences were observed in the mean number of adults captured among the tested attractants. Traps baited with absolute ethanol significantly attracted a greater number of adults compared to other attractants with 10.35 beetles/trap/week (Fig.1). Distillery ethanol-baited traps attracted the next highest number of adults, and it is on par with the Robusta twig extract in ethanol. No significant difference in the attraction was observed in the other tested attractants with the water-baited control.

Trial 2

Based on the preliminary observations, follow-up trials were conducted on the efficacy of different concentrations of distillery ethanol and absolute ethanol at CCRI. A comparison of the efficacy of ethanol (distillery and absolute) in different concentrations in trials conducted between December 2019 and February 2020 showed significant differences in the capture level between the distillery and absolute ethanol and different proportions. The comparative experiment indicated that absolute ethanol attracted a greater number of shot hole borer adults than distillery ethanol in all the concentrations. Whereas among the absolute ethanol, 50% concentration attracted a significant number of shot hole borer adults compared to all other proportions. Among the distillery ethanol, more attraction was recorded for 50% ethanol and the results are on par with 100% distillery ethanol (Fig.2). The trap catches increased with a concentration in both distillery ethanol and absolute ethanol up to 50%.

Table.1. Multi-location field evaluation of different attractants against *X. compactus*

Treatments	Mean no. of SHB adults attracted/week at different locations				Mean catch*
	CCRI	Mudigere	Sakaleshpura	Kalasa	
40 % DE	13.50 ± 1.94	4.75 ± 0.85	6.75 ± 0.48	14.00 ± 1.47	9.75 ± 2.35b
50 % DE	17.50 ± 2.96	6.00 ± 0.91	8.50 ± 0.96	17.00 ± 1.68	12.25 ± 2.93b
100 % DE	14.00 ± 2.83	5.25 ± 0.63	7.50 ± 0.29	12.75 ± 2.56	9.88 ± 2.09b
40 % AE	16.50 ± 1.55	7.75 ± 0.25	8.50 ± 0.65	16.75 ± 1.55	12.38 ± 2.46b
50 % AE	27.00 ± 2.08	26.75 ± 2.06	28.50 ± 1.26	27.75 ± 1.55	27.50 ± 0.40a
100 % AE	17.25 ± 0.48	12.50 ± 0.87	15.75 ± 2.17	14.75 ± 2.43	15.06 ± 1.00b
Water (Control)	0.25 ± 0.25	0.25 ± 0.25	0.00 ± 0.00	0.25 ± 0.25	0.19 ± 0.06c

Trial 3.

Based on the results of trials 1 and 2, multi-location field trials were conducted from September 2020 to January 2021 using the effective proportions of distillery ethanol and absolute ethanol to find out an efficient and economic attractant for *X. compactus*. The multi-location field trials showed that 50 percent absolute ethanol attracted the maximum number of shot hole borer beetles to the traps (Fig. 4b) in all the tested locations. The overall result of the experiment indicated 50% absolute ethanol attracted a significant number of beetles which was approximately twofold more compared to all other evaluated concentrations. This trend in trap catches has been observed throughout the study period in all the tested locations.

DISCUSSION

This work was aimed to identify the best attractant and thus to develop a trapping technique for managing shot hole borer infestation in coffee plantations in India. Field evaluation was carried out from November to January when the shot hole borer incidence was more in the coffee estates. Ethanol extracts from wood and bark act as the primary attractant of several ambrosia beetles and is responsible for increasing their attack rate (Moeck 1970, Bhagwandin 1992, Shore and Lindgren 1996). Concentrations and release rates are the factors that have might have affected the shot hole borer attraction, therefore, treatments like coffee twig extracts in ethanol and methanol along with commercial ethanol and methanol were tested in different concentrations. The finding of our study revealed that higher capture rates were obtained for ethanol in the preliminary studies which confirms the previous observations by Moeck 1970, Oliver and Mannion 2001, Hoagland and Schultz 2006, Miller and Rabaglia 2009, Burbano *et.al.,* 2012 and Avinash *et.al.,* 2021. Further field trials on the different concentrations of absolute and distillery ethanol clearly indicate the significant attraction for absolute ethanol compared to distillery ethanol. Our results demonstrated, *X. compactus* was significantly more attracted to 50% Absolute ethanol than either distillery ethanol or methanol. The difference in attraction between the distillery and absolute ethanol could be because of the difference in the degree of purity of ethanol. Our finding is in contrast with Avinash *et.al.,* 2021 who reported *X. compactus* was attracted more towards 95% absolute ethanol rather than 50% absolute ethanol.

Effective attractants can be used to set up a trapping program to examine this beetle's seasonality and flying behaviour. Traps can be used as an early warning system to identify the presence of *X. compactus*, as well as scheduling time for pesticide application. If a powerful attractant is available, traps may be effective as a mass trapping technique. In coffee plantations, an effective trapping system might be part of an integrated management approach for *X. compactus*. Hence, 50% absolute ethanol might facilitate implementing the strategy for management purposes.

ACKNOWLEDGMENT

The authors are thankful to the Coffee Board, Ministry of Commerce and Industry, Government of India, for the financial support provided. The authors are also grateful to the planters for providing all the facilities in their estates for large-scale multi-location studies.

REFERENCES

Avinash, Revenna, R and L. Hanumantharya. 2021. A study on efficiency of ethyl alcohol traps against Coffee shot hole borer *Xylosandrus compactus* in Robusta coffee plantation across seasons. *Indian Journal of Entomology*. **83**. Online published Ref. No. e20275. DoI No.: 10.5958/0974-8172.2020.00240.0

Bhagwandin, Jr., H. O. 1992. The shot hole borer: an ambrosia beetle of concern for chestnut orcharding in the Pacific Northwest. The 93rd Annual Report of the Northern Nut Growers

Association 168Ð177. (http://www.wcga.net/shb.htm).

Burbano E G, Wright M G, Gillette N E, Mori S, Dudley N, Jones T, Kaufmann M. 2012. Efficacy of traps, lures and repellents for *Xylosandrus compactus* (Coleoptera: Curculionidae) and other ambrosia beetles on Coffea arabica plantations and Acacia koa nurseries in Hawai. *Environmental Entomology*, **4** (1): 133-140.

Chacko, M. J. 1978. Control of certain devastating pests of coffee. *Indian Coffee*, 42: 265-268.

Chong, J. H., L. Reid, and M. Williamson. 2009. Distribution, host plants, and damage of the Black twig borer, *Xylosandrus compactus* (Eichhoff), in South Carolina. *Journal of Agriculture and Urban Entomology*, **26:** 199–208.

Egonyu, J. P., Kucel, P., Kangire, A., Sewaya. F., Nkungwa. C. 2009. Impact of the black twig borer on Robusta coffee in Mukono and Kayunga districts, Central Uganda. *Journal of Animal and Plant Science,* **2**(4):163-169.

Gao L, Wu S, Lu G et al (2017) Morphological characteristics and damage survey of Xylosandrus compactus. *Forest Pest and Diseases,* **36:**31–34.

Greco, E. B., and M. G. Wright. 2015. Ecology, Biology, and Management of *Xylosandrus compactus* (Coleoptera: Curculionidae: Scolytinae) with Emphasis on Coffee in Hawaii. *J. Integ. Pest Mngmt*. 6(1): 7; DOI: 10.1093/jipm/pmv007.

Hara, A. H., and Beardsley, J. W. Jr. 1979. The biology of the black twig borer, *Xylosandrus compactus* (Eichhoff), in Hawaii. *Proceedings of Hawaii Entomological Society*, **18:** 55–70. 18: 55–70.

Hayato, M. 2007. Note on the dieback of *Cornus florida* caused by *Xylosandrus compactus*. *Bull. For. Prod. Res. Inst.* **6:** 59–63.

Hoagland, T. P., and P. B. Schultz. 2006. Using ethanol baited traps to minimize risk of Asian ambrosia beetle, *Xylosandrus crassiusculus*, attack to hardwood trees in Virginia nursery production. SNA research conference 51: 132Ð133 (http://www.sna.org/pdf/2006/03Entomology. pdf).

Karunaratne, W.S., Kumar, V., Pettersson, J & N. S. Kumar. 2008. Response of the shot-hole borer of tea, *Xyleborus fornicatus* (Coleoptera: Scolytidae) to conspecifics and plant semiochemicals. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, **58:**4, 345-351, DOI: 10.1080/09064710701788802.

Matsumoto, K. 2002. Insect pests of Mahogany in Indonesia and Malaysia. *Tropical Forest,* **55:** 29–36.

Miller, D. R., and R. J. Rabaglia. 2009. Ethanol and (-)-α- pinene: attractant kairomones for bark and ambrosia beetles in the southeastern US. *Journal of Chemical Ecology,* **35**: 435-448.

Moeck, H. 1970. Ethanol as the primary attractant for the ambrosia beetle, *Trypodendron lineatum* (Coleoptera: Scolytidae). *Canadian Entomologist*, **102**: 985-995.

Oliver, J. B., and C. M. Mannion. 2001. Ambrosia beetle (Coleoptera: Scolytidae) species attacking chestnut and captured in ethanol baited traps in middle Tennessee. *Environmental Entomology*, **30**: 909-918.

Roobak Kumar, A., Uma, M. S., Manjunatha Reddy, G. V., Krishna Reddy, P., Seetharama, H.G., Dhanam, M., Surya Prakash Rao, N. and Y. Raghuramulu. 2019. Shot hole borer, flare ups: an issue of concern in coffee. *Indian coffee,* **83** (1-2): 12-13

Shore, T., and B. Lindgren. 1996. Effect of ethanol and -pinene on response of ambrosia beetle, *Trypodedron lineatum*, to lineatin-baited funnel and drainpipe traps. *Journal of Chemical Ecology*, **22:** 2187-2196.

SPSS. 1999. SPSS for Windows. Release 10.0.1. Standard Version, Copyright 1999 by SPSS Inc.

Vasquez, L. L., N. Tur, and S. Monteagudo. 1996. Insectos de la familia Scolytidae (Coleoptera) que atacan al cafeto en Cuba. *Revista de Proteccion. Vegetal,* **11**: 5–7.

Venkataramaiah, G., and P. Sekhar. 1964. Preliminary studies on the control of the shot hole borer, *Xylosandrus compactus* (Eichhoff) *Xyleborus morstatti* (HGDN). *Indian Coffee.* **28**: 208–210.

Wu Y, Yu J, Li X. 2007. A new pest *Xylosandrus compactus* in landscape plants. *Subtrop Plant Science,* **36**:13–16.

MS Received: 02 November 2022
MS Accepted : 12 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 115-118 (2022)

DOI Number: 10.5958/0974-4541.2022.00048.0

Plant Health Clinic (PHC) startups as a viable extension model for transfer of biocontrol products in horticultural crops

ABRAHAM VERGHESE*[1,2] and M. A. RASHMI[1]

[1] Rashvee International Phytosanitary Research and Services, Bengaluru 560024, India

* [2] Formerly with ICAR-National Bureau of Agricultural Insect Resources and ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, Karnataka, India

***E-mail :** abraham.avergis@gmail.com

ABSTRACT: In recent past, public funded institutions are entering into commercialization of their products especially in the field of crop protection like biopesticides and botanical products. This has paved way for emergence of 'startup culture' in agriculture with young entrepreneurs with sound technical background showing keen interest. This article highlights the scope of these startups doubling up as plant health clinics and how they reach out to farmers with a case study of an emerging startup being promoted by entomologists.

Keywords: Agri-startup, farm inputs, biopesticides, plant health clinic

INTRODUCTION

Biocontrol has been in existence in India for over a hundred years. The early phase of biocontrol was dependent on parasitoids and predators supplied by public labs. These bio-agents used to survive in crops which were less sprayed like coconut, papaya, sugarcane, eucalyptus, mulberry etc. But in horticultural crops where insecticide pressure was high like many of the commercial vegetables and fruits, the parasitoids and predators would be killed. Further the commercial availability of these was also low. However in the last 15 years the availability of commercial entomopathogenic fungi/nematodes/viruses/bacteria, many of which are products from ICAR institutes and state agricultural universities, have come to the market.

MATERIALS AND METHODS

To understand the knowledge levels on biocontrol among farmers of south Karnataka (Bangalore Rural, Chikballapur, Kolar districts) a survey was conducted between 2019 and 2022. A simple one page questionnaire was prepared. 240 farmers were interviewed through snowball sampling. As this was the Covid phase, interviews were conducted mostly over the phone. Apart from personal details, answers elicited were crops grown, awareness of biocontrol, biocontrol agents, use of bioagents like *Trichogramma*, *Cryptolaemus*, etc., ecosystem services by birds, reptiles, etc and knowledge/ information contact points. The data obtained were subjected to analysis to know the adoption levels of these commercially available biocontrol products. In 2022 a market survey was conducted among 12 randomly selected input suppliers (both private and public) of the same districts to know the commercial availability of biopesticides. The data were classified and four main responses were tabulated (Table1). Based on these foundational information further extensional strategisations were made.

RESULTS AND DISCUSSION

It was found that majority (94%) of the horticultural farmers were aware of 'biocontrol' and among the biocontrol products, they were mostly aware of Trichoderma (78%) followed by Bt (*Bacillus thuringiensis*) and entomopathogenic nematodes. Trichoderma is familiar as it is available commercially even in public labs of DDPQ, KVK's, ICAR, SAU's and certain NGO's. Some public labs distribute them free of cost. As part of the survey, feedback on Tricho cards and *Cryptolaemus* showed that 36% and 64% respectively, have not even heard of them. Main reason for this was that these were commercially not available. For the extent of ecosystem services through insectivorous birds, reptiles and frogs, farmers were not sure of it. Though (*Trichoderma*) was widely available, only about 16% of the farmers were aware of it and about 4% used it, and when it comes to other products like Bt, EPF, EPN the percentage of use was less than 2 percent. Even when used these were not appropriately used or timed well. Overall, among the horticulture farmers adoption of these commercially available biocontrol products as of 2020-22 was less than 5%.

Our further interactions showed that farmers were not aware what biocontrol products to be used for what pests and the dose and the frequency of application. There was the problem of quality as some products showed low efficacy (hence a distrust). We found that the entomopathogenic nematodes, *Beauveria bassiana,*

Table 1. Interview response of farmers from Karnataka (n=240 farmers)

Sl. No.	Questions	Response	Proportion (%)
1	Are you aware of biocontrol?	Yes	94
2	Which biocontrol agent do you know?	Trichoderma	16
3	Do you use any biocontrol agent?	Trichoderma (Recommended by dealer)	4
4	Whom do you consult in case of farm needs?	input dealers	100

Metarhizium anisopliae, Verticillium lecanii, Trichoderma viride, Pochonia, Paecilomycaes fumosoroeus, Pseudomonas florescence, Bacillus thuringiensis, Nuclear polyhedrosis viruses (NPV for *Spodoptera* and *Helicoverpa*) were the common biocontrol agents commercially available to farmers. Therefore, in our transfer of technology, we decided to concentrate on these, as transfer of technology (TOT) is possible only for biocontrol products on market shelf. Systematic extension activity was carried out using audio visual aids, social media, Facebook, Twitter, WhatsApp, YouTube, field demonstrations, farm melas, one to one contacts and field visits, to apprise the farmers of the benefits of the commercially available biopesticides. In order to facilitate a better adoption, a plant health clinic was started for diagnosis and attached to it an input supply system was created to ensure that farmers have free access to commercially available biopesticides which were tested for quality and efficacy.

The scope for TOT depended on a professional extension team (agriculture degree holders) who could constantly liaise with farmers literally 7 X 365 days. Such a team of seven qualified post graduates were formed. A plant health clinic (PHC) with complete diagnosis and input supply facility was started as a startup in 2021. This was recognized by the Ministry of Commerce and Primary industries (GoI) and DDPQ (GoI). Next to win farmers confidence meant a professional approach involving regular field visits, diagnosis of problems, finding solution, fixing problems with appropriate BC products on the market shelf, convincing the farmers of efficacy through lab bioassays and one window supply of BC products along with other inputs like seeds, fungicides, manures, etc. On an average 82 -112 farmers visited the PHC per day in the year 2022. Of these majority, visited between 8 and 11 am in the morning and between 5 and 8 pm in the evening (Fig.1). The activities of the PHC are summarized in Figs 2 & 3.

Transforming subsistence agriculture into profitable agriculture is a great challenge before the extension functionaries in India. It is a well-known fact that farmers' need for the latest knowledge has risen as the focus has shifted from subsistence to profitable agriculture, whereas, the knowledge of the public extension functionaries of the galloping input and agri-product markets and exports have not matched adequately. A unstructured interaction with more than a dozen serving research plant protectionists, cursory though, showed inadequacy of latest agri-products on the market shelf, though being used by farmers! The knowledge gap between FMCG in agriculture and extension, is mainly of combo and bio products.

Agri-input dealers in the country are a prime source of farm information to the farming community, besides the supply of inputs and credit. In India, there are about 2.82 lakh practicing Agri-input dealers, who are the prime source of farm information to the farming community. The first contact point for majority of farmers is the Agri-input dealer (National Institute of Agricultural Extension Management, MANAGE, Hyderbad). Our results fully corroborates this (Table1). In our agri startup which included laboratory and plant health clinic Rashvee-International Phytosanitary Research and Services Pvt Ltd., (R-IPRS) and input supply, we could do a quick diagnosis, followed by quick bioassay of all the commercially available biocontrol products before we recommend to the farmers because several brands were available in the market. Then, especially for grape, pomegranate and vegetable growers who were not willing to forgo insecticides and go for an organic approach we thought of a residue free approach using biocontrol agents after fruiting begins. Almost all farmers seemed to agree on this, provided we guaranteed quality fruits. We knew for sure that our extension approach should be that the commercial products should be compatible with insecticides and fungicides if not in combination but in time and space. The products we recommend should always be available and commercial products (Table 2) should have quality guarantee from us being experts and agriculture graduates. Further, it should also be cost

effective. Our field trials and demonstrations in farmers' fields at pre-harvest, excluding synthetic insecticides, but including tested biopesticides, gave confidence to farmers to adopt. We also had farmers involving in demonstrations in their own fields. They gave us 0.5 to 1.5 acre of their crop fields for short trials. The repeated demand for recommended biopesticides was taken as successful adoption and hence successful TOT. We introduced biological control at pre-harvest and the farmers' feedback was compiled and studied. From these we formulated and upscaled our recommendations.

Example of recommendations of biopesticides: In grapes one month prior to harvest we began to recommended Bt and NPV for *Spodoptera* and *Helicoverpa.* Immediately after pruning we recommended *Trichoderma* + vermicompost, and IIHR's Arka Microbial Consortia. Same was recommended for pomegranate soon after pruning and after the fruits set, mixtures of *Verticillium lecanii, Beauveria bassiana,* and *Metarhizium anisopliae,* mixed with Rashvee herbal liquid soap kept the thrips and mites away and also resulted in significantly lower volume of diseases. In tomato during fruiting, we demonstrated and recommended *Bt* + EPN + herbal repellants and *Phthorimaea* (=Tuta) traps were effective for controlling thrips, mites and *Tuta.* In mango, during flowering *Beauveria bassiana* + *Metarhizium anisopliae* effectively controlled caterpillars and reduced the hoppers (Table 2). These were compatible with Imidacloprid and L-cyalothrin.

Table 2. Some of biocontrol products and biopesticides available in the local market*

Biocontrol agent	Commercial product
Beauveria bassiana	BABA, Utakarsh, Holly green, Mycota, Green heal, Daman L,
Metarhizium anisopliae	Meta king, Kalichakra
Verticillium lecanii	Verti king, Varunastra, vertifire
Trichoderma viride	All round , Nisarga
Pochonia chlamydosporia	Nematofree
Paecilomycaes fumosoroeus	Niyantran
Pseudomonas florescence	All round, Sprash
Bacillus thuringiensis, B. subtilis	Basi rich, Mahastra
Nuclear polyhedrosis viruses (NPV for *Spodoptera* and *Helicoverpa*)	Biovirus-S

*List not exhaustive

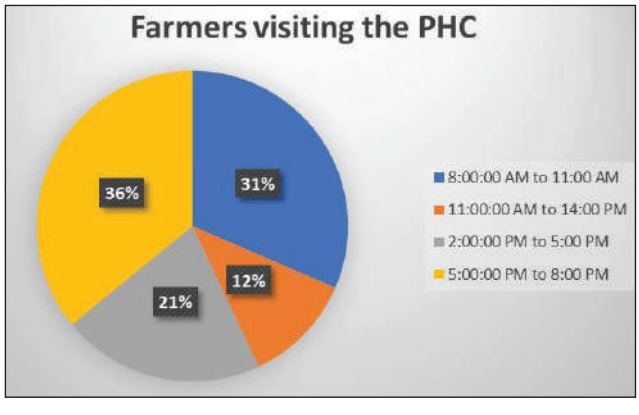


Fig. 1. Daily timeline of the farmers' footfall to the plant health clinic (PHC) for consultation, diagnosis and input purchase

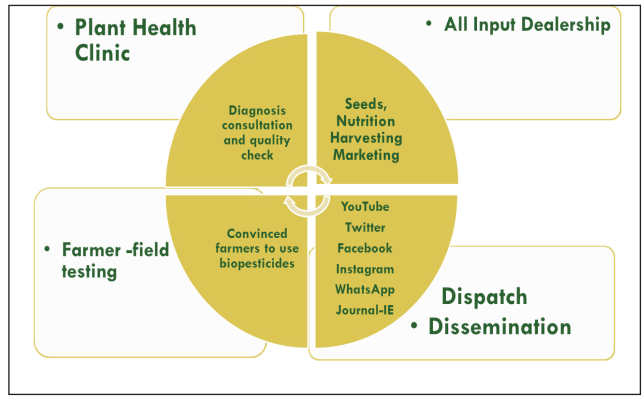


Fig. 2. Integrated approach of plant health clinic for transfer of technology

Our plant health clinic (RIPRS) has all the basic equipment like microscopes, BOD, bioassay facilities, autoclave, etc. and has been recognized by the Ministry of Commerce as a Startup and accredited by DPPQ. We have integrated approach to the farmers with diagnosis, consultation, supply of seeds/ planting material, plant protection requirements and marketing. The inputs supplied undergo quality check in the

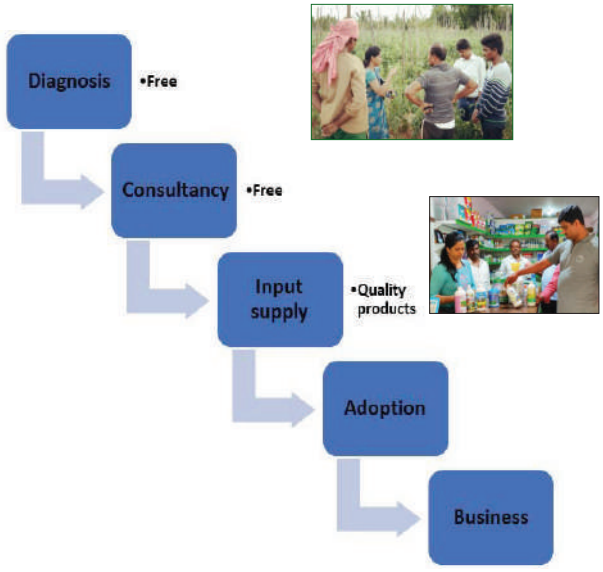


Fig. 3. Step by step activities of Plant health clinic - viable extension model for innovation and adoption

farmers field demonstrations which adds to the increased implementation of the biopesticides. The information is disseminated to large number of farmers with the help of the social media, audio visual aids (Fig. 2). We stressed that for quality fruits for export and table purpose we need residue-free products. So, one or two months prior to harvest depending on the crop the insecticides were tapered off and those EPNs and EPFs, pheromone traps and botanical herbals, all commercially available on the shelf were transferred and supplied to farmers. The steps involved and the activities carried out can be seen in the form of flowchart in Fig. 3 (Verghese and Rashmi, 2022).

CONCLUSION

Therefore as a way forward, it is suggested that industry as a vital loop and professional interfacing by agriculture graduates in the field will assure adoption of biocontrol products on the market shelf by the farmers. Using startups for business and transfer of technology should be the future of extension in the country. Professional interfacing by agriculture graduates in the field instilled confidence in farmers and assured supply of quality biocontrol products on the market shelf boosted biocontrol adoption. This way of using startups for business and transfer of products will be realistic and pragmatic extension for horticulture in the country.

REFERENCES

DPPQS, DAC, Ministry of Agriculture & Farmers Welfare, Government of India

NIAEM, MANAGE, 2023, https://www.manage.gov.in/daesi/daesi.asp

Soumya, B. R., Rashmi, M. A. and Verghese, A. 2021 Bioecology of the mango inflorescence caterpillar, *Perixera illepidaria* Guenée and record of its feeding on mango fruits, *Pest Management in Horticultural Ecosystems,* **27**(1) :22-26.

Verghese, A. and Soumya B. R. 2021. Threat of loopers (*Perixera illepidaria*). *Insect Environment*, 20 March, Blog. *www.insectenvironment.com*

Verghese, A. and Rashmi, M.A. 2022. Agri-startups as a viable extension model for innovation and adoption of biocontrol strategies: case studies in horticultural crops. *Proc. Seventh National Conference on Biological Control 75 Years of Biological Control of Pests and Diseases in Agriculture: Challenges and Way Forward 2022*, pp 176.

MS Received: 02 November 2022
MS Accepted : 14 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 119-128 (2022)

DOI Number: 10.5958/0974-4541.2022.00049.2

Escape resistance in fieldpea against *Phytomyza horticola* Goureau, as influenced by sowing time and its correlation with plant metabolites

RAJEEV KUMAR YADAV[1], ANUP CHANDRA[2]*, ASHOK KUMAR PARIHAR[2], SUJAYANAND G.K.[2], VAIBHAV KUMAR[2] , SANJAY M. BANDI[2], MEENAKSHI ARYA[1] and USHA[1]

[1]Rani Lakshmi Bai Central Agricultural University, Jhansi 284003, Uttar Pradesh

[2]ICAR-Indian Institute of Pulses Research, Kalyanpur, Kanpur 208024, Uttar Pradesh

*Email: anup.ento@gmail.com

ABSTRACT: Fieldpea or dry pea (*Pisum sativum* L.) is one of the important, highly productive cool season food legume crops and pea leaf miner, *Phytomyza horticola* Goureauis is a pest of high economic importance, associated with the crop**.** Twenty eight diverse field pea genotypes including checks were screened for their susceptibility to the pest and to study the influence of sowing time on the extent of infestation of pea leaf miner. The correlation of infestation with the host primary and secondary metabolites was also examined. In late sown condition, only eight genotypes were categorized as 'Susceptible', 2 genotypes under 'Moderately Resistant', 3 genotypes under 'Intermediate', whereas 15 genotypes were categorized as 'Moderately Susceptible'. None of the genotypes could be categorized as 'Very Highly Resistant', 'Highly Resistant', 'Resistant', 'Highly Susceptible' and 'Very Highly Susceptible' in case of late planting. In case of timely planting, only 9 genotypes were categorized under 'Moderately Susceptible', 7 genotypes each under 'Moderately Resistant' and 'Intermediate' and 5 genotypes grouped under 'Highly Resistant' category. None of the genotypes categorized as 'Susceptible' or beyond that in case of timely planting. Most of the genotypes which showed susceptibility under late sown condition had expressed more level of resistance in the timely sown crop. Although, different fieldpea genotypes differed significantly in terms of quantity of primary and secondary metabolites estimated, only the total phenol content was found significantly and positively correlated with the per cent infestation in the late sown genotypes.

Keywords: Genotypes, leaf miner, *Pisum sativum* L. resistance, screening, susceptibility

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the oldest domesticated crops on the planet (Ambrose, 1995; Zohary and Hopf, 2000) as well as one of the important, highly productive cool season food legume crops grown around the world to consume as food, feed and fodder (Rubiales *et al*., 2019). Since it is an excellent source of protein, starch, fibre and micro-nutrients, hence, widely used as an ingredient in many food industries around the world (Burstin*et al*., 2011; Dixit *et a*l., 2014; Gupta and Parihar, 2015; Parihar *et al*., 2016). Seeds also contain vitamins, minerals, polyphenolics, galactosides, saponins, and phytic acid which are being studied for their health-promoting properties (Arnoldi *et al*., 2015; Dahl *et al*., 2012; Marles *et al*., 2013; Mitchell *et al*., 2009). It is a prominent pulse crop and an important part of sustainable cropping system (Duc *et al*., 2010; Nemecek *et al*., 2008).

Among the various biotic stresses associated with the Fieldpea crop, pea leaf miner, *Phytomyza horticola* Goureau, taxonomically described under the family Agromyzidae of the Order Diptera, is a pest of high economic importance (Spencer, 1973). This insect is highly polyphagous, with over 127 recognized host plants (Singh and Havi, 1982) recorded from 268 genera from 36 families but most commonly on Brassicaceae, Fabaceae and Asteraceae (Spencer, 1990). It is one amongst the major insect pests of pea crop (Singh *et al*., 1992) widely distributed over Africa, Asia and Europe (Crop Protection Compendium, 2007). The pea leaf miner is a serious and persistent pest of peas and Brassicas in northern India, wreaking havoc on these crops (Atwal *et al*., 1969; Bhalla and Pawar, 1977; Prasad *et al*., 1984). It can cause up to 90% damage to the crop by mining young leaves which results in stunting and low flower production (Tariq *et al*., 1991). The tiny adults are two winged flies having greyish black mesonotum. The frons is yellow in colour. Eggs are laid by making punctures through inserting ovipositor into the leaf tissues. The adult females' activity, which punctures fragile leaves in several places with their sharp and pointed ovipositor for the purpose of oviposition or eating, is the first sign of damage to the leaves (Ahmad and Gupta, 1941). The damaging stages are larvae which are minute and slender and form a narrow, linear mine on the upper or lower leaf surface (Spencer, 1973) and feed on mesophyll between the upper and lower epidermis. Maggots that mine into the leaves, eating through the mesophyll while leaving the two epidermal layers intact, causes the most catastrophic harm to the crop (Ahmad and Gupta, 1941; Ancev and

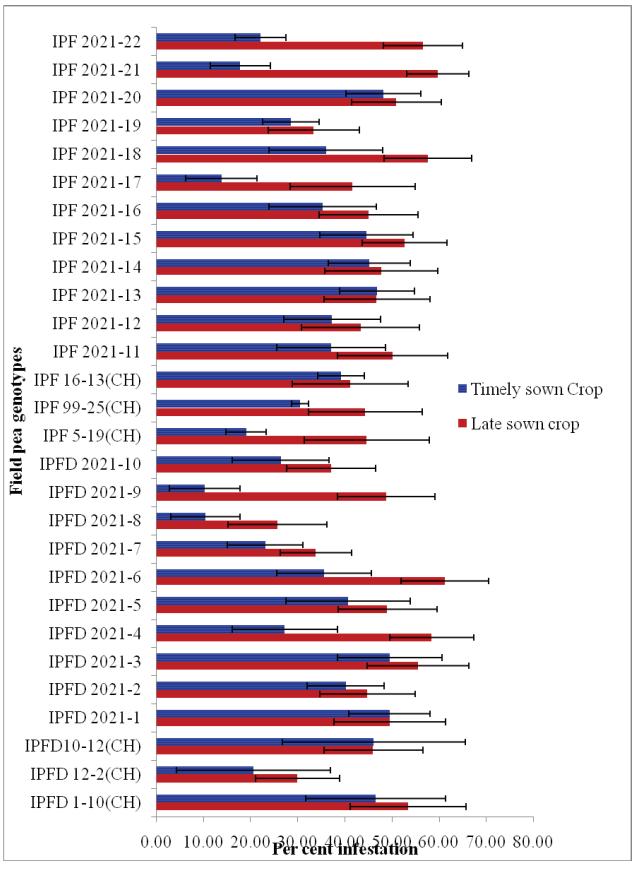


Fig 1. Per cent leaf infestation of different fieldpea genotypes caused by pea leaf miner in timely and late sown condition

Postolovski, 1978). Mining by larvae results in loss of chlorophyll which, in turn, affects the proper growth of plant and crop yield. Photosynthetic activity is severely hampered, and in severe infestations, leaves wither completely. The ability of afflicted plants to bloom and produce fruit is harmed. Under severe infestations, leaf miners cause withering of leaves and reduced flowering and fruiting (Molitas and Gabriel, 1978).

The majority of pest management methods are insecticidal in nature (Chopade, 1975; Dash, 1990; Khajuria and Sharma, 1995; Mehta *et al*., 1995). However, due to their limits, insecticide spraying on vegetable crops has been severely restricted in recent years. As a result, there is a pressing need to investigate and implement new environmentally benign pest management strategies, such as the use of resistant/tolerant types to reduce the use of harmful chemicals. In the present scenario of preferring ecofriendly management avenues over chemical insecticides, development of varieties resistant to insect pest always gets the first thought. Deployment of host plant resistance in insect pest management strategies will render reduced losses, less insecticide use, better crop yields and safer environment (Howe and Jander, 2008). Selections of varieties less prone to insect attack and studies related to adjustment in the date of sowing to gain escape resistance is the present day way forward (Chandra *et al*., 2021).The mechanism of plant resistance rotates around biochemical host attributes too which affects the oviposition and feeding preferences of an insect pest. In view of this, the study was taken up to investigate how the fieldpea crop escapes leaf miner infestation by changing the time of its sowing and to see the correlation of infestation with some primary and secondary plant metabolites.

MATERIALS AND METHODS

Screening of fieldpea genotypes

A field experiment was conducted during rabi 2020-21 at the research farm of ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh, India, to study the influence of sowing time on the level of infestation of pea leaf miner, *Phytomyza horticola* Goureau. The site of experiment was geographically situated between 26.49188^0 N and 80.27748^0 E with an altitude of 128 m above mean sea level. A panel of twenty eight diverse field pea genotypes including checks was selected for the trial. Timely sowing was done on November 1, whereas another set for late sowing was done on December 9. The experiment was executed in randomized complete block design with three replications. In each replication, genotypes were planted in three rows of 4.0 m length with inter and intra row spacing of 60 cm and 10 cm, respectively. The crop was raised following recommended package of practices to maintain a normal healthy crop. No control measures adopted for insect pest control.

The incidence of leaf miner in field pea crop noticed from first week of February, at the time crop reached the flowering stage. For estimation, done at weekly interval, ten plants were tagged randomly in each genotype. The per cent leaf infestation was calculated by counting the infested leaves out of the total number of leaves present in the plant. On the basis of per cent leaf infestation, the genotypes were categorized using a scale of 1-9 ('1' being 'Very highly resistant' and '9' being 'Very highly susceptible'), following the method given by Singh and Weigand (1994).

Estimation of plant metabolites

Protein content, total sugar, total phenol and tannin content were quantified in randomly selected 10 different field pea genotypes. Protein present in sample was estimated using Lowry's method (Lowry *et al*., 1951). The principle behind the Lowry method of determining protein concentrations lies in the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline

Table 1. Categorization of fieldpea genotypes on the basis of degree of susceptibility against pea leaf miner during different planting time

S.N.	Field Pea Genotypes	susceptibility category	
		Timely sown	Late sown
1	IPFD 1-10(CH)	MS	S
2	IPFD 12-2(CH)	MR	MR
3	IPFD 10-12(CH)	MS	MS
4	IPFD 2021-1	MS	MS
5	IPFD 2021-2	I	MS
6	IPFD 2021-3	MS	S
7	IPFD 2021-4	MR	S
8	IPFD 2021-5	MS	MS
9	IPFD 2021-6	I	S
10	IPFD 2021-7	MR	I
11	IPFD 2021-8	HR	MR
12	IPFD 2021-9	HR	MS
13	IPFD 2021-10	MR	I
14	IPF 5-19(CH)	HR	MS
15	IPF 99-25(CH)	MR	MS
16	IPF 16-13(CH)	I	MS
17	IPF 2021-11	I	MS
18	IPF 2021-12	I	MS
19	IPF 2021-13	MS	MS
20	IPF 2021-14	MS	MS
21	IPF 2021-15	MS	S
22	IPF 2021-16	I	MS
23	IPF 2021-17	HR	MS
24	IPF 2021-18	I	S
25	IPF 2021-19	MR	I
26	IPF 2021-20	MS	MS
27	IPF 2021-21	HR	S
28	IPF 2021-22	MR	S

VHR = Very highly resistant; 2. HR = Highly resistant; 3.R = Resistant; 4.MR = Moderately resistant; 5.I = Intermediate; 6. MS = Moderately susceptible; 7. S = Susceptible; 8. HS = Highly susceptible; 9. VHS = Very highly susceptible

Table 2. Quantity of protein, total sugar, total phenol and tannin in different field pea genotypes

Field pea genotypes	Protein (%)	Total sugar(mg/g of Leaf sample)	Total phenol (mgGAE/g of leaf sample)	Tannin (mgTAE/g of leaf sample)
IPED 1-10(CH)	5.63 (13.71)	13.16 (3.76)	2.81 (1.95)	3.31 (2.07)
IPFD 2021-1	5.36 (13.38)	14.88 (3.98)	2.31 (1.82)	2.72 (1.92)
IPFD 2021-3	3.65 (10.99)	6.65 (2.76)	2.25 (1.80)	2.80 (1.94)
IPFD 2021-5	4.20 (11.81)	13.16 (3.76)	2.31 (1.81)	3.09 (2.02)
IPFD 2021-8	5.50 (13.54)	5.77 (2.60)	2.15 (1.77)	3.17 (2.04)
IPF 2021-11	5.28 (13.27)	19.31 (4.50)	3.23 (2.05)	3.61 (2.14)
IPF 2021-15	6.22 (14.42)	29.53 (5.52)	3.16 (2.04)	3.97 (2.22)
IPF 2021-16	5.38 (13.39)	31.59 (5.70)	2.89 (1.97)	3.65 (2.15)
IPF 2021-21	5.40 (13.43)	18.21 (4.38)	3.29 (2.07)	3.64 (2.15)
IPF 2021-22	4.92 (12.80)	17.71 (4.32)	2.80 (1.94)	3.45 (2.11)
C.D	0.563	0.118	0.030	0.029
SE(m)	0.188	0.039	0.010	0.010
SE(d)	0.266	0.056	0.014	0.014
CV	2.490	1.651	0.911	0.807

Figures under parenthesis, corresponding to protein values,are arcsine transformation of the original percent values; Figures under parenthesis, other than protein values, are square root transformation of the original values.

conditions and the subsequent reduction of the Folin-Ciocalteu phosphomolybdic phosphotungustic acid to heteropoly molybdenum blue by the copper-catalyzed oxidation of aromatic acids. The absorbance was taken at 660 nm in spectrophotometer. A calibration curve was plotted using BSA (Bovine serum albumin) as standard and the protein content in the sample was calculated in percentage using plotted standard curve of BSA. Total phenolic content of the sample extracts was determined using Folin–Ciocalteu reagent following the method of Singleton and Rossi (1965).The absorbance of the samples was measured at 650 nm against reagent blank in spectrophotometer. The Calibration curve was plotted using gallic acid as standard and phenols in the sample was calculated as gallic acid equivalents (mgGAE/g sample) using standard curve of gallic acid.Tannins in the sample was estimated using Folin–Denis method based on the principle that tannin like compounds reduce Phosphomolybdic acid in alkaline solution to produce highly coloured blue solution, the intensity of which is proportional to the amount of tannins (Schanderl, 1970). The absorbance of the samples was taken at 700 nm in spectrophotometer. The calibration curve was plotted using tannic acid as standard and the content in the sample was calculated as tannic acid equivalents (mgTAE/g sample) using standard curve of tannic acid. The total sugar in the sample was estimated by the method of Dey (1990). The absorbance was recorded at 490 nm in spectrophotometer. The calibration curve was plotted by using glucose as standard. The total soluble sugar content in the sample was calculated in mg/g of leaf sample using standard. The chlorophyll content in all the 28 field pea genotypes was estimated using SPAD-502 and expressed in relative SPAD meter values which are proportional to the chlorophyll content present in the leaf (Ling *et al*., 2011).

Table 3. Correlation matrix showing Pearson's correlation coefficient amongst metabolites and per cent leaf infestation in field pea genotypes

	Chlorophyll	Per cent Infestation (late sown genotypes)	Per cent Infestation (timely sown genotypes)	Protein	Total sugar	Total Phenol
% Infestation (late sown genotypes)	0.285 NS					
% Infestation (timely sown genotypes)	0.232 NS	0.127 NS				
Protein		-0.017 NS	-0.165 NS			
Total sugar		0.259 NS	0.097 NS	0.538 NS		
Phenol		0.636*	-0.121 NS	0.538 NS	0.703*	
Tannin		0.324 NS	-0.312 NS	0.632*	0.768**	0.864**

Correlation analysis

Correlation analysis was done by calculation of Pearson's correlation coefficient through the OPSTAT analytical software to see relation, if any, between per cent leaf infestation done by pea leaf miner with quantity of protein, total phenol, tannin, total sugar and chlorophyll.

RESULTS AND DISCUSSION

Screening of field pea genotypes

The per cent leaf infestation of 28 field pea genotypes and their comparison under timely and late sown conditions has been depicted in the Figure 1. In timely sown condition, the per cent leaf infestation ranged from 3.44 (IPFD 2021-9) to 49.44 (IPFD 2021-1 and IPFD 2021-3). Other genotypes observed with comparatively higher per cent leaf infestation in timely sown condition were IPF 2021-20 (48.19%), IPFD 1-10 (Check) (46.53%), IPFD 10-12 (Check) (46.11%), IPF 2021-13 (46.81%), IPF 2021-14 (45.14%) and IPF 2021-15 (44.58%). Range of leaf infestation was found increased (25.67 for IPFD 2021-8 to 61.17 for IPFD 2021-6) in case of late sown crops.

Kooner and Singh (1980) observed the per cent infestation of pea leaves by leaf miner which was ranged from 2.0 to 31.7 in different varieties whereas Kashyap *et al*. (1982) found 33.6, 20.3, and 22.9 per cent pea leaf miner infestation on Bonneville, Arkel and Harabona-B cultivars of field pea, respectively. In the present study, IPFD 12-2 (Check), IPFD 2021-7, IPFD 2021-10 and IPFD 2021-19 were some of the genotypes observed with low per cent leaf infestation of 29.92, 33.83, 37.08 and 33.33, respectively. On the other hand, genotypes like IPFD 1-10 (Check), IPFD 2021-3, IPFD 2021-4, IPF 2021-11, IPF 2021-15, IPF 2021-18, IPF 2021-20, IPF 2021-21 and IPF 2021-22 were recorded with high per cent leaf infestation of 53.42, 55.50, 58.42, 50.08, 52.67, 57.58, 50.92, 59.75 and 56.58, respectively. Genotypes when sown under late condition, had recorded higher per cent leaf infestation as compared to timely sown genotypes. In both timely and late sown genotypes, it was observed that the per cent leaf infestation increased with progress of time.

In timely sown crops, mean per cent leaf infestation was recorded minimum on 8 February with only 5.96, followed by 14.98 on 15 February, 23.99 on 24 February and maximum of 41.48 on 1 March 2021. Similarly, in late sown condition, minimum per cent leaf infestation of 7.13 was observed on 3 February, which increased to 12.25 on 10 February, to 21.05 on 17 February, to 36.29 on 24 February and a maximum of 57.16 was recorded on 3 March 2021.

All the 28 field pea genotypes were rated using 1-9 scale and were categorized from Very highly resistant (1) to Very highly susceptible (9). In case of timely planting,

only 9 genotypes were categorized under 'Moderately Susceptible', 7 genotypes each under 'Moderately Resistant' and 'Intermediate' and 5 genotypes grouped under 'Highly Resistant' category (Table 1). None of the genotypes categorized as 'Susceptible' or beyond in case of timely planting. In late sown condition, among 28 genotypes, 8 genotypes grouped under 'Susceptible' category, 2 genotypes under 'Moderately Resistant', three genotypes under 'Intermediate', whereas 15 genotypes were categorized as 'Moderately Susceptible'. None of the genotypes could be categorized as 'Very Highly Resistant', 'Highly Resistant', 'Resistant', 'Highly Susceptible' and 'Very Highly Susceptible' in case of late planting.

Kashyap *et al*. (1982) reported no substantial differences in resistance to this pest among the various potential pea cultivars. Mahobe and Narsinghani (1986) found none of the pea lines with desired level of resistance to this miner; however, they reported some degree of resistance in the cultivars JP-179, JP-854, JP-747, JP-169-1 and JP-Batri Brown. Also, Dash *et al*. (1988) found no cultivars resistant to pea leaf miner attack; however, "early wonder" showed considerable resistance, followed by Arkel. Bhat (1988) examined 20 lines of pea for resistance to *C. horticola*, *Lampides boeticus* L., and *Bruchus pisorum* L. in Kashmir and found that different cultivars had various levels of infestation, but no variety was totally resistant to this pest. Sharma and Sharma (1991) classified 8 pea cultivars (IP-3, JP-169, PG-2, S-143, Sel-35, Sel-93, Sel— 3487, VG-1) as resistant to pea leaf miner, 22 as moderately susceptible and 11 as highly susceptible in field trials conducted in Himachal Pradesh. Thakur and Patial (2019) screened ninety-two pea germplasm against pea leaf miner where six pea genotypes (DPP 25G, DPPLMR 41, JI 1766 (2), JP 179, LMR 100, S143) exhibited high resistance, nineteen as resistant and seventeen as moderately resistant to the pest. In the present study, only two genotypes i.e. IPFD 2021-8 and IPFD 12-2 could demonstrate good level of resistance in both late and timely planting. These identified genotypes after further validation can be used as donor in resistance breeding program.

Quantification of plant metabolites in field pea germplasm

The estimated values of protein, total sugar, total phenol and tannin in selected field pea genotypes are shown in Table 2.

The difference in the contents of these bio-chemicals among the genotypes studied was found statistically significant.The protein content was estimated highest in IPF 2021-15 with 6.22 per cent and lowest in IPFD 2021-3 with only 3.65 per cent. The total sugar was observed maximum in IPF 2021-15 with 29.53 mg/g leaf sample while minimum quantity was observed in IPFD 2021-8 and IPFD 2021-3 with 5.77 and 6.65 mg/g of leaf sample, respectively. Sepehya *et al*. (2015) reported maximum sugar content of 4.21 mg/g in field pea. Minimum content of total phenol, 2.15 mg GAE/g leaf sample was estimated in IPFD 2021-8 to the maximum of 3.29 mg GAE/g leaf sample in IPF 2021-21. Wang *et al*. (1998) estimated total phenolics in field pea which differed significantly among cultivars, ranging from 162 mg/kg DM (dry matter) (CE, catechin equivalents) to 325 mg/kg DM (CE). Zia-ul-Haq *et al*. (2013) showed that the contents of total phenols, flavonoids, and condensed tannins in seeds of four Pakistani pea cultivars ranged between 0.84–0.99, 0.09–0.17, and 0.57–0.68 mg/g, respectively. Hegedusova *et al*. (2015) reported the total polyphenol contents of six garden pea varieties as 1179.995±28.081 mg GAE/kg as the highest value and the lowest value as 674.505± 26.541 mgGAE/kg. The tannin content was found minimum in IPFD 2021-1 with only 2.72 mgTAE/g of leaf sample while maximum tannin content was recorded in IPF 2021-15 with 3.97 mgTAE/g leaf sample. Overall, it was observed that IPFD 2021-15 was the genotype observed with maximum content of protein, total sugar, total phenol and tannin content, whereas, IPFD 2021-3 and IPFD 2021-8 was found low in all of these parameters.

The chlorophyll contents in all the 28 field pea genotypes were estimated and expressed in relative SPAD meter values which remain proportional to the chlorophyll content present in the leaf (Ling *et al*., 2011). The SPAD meter values ranged from 26.63, 27.41 and 28.99 in IPF 16-13 (Check), IPF 99-25 (Check) and IPF 2021-11 to maximum of 51.02, 48.71 and 47.68 in IPFD 2021-3, IPFD 10-12 (Check) and IPFD 2021-4, respectively. Golawska *et al*. (2010) reported chlorophyll ranging from 36.57 to 39.82 (SPAD meter values) in *P. sativum*. The difference among genotypes in terms of chlorophyll content was found statistically significant.

Correlation between per cent infestation and physiological attributes

Correlation analysis were done among per cent infestation observed in timely sown crops, late sown crops, their protein content, total sugar, total phenol and tannin content in randomly selected 10 genotypes. Correlations of chlorophyll content of all 28 genotypes were analysed with the corresponding per cent leaf infestation in both timely and late sown conditions. Of all the correlations, only per cent infestation observed in late sown condition was found significantly and positively correlated with the total phenol content with a correlation coefficient of 0.64 (Table 3).

Also, Sharma and Aggarwal (1983) observed positive correlation between population of jassid, *Amrasca biguttula biguttula* Ishida, with free phenol and the tannin content of cotton. On the other hand, Woodhead *et al*., (1980) found high phenolic acid concentrations linked to reduce eating by several grasshoppers and the plant hoppers. Dass and Odak (1987) reported total phenols to be adversely linked with pod fly (*Melanagromyza obtusa* Malloch) infestation in *Cajanus cajan*. Nomura and Itioka (2002) discovered that tannin inhibits cutworm larvae from growing and that the inhibitory impact was proportional to the amount of tannin consumed. According to Chandra (2012), total chlorophyll, carotenoid and sugar in maize germplasm were significantly and positively correlated with the Leaf Injury Rating (LIR) recorded with respect to *Chilo partellus*. Contrastingly, Singh (1984) reported that chlorophyll content of a genotype was not related to resistance or susceptibility in *Brassica* strains to pea leaf miner. In another study, Padmavathi *et al*. (2013) found leaf folder damage leading to 57% loss in chlorophyll content, a 23% drop in PS II activity and a 23% reduction in relative water content. Saheb *et al.* (2018) reported proteins with a significant negative correlation with incubation period and fifth instar larval duration, phenols with a significant positive correlation with fifth instar duration, reducing sugars showed positive correlation with incubation period, fifth instar duration and a negative significant correlation with post-oviposition period of leaf bud borer in groundnut. Saleem *et al*. (2019) observed significant negative correlation between *S. litura* damage and protein and phenol content while significant positive correlation was found with reducing and total sugar.

CONCLUSION

Although, there exist a large variation in results pertaining to the nature of correlation between plant biochemical and their impact on biology of insect pests; in the present study, physiological attributes like protein, total sugar, tannin, chlorophyll content found no significant correlation with per cent infestation in the field pea crop. The significant positive correlation observed between per cent leaf infestation in late sown condition and phenol content of the genotypes may also be seen as an induction or plant response to higher infestation of leaf miner in late sown crop. Secondary metabolites like phenol may prolong the life stages of the insect, facilitating prolonged feeding and thereby increase the level of infestation. Most of the genotypes which showed susceptibility under late sown condition had expressed more level of resistance when timely sowing was done.

ACKNOWLEDGMENT

The authors acknowledge the facilities provided by the Indian Council of Agricultural Research-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh, India. The authors also acknowledge the support provided by the Rani Lakshmi Bai Central Agricultural University, Jhansi, Uttar Pradesh, India.

REFERENCES

Ahmed, T. and Gupta, R. L. 1941. The pea leaf-miner, Phytomyza atricornis (Meigen), in India. *Indian Journal of Entomology,* 3(1): 37- 49.

Ambrose, M. J. 1995. From Near East center of origin, the prized pea migrates throughout world. *Dıversıty-Arlıngton Then Washıngton,* 11:118-119.

Ancev, E. and Postolovski, M. 1978. Contribution to knowledge of Phytomyza horticola Goureau (Diptera: Agromyzidae). *Zastita Bilja,* 29(3): 273-277.

Arnoldi, A., Zanoni, C., Lammi, C. and Boschin, G. 2015. The role of grain legumes in the prevention of hypercholesterolemia and hypertension. *Critical Reviews in Plant Sciences*, 34 (1-3): 144-168.

Atwal, A. S., Chaudhary, J. P. and Ramzan, M. 1969. Studies on the biology and control of pea leafminer, Phytomyza atricornis Meigen. (Agromyzidae: Diptera). *Punjab Agricultural University Journal Research,* 8 (1): 163-169.

Bhalla, O. P. and Pawar, A. D. 1977. A survey study of insect and non-insect pests of economic importance in Himachal Pradesh. *A survey study of insect and non-insect pests of economic importance in Himachal Pradesh.* D. N. Road, Bombay, India: Tiku and Tiku, Kitab Mahal, 15-17.

Bhat, A. A. 1988. Preliminary screening of some pea cultivars against insect pests in Kashmir. *Progressive Horticulture,* 20(1-2): 167-168.

Burstin, J., Gallardo, K., Mir, R. R., Varshney, R. K. and Duc, G. 2011. 20 Improving Protein Content and Nutrition Quality. In Biology and breeding of food legumes Ed. Pratap, A., and Kumar, J. CABI, pp. 314. https://doi.org/10.1079/9781845937669.0314.

Chandra, A. 2012. Study on secondary metabolite (DIMBOA) and some other primary metabolites (chlorophyll, protein, sugar) in maize germplasm and their relation to *Chilo partellus* (Swinhoe)

susceptibility. PhD, Indian Agricultural Research Institute, New Delhi, India.

Chandra, A., Sujayanand, G. K. and Kumar, R. 2021. Influence of Sowing Dates and Host Crops on Population Incidence of Whitefly, *Bemisia tabaci* (Gennadius) in Greengram and Blackgram. *National Academy Science Letters,* 44 (5): 389-391.https://doi.org/10.1007/s40009-020-01033-8.

Chopade, H. M. 1975. Chemical control of some major pests of field pea, *Pisum sativum*. *Entomologists News,* 5 (2): 17.

Crop Protection Compendium. 2007. Edition © CAB International, Wallingford, UK.

Dahl, W. J., Foster, L. M. and Tyler, R. T. 2012. Review of the health benefits of peas (*Pisum sativum* L.). British *Journal of Nutrition,* 108 (S1): S3-S10. https://doi.org/10.1017/S0007114512000852.

Dash, A. N. 1990. Evaluation of some insecticides for their efficacy against the pea leaf miner, *Phytomyza atricornis* (Meigen). *Indian Journal of Plant Protection*, 18(2): 295-297.

Dash, A. N., Patnaik, N. C. and Nandi, A. 1988. Reaction of some garden pea cultivars to the pea leaf mine (*Phytomyza atricornis* Heigen) (Diptera: Agromyzidae) in Orissa. *Environmental Ecology*, 6 (2): 530-531.

Dass, S. B. and Odak, S. C. 1987. Biochemical basis of resistance in pigeonpea pod walls to pod fly *Melangromyza obtusa* Malloch (Diptera: Agromyzidae). *Crop improvement*, 14 (1): 64-68.

Dey, P. M. 1990. Oligosaccharides. In Methods in plant biochemistry, Academic Press.,189-219.https://doi.org/10.1016/B978-0-12-461012-5.50011-2.

Dixit, G. P., Parihar, A. K. and Gupta, S. 2014. Perspective for increasing field pea production in India. Handbook on minor and imported pulses of India. Commodityindia.com. 37-39.

Duc, G., Blancard. S., Hénault, C., Petit, M. S., Bernicot, M. H., *et al.* 2010. Prominent cool season pulse crop and an important part of sustainable cropping system of agriculture durable in Bourgogne. *Innovations Agronomiques*,11: 157–173.

Golawska, S., Krzyzanowski, R. and Lukasik, I. 2010. Relationship between aphid infestation and chlorophyll content in Fabacea species. *Acta Biologica Cracoviensia. Series Botanica*, 52(2). https://doi.org/10.2478/v10182-010-0026-4.

Gupta, S. and Parihar, A. K. 2015. Broadening the genetic base of pulse crops. *Pulses: Challenges & Opportunities,* 86.

Hegedűsová, A., Mezeyová, I., Timoracká, M., Šlosár, M., Musilová, J. and Juríková, T., 2015. Total polyphenol content and antioxidant capacity changes in dependence on chosen garden pea varieties. *Potravinarstvo,* 9(1):https://doi.org/10.1038/hortres.2017.17.

Howe, G. A. and Jander, G. 2008. Plant immunity to insect herbivores. *Annual review of plant biology,* 59 (1): 41-66.https://doi.org/10.1146/annurev.arplant.59.032607.092825.

Kashyap, R. K., Bhanot, J. P. and Verma, A. N. 1982. Relative susceptibility of pea cultivars against leaf miner, *Melanogromyza conavistae* Greathead (Diptera: Agromyzidae). *Haryana Agricultural University Journal of Research.* 12(1): 128-129.

Khajuria, D. R. and Sharma, J. P. 1995. Efficacy of insecticides in controlling pea leafminer (*Chromatomyia horticola*) on seed crop of pea (*Pisum sativum*). *Indian Journal of Agricultural Sciences*,65(5): 381-384.

Kooner, B. S. and Singh, H. 1980. Performance of Some Cultivars of Field-Peas Against Leaf-Miner (*Phytomyza Horticola* Gour.). *Indian Journal of Horticulture,* 37(1):101-102.

Ling, Q., Huang, W. and Paul, J. 2011. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana, Photosynthesis Research* 107(2):209-214.https://doi.org/10.1007/s11120-010-9606-0.

Lowry, C. O., Rosebrough, N., Farr, A. and Randall, R. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry,* 193(1): 265-275. https://doi.org/10.1016/S0021-9258(19)52451- 6.

Mahobe, J. and Narsinghani, V. G. 1986. Screening of pea varieties against leaf miner (*Phytomyza atricornis* Meigen). *Vegetable Science*, 13(1): 387-388.

Marles, M. S., Warkentin, T. D. and Bett, K. E. 2013. Genotypic abundance of carotenoids and

polyphenolics in the hull of field pea (*Pisum sativum* L.). *Journal of the Science of Food and Agriculture*, 93(3): 463-470.https://doi.org/10.1002/jsfa. 5782.

Mehta, P. K., Vaidya, D. N. and Kashyap, N. P. 1995. Efficacy and economics of some insecticides against pea leaf miner, *Chromatomyia horticola* (Goureau) on pea. *Journal of Insect Science,* 8:116-117.

Mitchell, D. C., Lawrence, F. R., Hartman, T. J. and Curran, J. M. 2009. Consumption of dry beans, peas, and lentils could improve diet quality in the US population. *Journal of the American dietetic association*, 109 (5): 909-913.https://doi.org/10.1016/j.jada.2009.02.029.

Molitas, L. P. and Gabriel, B. P. 1978. Biology of the pea leaf miner, *Phytomyza horticola* G. (Diptera: Agromyzidae) in Philippines. *Philippine Entomologist*, 3: 89-108.

Nemecek, T., Richthofen, J. S. V., Dubois, G., Casta, P., Charles, R., *et al.* 2008. Environmental impacts of introducing grain legumes into European crop rotations. *European Journal of Agronomy,* 28 (3): 380 - 393. https://doi.org/10.1016/j.eja.2007.11.004.

Nomura, M. and Itioka, T. 2002. Effects of synthesized tannin on the growth and survival of a generalist herbivorous insect, the common cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Applied Entomology and Zoology, 37 (2): 285 - 289. https://doi.org/10.1303/aez.2002. 285.

Padmavathi, C., Katti, G., Padmakumari, A. P., Voleti, S. R. and Subba Rao, L. V. 2013. The effect of leaffolder *Cnaphalocrocis medinalis* (Guenee) [Lepidoptera: Pyralidae] injury on the plant physiology and yield loss in rice. *Journal of Applied Entomology,* 137(4): 249-256.https://doi.org/10.1111/j.1439-0418.2012.01741.x.

Parihar, A. K., Bohra, A. and Dixit, G. P. 2016. Nutritional benefits of winter pulses with special emphasis on peas and rajmash. In *Biofortification of food crops* (pp. 61-71). Springer, New Delhi.pp.61-71.https://doi.org/10.1007/978-81-322-2716-8_6.

Prasad, D., Singh, K. M., Katiyar, R. N. and Singh, R. N. 1984. Incidence of insect pests in early maturing high yielding variety of pea, Pisum sativum Linn. *Indian Journal of Entomology,* 46 (3): 353 - 362.

Rubiales, D., Gonzalez - Bernal, M. J., Warkentin, T. D., Bueckert, R., Vaz Patto, M. C. *et al*. 2019. Advances in breeding of peas (Fabaceae). InAchieving sustainable cultivation of vegetables Ed. Hochmuth, G., Burleigh, Dodds Science Publishing. https://doi.org/10.19103/AS.2019.0045.28.

Saheb, Y. P., Hari-Prasad, K. V., Swarajya, K. L. and Rani, J. S. 2018. Mechanisms of resistance in groundnut genotypes against leaf bud borer, *Anarsia ephippias* (Meyrick). *Journal of Entomology and Zoology Studies*, 6 (2): 694 - 705.

Saleem, M. A., Gopalakrishna, N. K., Tippannavar, P. S. and Nadaf, H. L. 2019. Biophysical and biochemical mechanism of resistance to *Spodoptera litura* in groundnut (*Arachis hypogaea* L). *Journal of Entomology and Zoology Studies,*4: 86-96.

Schanderl, S. H. 1970. Method in food analysis. New York: Academic Press, 709.

Sepehya, S., Bhardwaj, S. K. and Dhiman, S. 2015. Quality Attributes of Garden Pea (*Pisum sativum* L.) as Influenced by Integrated Nutrient Management Under Mid Hill Conditions. *Journal of Krishi Vigyan*, 3 (2): 78 - 83. https://doi.org/10.5958/2349-4433.2015.00017.3.

Sharma, H. C. and Agarwal, R. A. 1983. Role of some chemical components and leaf hairs in varietal resistance in cotton to jassid, *Amrasca biguttula biguttula Ishida. Journal of entomological research,* 7:145-149.

Sharma, I. D. and Sharma, K. C. 1991. Screening of pea germplasm for resistance to leaf miner, *Phytomyza atricornis* Meigen. *Indian Journal of Plant Protection,*19 (2): 206.

Singh, P.1984. Morphological, anatomical and biochemical basis of resistance in rapeseed and mustard to the leaf miner, *Phytomyza horticola* Goureau. PhD Thesis, Punjab Agricultural University, Ludhiana, Punjab, India.

Singh, S. K., Rahman, S. J., Gupta, B. R. and Kalha, C. S. 1992. An integrated approach to the management of the major diseases and insect pests of peas in India. *International Journal of*

Pest Management, 38(3):265-267. https://doi.org/10.1080/09670879209371706.

Singh, P., and Havi, G. S. 1982. A critical review on the distribution and host plant index of the leaf miner, *Phytomyza horticola* Goureau. *Bulletin of Pure and Applied Sciences,* 1: 45-51.

Singh, K. B. and Weigand, S. 1994. Identification of resistant sources in *Cicer* species to *Liriomyza cicerina. Genetic Resources and Crop Evolution,* 41:75–79.

Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture,* 16 (3): 144-158.

Spencer, K. A.1973. Leaf miners on cereals and other graminaceous crops. InAgromyzidae (Diptera) of Economic Importance. Springer, Dordrecht, 236-297. https://doi.org/10.1007/978-94-017-0683-4_9.

Spencer, K. A. 1990. Host Specialization in the World Agromyzidae (Diptera) with Illustrations by Ann Spencer. https://doi.org/10.1007/978-94-009-1874-0.

Tariq, M., Khokhar, K. M., Farooq, M. and Mohammad, A. 1991. Larval fluctuation of pea leaf miner on pea crop and effect of abiotic factors on its dynamics. *Pakistan Journal of Agricultural Research,* 12(3): 202-205.

Thakur, A. and Patiyal, M. 2019. Studies on host plant resistance in pea against pea leaf miner, *Chromatomyia horticola (Goureau). International Journal of Tropical Agriculture,* 37(2): 271-279.

Wang, X., Warkentin, T. D., Briggs, C. J., Oomah, B. D., Campbell, C. G. and Woods, S. 1998. Total phenolics and condensed tannins in field pea (*Pisum sativum* L.) and grass pea (*Lathyrus sativus* L.). Euphytica, 101(1): 97-102.

Woodhead, S., Padgham, D. E. and Bernays, E. A. 1980. Insect feeding on different sorghum cultivars in relation to cyanide and phenolic acid content. *Annals of Applied Biology,* 95(2):151-157. https://doi.org/10.1111/j.1744-7348.1980.tb04733.

Zia-Ul-Haq, M., Amarowicz, R., Ahmad, S. and Riaz, M. 2013. Antioxidant potential of some pea (*Pisum sativum* L.) cultivars commonly consumed in Pakistan.*Oxidation Communications,* 36(4): 1046-1057.

Zohary, D. and Hopf, M. 2000. Domestication of plants in the Old World: The origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. Oxford university press.

MS Received: 17 November 2022
MS Accepted : 23 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 129-132 (2022)

DOI Number: 10.5958/0974-4541.2022.00050.9

Integrated management of sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae)

GOURI SHANKAR GIRI[1]*, KAUSHAL KISHOR[2], VINOD KUMAR DUBEY[2] and SOURAV MAJI[2]

[1]Tirhut College of Agriculture, Dholi, Muzaffarpur, Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur) 848125 Bihar, India

[2]Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur) 848125 Bihar, India

***E-mail:** gsgiri@rpcau.ac.in

ABSTRACT: Sweet potato weevil, *Cylas formicarius* (Fabricius) is the most important and serious pest of sweet potato. Being an internal feeder and soil dweller, it's very difficult to manage by a single management practice. Hence, an integrated approach is ideal. Studies were conducted to evaluate management modules involving soil solarization, vine treatment with imidacloprid at the time of planting, earthing up at 30 days after planting, spraying of indoxacarb @ 1.5 ml/l of water at 30, and 60 days after planting, alone or in combination were evaluated against sweet potato weevil. Results revealed that, soil solarization and vine treatment were equally effective for suppression of weevil population during early growth period. Among different modules formulated, vine treatment with imidacloprid 17.8 SL @ 1ml/l of water followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 days after planting and timely harvest of tuber was found most effective in managing weevil population.

Keywords: sweet potato weevil, vine treatment, soil solarization, earthing up, insecticides

INTRODUCTION

The sweet potato (*Ipomea batatas* L.) commonly known as "Sakarkand" is one of the most extensively produced tuber crops. The sweet potato could be a great new source of natural health promoting compounds like β-carotene and anthocyanins for the functional food market. Sweet potato leaves and roots have protein levels ranging from 4.0% to 27.0% and 1.0% to 9.0%, respectively (Bovell Benjami, 2007). In India, Odisha is the leading state to produce sweet potato in both area and production followed by Kerala, Bihar, and West Bengal. Among several insect pests attacking sweet potato, the sweet potato weevil, *Cylas formmicarius* (Fabricius) is the major pest. The four most damaging sweet potato weevil species *viz., Euscepes postfasciatus* (Fairmaire), *Cylas formicarius* (Fabricius), *Cylas puncticollis* (Boheman), and *Cylas brunneus* (Fabricius) (Chalfant *et al.,* 1990). The losses caused by sweet potato weevil are reported around 5-80% depending on the length of crop remain on the ground (Sutherland, 1986). Therefore, we have to manage the pest efficiently to control its damage and yield loss. An integrated Pest Management (IPM) programme is ideal involving cultural, mechanical, physical and other methods along with the chemical method to control the pest population. Therefore, a proper IPM model is prepared which can be used to manage the sweet potato weevil population effectively.

MATERIALS AND METHODS

An experiment was conducted at Research Farm, Tirhut College of Agriculture, Dholi, Odisha, India during September to January of 2020-21 and 2021-22. The experiment was laid down in Randomised Complete Block Design (RCBD) with seven treatments each replicating thrice. The treatment consisted cultural, physical and chemical control of all the suitable techniques in a compatible manner as possible and includes all aspects of pest management. The study tries to evaluate in a compatible manner as possible. The treatment details were presented in (Table 1).

The efficacy of management practices was determined on the basis of vine and tuber infestation. Vine infestation was recorded at 15 days interval starting from 30 days after planting whereas tuber infestation was recorded at the time of harvesting. Vine infestation was recorded by dividing the number of infested vine to total number of vine whereas tuber infestation was recorded by dividing the weight of infested tuber to weight of healthy tuber. Per cent vine and tuber infestation was then calculated by multiplying with 100. The data were then subjected to analysis of variance. All the statistical analysis was performed through SPSS version 20.0.

Table 1. Management modules used in the study

Treatment No.	Treatment details
T_1	Untreated control
T_2	Vine treatment with Imidacloprid 17.8 SL @ 1ml/l of water + timely harvest
T_3	Vine treatment with Imidacloprid 17.8 SL @ 1 ml/l of water + spraying of Indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP* + timely harvest
T_4	Vine treatment with Imidacloprid 17.8 SL @ 1ml/l of water + earthing up + spraying of Indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP + timely harvest
T_5	Soil solarisation + vine treatment with Imidacloprid 17.8 SL @ 1ml/l of water + earthing up + spraying of Indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP + timely harvest
T_6	Soil solarisation + earthing up + spraying of Indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP + timely harvest
T_7	Spraying of Chloropyriphos 20 EC @ 2 ml/l of water at 30 and 60 DAP + timely harvest

*DAP: Days after planting

RESULTS AND DISCUSSION

The efficacy of different treatments against per cent vine and tuber infestation during 2020-21 was given in (Table 2). From Table 2, it was found that average vine infestation was minimum in plot where soil was solarised and vine were treated with imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (10.08 %). This was found statistically at par with plot where vine were treated with Imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (10.17 %) or plot where vine were treated with Imidacloprid 17.8 SL @ 1 ml/l of water followed by spraying of Indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (13.08 %) or plot soil was solarised followed by earthing up and spraying of Indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (13.83 %). From tuber yield point of view, maximum tuber yield was recorded in plot where vine were treated with imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (20.42 t/ha). This was found statistically at par with plot where soil was solarised and vine were treated with imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of Indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (20.40 t/ha) or plot where vine were treated with imidacloprid 17.8 SL @ 1 ml/l of water followed by spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (20.22 t/ha) or plot soil was solarised followed by earthing up and spraying of Indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (19.24 t/ha). As per cent tuber infestation was concerned, minimum tuber infestation was recorded in plot where vine were treated with imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (6.45 %) which was found statistically at par with plot where soil was solarised and vine were treated with imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (6.71 %).

The efficacy of different treatments against per cent vine and tuber infestation during 2021-22 was given in (Table 3). From Table 3, it was found that average vine infestation was minimum in plot where soil was solarised and vine were treated with imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (7.83 %) and was found statistically at par with plot where vine were treated with imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of Indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (8.67 %). This was followed by plot where vines were treated with Imidacloprid 17.8 SL @ 1 ml/l of water followed by spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (12.00 %) and plot where soil was solarised followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (13.33) and plot where vine were sprayed with indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (15.33 %). From tuber yield point of view, maximum tuber yield was recorded in plot where soil was solarised and vine were treated with Imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (20.48 t/ha). This was found statistically at par with plot where vines were treated with Imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of Indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (20.43) or plot where vine were treated with imidacloprid 17.8 SL @ 1 ml/l of water followed by spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (19.85t/ha).

Table 2. Efficacy of pest management module against sweet potato weevil (2020-21)

Treatment	Per cent vine infestation					Tuber infestation		
	30 DAP*	45 DAP	60 DAP	75 DAP	Mean vine infestation	Tuber yield (t/ha)	Infested tuber yield (t/ha)	Tuber infestation(%)
T_1	17.33	26.00	32.00	45.33	30.17	12.84	7.57	58.92
T_2	10.00	18.67	27.33	36.00	23.00	18.01	5.22	28.93
T_3	8.67	5.33	14.33	24.00	13.08	20.22	3.79	18.68
T_4	8.67	3.33	14.00	14.67	10.17	20.42	1.32	6.45
T_5	9.67	4.00	12.67	14.00	10.08	20.40	1.37	6.71
T_6	12.00	7.33	14.67	21.33	13.83	19.24	4.72	24.55
T_7	19.33	10.67	20.00	26.00	19.00	15.97	5.11	31.96
SE(m)	0.80	0.90	1.79	1.51	1.25	0.47	0.34	1.99
C.D.	2.50	2.79	5.59	4.70	3.90	1.46	1.06	6.19
C.V.	11.24	14.43	15.53	10.09	12.82	4.73	14.22	13.24

DAP*: Days after treatment

Table 3. Efficacy of pest management module against sweet potato weevil (2021-22)

Treatment	Per cent vine infestation					Tuber infestation		
	30 DAP	45 DAP	60 DAP	75 DAP	Mean vine infestation	Tuber yield (t/ha)	Infested tuber yield (t/ha)	Tuber infestation(%)
T_1	13.33	23.33	29.33	37.33	25.83	12.84	7.96	62.16
T_2	8.67	19.33	25.33	36.67	22.50	16.57	4.89	29.51
T_3	6.00	6.00	15.33	20.67	12.00	19.85	4.08	20.59
T_4	6.67	4.67	10.67	12.67	8.67	20.43	1.02	5.01
T_5	4.67	2.67	10.67	13.33	7.83	20.48	1.11	5.40
T_6	12.00	8.00	12.67	20.67	13.33	17.75	4.49	25.29
T_7	13.33	9.33	17.33	21.33	15.33	17.23	5.19	30.12
SE(m)	1.13	0.72	0.94	0.98	0.94	0.43	0.18	1.55
C.D.	3.53	2.24	2.94	3.04	2.94	1.34	0.57	4.82
C.V.	21.22	11.91	9.42	7.27	12.45	4.17	7.56	10.25

This was followed by plot where soil was solarised followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (17.75 t/ha) and plot vine were sprayed with indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (17.23 %). As per per cent tuber infestation was concerned, minimum tuber infestation was recorded in plot where vine were treated with Imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (5.01 %) which was found statistically at par with plot where soil was solarised and vine were treated with imidacloprid 17.8 SL @ 1 ml/l followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 DAP (5.40).

By analysing the individual component, during both the years, it was observed that after 30 days of planting, minimum tuber infestation was recorded in plot in which vine were treated with imidacloprid 17.8 SL @ 1 ml/l alone or plot in which the soil was solarised and vine were treated with imidacloprid 17.8 SL @ 1 ml/l. Similar results were also reported by (Reddy et al., 2022) who found that vine treatment with chlorpyriphos 20 EC provide protection at the early-stage growth of the crop. Soil solarisation is a chemical free method usually used to manage soil insect pest as well as weed population (Katan, 1987; McGovern and McSorley, 1997; Gill et al., 2009). After 45 days of planting minimum tuber infestation were recorded in plot where the plants were sprayed with indoxacarb @ 1.5 ml per litre of water at 30 Days after planting. After that vine infestation was increased slightly in all the plots at 60 days after planting then decreased in all the plots at 75 days after planting where the plants were sprayed with indoxacarb @ 1.5 ml per lit of water at 60 Days after planting. The tuber yield was recorded to be highest in all plots where vines were treated with imidacloprid 17.8 SL @ 1 ml per litre followed by spraying of indoxacarb @ 1.5 ml per lit of water at 30 and 60 DAP. By comparing T_3, T_4 and T_5, It was observed that earthing up may be responsible for reducing the tuber infestation to some extent as except earthing up, all other components were same for all these three. Infestation of weevil was initially low in plot where vine were treated with imidacloprid 17.8 SL @ 1ml per lit of water while after 30 days of planting, infestation was more or less same with untreated control plot. Prasad et al. (2022) [4] reported vine treatment followed by application of chemicals at 30 and 60 days after planting considerably reduce the weevil population, hence reduce the per cent vine infestation.

CONCLUSION

During both the years, it was observed that vine treatment with imidacloprid 17.8 SL @ 1 ml/l or soil solarization helps in reducing the vine infestation at early stage of growth. After that earthing up followed by spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 days after planting reduces the vine infestation as well as tuber infestation. Based on two years experiment, it may be conclude that vine treatment with imidacloprid 17.8 SL @ 1ml/l of water followed by earthing up and spraying of indoxacarb @ 1.5 ml/l of water at 30 and 60 days after planting and timely harvest of tuber will be helpful for farmers in minimizing the infestation of sweet potato weevil under field conditions.

ACKNOWLEDGEMENTS

The authors are thankful to the Professor and Head, Dr. P. P. Singh, Department of Entomology, Tirhut College of Agriculture, Dholi for providing all the facilities to carry out this experiment smoothly.

REFERENCES

Bovell Benjamin, A. C. 2007. Sweet potato: a review of its past, present, and future role in human nutrition. Advances in food and nutrition research, 52: 1-59.

Chalfant, R. B., Jansson, R. K., Seal, D. R., and Schalk, J. M. 1990. Ecology and management of sweet potato insects. Annual review of entomology, 35(1): 157-180.

Sutherland, J. A. 1986. A review of the biology and control of the sweet potato weevil Cylas formicarius (Fab). International Journal of Pest Management, 32(4): 304-315.

Reddy, P. R., Narayan, N. D., Alam, A. T., Giri G. S. and Singh P. P. 2022. Field efficacy of botanical insecticides against sweet potato weevil, Cylas formicarius Fab. (Coleoptera: Brentidae). The Pharma Innovation Journal, 11(7): 3243-3246.

Katan, J. 1987. "Soil solarization" in Innovative Approaches to Plant Disease Control ed. Chet I. (New York, NY: John Wiley & Sons) pp. 77–105.

McGovern, R. J., McSorley, R. 1997. "Physical methods of soil sterilization for disease management including soil solarization," in Environmentally Safe Approaches to Crop Disease Control eds Rechcigl N. A., Rechcigl J. E. (Boca Raton, FL: CRC Press, Inc.) pp. 283–313.

Gill, H. K., McSorley, R., Treadwell, D. D. 2009. Comparative performance of different plastic films for soil solarization and weed suppression. Horticultural Technology, 19: 769–774.

MS Received: 24 November 2022
MS Accepted : 26 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 133-137 (2022)

DOI Number: 10.5958/0974-4541.2022.00051.0

Modified tree trunk banding technology for mango mealybug, *Drosicha mangiferae* (Green) management: A techno-economic analysis

H. S. SINGH, GUNDAPPA BARADEVANAL* and DHARAM BEER

ICAR- Centrals Institute for Subtropical Horticulture, Rahmankhera, PO, Kakori
Lucknow -226101, India

***E-mail:** Gundappa@icar.gov.in

ABSTRACT: Mango mealybug *Drosicha mangiferae* (Green) is a univoltine seasonal pest, affecting a series of trees from December to April in north Indian conditions. Once it crawls up the tree in the season, insecticidal control becomes cumbersome and costly therefore tree trunk banding with plastic, supplemented with a sticky barrier was recommended long back for the management of this pest. Owing to various factors, this old technology is obsolete now and many farmers have shifted to insecticide dust-based trunk banding which is costly and environmentally harmful. We modified the old technology, replaced the materials and their specification and worked out its techno-economic studies. Modified banding technology has been found highly effective, its tree application rate is very fast; a team of two persons can apply the band to more than 100 trees in a day at a total cost of Rs 1750 whereas, in the old method of banding, it comes around Rs 4775 and that of in dust application, around Rs 2375 which ecologically also costly.

Key words: Mango, mealybug, banding, management

INTRODUCTION

Mango is well known for its excellent exotic flavor and usually referred to as the king of fruit (Sivakumar *et al.* 2011). The major mango-growing states are Andhra Pradesh, Uttar Pradesh, Karnataka, Bihar, Gujarat, and Tamil Nadu. Uttar Pradesh ranks first in mango production with a share of 23.47 % and the highest productivity (APEDA, 2021). Insect pests attack the mango and consume the nutrients from various parts of the plant system. Tandon and Varghese (1985) reported mealybug, *Drosicha mangiferae* (Green) as dangerous for the mango crop. It is not only the pest of mango but also attacks more than 70 other plants (Tandon and Lal 1978: Narula, 2003; Bandana *et al.*, 2017). It is a serious, dilapidating, polyphagous, dimorphic, and notorious insect pest of mango in the Indian sub-continent, distributed in Indo-Gangetic plains, feeding on other fruits crops, forest trees, ornamental plants, and weeds. During peak infestation, mango mealybug has been reported in different forest trees and an enormous number of crawlers and females get approach to tree *Dalbergia sissoo, Bombyx ceiba, Ficus religiosa*, and *Populus alba* via stem (Khan, 2001).

The eggs are laid in the soil around the tree trunk. There is great variation in the time of hatching of eggs (December to January) in different states due to variation in soil conditions. First instar nymphs are found during December-January and third instar females are found from March to the middle of April. Fertilized females start reverse migration from the third week of April to May to soil. Generally, the females migrate through the main stem but some of them also fall on the ground directly from the infested panicles and lay eggs in the soil around the tree trunk. The eggs remain in diapause in soil from May to December. Just after hatching, the minute pink to brown-coloured nymphs crawls up the tree. Nymphs and adult females suck the sap from tender leaves, shoots, and inflorescence. The insect also secretes honeydew over which sooty mold develops as a result, leaves and inflorescence become shiny black and sticky (Gundappa *et al.,* 2018).

The presence of a large alternative host range of mealybug makes them a great threat in orchard. The long-term control strategy is required in repeated approach due to its hiding habit and protective body covering. For this reason, chemical treatment is not advised till satisfactory control is achieved by other alternative methods. Alteration of environmental factors may affect their life cycle thereby affecting the time of infestation. By following the life cycle and seasonal dynamics, the mango mealybug population can be checked, therefore, the spatial and temporal separations in the life cycle of this insect have been worked out that provides an opportunity to apply a range of cultural, biological, and chemical control measures alone or in combinations.

Keeping the habit of tree ascending of mealy bugs and congregation, some of the management technologies like tree banding with plastic, chemical tree trunk banding, and insecticidal spray were developed by various workers. Banding of tree trunks with a polythene sheet (400-gauge, 30 cm wide) at a height of about 30 cm from the ground level and grease applied at the lower edge of the band during the 3rd/4th week of December was

recommended in the eighties. Due to cumbersomeness and cost, this technology became obsolete and another method of insecticide banding was introduced wherein, the tree trunk is mounted with raked soil up to a height of 6 -8" from the ground level followed by the application of 1.5 percent chlorpyriphos dust @ 250 g/ tree around tree trunk preferably in 3rd or 4th week of December. This method has its limitation such as ineffectiveness in rains and the related application and ecological costs. Nowadays readymade sticky bands (brown cello tape) are available in the market which may be equally effective. However, it needed standardization before recommendation hence, an attempt was made to economize the trunk banding technology in the present investigation.

MATERIALS AND METHODS

The body size of the mealy bug ranges from a few mm (at early instar) to 18 mm (last instar). In the first instar, their leg size is a few mm and hence, it covers the very small surface area on the tree trunk while crawling up. The tree banding with polythene provides a slippery surface that does not allow them to crawl up. Since their leg expansion is too little, a smaller slippery surface (3-4 inches) may give the same efficacy as given by 30 cm wide polythene sheet recommended in old technology, hence there was a scope of cost reduction on polythene by reducing its size. In old technology, mud application on the whole banding area is recommended before the fixing of polythene to seal the cracks and crevices below the sheet so that bugs don't find a way to crawl up. It was presumed that whole area mud application is not needed rather a band of sticky mud may seal the cracks and crevices effectively and therefore, the cost of labour on the application of mud can be reduced. In the old method, both the end of the polythene sheet needs to be fastened with twine. The grease application at the lower end of the band in old technology is recommended to seal the cracks and crevices so that mealybug does not find a way to crawl up from inside of the band, however, getting grease at village level and its associated cost is inhibitory in the application of this technology. Keeping this assumption in view, the experiment was designed and carried out at ICAR-CISH, Rahmankheda mango farm during 2019-2021. Various combinations of wrapping materials, soil paste, and sticky bands were applied during the 3rd week of December as below:

T1- Polythene banding (existing technology): Banding of tree trunks with a polythene sheet (400-gauge, 30 cm wide) at a height of about 30 cm from the ground level with grease banding at the lower edge.

T2- Brown cello tape wrapping over the soil band with grease band on the upper edge.

T3- Brown cello tape wrapping over the soil band with grease paste at the lower edge.

T4- Brown cello tape wrapping over the soil band with glue band at the lower edge

T5- Brown cello tape wrapping over the soil band with glue band on the upper edge.

T6- Chemical banding: Chemical raking in tree basin, the tree trunk mounting and raking soil up to a height of 6 -8" from the ground level than the application of 1.5 percent chlorpyriphos dust @ 250 g/ tree around the tree trunk.

T7- Control

In T2-T5, the materials used were i) locally available brown cello tape band of 4 inches wide having glue on its inner surface, ii) mixture of 1 kg clay soil with 50 ml of burnt Mobil oil, 250 gm POP, and water, kneaded to make it a dough (like loosely kneaded atta) paste, iii) a locally designed hand tool to remove the old and dead bark and iv) glue embedded twine.

To apply the treatment in T2-T5, around 7–8-inch bark area was cleaned on the tree trunk above 30 cm or any approachable trunk height by using bark remover to reduce the cracks and crevices on the trunk surface. Wherever the trunk surface was smooth, this exercise was avoided. Around about the center of the cleaned tree trunk area, the soil paste was applied in the form of a band of 2-inch width. The cello tape was wrapped wrinkle-free over the soil band in such a way that the soil band comes in the middle of the wrapped sheet. The cello tape was rolled twice tightly. The glue embedded twine was tied as per the treatment requirement.

All the treatments (except in control treatment) were supplemented with a second sticky band with brown cello tape over soil mud as data recording band, a foot above the treatment band on the tree trunk to count the number of mealybugs that succeeded in crossing the treatment band.

Each treatment was replicated 3 times in a randomized block design. The experiment was continued up to April when the bugs were matured and started reverse migration from the tree. The number of nymphs congregated below the first band as well as on the second band (data recording band) was recorded weekly whereas, on buds it was recorded when most of the bugs completed their crawl up. The cumulative population was subjected to analysis. The relative merit of the three most effective

Table 1. Efficacy of different banding methods in restricting the mealybug ascending on tree trunk and canopy

Treatments	Mean no. of mealybugs restricted at a lower band	Mean no. of Mealybugs succeeded to reach the second band	Mean no. of mealybugs / random buds
T1- Polythene banding (old technology).	73.17^{ab} (7.09)	61.54^{ab} (5.34)	0.0^{b} (0.50)
T2- Brown cello tape wrapping over the soil band with grease band on the upper edge.	172.5^{ab} (7.61)	118.83^{a} (6.60)	1.04^{b} (0.84)
T3- Brown cello tape wrapping over the soil band with grease paste at the lower edge.	120.17^{ab} (8.87)	22.5^{b} (3.46)	0.0^{b} (0.50)
T4- Brown cello tape wrapping over the soil band with glue band at the lower edge.	248.08^{a} (11.86)	0.54^{b} (0.79)	0.12^{b} (0.57)
T5- Brown cello tape wrapping over the soil band with glue band on the upper edge.	30.54^{b} (5.01)	7.42^{b} (2.42)	0.0^{b} (0.50)
T-6 Chemical banding	0.0^{b} (0.50)	0.0^{b} (0.50)	0^{b} (0.50)
T-7 Control	0.0^{b} (0.50)	0^{b} (0.50)	23^{a} (3.47)

Means with the same letter are not significantly different in Tukey's Honest Significant Difference (HSD) Test; Values in parenthesis are square-root transformed

Table 2. Comparison of application issues of modified banding method under field condition as against prevailing technologies.

.

Parameters	T1- Polythene banding (old technology)	T4- Cello tape wrapping over the soil band with glue band at lower edge	T-6 Chemical banding
Amount of toxic chemicals added to the ecosystem	Nil	Nil	25 kg per ha
Application frequency	Once	Once	Repetition may be needed
Cost per 100 trees	4775	1750	2375 (ecological cost is high)
Application easiness	Labour intensive Needs re-sizing of polythene for application	Less labour, the required size is a market available.	Labour intensive Market available
Efficacy of the technology	No crawling up	No crawling up	No crawling up, but fails if rains and therefore re-application is needed
Alertness in timing of application	High	High	Very high

treatments and their associated cost was also worked out.

RESULTS AND DISCUSSION

The results indicated that the number of mealybugs ascended and assembled at the lower band (lower edge of treatment band) was found significantly different among the treatments ($F_{6, 159}$ =4.69; p<0.001). The highest number of mealybugs (248) stopped at the lower edge was found in T4 (cello tape wrapping over the soil band with glue band at lower edge)followed by T2 (cello tape wrapping over the soil band grease band on upper edge) numbering 172. The number of mealybugs congregated at the upper band (data recording band) was also found different among the treatments ($F_{6, 159}$ =4.71; p<0.001. The lowest number (0.54) was found in the treatment T4 (cello tape wrapping over the soil band with glue band at lower edge)followed by T5 (cello tape wrapping over the soil band glue band on upper edge) numbering 7.42. The number of mealybugs found in the buds of mango was also found significant among the treatments ($F_{6, 159}$ =7.92<0.001). Among the banding methods compared, mealybug was found (1.04) only in T2 (cello tape wrapping over the soil band and grease band on upper edge when compared to control (23 mealy bugs /bud) (Table 1). Very few numbers of mealybugs were found on buds in most of the treatments except control because they were prevented by a second band fixed a foot above the treatment band to restrict them for data purposes. Among the banding methods compared mealybug was found only in modified method with cello tape banding without upper restriction (1.04) when compared to control (23 mealy bugs /bud) (Fig 2). These findings were in agreement with Yousuf (1993) Mohmmad *et al*. (2004) who also found similar results of tree banding treatments of another kind for the control of mango mealybug.

The system of banding in T4 recorded maximum nymphs as they failed to crawl up and congregated at the bottom due to reasons such as i) closing bark cracks and crevices by soil mud band prevented the first and second instar nymphs crawling through cracks and crevices, ii) soil mud band curve formation with slippery surface changed center of gravity of crawling mealybug, therefore, they fell from the trunk in the later instars and iii) sticky band with glue dipped twine at lower edge prevented the crawling of young ones at the base of the band.

The superiority of the most effective treatment (T4) is presented in Table-2 which indicates that the technology requires less labor, the material is available in the market, all the stages of the mango mealybug are perfectly prevented from crawling on the trunk and no re-sizing of wrapping material is needed, hence can be recommended as modified tree trunk banding technology as an alternative to old banding technology. The cost estimation indicated that application and material cost for 100 trees comes around Rs 1750 in this modified tree trunk banding technology whereas, in the old method of banding, it comes around Rs 4775 and that of in dust application, around Rs 2375 which ecologically also costly. Adoption of this technology may be useful for the eco-friendly management of the mealybug.

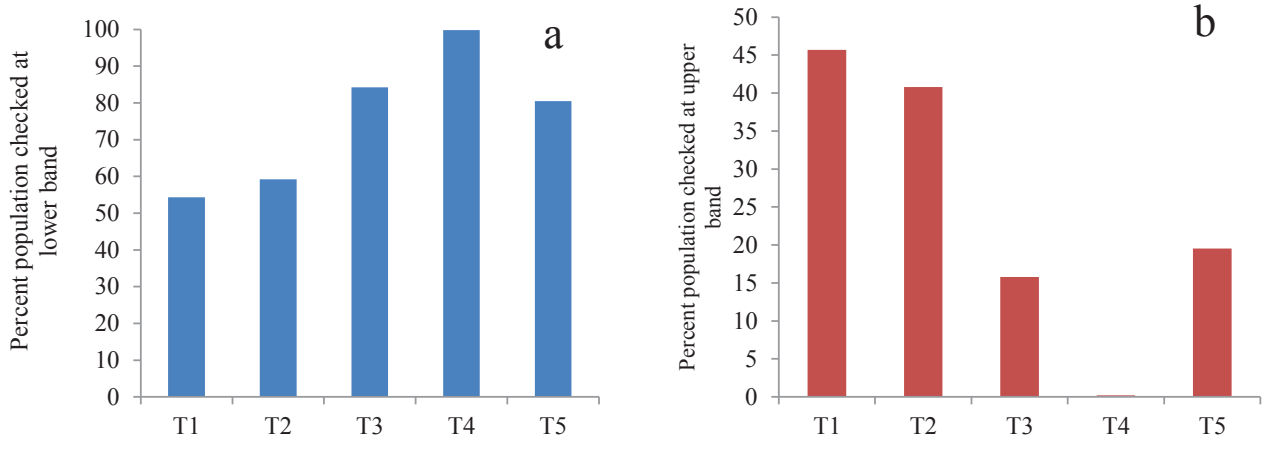


Fig. 2 Per cent mealy bug population checked at lower (a) and upper(b) band

REFERENCES

APEDA.2021.Mango: http://apeda.gov.in/apedawebsite/SubHead_Products/Mango.htm

Bandana Bhau, Uma Shankar and D.P. Abrol. 2017. Studies on Host Range and Biology of Mango Mealy Bug (*Drosichamangiferae*) in Jammu Region. *International Journal of Current Microbiology and Applied Sciences,* **6** (9): 230-240.

Gundappa, Adak T, Shukla, PK. 2018. Population dynamics of mango mealybug (*Drosicha mangiferae*)(Margorididae: Hemiptera) and its relation with weather parameters in subtropical climatic conditions. *Indian Journal of Agricultural Sciences*, **88**(6): 865-870.

Khan MA. 2001. Control of insect pests of mango. Proc. Inter. Mango workshop 27 Feb.-1 Mar. Direct. Agric-Multan Region, Punjab:224.

Muhammad Ishak, Muhamad Usman, Muhammad Asif and I.A. Khan. 2004. Integrated Pest Management of Mango against Mealy Bug and Fruit Fly *International Journal of Agriculture and Biology,*6: 452–454.

Narula S. 2003. Time to tackle bugs that attack mango trees.Lead article. *The TribuneSpectrum*, **Sunday, January 12, 2003**

Sivakumar D, Jiang Y, Yahia EM. 2011 Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. *Food Research International*:44:1254-1263.

Tandon PL, Lal B. 1978. The mango coccid, *Rastrococcusiceryoides* Green (Homoptera: Coccidae) and its natural enemies. *Current Science*:13:46-48.

Tandon PL, Verghese A. 1985. World List of Insect, Mite, and other Pests of Mango. Technical Document No. 5, IIHR, Banglore:22.

Yousuf M. 1993. Mango mealybug control with polyethylene bands. *Pakistan Entomologist*:15:129.

MS Received: 27 November 2022
MS Accepted : 29 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 138-143 (2022)

DOI Number: 10.5958/0974-4541.2022.00052.2

Biochemical basis of resistance against root knot nematodes in chilli (*Capsicum annuum* L.)

G. SANTHOSH[1], R. UMA MAHESHWARI[2*], K. S. SHIVASHANKARA[3], NARESH PONNAM[4], D. C. LAKSHMAN REDDY[3], D. K. NAYAK[1] and B. K. DASH[1]

[1]Department of Nematology, Odisha University of Agriculture and Technology, Bhubaneswar, India

[2]Division of Crop Protection, [3]Division of Basic Science, [4]Division of Vegetable Crops,

ICAR - Indian Institute of Horticultural Research, Bengaluru, India

*** E-mail:** umanema369@gmail.com

ABSTRACT: Chilli (*Capsicum annuum* L.) is an important and versatile vegetable cum spice crop. A study was conducted to understand the biochemical mechanism in two resistant advanced lines developed (ACRIL 90, ACRIL 70) along with two susceptible genotypes (Arka Mohini, Arka Suphal) , with and without incouation of RKN, *M. incognita*. The results showed that there was a significant increase in antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (PO), and polyphenol oxidase (PPO) in resistant genotypes compared to susceptible genotypes upon inoculation of nematodes. Resistant genotypes also recorded significant higher contents of lignin, phenol, and protein than the susceptible ones. Thus the current study proves that antioxidant enzymes, lignin and phenol contents play a significant role in inducing resistance in host plants against *M. incognita*.

Keywords: Capsicum, Root knot nematode, SOD, PO, PPO, lignin, phenols and protein

INTRODUCTION

Chilli (*Capsicum annuum* L.) is a versatile plant that is cultivated as a vegetable and spice crop. The yield, quality and growth of plants are limited by many biotic and abiotic factors (Naresh *et al*., 2019). India is the major producer of dry chilli with an annual production of 1.2 million tonnes followed by China with around 0.25 million tonnes (FAOSTAT, 2020). Nematodes are devastating parasites of crop plants in agricultural production and certainly contribute significantly to net reduction in yield. Nearly every crop in the world is attacked by root-knot nematodes (RKN), making them the most commercially significant group of plant parasitic nematodes (Sasser and Freckman, 1987). *M. incognita* infection severely damages the root system and cause huge economic losses in pepper (Thies *et al*., 1998).

Managing RKN through host plant resistance is a cost-effective farmer-friendly and eco-friendly approach.. Resistance to phytoparasitic nematodes is commonly associated with hypersensitive reaction (HR), a rapid and localised cell death in the sick plant in response to nematode attack. Reactive oxygen species (ROS) are essential for plant defense mechanism, and resistant plants frequently have higher levels of ROS-detoxifying enzymes like peroxidase (PO) and catalase (CAT) when a pathogen attack is occurring. Plants produce more ROS as a result, and when these ROS build up in plant cells, HR occurs. Hydrogen peroxide plays a crucial role in the initiation of HR in interactions that are incompatible. Antioxidant enzymes including peroxidase (PO), phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), super oxide dismutase (SOD), and catalase (CAT) are among the most important protective enzymes involved in the removal of free radicals and activated oxygen species and associated with disease resistance mechanisms (Chandrawat *et al,.*2020).

Polyphenols are believed to seal wounds or diseased tissue, thereby preventing secondary infection or infection spread. Polyphenols cause the darkening of tissue during lesion development (Vaughn *et al.,* 1988; Mayer and Harel, 1979). Plants' phenol metabolism and hypersensivity response are defense systems against invading harmful organisms such as nematodes. The increase in polyphenol oxidase following nematode entry attributes to pathogen-induced phenol oxidation mechanism (Maraite, 1973).Also, plants produce a number of proteins in response to pathogen attack. Pathogenesis-related (PR) proteins are host-encoded proteins generated in response to pathogen invasion, stress, or elicitor treatment (Van Loon, 1997). Systemic acquired resistance has been connected with the creation of PR proteins (SAR).

Since chilli plants are vulnerable to RKN damage, identifying the resistant lines and understanding their mechanism of action helps in efficient management of RKN. Further identified lines can also be explored as root stocks for cultivation of bell/sweet peppers under protected cultivation. Keeping this in view, the current

research aims to assess the biochemical mechanism of resistance exhibited in advanced resistant lines as a function of activities of antioxidant enzyme and changes in biochemical composition, when infected by nematodes.

MATERIALS AND METHODS

The studies were carried out at the Division of Crop Protetion, ICAR - Indian Institute of Horticultural Research (IIHR), Bengalurufrom July 2021 to September 2022 in Bengaluru. Two identified resistant advanced breeding lines (ACRIL90, ACRIL70) and susceptible (Arka Mohini, Arka Suphal) lines were studied to elucidate the mechanism of action of resistant lines against *M. incognita.*

Nematode collection

Egg masses were collected from culture plants of tomato (cv. Arka Rakshak) maintained in Nematology glass house, Division of Crop Protection, ICAR –IIHR, Bengaluru. Second stage juveniles (J2) hatching out from the eggs were harvested every day, and only J2 not less than 5 days old were utilized for inoculation.

Nematode inoculation

In a completely randomised design with six replications, two resistant lines were sown in polybags in the net house with inoculated and uninoculated treatments. These plants were uprooted 15 days and 30 days after inoculation, and the following chemical compositions were estimated in both resistant and susceptible plants under both control and inoculated conditions: The biochemical changes occurring in resistant lines were analysed by studying the total phenols, lignin content, protein content and defence enzyme activities.

The seeds of the selected resistant advanced breeding lines (ACRIL90, ACRIL70) along with susceptible varieties (Bell pepper; Arka Mohini, chilli; Arka Suphal) were surface sterilized for 5 minutes with 0.1% $HgCl_2$, then carefully rinsed with sterile water and air dried. They were then sowed in Coco peat trays and maintained with regular watering. Four weeks old seedlings were transplanted to black nursery polybags (1 kg capacity) filled with sterilized potting mixture containing red soil, FYM and sand in 1:1:1 proportion. In a completely randomised design with six replications, the lines were arranged with nematode inoculated and uninoculated treatments, For challenge inoculation with nematodes, second stage juveniles of *M. incognita* were inoculated into three 2 cm deep holes in the soil around the stem base @ 1000 J2 per plant. After 15 and 30 days, the plants were pulled out and the roots were rinsed, and the following enzymatic and biochemical composition were measured to evaluate their response to nematodes.

Enzyme extraction

Frozen root sample (1 g) was grinded in a mortar and pestle with 10 mL of 0.05 M phosphate buffer (pH 7.0) containing 10% (w/v) polyvinylpyrrolidone (PVPP) and 0.1 M EDTA. Homogenates were centrifuged (15000g, 15 min, 4 °C) and supernatants used for enzyme assays.

Superoxide Dismutase (SOD)

Methionine, nitroblue tetrazolium (NBT), EDTA, Na_2CO_3, phosphate buffer, and distilled water comprised the reaction mixture. The enzyme extract was added last, followed by addition of 0.1 ml of riboflavin to initiate the reaction. The intensity of the produced colour was measured at 560nm (Xing *et al.,*2008)**.**

Polyphenol Oxidase (PPO)

To facilitate enzyme extraction, phosphate buffer was added. The reaction was started by adding 2% of catechol. PPO activity was assayed by measuring the linear increase in absorbance at 410 nm by following the method of Augustin *et al*. (1985).

Peroxidase (PO)

Enzyme extract was added to chilled guaiacol The reaction was started by adding H_2O_2 and the rate of decrease in absorbance at 470 nm was measured at 30 seconds intervals for 3 mins. The unit of enzyme is defined as a decrease in O.D by 1.0 under standard conditions by following Shannon *et al*. (1966) method.

Determination of total phenol content

Total phenol content was estimated by the method described by Skerget *et al.* (2005). Plant extract was prepared by adding 400 mg of dried plant sample to 60% ethanol. Then, 2 mL of plant extract was added to Folin-Ciocaltaeu reagent and 8 mL of Na_2CO_3. Incubation was performed for 2 h, and absorbance was recorded at 765 nm.

Determination of protein content

About 0.5g of root tissue was taken and thoroughly grinded with 5ml of water. The extract was centrifuged for 10 min at 10000 rpm. Then, 0.1ml of supernatant was taken in clean test tubes and the volume made up to 1ml by adding distilled water. To this was added 5 ml of reagent 4 (Alkaline copper solution), mixed thoroughly and allowed standing at RT for 10 min. The, 0.5 ml Folin-Ciocalteau's reagent (1:1) was added and incubated all the tubes for 30 min at dark. The intensity of the blue colour developed was read by measuring the

absorbance at 750 nm. Results were expressed as mg of bovine serum albumin (BSA) equivalents per 100 g of fresh weight (Lowry *et al.*, 1951).

Quantification of lignin

The ash content of all samples was determined by burning the insoluble fraction for 4 hours in a muffle furnace at 550 °C. Lignin from the insoluble fraction was calculated by the difference in the weight of the dry mass and the total ash for each sample. The lignin content was determined by the sum of the insoluble and soluble lignin and was expressed as mg/g21 cell wall following the method of Abdelrahman (2018).

Results and discussion

The results on defense enzymes revealed significantly higher acitivity in resistant lines than the susceptible ones, with enhanced expression when inoculated with nematodes than the uninoculated plants.

Super oxide dismutase (SOD)

The analysis of nematode resistant and susceptible rootstocks for super oxide dismutase activity revealed that the inoculated resistant lines expressed higher activity with 10.009mg and 16.923mg in ACRIL 70; 9.987 mg and 14.363mg in ACRIL 90 at 15 and 30 days after inoculation, respectively. The uninoculated resistant lines showed lower SOD activity signifying the elevation of SOD activity for defense during the time of infection.

Even in the susceptible varieties, inoculated Arka Mohini and Arka Suphal exhibited greater SOD activity than the un-inoculated plants with 7.908 mg and 8.783 mg, respectively, at 15 DAI. However, at 30 DAI, the SOD activity was recorded to be higher in the susceptible uninoculated plants with 10.245 mg and 11.512 mg than the inoculated plants with 9.08 mg and 9.236 mg values for Arka Mohini and Arka Suphal, respectively (Table 1 and 2). Similar results were also reported by Kashyap *et al.,* (2021), where SOD activity was raised in all treatments 24 hours after pathogen injection. Neena Chawla *et al*. (2013) also reported similar results, indicating that SOD activity increased in the roots and leaves of inoculated and uninoculated plants of resistant and susceptible genotypes.

Peroxidase

The resistant (ACRIL 70 and ACRIL 90) lines inoculated with nematodes recorded with higher peroxidase activity values compared to both the uninoculated resistant lines as well as the susceptible check varieties. The results showed that at 15 and 30 DAI, in both the resistant genotypes, enzyme activity was increased than that of susceptible genotypes in both inoculated and uninoculated treatments. While in susceptible genotypes decreased activity was observed when compared with resistant genotypes.

The resistant inoculated lines showed peroxidase activity as high as 9.233µg in ACRIL 70 and 8.905µg in ACRIL 90 after 15 days and still higher as 14.312µg (ACRIL 70) and 11.276µg (ACRIL 90) after 30 days of inoculation. Concurrently the uninoculated ACRIL 70 recorded 4.428µg, 8.879µg and ACRIL 90 recorded 4.264µg, 8.106µg at 15 and 30 days after inoculation, respectively. Among the susceptible Arka Mohini and Arka Suphal also, the inoculated varieties recorded higher peroxidase activity than the uninoculated, both at 15 and 30 DAI (Table 1 and 2).

This strengthens the point that the nematode infection elevates the defense mechanism of peroxidase activity that helps to speed up the polymerization process by which phenolic chemicals are transformed into lignin as observed by Gaspar *et al.* (1982). When a pathogen attacks, a plant's primary defense is to begin producing new cell walls. Plants contain many peroxidase isoenzymes that vary in how they react with substrates and how they are constructed. These results are in line with the findings of Pankaj *et al*. (1994) in which the highest compared to DL 482, the susceptible line.

Poly phenol oxidase (PPO)

PPO is a part of primary defense mechanism, thus the same has been exhibited in the present study. Table 1 and 2 demonstrates the obtained results for the PPO content. The inoculated resistant lines, ACRIL 70 and ACRIL 90 showed a higher accumulation of PPO i.e. 2.006mg, 1.730mg and 3.213mg, 3.010mg at 15 and 30 DAI, respectively. Meanwhile, the uninoculated resistant ACRIL 70 (1.260mg, 1.026mg) and ACRIL 90 (2.486mg, 2.180mg) had lower PPO content.

On the other hand, significantly lesser PPO content was observed in the susceptible varieties, Arka Mohini (1.310mg,1.023mg) and Arka Suphal (1.416 mg,1.320 mg) both at 15 and 30 DAI leading to the conclusion that susceptible varieties succumbed to the infection and hence, lacked this defense mechanism. The variation can be clearly understood within the inoculated and uninoculated susceptible varieties showing reduction in PPO content in inoculated plants than in the uninoculated ones. These results were positively correlated with the findings of Kosuge (1969) that PPO catalyzes the hydroxylation of mono phenols to diphenols and the oxidation of diphenols to quinones which rapidly polymerize to produce black or brown pigments

Table 1. Biochemical changes in resistant and susceptible genotypes at 15 DAI of root knot nematodes

15 DAI	Phenols (mg/g FW)	Lignin (Ash %)	Proteins (µg/g FW)	PO (units/µg protein)	SOD (units/mg protein)	PPO (units min-1mg-1 FW)
Resistant						
ACRIL-70 U	0.619	65.3	0.551	4.428	7.377	1.26
ACRIL-70 I	0.716	61.1	0.575	9.233	10.009	2.006
ACRIL-90 U	0.581	68.2	0.513	4.264	6.87	1.026
ACRIL-90 I	0.692	50.7	0.557	8.905	9.987	1.73
Susceptible						
Arka Mohini U	0.308	49.7	0.326	3.314	5.868	2.23
Arka Mohini I	0.581	31.4	0.471	6.413	7.908	1.31
Arka Suphal U	0.341	52.3	0.391	3.338	5.984	2.64
Arka Suphal I	0.524	32.23	0.489	6.604	8.783	1.416
CD at 5%	0.009	3.551	0.0056	0.034	0.491	0.122
SEm	0.003	1.174	0.003	0.011	0.162	0.04

I= Inoculated U= Un inoculated FW= Fresh Weight; DAI – Days after Inoculation

(polyphenols). PPO activity increases in virus, bacteria, fungi and nematode infected tissues and similar findings was observed by Brueske and Dropkin (1973).

Phenol content

The study revealed that the phenol content was higher in resistant lines i.e., ACRIL 70 and ACRIL 90 both at 15 and 30 DAI of nematodes with 0.716mg, 0.920mg and 0.692mg, 0.980mg, respectively. In comparison the susceptible lines under the experiment, Arka Mohini and Arka Suphal recorded only 0.581 mg, 0.618mg and 0.524mg, 0.606mg at 15 and 30 DAI, respectively. The variation in the phenol content within the resistant lines revealed that the inoculated lines showed higher phenol accumulation, than the uninoculated lines both at 15 and 30 days intervals (Table 1 and 2). The trend of higher phenol accumulation in inoculated plants was followed in the susceptible lines as well, indicating increase in phenol content as a mechanism favouring resistance. Similar kind of results were observed by Pandey *et al.* (2016) that increased amount of phenol content was observed in resistance genotypes than susceptible genotypes during infection by RKN in greengram.

Protein content

Higher protein content was observed in nematode inoculated resistant lines than susceptible lines after 15 and 30 DAI. In inoculated resistant lines with 0.575 mg, 0.557 mg (15 DAI) and 0.684 mg, 0.667 mg (30 DAI) as against the uninoculated resistant lines with 0.551 mg, 0.513 mg (15 DAI) and 0.487 mg, 0.472 mg (30 DAI) (Table 1 and 2). The results were on par with the findings of Gopinath *et al.* (2002) in which the moderately resistant tomato cultivars, Vivek and Radha recorded maximum concentration of proteins, which confer resistance to RKN infection whereas in susceptible cultivar Pusa Ruby the protein concentration is less as compared to the resistant cultivars.

Lignin content

Lignin concentration was relatively higher in resistant lines compared to the susceptible lines however uninoculated lines revealed more lignin concentration than the inoculated ones. . This consequently establishes the fact that the nematodes on infection damage the cell wall and ultimately leads to reduction in the lignin content. The damaged cells also show less pronounced translation subsequently producing lower levels of protein. The resistant lines ACRIL 70 and ACRIL 90 showed lignin content with 61.1% ash and 50.7% ash in the inoculated lines, while the uninoculated lines had 65.3 % ash, 68.2 % ash of lignin at 15 DAI. At 30 DAI lignin content of 60.2 % ash, 54.0 % ash in inoculated and 65.2 % ash, 63.8 % ash in uninoculated lines, respectively were recorded. These results were coincided with the results obtained by Tian *et al.* (2022) revealing that *C. chinense* showed higher lignin content in resistant genotype than susceptible after the *M. enterolobii* inoculation.

CONCLUSION

Understanding biochemical basis of resistance in roots against root knot nematodes will facilitate in identification of candidate biochemical markers for indirect selection of genotypes with resistance. Based on the results we can conclude that the defense enzymes (SOD, PPO and PO) and biochemical constituents

Table 2. Biochemical changes in resistant and susceptible genotypes at 30 DAI of root knot nematodes

30 DAI	Phenols (mg/g FW	Lignin (Ash %)	Protiens (µg/g FW)	PO (units/µg protein)	SOD (units/mg protein)	PPO (units min-1mg-1 FW)
Resistant						
ACRIL-70 U	0.780	65.2	0.487	8.879	14.713	2.486
ACRIL-70 I	0.920	60.2	0.684	14.312	16.923	3.213
ACRIL-90 U	0.683	63.8	0.472	8.106	13.42	2.18
ACRIL-90 I	0.980	54	0.667	11.276	14.363	3.01
Susceptible						
ArkaMohini U	0.423	56.3	0.221	5.577	10.245	2.003
Arka Mohini I	0.618	32.6	0.491	9.843	9.087	1.023
Arka Suphal U	0.440	52.7	0.277	5.979	11.512	2.106
Arka Suphal I	0.606	27.3	0.494	9.986	9.236	1.32
CD at 5%	0.008	3.299	0.0042	0.072	0.424	0.07
SEm	0.003	1.091	0.004	0.024	0.14	0.023

I= Inoculated U= Uninoculated FW= Fresh Weight; DAI – Days after Inoculation

(Phenols, lignin and protein) were increased in the roots of resistant lines compared to that of susceptible genotypes after RKN inoculation. Advanced breeding lines ACRIL 70 and ACRIL 90 showed increased resistance to RKN both phenotypically and biochemically it can be further utilized for the breeding to incorporate the resistance into the elite genotypes. These genotypes can serve as potential root stock for chilli/capsicum to mitigate the root knot nematode problem in the infected soils.

REFERENCES

Abdelrahman, Nour and Galiwango, Emmanuel. 2018. Klason Method: An Effective Method for Isolation of Lignin Fractions from Date Palm Biomass Waste. *Journal of Food Process Engineering*, **57**. 46-58.

Augustin, M. A., Ghazali, H. M., and Hashim, H. 1985. Polyphenol oxidase from Guava (Pisidum guajava L.). *Journal of the Science of Food and Agriculture*, **36**: 1259-1265.

Brueske, C. H., and Dropkin, V. H. 1973. Free phenols and root necrosis in nematex tomato infected with root knot nematode. *Phytopathology*, **63**: 329-334.

Chandrawat, B. S., Siddiqui, A. U., Bhati, S. S. and Saharan, V. (2020). Bio-agents: A source for initiation of defence enzymes in chilli infected with root-knot nematode, Meloidogyne incognita. *Journal of Entomology and Zoology Studies*, **8**: 1684-1688

FAOSTAT. 2020. Available from: http://www.fao org/faostat/ en/#home (accessed on 2021 November).

Gaspar, T., Penelc. L., Tiiorpet. and Greppin, H. 1982. Peroxidases 1970-1980. A Survey of Their Biochemical and Physiological Roles in Higher Plants. University de Geneve: 89-112.

Gopinatha, K. V., Nagesh, M. and Nanjegowda, D. 2002. Biochemical estimation of resistance in tomato cultivar to root-knot nematode, Meloidogyne incognita, *Indian Journal of Nematology*, **32**: 183-233.

Kosuge, T. 1969. The role of phenolics in host response to infection. Annual Review of Phytopathology, **7**: 195-222.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the fo1in phenol reagent. ~. *Biological Chernobyl*. **173**: 265-275.

Maraite, H.1973. Changes in polyphenol oxidase and peroxidase in muskmelon (Cucumis melo L.) infected by *Fusarium oxysporum f.sp. melonis*. *Physiol.PIant Pathology*, **3**: 29- 49.

Mayer, A. M., and Harel, E. 1979. Polyphenol oxidases in plants. *Phytochemistry*, **18**: 193–215.

Naresh, P., Meenu, K., Acharya, G. C., Reddy, A. C. and Reddy, D. C. L. 2019. Genetics and molecular markers for resistance to major soil

borne pathogens in chilli (*Capsicum annuum* L.).*Research Journal of Biotechnology,* **14** (1): 101-105.

Pandey, R. K., Nayak, D. K., Lepcha, R. and Kar, R. K. 2016. Biochemical changes in susceptible and resistant black gram cultivars induced by root-knot nematode, Meloidogyne incognita, *Agricultural Science Digest*, **36**(4): 326-328.

Pankaj., Dhawan, S. C. and Dasgupta, D. R. 1994. Peroxidase (E.C.1.11.1.7) activity in resistant and susceptible barley cultivars infected with cereal cyst nematode, *Heterodera avenae*. *Indian Journal of Nematology*, **24**: 80-82.

Satyaprakash Barik., Naresh Ponnam., Anand .C. Reddy., Lakshmana Reddy, D. C., Koushik Saha, Acharya, G. C. and Madhavi Reddy, K. Breeding peppers for industrial uses: Progress and prospects,Industrial Crops and Products, 178, 2022.

Sasser, J. N. and Freckman, D. W. 1987. A world perspective on nematology: the role of the society. In: Veech, J.A., and Dickson, D.W. (eds) Vistas on Nematology. *Society of Nematologists, Hyattsville*. 7-14.

Shannon, L. M., Kay, E. and Lew, J. Y. 1966. Peroxidase Isozymes from Horseradish Roots. 1. Isolation and Physical Properties. *Journal of Biological Chemistry*, **241**, 166-2172.

Škerget, M., Kotnik, P., Hadolin, M., Hras, A. R. and Simonic M, Knez, Z. 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem* **89**:191–198.

Thies, J. A., Mueller, J. D. and Fery, R. L. 1998. Use of a resistant pepper as a rotational crop to manage southern root-knot nematode. *Hort Science,* **33**: 716–718.

Tian Xiaoxiao, JIANG Bingzheng, CAO Zhenmu, LIU Ziji, LING Peng, XIE Shangqian, ZHU Jie, , (2022). Identification of *Capsicum chinense* Germplasms Resistant to *Meloidogyne enterolobii* and Preliminary Analysis on Resistance Mechanism [J]. *Chinese Journal of Tropical Crops*, **43**(1): 165-172.

Van Loon, L.C., Pierpoint, W. S., Boller, T. and Conejero, V. 1994. Recommendations for naming plant pathogenesis-related proteins. *Plant Molecular Biology Reporter*, **12**: 245-264.

Vaughn, K. C., Lax, A. R. and Duke, O. 1988. Polyphenol oxidase: The chloroplast oxidase with no established function. *Plant Physiology*, **72**: 659–665.

Xing, G., Romanyukha, A. and Bunger, R. 2008. Reactive oxygen species (ROS) in human breast cancer cell lines differing in malignancy, an electron paramagnetic resonance (EPR) Study. *FASEB J*, **22**, 79410.

MS Received: 11 November 2022
MS Accepted : 19 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 144-152 (2022)

DOI Number: 10.5958/0974-4541.2022.00053.4

Survey, characterization and management of leaf blight of Chrysanthemum caused by *Alternaria alternata* (Fries) Keissler

H. G. SUJATHA[1], B. ANJANEYA REDDY[2], R. K. RAMACHANDRA[3] and G.K. SEETHARAMU[4]

[1,4]College of Horticulture, [2] Regional Horticultural Research and Extension Center, University of Horticultural Sciences, GKVK, Bengaluru 560 065, Karnataka, India

[3]Horticulture Research and Extension Center, Hogalagere, Srinivasapura 563138, Karnataka, India

***E-mail**: arb_agri@yahoo.co.in, ajreddyb007@gmail.com

ABSTRACT: Leaf blight caused by *Alternaria alternata* is one of the most important foliar diseases hindering the cultivation of chrysanthemum as it infects both leaves and flowers. The survey conducted in eastern dry zone of Karnataka, India revealed highest disease severity at Chikkanahalli in Tumakuru district and the least disease severity (8%) at Mavahalli in Kolar district. The pathogen produced conidia which are typically muriform, dark brown, thick walled. The conidium was up to 33.93 – 57.42 µm long and 10.44 – 18.27 µm wide with 6 - 7 transverse septa and 1 - 3 longitudinal septa. (Majority of conidia are non-beaked few with short rudimentary dark brown beaks, with a range of 13.05 - 26.10 µm length). Molecular confirmation of the causal organism was done through PCR amplification and sequencing of ITS region. *In-vitro* evaluation of fungicides revealed, among contact, systemic and combi-products tested copper oxy chloride 50%WP, hexaconazole 5% SC and Zineb 68% + hexaconazole 4% WP and tricyclazole 18% + mancozeb 62% WP respectively were effectively inhibited the pathogen. *In-vivo* evaluation of fungicides revealed, foliar application of copper oxy chloride 50% WP @ 0.3 per cent proved to be highly effective in arresting spread of the disease.

Keywords: Chrysanthemum, *Alternaria alternata*, leaf blight, ITS (Internal transcribed Spacer), characterization

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflora* Ramat.) is a multi-use flower crop belonging to Asteraceae family and gaining more popularity as a cut flower, loose flowers and pot plant. Chrysanthemum is commonly known as Queen of East produces very attractive flowers of different shape, size and colours. It is an important commercial flower next to rose in the international florist's trade and grown throughout the world (Kher, 1990). In Karnataka, it is being cultivated extensively in Bengaluru Urban, Bengaluru Rural, Mysore, Tumkur, Kolar, Chikkaballapur, Dharwad and Belgaum districts with an area of 4978 ha with 6006 metric tonnes (MT) of production and with an average productivity of 11.60 metric tonnes per hectare (Anon., 2015).

Chrysanthemum flowers are mainly used as loose flowers in garland making and cut flowers for bouquet making. The crop is cultivated both in open field and green houses. Chrysanthemum is prone to infection by several pathogens including fungi, bacteria, virus, viroid and nematode, which cause damage to roots, stem, leaves and flowers. The different fungal diseases are leaf spot (*Alternaria alternata*), white rust (*Puccinia horiana*), Dry rot/ Crown rot (*Rhizoctoni solani*), wilt (*Verticilium* spp., *Fusarium oxysporum*), root rot (*Pythium* spp., *Phytophthora* spp.), leaf spot (*Septoria chrysanthemella*), gray mould (*Botrytis cineria*), black rot (*Ascochyta* spp.), stem rot (*Fusarium solani*), powdery mildew (*Spacelotheca* spp.). Bacterial diseases *viz*., bacterial leaf spot (*Pseudomonas cichori*), crown galls tumors (*Agrobacterium tumefaciens*) and viral diseases like mosaic and stunt (Pradeep kumar *et al*., 2008) are the few common diseases.

Among these diseases, the chrysanthemum blight caused by *Alternaria alternata* (Fries.) Keissler has been found to be the most important disease, adversely affecting quality and yield loss upto 80 to 90 per cent in field as well as in market conditions (Kumar, 2008). Since there are no sources of resistance available for the disease management, farmers are largely depended on use of fungicides to manage this disease. Looking in to these bottle necks and also to tackle the problem of fungicidal resistance the present investigation was undertaken to identify the new effective fungicides, which derive maximum benefit to the farmers.

MATERIALS AND METHODS

Survey for leaf blight of chrysanthemum in major growing areas in the eastern dry zone of Karnataka

Roving survey was conducted in the chrysanthemum growing areas of Kolar, Chikkaballapur, Tumkur and Bengaluru Rural districts of Karnataka during

September, 2018 to January, 2019. For field survey, the places were selected spreading across different districts mentioned (Table 1). During survey the disease severity was estimated by recording symptom severity using 0-5 scale (Mridha *et al*., 2007; Ghosh *et al*., 2009) in plants in the field, where ten plants were randomly selected in $10m^2$ area and five such areas were selected for one acre crop. Where, 0 - No disease symptoms, 1 - A few spots towards tip covering 10 per cent leaf area, 2 - Several dark brown patches covering upto 20 per cent leaf area, 3 - Several patches with paler outer zone covering upto 40 per cent leaf area, 4 - Leaf blight covering upto 75 per cent leaf area or breaking of the leaves from centre, 5 - Complete drying of the leaves or breaking of the leaves from centre.

The recorded grade values were converted into Per cent Disease Index (PDI) by using following formula proposed by Wheeler (1969).

$$PDI = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings x maximum disease grade recorded}} \times 100$$

During survey, the information on the following aspects was recorded namely, District, taluk, village, farmer's name, crop stage, name of the cultivar, and soil type, open or polyhouse conditions, the other pests and diseases noticed and plant protection measures followed.

Isolation of the pathogen

The samples brought from the field, showing typical symptoms of leaf blight disease were subjected for dissection and microscopic observation and the pathogens were identified morphologically. The pathogen that occured repeatedly in higher number of fields surveyed was tried to cultured on PDA medium. The standard tissue isolation procedure was followed to isolate the pathogen. The infected leaf bits were surface sterilized with 1:1000 sodium hypochlorite solution for 30 seconds and repeatedly washed separately in sterilized distilled water to remove the traces of chemical solution if any and then transferred to sterilized petri plates (1-2 leaf bits per Petri dish) containing potato dextrose agar (PDA).

The petri plates were incubated at room temperature (27±1°C) and observed periodically for the growth of the fungus. Bit of fungal growth developed from the infected tissue was transferred to PDA slants and incubated at 27°±1°C for 12 days. Then such slants with pure culture were used for further studies.

Identification and characterization of the pathogen

Identification of the fungus was made after examining about one hundred conidia under microscope (under 10x) from mature pure culture of the fungus obtained from infected leaves of chrysanthemum. To study the morphological characters, stage and ocular micrometry were used to measure the length and breadth of conidium and beak length and septal number. These observations were compared with those of the standard measurements given by Ellis (1971) to identify the pathogen.

Molecular characterization

Isolation of total genomic DNA and amplification

Total DNA was isolated from seven days old pathogen culture grown on potato dextrose broth under continuous agitation at 26 °C using Cetyl-Trimethyl Ammonium Bromide (CTAB) method (Kajal *et al*., 2018). Amplification was done using ITS_1 (5'- TCCGTAGGTGAACCTGCGG -3') and ITS_4 (5'- TCCTCCGCTTATTGATATGC -3') primers with denaturation at 94°C for 6 min., annealing temperature of 55°C for 1 min. and final extension at 72°C for 30 min. The amplified PCR product was cut from the gel and purified using minicolumn, purification resin and buffer, according to the manufacturer's protocols (High Pure PCR Product Purification Kit; Roche Mannheim, Germany). The purified amplicon was sent for sequencing along with details (host name, primers used and quantity of product).

Phylogenitic analysis

The sequences so obtained were used for NCBI – Blast analysis and using Bioedit, ClustalW and phylogenetic relationship of *A.alternata* was established. The dendrogram was constructed using MEGA–X (https;// www.megasofware.net) after alignment of sequences through Clustal - W.

***In vitro* evaluation of fungicides**

The efficacy of contact, systemic and combi product fungicides against blight pathogen were assessed by poisoned food technique. Required quantities of individual fungicides were added separately into molten and cooled potato dextrose agar so as to get the desired concentration of the fungicides. Later 20 ml of the poisoned medium was poured into sterile petri plates. Mycelial discs of 5 mm size from actively growing culture of the fungus were cut out by a sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicides to the medium. Each treatment of contact fungicide and

combi products was replicated thrice while the systemic fungicides with five replications. Then such plates were incubated at room temperature for eight days and radial colony growth was measured. The efficacy of a fungicide was expressed as radial growth of mycelium over control and per cent inhibition of mycelial growth over control was calculated by using the formula given by Mridha *et al.* (2007). The growth values were subjected to square root transformations and the data were analyzed statistically.

***In vivo* evaluation of fungicides**

The field experiment was laid out during *kharif / rabi* 2018-19 in Randomized Block Design (RBD) with three replications under natural conditions. Totally three sprays were given at 15 days interval, starting from the initiation of the disease. The observations on leaf blight disease were recorded before each spray, where six plants were selected and labeled from each treatment and a 0-5 scale (Mridha *et al*., 2007; Ghosh *et al*., 2009) was used for recording the disease severity of leaf blight (Table1). The Per cent disease index (PDI) was calculated by using the formula of Wheeler (1969).

RESULTS AND DISCUSSION

Distribution of the disease: The roving survey carried out during *rabi* 2018-19 in four major chrysanthemum growing districts in Eastern dry zone of Karnataka *viz*., Kolar, Chikkaballapur, Tumakuru and Bengaluru rural revealed that the *Alternaria* leaf blight disease was severe in all the surveyed districts and disease severity ranged from 8.00 to 86.00 per cent. The highest severity (86%) of *Alternaria* leaf blight was noticed in the fields of Chikkanahalli village in Tumakuru district, whereas least disease severity (8%) was recorded at Mavahalli village in Kolar district (Table 1). The highest district average of the per cent disease index (PDI) was recorded in Tumakuru (45.66%) followed by Kolar (42.66%), Chikkaballapur (40.33%) and the least PDI was recorded at Bengaluru rural (38%). The variation in the disease severity in different districts may be due to variation in cultural practices, distribution of the rainfall and plant protection measures followed. Kolte, (1984) reported that higher rainfall and relative humidity was reported to cause severe epidemics of *Alternaria* blight of sunflower. Continuous cultivation of any crop over the season and years build up inoculum level to such an extent that the epidemics become a common phenomenon (Kumar, 2008).

The symptoms of *Alternaria alternata* observed in all growing areas infecting on aerial parts of the plant and produced symptoms on foliar parts as minute brown circular spots, which enlarge at later stages of infection (Plate 1).

Morphological features of *Alternaria*

In culture, the fungal growth was initially white, cottony with profuse aerial mycelium which gradually turned greenish brown. Aged culture appeared completely black with no aerial mycelium. Conidia were observed to measuring about 33.93 – 57.42 µm long and 10.44 – 18.27 µm wide. Conidia are typically muriform, dark brown, thick walled. Majority of the conidia were non-beaked few with short rudimentary dark brown beaks, with a range of 13.05 - 26.10 µm length, conidia had 6 - 7 transverse septa and 1 - 3 longitudinal septa (Table 2 and Plate 2). Based on the characters of the colony

Symptoms on leaves

Symptoms on flowers

Plate 1: Symptoms of blight caused by *Alternaria alternata* in chrysanthemum

Table 1. Per cent disease index of leaf blight of chrysanthemum in different parts of Eastern dry zone of Karnataka during *rabi* 2018-19

District	Taluk	Village	Variety grown	Stage of crop (DAP)	Per cent disease index (PDI)		Other disease
					Alternata leaf blight	Septoria leaf blight	
Kolar	Bangarpet	Nayakarahalli	Poornima white	115	74.00		-
		Madamangala	Poornima white	80	50.00	36.00	White rust
		Nernahalli	Green valley marigold	98	66.00	16.00	White rust
	Kolar	Mavahalli	Green valley marigold	28	8.00	-	-
		Malandlahalli	Poornima yellow	57	36.00	10.00	White rust
		Mattikunte	Poornima yellow	62	40.00	10.00	-
	Mulbagal	Arati	Poornima white	45	22.00	-	-
		Soorakunte	Green valley marigold	74	48.00	18.00	-
		Gummakullu	Green valley marigold	58	40.00	24.00	-
Average PDI					42.66	19.00	
Chikkaballapur	Gudibande	Bogenahalli	Green valley marigold	50	26.00	-	-
	Chikkaballapur	Hosahudya	Green valley marigold	65	58.00	32.00	-
		Giddaganahalli	Green valley marigold	48	18.00	-	-
		Kurappalli	Green valley marigold	55	28.00	-	-
	Chintamani	Vaddahalli	Green valley marigold	60	32.00	-	-
		Barlahalli	Poornima white	105	66.00	20.00	White rust
Average PDI					40.33	26.00	
Bengaluru rural	Devanahalli	Lalagondahalli	Poornima white	72	40.00	-	-
		Hosakurubarakunte	Poornima white	68	48.00	-	White rust
		Koramangala	Green valley marigold	50	26.00	-	-
Average PDI					38.00	0.00	
Tumkur	Sira	Bargur	Green valley marigold	60	28.00	-	-
	Gubbi	Bukkapatna	Green valley marigold	60	22.00	-	-
		Chikkanahalli	Green valley marigold	125	86.00	14.00	White rust
		Chelur	Poornima white	102	64.00	22.00	White rust
		Hosakere	Green valley marigold	58	30.00	-	-
		Hagalvadi	Green valley marigold	60	44.00	-	-
Average PDI					45.66	18.00	

*DAP- Days after planting

and morphological characters of conidia, the fungus was identified as *Alternaria alternata*. Conidia varied from 22.75 to 63.70 µm in length and 13.65-18.20 µm in width. Conidia had 2-3 transverse septa and usually several longitudinal septa (Ellis, 1971). Basim *et al*. (2017) stated that colonies of *Alternaria* isolates were white-grey mycelium with the mixture of green and dark brown conidia in chains ranged from 7 to 45.9 µm in length.

Table 2. Morphology of *Alternaria alternata* causing leaf blight of chrysanthemum

Conidia		Beak length(µm)	Conidia	Color	No. of septations	
Length (µm)	Width(µm)				Cross	Transverse
33.93-57.42	10.44-18.27	13.05- 26.10	Muriform	Dark brown	6-7	1-3

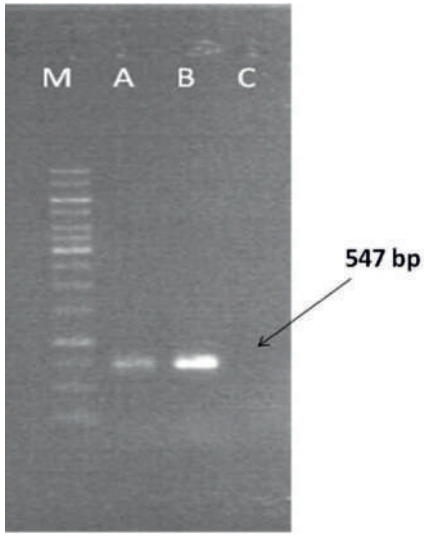


Plate 2. The Agarose gel showing the PCR product of *Alternaria alternata* (Fr.) Keissler M – 1000bp marker; A & B - 547bp amplicons, C- Control

Molecular confirmation of the pathogen

The total genomic DNA was isolated and purified. The genomic DNA was amplifiifed using ITS1 and ITS4 primers, which yielded an amplicon size of 547 bp (Plate 3). After amplification and subsequent confirmation, the PCR product was sequenced. The alignment of the sequence was done using Clustal W and dendrogram was constructed using MEGA X (https;//www.megasoftware.net). The sequence analysis and BLAST search results revealed that the sequence showed 99.30 per cent homology with *Alternaria alternate* infecting *Citrus reticulata* (MH879769). The phylogenetic analysis revealed that *Alternaria alternata* under study had close relationship with Gen Bank accessions MH879769 (Pakistan isolate *Alternaria alternata*) and others *viz.,* MK409081 (USA), JQ625589 (India), MF475920 (Portugal) and DQ323694 (Puerto Rico). Similarly, Shamala and Janardhana (2015) confirmed *A. alternata* causing leaf blight of chrysanthemum using the ITS primers, where they could get an amplicon size of 550bp and subsequent BLAST analysis revealed 99% homology with other isolates.

Effect of fungicides against leaf blight of Chrysanthemum

In vitro* evaluation of fungicides against *A. alternata

Four contact fungicides and three combi products were evaluated at four concentrations i.e. 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm for their efficacy to suppress mycelial growth of *A. alternata* on potato dextrose agar amended with the prescribed concentrations of fungicides by poisoned food technique. All the three combi product fungicides tested have significantly inhibited the mycelial growth of *Alternaria alternata*. The T_5- carbendazim 12% + mancozeb 63% WP, T_6-Zineb 68% + hexaconazole 4%WP, and T_7-Tricyclazole 18% + mancozeb 62%WP were effective at all the concentrations tested (500, 1000, 1500 and 2000 ppm) when compared with contact fungicides but at 1500 ppm and above was highly effective resulting in 100 per cent inhibition of mycelial growth in all the three products tested. Among these three T_6-Zineb 68% + hexaconazole 4%WP, and T_7-Tricyclazole 18% + mancozeb 62%WP were able to give 100 per cent inhibition even at 500 ppm (Table 3).

Among the contact fungicides tested, T_4- Copper oxy chloride 50%WP was highly effective at all the concentrations, with least mycelial growth (6.67 mm) and 92.16 per cent inhibition followed by T_3-Propineb 70% WP with 30.33 mm growth and 63.31 per cent inhibition at 2000 ppm (Table 3). Least inhibition was found with T_2-Chlorothalonil 75%WP (43.14%) with 48.33 mm growth. Similar trend was observed at all the other concentrations tested. Similarly, Shamala and Janardhana

Table 3. *In vitro* evaluation of contact fungicides and combi-products against *Alternaria alternata*

Treatment details	Mycelial growth (mm)							
	Concentration							
	500 ppm	Per cent inhibition	1000 ppm	Per cent inhibition	1500 ppm	Per cent inhibition	2000 ppm	Per cent Inhibition
T_1 Mancozeb 75%WP	76.00 (8.75)	10.59	57.00 (7.58)	32.94	41.33 (6.47)	51.37	37.67 (6.18)	55.69
T_2 Chlorothalonil 75%WP	65.00 (8.09)	23.53	51.67 (7.22)	39.22	50.33 (7.13)	40.78	48.33 (6.99)	43.14
T_3 Propineb70%WP	54.00 (7.38)	36.47	36.33 (6.07)	57.25	31.00 (5.61)	63.53	30.33 (5.55)	64.31
T_4 Copper oxychloride 50%WP	21.33 (4.67)	74.90	13.00 (3.67)	84.71	9.33 (3.14)	89.02	6.67 (2.68)	92.16
T_5 Carbendazim 12% + Mancozeb 63%WP	61.33 (7.86)	27.84	46.33 (6.84)	45.49	0.00 (0.71)	100.00	0.00 (0.71)	100.00
T_6 Zineb 68% + Hexaconazole 4% WP	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00
T_7 Tricyclazole 18%+ Mancozeb 62%WP	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00
T_8 Control	85.00 (9.25)	0.00	85.00 (9.25)	0.00	85.00 (9.25)	0.00	85.00 (9.25)	0.00
S.Em±	**1.14**		**2.59**		**0.72**		**0.67**	
CD@ 1%	**4.82**		**10.93**		**3.06**		**2.86**	

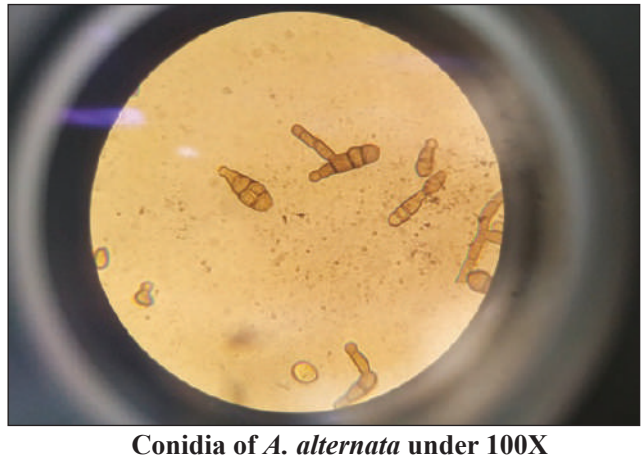
***Values in parenthesis are square root transformed.**

(2015) reported the efficacy of Carbendazim+Mancozeb (2.0%) with 95.65 per cent inhibition followed by Carbendazim at 0.25 per cent concentration with 32.17 per cent inhibition against *Alternaria alternata* of chrysanthemum.

In vitro evaluation of systemic fungicides against *A. alternata*

Three systemic fungicides along with a control were evaluated at four concentrations i.e. 250 ppm, 500 ppm, 750 ppm and 1000 ppm for their efficacy towards inhibition of the mycelial growth of *A. alternata* on potato dextrose agar amended with their prescribed concentrations of fungicides by the poisoned food technique and replicated five times. All the systemic fungicides tested significantly inhibited the mycelia growth of *Alternaria alternata* (Table 5). T_1-Hexaconazole 5% SC and T_3-Tricyclazole 75% WP were very effective at all the concentrations tested and statistically superior over T_2-Azoxystrobin 23% SC (34.40 mm) at 1000 ppm. Devaraja (2011) reported the efficacy of systemic fungicies *viz*., hexaconazole, propiconazole, difenoconazole and penconazole at 0.1 per cent concentrations completely inhibited the mycelial growth of *A. alternata.*

Culture of *A. alternata*



Conidia of *A. alternata* under 100X

Plate 3. Photographs showing culture and conidia of *A. alternata*(Fr.) Keissler

Table 4. *In vitro* evaluation of systemic fungicides against *Alternaria alternata*

Treatment No.	Treatment details	Mycelial growth (mm) Concentration							
		250 ppm	Per cent inhibition	500 ppm	Per cent inhibition	750 ppm	Per cent inhibition	1000 ppm	Per cent inhibition
T_1	Hexaconazole 5%SC	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00
T_2	Azoxystrobin 23% SC	54.20 (7.40)	35.63	48.00 (6.96)	42.99	45.20 (6.76)	46.32	34.40 (5.89)	59.14
T_3	Tricyclazole75%WP	67.80 (8.24)	19.48	23.80 (4.93)	71.73	0.00 (0.71)	100.00	0.00 (0.71)	100.00
T_4	Control	84.20 (9.20)	0.00	84.20 (9.20)	0.00	84.20 (9.20)	0.00	84.20 (9.20)	0.00
	S.Em±	**2.68**		**0.90**		**0.69**		**1.27**	
	CD@ 1%	**11.61**		**3.92**		**3.01**		**5.49**	

***Values in parenthesis are square root transformed.**

In vivo* evaluation of fungicides and a bioagent against *A. alternata

The experiment was conducted to evaluate the relative efficacy of different fungicides and a bioagent as foliar spray against Alternaria leaf blight of chrysanthemum. The experiment was conducted during *kharif/rabi* season of 2018-19 with ten fungicides and one bioagent. Totally three sprays were given at 15 days interval starting from the onset of the disease (40 days after planting). The observations on Alternaria leaf blight was recorded before each spray. Using 0-5 disease scale and converted into per cent disease index (PDI) using the formula given by Wheeler (1969).

From the data it is evident that the T_3- Copper oxy chloride@ 0.3 per cent was very effective in controlling the disease (41.79% disease suppression) with 43.33 per cent disease index and effective in increasing the yield (12.71 q/h) followed by T_5-Zineb 68% + hexaconazole 4%WP (35.82 % disease suppression) with 47.78 per cent disease index and yield of 12.49 q/ha (Table 6). Least disease control of 7.46 per cent was noticed in T_{11}- *Pseudomonas fluorescens* with 68.89 per cent disease index and less yield (5.97 q/ha) and was followed by T_2 – Chlorothalonil @ 0.2 per cent with the PDI of 65.56 and disease suppression of 11.94 per cent with an yield of 6.56 q/ha. The efficacy of Copper oxy chloride @ 0.3 per cent and Chlorothalonil @ 0.2 per cent was well documented by earlier workers, Kamanna *et al*., (2010) and Gangawane (2011). The new product, hexaconazole+zineb @ 0.1% can be used as an alternative to manage the resistance development in the intensive crop production system.

CONCLUSION

The causal organism of leaf blight of Chrysanthemum was found to be *Alternaria alternata* and the highest disease severity was at Chikkanahalli in Tumakuru district and the least disease severity (8%) was recorded at Mavahalli in Kolar district in eastern dry zone of Karnataka. The pathogen produced conidia which are typically muriform, dark brown, thick walled. Majority of conidia are non-beaked few with short rudimentary dark brown beaks. The conidiaum varied in length from 33.93 to 57.42 µm and 10.44 to 18.23 µm. Molecular confirmation of the causal organism was done through PCR amplification and sequencing of ITS region. *In-vitro* evaluation fungicides revealed that, among contact, systemic and combi-products tested Copper oxy chloride 50%WP, Hexaconazole 5% SC and Zineb 68% + hexaconazole 4%WP and Tricyclazole 18% + mancozeb 62%WP respectively were effectively inhibited the pathogen. *In-vivo* evaluation of fungicides revealed, foliar application of Copper oxy chloride 50%WP @ 0.3 per cent and Zineb 68% + hexaconazole 4%WP proved to be highly effective in arresting spread of the disease.

Table 5. *In-vivo* efficacy of different fungicides and a bioagent against *Alternaria* leaf blight of chrysanthemum during *rabi* 2018-19

Treatment No.	Treatment details	Per cent disease index				Per cent disease control After 3rd spray	Yield (q/ha)
		Before spray	After 1st spray	After 2nd spray	After 3rd spray		
T_1	Hexaconazole (0.1%)	43.33 (41.07)*	37.78 (37.71)	50.00 (33.51)	53.33 (46.94)	28.35	7.87
T_2	Chlorothalonil (0.2%)	44.44 (41.75)	44.44 (41.80)	55.56 (36.90)	65.56 (54.14)	11.94	6.56
T_3	Copper oxy chloride (0.3%)	41.11 (39.56)	34.44 (34.35)	45.56 (32.29)	43.33 (41.10)	41.79	12.71
T_4	Mancozeb (0.25%)	25.56 (30.24)	38.89 (38.46)	44.44 (33.21)	54.44 (47.61)	26.86	7.29
T_5	Zineb + Hexaconazole (0.2%)	18.89 (25.62)	34.44 (35.86)	43.33 (35.47)	47.78 (43.70)	35.82	12.49
T_6	Azoxystrobin (0.1%)	36.67 (36.91)	35.56 (36.51)	54.44 (43.49)	52.22 (46.28)	29.85	8.02
T_7	Tricyclazole + Mancozeb (0.2%)	33.33 (35.00)	48.89 (44.36)	45.56 (37.07)	52.22 (46.28)	29.85	9.52
T_8	Carbendazim + Mancozeb (0.2%)	38.89 (38.34)	37.78 (37.14)	51.11 (37.65)	53.33 (47.08)	28.35	7.83
T_9	Tricyclazole (0.1%)	43.33 (41.14)	48.89 (44.36)	54.44 (41.71)	62.22 (52.09)	16.41	6.88
T_{10}	Propineb (0.2%)	44.44 (41.75)	52.22 (46.33)	54.44 (43.68)	61.11 (51.65)	17.91	6.96
T_{11}	*Pseudomonas fluorescens* (1.0%)	28.89 (32.41)	51.11 (45.64)	55.56 (45.07)	68.89 (56.47)	7.46	5.97
T_{12}	Control	41.11 (39.87)	50.00 (44.95)	61.11 (48.25)	74.44 (59.76)	0.00	5.20
	S.Em±	**5.202**	**4.947**	**3.987**	**6.65**		**2.99**
	CD@ 0.05%	**15.25**	**14.51**	**15.89**	**19.51**		**8.78**

*Values in parenthesis are arcsine transformed

REFERENCES

Anonymous. 2015. Indian Horticulture Database (2015-16), www.nhb.gov.in/areapro/database.

Basim, E., Basim, H., Abdulai, M., Baki, D. and Ozturk, N. 2017. Identification and characterization of *Alternaria alternata* causing leaf spot of olive tree (*Olea curopaea*) in Turkey. *Crop Protection*, **92**: 79-88.

Broerties, C., Koene, P. and Van Heen J. W. H. 1980. Chrysanthemum *Euphytica*, **29**: 523-530.

Devaraja, O. 2011. Investigations on chrysanthemum leaf blight caused by *Alternaria alternata* (Fr.) Keissler. *M.Sc. (Agri.) Thesis*, University of Agricultural Sciences, Dharwad (India).

Ellis, M. B. 1971. *Dematiaceous hyphomycetes*. *Commonwealth. Mycology Institute*, Kew, Surrey, England. p. 608.

Ghosh, P. P., Mandal, D., Laha, S. and Dasgupta, M. K. 2009. Dynamics and severity model in managing fungal diseases. *Journal of Plant Protection Sci*ence, **1**(1): 55-59.

Kajal, J., Ekta, B., Someshree, M., Divya, J. and Ashwini, K. 2018. Molecular characterization of *Alternaria alternata* causing fruit rot of chilli through RAPD marker. *International Journal of Current Microbiology and Applied Science*, **7(**03): 292-298.

Kher, M. A. 1990. Chrysanthemum "Queen of the East'. Indian Horticulture, **35** (1): 10-13.

Kolte, S. J. 1984. Diseases of annual edible oilseed crops. Vo.-III, CRC Press, Florida, pp. 996.

Kumar, A. G. S. 2008. Studies on leaf blight of chrysanthemum caused by *Alternaria alternata* (Fr.) Keissler. *M.sc. Thesis,* University of Agricultural Sciences, Dharwad (India).

Mridha, M. A. U., Jabbar, F., Bhuiyan, M. K., Rahman, M., Akter, F. and Dewan, S. 2007. The severity and cause of leaf spot disease of *Pongamia pinnata* L. and fungicidal control of the pathogen. *Journal of Forestry Research*, **18** (3): 236-240.

Pradeep Kumar, T., Suma, B., Jyotibhaskar and Satheesan, K. N. 2008. Management of horticultural crops. Published by IARI, New Delhi. 440.

Shamala, G., Janardhana, G. R. 2015. Prevalence and severity of leaf blight disease of chrysanthemum in southern regions of Karnataka and *in-vitro* fungicidal effect on *Alternaria alternata. International Journal of Life Science,* **9** (3): 38-45.

Wheeler, B. E. J. 1969. An introduction to plant diseases. John Wiley and Sons Ltd., London.

MS Received: 21 July 2022
MS Accepted : 04 October 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 153-159 (2022)

DOI Number: 10.5958/0974-4541.2022.00054.6

Screening of betel vine inter-specific hybrids for resistance to *Phytophthora nicotianae*

G. P. MOHANKUMAR[1,2*], K. HIMA BINDU[2], S. SRIRAM[3], SUBHASH SRI SANJAY MALEMPATI[4]

[1]Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Horticulture, UHS Campus, Bengaluru-560065, India

[2]Division of Flower and Medicinal Crops, [3]Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bengaluru- 560089, India

[4]Department of Plant Pathology, JNKVV, Jabalpur- 482004, India

***E-mail**: mohangp2794@yahoo.com

ABSTRACT: An experiment was conducted to standardize the isolation and screening protocol *Phytophthora nicotianae* on betel vine to identify the resistance source. The artificial inoculation was carried out by detached leaf assay (pin prick method) and drenching with spore suspension for the root. The result showed that carrot agar was suitable for active culture subsistence, and the pin prick method was the best method for inoculation and screening. Among the inter-specific hybrids used in the present investigation, IIHRPBIH9 recorded significant resistance to Phytophthora foot rot compared to the susceptible check IIHRBV170. Thus, screening of inter-specific hybrids for this disease through artificial inoculation helps to identify resistance sources for the breeding programme, thereby meeting the demand for superior quality, disease-free planting materials and effective disease management. This is the first report for identifying the resistance source in betel vine against *P. nicotianae*.

Keywords: Betel vine, Inter-specific hybrids, *Phytophthora*, Resistance, Screening

INTRODUCTION

Betel vine, *Piper betle* L. (F: Piperaceae), is a dioecious, perennial creeper that originated from Central and Eastern Malaysia. In India, it is considered an important cash crop and stands top in the production of betel leaves in the world (Arulmozhiyan *et al.,* 2005), with an estimated area and production of 53,539 ha and 57,530 MT, respectively (Anon., 2019). Overall the country has exported 6158.39 MT of betel vine leaves worth 26.18 crores (Anon., 2020). Betel vine is considered a highly labour-intensive crop estimated to employ about 20.80 lakh people in the country throughout the year (Das *et al.,* 2017). The presence of eugenol, chavibetol, caryophyllene, and methyl eugenol gives the leaves their antioxidant, anti-apoptotic, anti-inflammatory, anti-cancerous, and anti-microbial characteristics, in addition to their chewing benefits.

The crop is highly susceptible to diseases and pests (Rahman *et al.,* 2020; Javaregowda, 2006). Foot rot is the major disease caused by *Phytophthora nicotianae*, which results in the complete death of the plant. The pathogen attacks the plant at the collar region and below the soil. The characteristic symptom of the disease is the disappearance of the lustre of leaves, followed by wilting and drooping of the vine. Meanwhile, underground parts of the plant rot completely. The extent of losses may vary from 5 to 90 per cent due to foot rot (Dasgupta *et al.,* 2008) and 20 to 40 per cent in case of leaf rot, leading to total crop failure (Dasgupta *et al.,* 2000).

Betel vine is a vegetatively propagated crop; hence it is easy to fix a favourable combination of important traits, unique chemotypes and morphotypes (Glemin *et al.,* 2006). Unfortunately, only some countable efforts have been made till now in identifying resistant sources or developing resistant hybrids. *Piper colubrinum* is a distant relative of the cultivated betel vine, showing high resistance to Phytophthora foot rot. At ICAR-Indian Institute of Horticultural Research, interspecific hybrids have been developed by crossing Phytophthora resistant species *Piper colubrinum* and *Piper betle* to develop resistant sources for this devastating disease (Turner, 1969; Purseglove, 1981). The standardization of *in-vitro* screening methods and protocols for foot rot (*Phytophthora nicotianae*) is important to understand the resistance mechanism and host-pathogen interaction, which aids in the identification of resistant sources for future crop development and disease management. In this view, the present study was focused on standardizing the protocol and screening of 13 interspecific hybrids through artificial inoculation for resistance to foot rot in betel vine.

MATERIALS AND METHODS

Plant material

Interspecific hybrids (Table 1) developed at the Division of Flower, and Medicinal Crops, ICAR-IIHR, Bengaluru, were maintained under polyhouse. The betel

vine stem cuttings collected from the selected mother vine were prepared by giving a slant cut at the proximal end and a horizontal cut at the distal end. A duly punched nursery polybags were filled with soil, sand and FYM in a 2:1:1 ratio, respectively. The cuttings were planted in nursery polybags of 200 gauge and size 4" × 6" for effective growth of cuttings. Stem cuttings with 3-5 nodes were used for propagation and planted so that one to two nodes were buried in nursery media and one node was kept above nursery media. The bags were then kept in polytunnels for 15 days to induce sprouting. After 30-45 days, rooted plants were shifted to pots filled with nursery mixture. Nursery management practices, *viz.,* regular watering, weeding and plant protection, were taken up periodically.

Table 1. List of Inter-specific hybrids used for screening of Phytophthora foot rot disease Reaction in betel vine

IIHR/Acc. No	Inter-specific hybrids
IIHRPBIH1	IIHRBV53/ *Piper colubrinum*
IIHRPBIH2	IIHRBV53/ *Piper colubrinum*
IIHRPBIH3	IIHRBV53/ *Piper colubrinum*
IIHRPBIH4	IIHRBV53/ *Piper colubrinum*
IIHRPBIH5	IIHRBV53/ *Piper colubrinum*
IIHRPBIH6	IIHRBV53/ *Piper colubrinum*
IIHRPBIH8	IIHRBV42/ *Piper colubrinum*
IIHRPBIH9	IIHRBV42/ *Piper colubrinum*
IIHRPBIH13	IIHRBV53/ *Piper colubrinum*
IIHRPBIH18	IIHRBV42/ *Piper colubrinum*
IIHRPBIH19	IIHRBV53/ *Piper colubrinum*
IIHRPBIH21	IIHRBV96/ *Piper colubrinum*
IIHRPBIH22	IIHRBV68/ *Piper colubrinum*
IIHRBV170*	Meetapan

*Check variety

Standardization of different media

Growth characters of *Phytophthora nicotianae* were studied on three semi-synthetic solid media *viz*., potato dextrose agar (PDA), V-8 Juice agar and carrot agar (CA). All the media were sterilized for 15 minutes at 121.6 ^{0}C of pressure 1.05 kg cm^{-2}. Further, growth was recorded on the Petri plate.

Fungal isolate for inoculation

Direct tissue isolation and isolation from leaf samples technique was employed to isolate wilt associated pathogens from betel vine showing typical symptoms of foot rot. In direct tissue isolation, the samples were collected from the field where the incidence of foot rot disease was high. Subsequently, the root samples were washed and treated with 0.1 per cent sodium hypochlorite, followed by drying, sectioning of root samples and plating onto PDA, CA, and V-8 juice agar medium and then incubated at 24 ± 2 0C for 07-10 days. In case of isolation from leaf samples, the infected leaf portion showing actively progressing lesions was cut into small pieces and sterilized with 0.1 per cent sodium hypochlorite, washed with distilled water and placed aseptically in sterile Petri plates containing medium and incubated at 24 ± 2 0C for 07-10 days.

Identification and maintenance of pure culture

Phytophthora culture was identified based on spore morphology and colony characters, referring to the description by Drenth and Sendall (2001). *Phytophthora nicotianae* was maintained on CA medium in the Petri plates by culturing a single bit of previously grown culture by hyphal tip isolation method to obtain a pure culture of a pathogen. The culture plates with pathogens were covered properly and kept at a low temperature (4 0C) to arrest further growth. The pathogen was sub cultured periodically for 10 to 15 days during the investigation.

Molecular identification of the pathogen

The fungal DNA was extracted from five days old cultures grown in culture plates by CTAB method. The mycelium from the culture plate was scraped out with the help of a sterile spatula. Around 0.1 g of sample was placed in a mortar and homogenized with 1 ml of extraction buffer and the homogenate was transferred to a 2 ml-microfuge tube. An equal volume of Phenol: Chloroform: Isoamlyalcohol (25:24:1) was added to the tubes and mixed well by gently shaking the tubes. The tubes were centrifuged at room temperature for 15 minutes at 14,000 rpm. The upper aqueous phase was transferred to a new tube and an equal volume of Chloroform: Isoamlyalcohol (24:1) was added and mixed. The resulting mycelium tissue homogenate was centrifuge at 14,000 rpm for 10 min and supernatant was transferred to a fresh tube. Add 0.1 volume of 3 M Sodium acetate (pH 7.0) and 0.7 volume of Isopropanol to precipitate the DNA from the solution. The tubes were centrifuged at 4 ^{0}C for 15 minutes at 14,000 rpm, after keeping them for incubation at room temperature for 15 minutes. The DNA pellet was washed twice using 70 per cent ethanol and then using 100 per cent ethanol and air

Table 2. Details of primers used for amplification

Oligo name	Sequence (5`and 3`)	Tm (^{0}C)	GC- Content (%)
ITS Forward	TCCGTAGGTGAACCTGCGG	57	63.15
ITS Reverse	TCCTCCGCTTATTGATATGC	53	45.00

Table 3. Percentage disease Index (PDI) of interspecific hybrids

Accession No.	Time Intervals			
	3rd dpi	5th dpi	7th dpi	Mean
IIHRPBIH1	24.45 (29.63)	44.43 (41.80)	71.20 (57.55)	46.70 (42.99)
IIHRPBIH2	28.84 (32.48)	42.25 (40.54)	67.13 (55.02)	46.07 (42.68)
IIHRPBIH3	26.67 (31.09)	54.88 (47.80)	73.23 (58.84)	51.59 (45.91)
IIHRPBIH4	22.23 (28.13)	48.89 (44.35)	57.44 (49.28)	42.85 (40.59)
IIHRPBIH5	28.87 (32.50)	55.47 (48.14)	77.83 (61.91)	54.06 (47.52)
IIHRPBIH6	24.41 (29.60)	57.66 (49.41)	73.40 (58.95)	51.82 (45.99)
IIHRPBIH8	15.59 (23.26)	37.87 (37.98)	53.25 (46.86)	35.57 (36.03)
IIHRPBIH9	0.00 (2.87)	2.25 (8.62)	11.14 (19.50)	4.46 (10.33)
IIHRPBIH13	20.08 (26.62)	42.27 (40.55)	64.43 (53.39)	42.26 (40.19)
IIHRPBIH18	13.15 (21.26)	31.16 (33.93)	42.10 (40.45)	28.80 (31.88)
IIHRPBIH19	22.28 (28.16)	33.28 (35.23)	55.56 (48.21)	37.04 (37.20)
IIHRPBIH21	24.45 (29.63)	46.67 (43.09)	66.26 (54.49)	45.79 (42.41)
IIHRPBIH22	17.79 (24.94)	40.00 (39.23)	62.50 (52.24)	40.10 (38.81)
IIHRBV170	40.00 (39.23)	71.35 (57.64)	97.75 (81.44)	69.70 (59.45)
Mean	22.06 (27.10)	43.46 (40.59)	62.37 (52.73)	
For comparison of mean	**S. Em ±**	**CD @ 5 %**	**CV (%)**	
Treatment (T)	0.62	1.78		
Condition (C)	0.29	0.82	4.46	
Interaction (T × C)	1.09	3.08		

*****Mean of three replication; Figures in the parenthesis are arc sine transformed values

Table 4. Per cent disease infection in interspecific hybrids

Accession No.	Time Intervals*			
	3rd dpi	5th dpi	7th dpi	Mean
IIHRPBIH1	9.85 (18.29)	24.76 (29.84)	16.25 (23.77)	16.95 (23.96)
IIHRPBIH2	1.24 (6.39)	3.93 (11.43)	19.92 (26.50)	8.37 (14.78)
IIHRPBIH3	9.03 (17.49)	26.02 (30.67)	21.19 (27.40)	18.75 (25.19)
IIHRPBIH4	4.29 (11.95)	8.08 (16.52)	18.39 (25.39)	10.25 (17.95)
IIHRPBIH5	7.13 (15.49)	21.91 (27.90)	37.73 (37.89)	22.26 (27.09)
IIHRPBIH6	8.80 (17.26)	28.33 (32.15)	22.22 (28.12)	19.78 (25.85)
IIHRPBIH8	2.11 (8.34)	3.53 (10.81)	18.61 (25.55)	8.08 (14.90)
IIHRPBIH9	0.00 (2.87)	0.16 (2.23)	0.68 (4.69)	0.28 (3.26)
IIHRPBIH13	1.95 (8.01)	4.92 (12.81)	20.59 (26.98)	9.15 (15.94)
IIHRPBIH18	3.25 (10.37)	3.90 (11.39)	10.24 (18.66)	5.80 (13.48)
IIHRPBIH19	3.40 (10.62)	10.64 (19.03)	19.95 (26.53)	11.33 (18.73)
IIHRPBIH21	1.87 (7.87)	7.68 (16.09)	27.94 (31.91)	12.50 (18.62)
IIHRPBIH22	2.47 (9.04)	9.62 (18.07)	24.72 (29.81)	12.27 (18.97)
IIHRBV170	9.32 (17.78)	28.31 (32.14)	64.57 (53.47)	34.07 (34.47)
Mean	4.62 (11.55)	12.99 (19.36)	23.07 (27.62)	
For comparison of mean	**S. Em ±**	**CD @ 5 %**	**CV (%)**	
Treatment (T)	0.19	0.54		
Condition (C)	0.08	0.25	4.24	
Interaction (T × C)	0.33	0.93		

*Mean of three replication; Figures in the parenthesis are arc sine transformed values

dried. The DNA was dissolved in TE (Tris-Cl 10 mM pH 8.0, EDTA 1 mM). To remove RNA, 5 µl of DNAse free RNAse (10 mg/ml) was added to the DNA.

Quantity and quality determination

The quantity of the extracted DNA was determined by measuring the absorbance at 260 nm using a spectrophotometer (Thermo Scientific NanoDrop 1000). The quality of DNA (2 µl) was accessed by subject to 1.5 per cent agarose gel electrophoresis. The DNA band was observed under the UV Trans illuminator gel documentation system. PCR amplification was performed using ITS1 and ITS4 primers to amplify the Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA in a thermal cycler (Table 2). The temperature profiles of 94^{0}C for 3 minutes of initial denaturation, followed by 30 cycles of denaturation at 94 ^{0}C for 3 min, with constant annealing at 50 ^{0}C for 1 minute and extension at 72 ^{0}C for 2 minutes with a final extension at 72 ^{0}C for 7 min (Source: GenBank ON358198).

Experimental design

The pathogenicity test was carried out in the division of crop protection, ICAR-IIHR, Bengaluru. For inoculation of a pathogen, betel vine leaves were used, and leaves were carefully washed with adequate tap water to remove

excess adhering materials before surface sterilization and wiped with 70 per cent ethanol, followed by air drying on the sterile filter papers. After the drying process, the leaves were inoculated with two different inoculation methods, *viz*., detached leaf (pin prick at the centre of the leaf) and spore suspension method (soil drenching and zoospore suspension spray on leaf), and observed for their effectiveness for screening using *Phytophthora nicotianae* with 13 interspecific hybrids of betel vine.

Detached leaf method of inoculation

Mycelium plugs of 8 mm in diameter from 7-day-old culture cultivated at 24± 2 0C were taken from the edge of an actively growing colony and placed on the detached leaf with the mycelial side facing down at the center of the leaves. While performing the pin prick method before inoculation, the leaves were washed with sterilized water and air-dried. The sharp, sterilized stainless steel paper pins were used to prick the leaves. The plug was gently pressed to ensure good contact with the leaf surface. Inoculated leaves were placed on moist blotting paper towels in transparent polystyrene plastic boxes to maintain humidity and incubated at 24± 2 0C. The uninoculated leaves were maintained as a control for comparison with the inoculated leaves. After 3 days of inoculation, disease symptoms were observed, and lesion size was measured at 3, 5 and 7-day intervals.

Spore suspension method

One to two-month-old betel vine plants were used for inoculating pathogens using the spore suspension method. Spores were harvested from the 7-day-old culture of the pathogen. Induction of zoospore release was achieved by flooding the colonies with 10 mL of sterile distilled water, then incubating at 4 ^{0}C for 15 minutes, followed by 30 minutes at room temperature. After chilling, 1 µL drops were viewed under a microscope to count the average zoospore with a hemocytometer. Finally, a concentration of 1.0×10^4 zoospores per mL was obtained, and 10 mL of this suspension was used for soil drenching. Spores were sprayed uniformly on the betel vine leaves using a sprayer, and plants were covered with polythene covers (above 51 microns) to generate humidity which helps with spore germination and infection. After 14 days of inoculation, symptoms were observed and recorded.

After symptom development, re-isolation of the pathogens was done from the artificially infected plants. The inoculated betel vine leaf with typical symptoms was selected for isolation. Thus obtained fungi culture was compared with the original culture for confirmation. These cultures were subsequently used for further investigations and were maintained in slants at 4 ^{0}C.

Microscopic observation and disease assessment

The plate containing culture was incubated under light for 24 to 48 h. The mycelial was taken off, and slides were prepared for microscopic observation for the presence of sporangia. The lesion size development was observed regularly on the detached leaf, inoculated with *Phytophthora nicotianae*. The disease severity was calculated on the seventh day by measuring the area covered by the pathogen by using the 0 to 5 scale as described by (Goswami *et al.,* 1993): 0= infection free or healthy leaf; 1= leaf area covered up to 5 per cent; 2= 6 to 15 per cent leaf area covered; 3= 16 to 30 per cent leaf area covered; 4= 31 to 50 per cent leaf area covered; 5= leaf area covered above 50 per cent. Disease severity scale (0-5) for foot rot disease of betel vine using detached leaf assay method. Thus the individual leaf ratings were recorded, the per cent disease index was calculated using the formula (Wheeler, 1969), and analysis was carried out using WASP-2.

RESULTS AND DISCUSSION

$$PDI = \frac{\text{Sun of all disease scores}}{\text{Number of leave observed} \times \text{Maximum disease score}} \times 100$$

Effect of media and inoculation methods

`In the present study, carrot agar was found to be suitable for the active subsistence of culture. The pin prick method was found to be the best method of detached leaf assay for inoculation and screening of Phytophthora foot rot disease compared to the spore suspension method. The disease system appeared on betel vine leaves 3 days after inoculation with the pin prick method at 24± 2 ^{0}C. However, the disease system appeared on roots by the spore suspension inoculation method only after 14 days. As the spore suspension method is laborious and time-consuming, the pin prick method was considered the best method for screening Phytophthora in betel vine as it is simple and rapid. Phytophthora disease development was observed on a leaf at the 3rd dpi, 5th dpi and 7th dpi. Leaf was incubated in water after 7th dpi, and microscopic observation was recorded accordingly to confirm spore development (Fig 1).

Percentage disease index (PDI)

Among the interspecific hybrids used, IIHRPBIH9 showed significant resistance to Phytophthora with a mean value of 4.46 per cent (Table 3), as IIHRPBIH9 at initial days doesn't develop any symptoms. But on 7 dpi, there was a trace amount of symptoms which was noticed to be 11.14 per cent. The key effectors triggering the resistance were hypersensitive response (HR) and

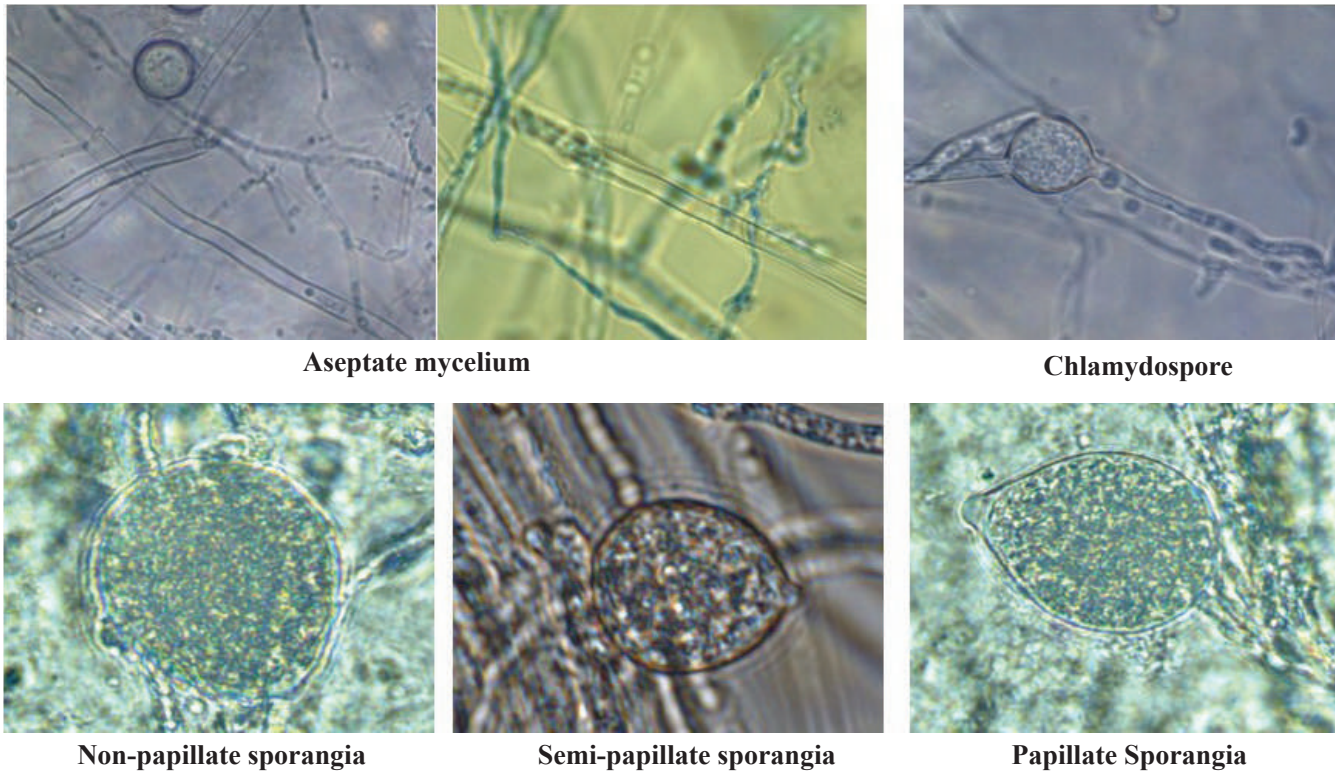


Fig 1. Morphometric observation of *Phytophthora nicotianae*

phenolic component. And it was seen that the necrosis was at a trance amount under hypersensitive defense responses compared to the susceptible check variety (IIHRBV170). The mean value of susceptible check was significantly higher at 69.70 per cent, and it was noticed that there was a gradual increase in the trend of disease development as compared to IIHRPBIH9 interspecific hybrid.

Assessment of disease progress of interspecific hybrids

The detached leaf assay was carried out with artificial inoculation under control conditions in order to check the disease progression. The total infected area of 3rd dpi, 5th dpi and 7th dpi was recorded manually and it was found that the inter-specific hybrid IIHRPBIH9 shows less of severity and the mean area of infection was found to be 0.28 per cent, ranging from 0.00 to 0.68 percent at different days interval (Table 4). Whereas the susceptible check variety (IIHRBV170), the mean area of infection was around 34.07 per cent, ranging from 9.32 to 64.57 per cent. This shows that if the infection is observed at early stages, rotting of leaves is observed and ultimately leads to the death of the plant.

CONCLUSION

The present investigation, inferred that IIHRPBIH9 shows the resistance against Phytophthora foot rot infection as the disease progression was found to be comparatively lesser than the susceptible check IIHRBV170. Screening of inter-specific hybrids for Phytophthora foot rot through artificial inoculation helps to identify resistance sources for breeding programme thereby meeting the demand of superior quality, disease free planting materials and effective management of the disease.

ACKNOWLEDGMENT

The authors are thankful to the Director, ICAR-Indian Institute of Horticultural Research, Bengaluru for providing the necessary research facilities.

REFERENCES

Anonymous, 2019. SHD Statistics, Department of Horticulture, Karnataka, India.

Anonymous, 2020. APEDA Statistics, Agricutural and Processed Foood Products Export Development Authority, Ministry of Commerce and Industry, India.

Arulmozhiyan, R., Chitra, R., Prabhakar, K., Jalaluddin, S. M. and Packiaraj, D. 2005. SGM. BV.2 – A new promising betel vine variety. *Madras Agricultural Journal,* **92:** 498-503.

Das, S., Pandey, A. K. and Kumar, P. 2017. Betel vine Cultivation: A New Avenue for Livelihood Security. *Hortflora Research Spectrum,* **6:**300-303.

Dasgupta, B., Mohanty, B., Dutta, P. K. and Maiti, S. 2008. Phytophthora diseases of betelvine (*Piper betle* L.): A menance to betelvine crop. *SMRC Journal of Agricultural Research*, **6:**71-89.

Dasgupta, B., Roy, J. K. and Chitreshwar, S. 2000. Two major fungal diseases of betel vine. In proceeding of *Diseases of plantation crops, spices, betel vine and mulberry*, 133-137.

Drenth, A. and Sendall, B. 2001. Practical guide to detection and identification of Phytophthora CRC for *Tropical Plant Protection Australia*, **1:** pp.1-42.

Glemin, S., Baszin, E. and Charlesworth, D. 2006. Impact of mating systems on patterns of sequence polymorphism in flowering plants. *Proceedings of the Royal Society Series B-Biological Sciences,* **273:** 3011-3019.

Goswami, B. K., Zahid, M.I. and Haq, M. O. 1993. Screening of *Colocasia esculenta* germplasm to Phytophthora leaf blight. Bangladesh Journal of Plant Pathology, **9:**21-24.

Javaregowda, 2006. Incidence of snail, *Achatina fulica* (Bowdich) in betel vine and its management. *Pest Management in Horticultural Ecosystems*, **12**(1); 41-43.

Purseglove, J. W., Brown, E. G., Green, C. L. and Robbins, S. R. J. 1981. Spices, Longman, London and New York. **1:** pp 1-439.

Rahman, M. H., Islam, M. R., Aminuzzaman, F. M, Latif, A. and Rahman, H. 2020. Survey on foot and root rot disease of betel vine (*Piper betle* L.) under prevailing environmental condition. *The Agriculturists,* **18:** 26-30.

Turner, G. L.1969. *Phytophthora paimivora* from *Piper betle* in Sarawak. *Transactions of the British Mycological Society,* **52:**411-18.

Wheeler, B. E. J. 1969. *An Introduction to Plant Diseases*, John Wiley and Sons Limited, London pp. 301.

MS Received: 15 November 2022
MS Accepted : 24 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 160-166 (2022)

DOI Number: 10.5958/0974-4541.2022.00055.8

Assessment and molecular detection of *Candidatus* Liberibacter *asiaticus* in mandarin orange and acid lime in Tamil Nadu

KONDA SAMEER and VAIKUNTAVASAN PARANIDHARAN*

[1]Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu - 641003, India

***E-mail**: paranidharan.v@tnau.ac.in

ABSTRACT: The Greening disease of citrus, popularly known as Huanglongbing (HLB), has been severe in India since late 1966. It is associated with infection by '*Candidatus* Liberibacter asiaticus,' a heat-sensitive, non-cultured, phloem-limited alpha-proteobacterium. Leaf samples exhibiting greening symptoms were picked up from 26 Mandarin orange trees from each 5 orchards in the Dindigul district and 13 Acid lime trees from each 5 farms of Nilgiris, Dindigul and salem districts of Tamil Nadu. Among ten amplicons were derived from nine samples. Nine samples among them yielded between 1, 100-bp to 1160bp amplicon indicative of '*Ca.* L. asiaticus' infection, the mandarin orange samples are amplified with A2 and J5 primers which showed 690bp amplicon size targeting (rplKAJL-rpoB) Beta operon gene of Las. The amplicons obtained from the samples were sequenced, and all showed approximately 1 160bp, identical to the cognate '*Ca.* L. asiaticus' NCBI GenBank. Based on the survey results, the phylogenetic tree was built and interpreted. It has been concluded that up to date, only '*Ca.* L. asiaticus' is the only strain associated with citrus greening (HLB) in commercial citrus Orchards of Tamil Nadu.

Keywords: HLB, Mandarin orange, Acid lime, *Candidatus* Liberibacter asiaticus, Singleplex PCR

INTRODUCTION

Mandarin orange, *Citrus reticulata* L. and acid lime, *Citrus aurantifolia* (Christm) Swingle (Family: Rutaceae) are the most popular and widely grown citrus fruits worldwide. Citrus production is affected by several biotic stress factors, namely bacteria, fungi, viruses, and nematodes. Among the bacterial diseases, citrus greening disease Huanglongbing disease is the most destructive disease that declines citrus yield in India and other countries of Asia, the Pacific, and Africa (Ahlawat, 1997; Bove, 2006; Lee, 1921; Mccleane*t al*., 1965). The symptoms of HLB on the leaves of infected citrus trees range from complete yellowing, asymmetrical blotchy mottling, and fruits with these symptoms have a small size, asymmetrical shape, inverted colour, aborted seeds, poor flavour, and excessive fruit drop (Anon, 1996; Lin 1956; Tolba and Soliman, 2015). In India, Huanglongbing, caused by *Candidatus* Liberibacter asiaticus (Varma *et al*., 1993), is one of India's most dangerous citrus diseases. HLB has a high negative impact on the yield of mandarin plants grown in warm, humid regions. The disease is caused by Gram-negative fastidious bacterium (Garnier *et al*., 1984), *Candidatus* Liberibacter asiaticus in Asia, *Candidatus* Liberibacter africanus in Africa (Jagoueix *et al*., 1994), and *Candidatus* Liberibacter americanus in South America (Teixeira *et al*., 2005).

The HLB is a phloem-limited, non-culturable alpha proteobacterium (Jagoueix *et al.,* 1994). The term "*Candidatus*" in Latin binomial indicates that the bacterium is not culturable in an axenic medium and is only characterized by molecular DNA-based techniques (Murray and Schleifer, 1994). Fraser and Singh (1968) reported that the decline in citrus production in Punjab was due to the Presence of Huanglongbing disease. In India, Dr Lilian R. Fraser first reported the citrus Huanglongbing in 1966 (Fraser *et al*., 1966). It was after that reported in different citrus-growing regions of Bihar, West Bengal, and Sikkim (Nariani and Raychaudhuri, 1968). Thus it would be very significant to detect the HLB-causing organism in mandarin orange orchards in south India and better manage them appropriately. Detecting this fastidious bacterium is difficult because of its non-culturability, low concentration, and uneven distribution in its natural hosts (Su and hang 1974; Graca 1991). The disease is diagnosed by biological indexing on indicator hosts (Nariani and Raychaudhuri, 1968), which is time-consuming, and symptoms depend on temperature. The disease, therefore, cannot be diagnosed easily by conventional procedures such as electron microscopic examination of ultrathin sections and bioassay on indicator plants. As a substitute, a reliable and rapid detection protocol by PCR was developed, giving quick results for early detection of HLB.

MATERIALS AND METHODS

Survey for huanglongbing disease at Various citrus growing groves of Tamil Nadu

A rapid roving survey was conducted in different citrus-growing areas of Dindugal and Thirunelveli, Tamil Nadu, from March 2018 to March 2019. During the surveys, symptom expressions on different citrus species

A

Lane1- TMOL1
Lane2- TMOL2

Fig. 1 A. Genomic DNA isolated from different samples of HLB affected mandarin orange plants. *TMOL1- Kanalkadu mandarin orange midrib Farm1 *TMOL2- Thandikudi mandarin orange midrib farm 2

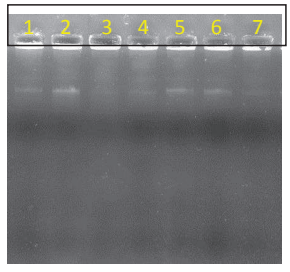
were noticed. Symptomatic samples presumed to have HLB infection were amassed and immediately stored in a cool box to avoid DNA degradation. The amassed samples were brought to the laboratory and processed promptly or stored for DNA extraction at -20°C or -80°C for future use.

Detection of CLas bacterium associated with huanglongbing disease of citrus in Tamil Nadu

Total DNA was extracted from the leaf lamina and midribs separately from the collected samples using Plant DNeasy mini kit (Qiagen, Germany). DNA was isolated following two validated protocols, DNeasy Plant Mini Kit (Qiagen, Germany) or CTAB extraction protocol. The CTAB method gave a good yield of DNA but a low quality compared to the QiagenDNeasy Plant Mini Kit. DNA isolation using the DNeasy Plant Mini kit was done following the manufacturer's protocol using a column for DNA isolation. For DNA extraction, 100 mg of samples were used. The quantity and quality of the isolated DNA were determined by taking OD values at 260nm and 280nm using a NanoDrop Spectrophotometer (NanoDrop, TNAU).

PCR amplification: Amplification was executed in Thermal cycler (Eurofins) through conventional PCR using primers set OI1F/OI2cR (Ahmad and sijam, 2009) targeting partial 16SrDNA (most specific region of the CLas genome). The reaction mixture was geared up for 25µl volume using 0.5µl of dNTPs (10mM),5µl of 10x buffers and 2.0µl MgCl2(25mM), 2.0µl of forward and reverse primers (10µM), DNA template of 5µl (100-200 ng/µl) and 0.3µl Taq polymerase (5 units/µl, Genei TM), and remaining volume was makeup with nuclease-free water. The thermal cycle conditions were: one cycle at95ºC for two minutes, followed by 35 cycles at 95ºC

B



Lane1- SALL1
Lane2- SALL2
Lane3- SALL3
Lane4- SALL4
Lane5- SALM1
Lane6-SALM2
Lane7-SALM3

*SALL-survey acid lime leaf
*SALM-survey acid lime midrib

Fig. 1 B. Genomic DNA isolated from different samples of HLB affected Acid lime plants

for 40 seconds, followed by 60ºC for one minute and 72ºC one minute, followed by a 72ºC extension for 10 min(Ahmad and sijam, 2009). The amplification product was examined at 1% agarose gel containing ethidium bromide in Tris-acetate EDTA buffer. The amplicons were looked over through UV illumination in a gel documentation system.

Phylogenetic analyses

Sequences retrieved in this work and all the 16S rRNA nucleotide sequences of '*Ca.* Liberibacter asiaticus available in the NCBI database (Table 1, 2, and Table 3) were aligned by using ClustalW software. For phylogenetic analyses, 16S rRNA gene sequences of all known '*Ca.* Liberibacter asiaticus' and related representative strains were analyzed by minimum evolution analysis using the Neighbor-Joining method and bootstrap analyzed by MEGA6.06 software (http://www.megasoftware.net/ index. HTML) (Tamura *et al.*, 2007).

RESULTS AND DISCUSSION

Huanglongbing disease survey at different citrus growing pockets of Tamil Nadu

Most of the samples were collected during the warmer season (March-April) of the year as the concentration of CLas bacterium was expected to be more during these periods. Throughout the survey, diverse symptoms varying from yellowing of leaves, and branches, rabbit ear-like appearance of leaves, irregular mottling on leaf lamina (blotchy mottle shoot with yellow patches), mineral deficiencies like symptoms (a regular pattern of yellowing or vein yellowing or clearing on leaf lamina) were observed. Interestingly, leaves with mineral deficiency-like symptoms and rabbit ear-like appearance

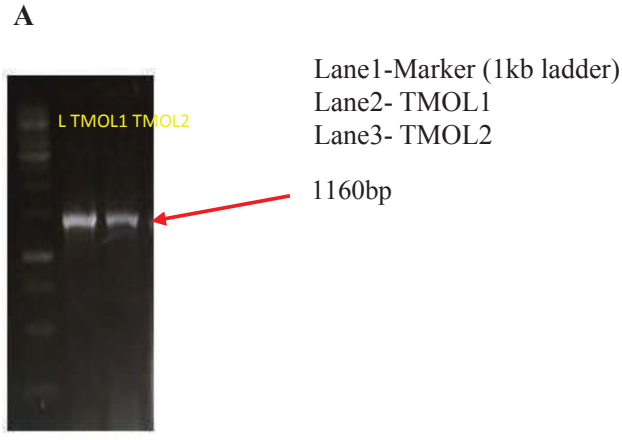


Fig 2A. Gel picture ofOi1 and Oi2c Primers PCR amplified mandarin orange midrib samples showing 1160 base pairs similar to *Candidatus* Liberibacter asiaticus.1160 base pairs similar to *Candidatus* Liberibacter asiaticus

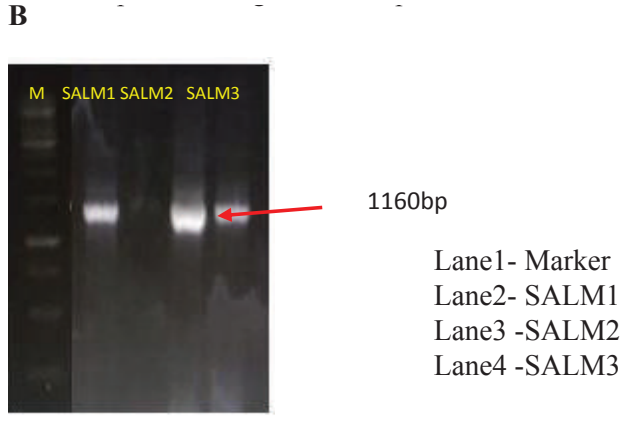


Fig 2B. Gel picture of Oi1 and Oi2c Primers PCR amplified Acid lime MID RIB samples showing 1160 base pairs similar to *Candidatus* Liberibacter asiaticus

were found positive for CLas. The irregular blotching called mottling and chlorosis is the peculiar symptoms of HLB (Baranwal, 2004). These determinations indicated that CLas isolates prevalent in Tamil Nadu exhibit different symptoms under field conditions. For example, all the citrus species collected from different screened areas were sensitive to HLB, as described earlier (Garnier and Bove, 1993; Garnier and Bove, 1994a). The contrasting symptoms observed under field conditions in the present study might be due to the pervasiveness of various strains/haplotypes of *Candidatus* Liberibacter asiaticus bacterium and susceptibility patterns of the citrus genotypes reported by Tsai *et al.* (2008).

CLas bacterium detection associated with huanglongbing disease of citrus in Tamil Nadu

The CLas bacterium was detected by PCR employing the primer targeting partial 16S rDNA (the most conserved region of the CLas bacterium genome). The expected amplicon of approx. 1160 bp was observed on the agarose gel in the citrus samples having CLas infections. Out of the fourteen samples collected from different locations of citrus growing pockets of Tamil Nadu, thirteen samples tested positive (90.00 per cent infection). The details of the samples, symptoms expression at the field level and host plant are given in Table 2. The single band of intact genomic DNA was visualized on the agarose gel (Fig. 1A, 1B, 2A, 2B and 2C).

PCR DETECTION

The 16S r RNA region of these bacterial isolates was amplified with universal primer pair FD1 and RP2 primer, and PCR fragments with the size of 1500 bp and 16s r DNA Las gene identifying OI1 and Oi2c specific primers were used. PCR fragments with the size of 1160bp were obtained respectively (Fig. 2B). Based on the 16s ribosomal sequence analysis, *Candidatus* Liberibacter asiaticus was identified in mandarin orange samples number1 and sample 2 from Thandikudi and kanalkadu field and Acid lime samples collected from various districts such as Dindigul, yercaud, sankarankoil and kallar districts which are samples 1,2,3 and sample number 4. The sequence has been submitted in NCBI GenBank and acquired the following accession numbers such as OP832019 and OP895022 amplified at 690bp by A2 and J5 primer set and MT671371 for Mandarin Orange amplified at 1100bp by Oi1 and Oi2c primer set. The sequence JQ867421.1 was taken from the NCBI database to compare Mandarin isolate and native isolates. OP895023 and OP895024 amplified at 690bp by A2 and J5 primer set and Accession numbers – MT671445, MT671446, MT671447, and MT671448. For Acid Lime Orange amplified at 1100bp by Oi1 and Oi2c primer set. MT671371 is the accession number obtained from the DNA sequence of the Mandarin orange midribs, whereas MT671445, MT671446, MT671447, and MT671448 are DNA sequences obtained from the Acid lime leaf and Midribs. All the samples were obtained through midribs. Interestingly leaf samples without midrib are also showed positive to CLas for acid lime samples (Table. 1)

Phylogeny analysis

The detected strains were compared with the other *Candidatus* Liberibacter asiaticus spp. ID in parenthesis Such as AB008366.1, KY990821.1,KU761591.1, JQ867432.1, DQ303210.1, AB038369.1, KY211974.1, LN795908.1, KY230624.1, KY008940.1, MK142766.1, AB008366.1, DQ303210.1,

C

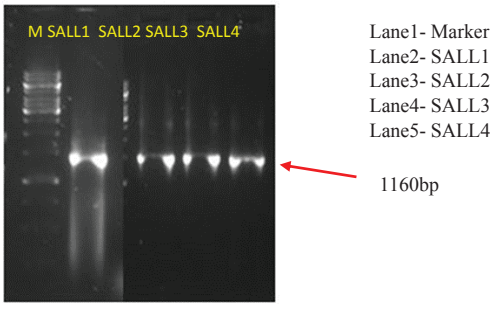


Fig. 2C. Gel picture of Oi1 and Oi2c Primers PCR amplified Acid lime LEAF samples showing 1160 base pairs similar to *Candidatus* Liberibacter asiaticus

D

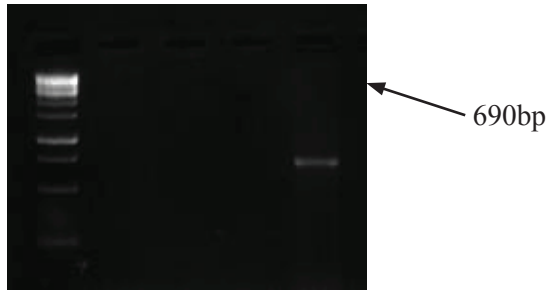


Fig. 2D. Gel picture of A2 and J5 Primers PCR amplified mandarin orange midrib sample showing 1160 base pairs similar to *Candidatus* Liberibacter asiaticus

KY008940.1, and also with the different strains of Candidatus genus, which were enormously infecting other continents such as North America and South Africa, are as follows KF170062.1(*Ca*. L. Solanacearum), L22533.1 (*Ca*. L. Africanus), AY742824.1 (*Ca*. L. americanum).

Interpretation of Phylogenetic analysis

The 16S rRNA amplicons obtained from nine representatives HLB affected citrus plants were sequenced using Specific Primers. The 1167 bp long sequences shared 100% nucleotide identity, indicating a low polymorphism level among strains from the same geographical region. The sequences had 99% sequence identity with previously reported nucleotide sequences of '*Ca.*Liberibacter asiaticus', confirming the PCR data and showed 100%sequence identity with '*Ca.* Liberibacter Americans, 78% with '*Ca.*Liberibacter Africanus-related isolates 75% with '*Ca.* Liberibacter solanacearum'. Minimum evolution phylogenetic analysis of 16S rRNA gene sequences revealed that '*Ca.* Liberibacter asiaticus isolates from Tamil Nadu clustered together in a phylogenetic subclade with known '*Ca.* Liberibacter asiaticus isolates (Fig. 3). Simultaneously, the 16sRNA sequences of 9 samples which were showing consistent positive result to CLas through PCR were compared with the NCBI retrieved sequences and they were correlated with each other. The similarity between the retrieved and isolated samples were 100% and the similarity between acid lime and mandarin orange isoalted samples is 88 percent.

The *Candidatus Liberibacter asiaticus* strains showed 99% similarity with the clade of CLas-affected *Diaphorina citri* nucleotide sequence, which was acquired from NCBI GenBank (AB038369.1). Liberibacter endosymbiont of *Diaphorina citri* gene, which gave evidence of Asia citrus psyllid (*Diaphorina citri*), was a perfect vector for transmission of greening disease from plant to plant.

***Sample Names:**

TMOL1 =*Candidatus* Liberibacter asiaticus Farm1 (Mandarin orange)

Assessment and prevalence of Las in Collected samples

The prevalence was observed for the present study; 12 samples were collected from 10 farms at various locations of citrus growing pockets of Tamil Nadu; 11 samples tested positive; the total samples showed positive for *Candidatus* Liberibacter asiaticus (90%). The samples from Sankarankoil, Ayyampalayam and kallar showed positive to both leaf and midribs. (Table. 1)

Assessment and prevalence of Las in Collected places

The characteristics of the samples, symptoms expression at the field level, and host plant are given in Table 2. Percentage infection of HLB was highest at Dindigul, sankarankoil and kallar districts (100%), followed by Salem district (75%), which was the lowest per cent infection was recorded amongst the samples

CONCLUSION

The survey has given a complete understanding of the prevalence of HLB occurrence in the citrus belts of Tamil Nadu. The PCR-based assay has overcome difficulties caused by the low concentration and uneven distribution of HLB in citrus. The diagnosis of HLB by PCR was excellent and essential for screening diseased plants and establishing disease-free citrus nurseries. Although the cost of PCR and DNA hybridization methods is higher than that of an immunological assay such as ELISA, it can provide reliable data quickly. HLB detection using PCR should facilitate epidemiological studies, aiding in HLB control. These findings are being used extensively

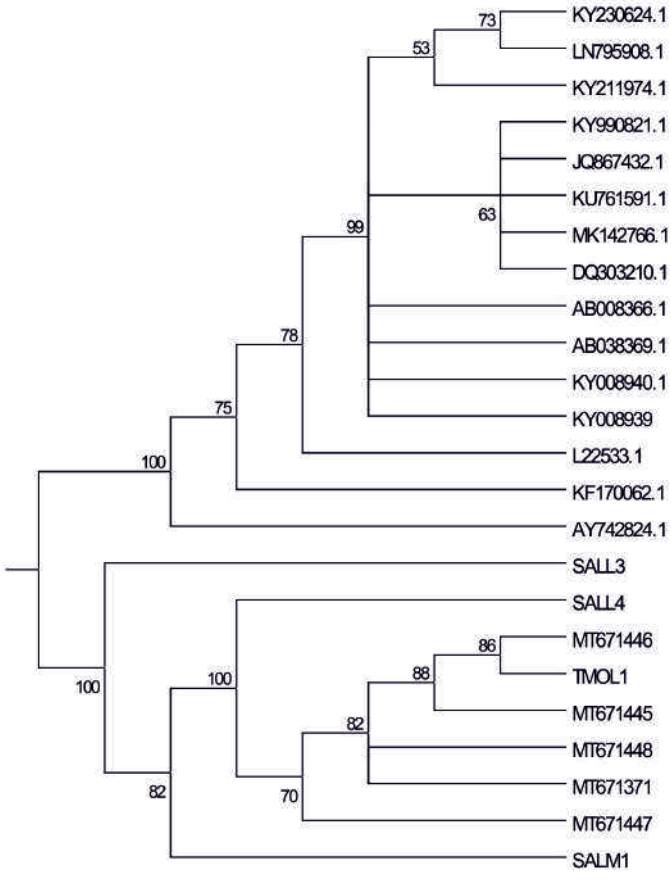


Fig 3. A. Phylogenetic experiments were carried out using 16S-rDNA sequences of Candidatus Liberibacter asiaticus MT6711371, MT671445, MT671446, MT671447, and MT671448 with 16S-rDNA sequences of other Candidatus Liberibacter asiaticus spp. ID in parenthesis indicates the GenBank number referring to the 16S-rDNA sequence of the corresponding organisms.

Table 1. List of leaves and midribs showing positive for Las extracted from Mandarin and Acid lime samples

Place	Crop	Samples	Farm No	Part extracted		*Candidatus* Liberibacter asiaticus Positive/negative
				leaf	midrib	
Thandikudi	Mandarin orange	TMOL1	1	-	midrb	Positive
Kanalkadu	Mandarin orange	TMOL2	2	-	midrb	Positive
Yercaud	Acid lime	SALL1	1	-	midrb	Positive
Sankaran Koil	Acid lime	SALL2/SALL3	2	leaves	midrb	Positive
Kallar	Acid lime	SALL3/SALM3	3	leaves	midrb	Positive
Ayyampalayam	Acid lime	SALL4/SALM4	4	leaves	midrb	Positive

*Total nine samples showed positivity

*Two samples are midribs of mandarin orange

* Four samples are midribs of acid lime, three samples are leaves of acid lime

Table 2. Pervasivenss of CLas bacterium based on 16S rDNA of both Acid lime and Mandarin orange

Sl.no.	Districts				No. of the sample tested positive for HLB	Incidence of HLB (%) *
		Acid lime	Mandarin orange	Total		
1	Dindigul	2	2	4	4	100
2	Yercaud	4		4	3	75
3	Sankarankoil	2		2	2	100
4	Kallar	2		2	2	100
Total	**four**			**12**	**11**	**90**

*PCR based detection

for HLB diagnosis in Tamil Nadu's citrus belts and help supply HLB-free seedlings to registered nurseries for multiplication in huge numbers.

ACKNOWLEDGMENTS

The first author is thankful to the professor and Head, Dr. Bala Kumudan, Horticulture Research Institute, Thadiyankudsai for providing facilities regarding accommodation and conducting the research in Kanalkadu. The authors are also thankful to the farmer, Mr. Paval Rajan from Kallankaadu village of Dindigul district for his cooperation during study period.

REFERENCES

Bove, J. M. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J. Plant Pathology, **88** (1):7–37.

Garnier, M., Danel, N. and Bove, J. M. 1984. Etiology of citrus greening disease. Ann. Microbiology, **135**A: 169 -179.

Jagoueix, S., Bove, J. M. and Garnier M. 1994.The phloem-limited bacterium of greening disease of citrus is a member of the alpha subdivision of the Proteobacteria. *Int. J. Syst. Bacteriol*, **44**(3): 379-386.

Tsai, C. H., Hung, T. H. and Su, H. J. 2008. Strain identification and distribution of citrus huanglongbing bacteria in Taiwan. *Botanical studies,* **49**: 49–56.

Baranwal, V. K., Mazumder, S., Singh, J., Suryanarayana, V., Ghosh, D. K. and Ahlawat, Y.S. (2004). PCR detection of *Candidatus Liberibacter asiaticus*, the agent of Huanglongbing or greening disease in citrus. *Indian Phytopath,* **57**: 164-168.

Garnier, M. and Bové, J. M. 1993. Citrus greening disease and the greening bacterium. *Proc. of the 12th Conference for Initiatives on Organ*, 212-219.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24** (8): 1596-1599.

Ahmad, K., Sijam, K., Hashim, H., Kadir, J., Omar, S. R. and Rastan, S. 2009. Characterization of '*Candidatus* liberibacter asiaticus' isolated from *Citrus grandis* and *Citrus reticulata*based on 16S rDNA and outer membrane protein (OMP) genes. *International Journal of Agriculture a Biology,* **11**(4): 401-407.

Tolba, I. H. and Soliman, M. A. 2015. Citrus Huanglongbing (greening disease) in Egypt: symptoms documentation and pathogen detection. *American-Eurasian Journal of Agricultural and Environmental Sciences,* **15** (10): 2045-2058.

Varma, A., Ahlawat, Y. S., Chakraborty, N. K., Garnier, M. and Bove, J. M. 1993. Detection of greening by electron Microscopy, DNA hybridization in citrus leaves with and without mottle from various regions in India. In *Proceedings 12th Conf.,* IOCV Riverside, California. Pp, 280-285.

Lee, H. A. 1921. The relation of stocks to the mottled leaf of citrus leaves. *Philosophical Journal of Science,* **18**: 85-95.

Mcclean, A. P. D. and P. C. J. oberholzer 1965. Greening disease of the sweet orange; evidence that a transmissible virus causes it. *South African Journal Agricultural Science*, **8**: 253-76

Teixeira, D., Saillard, C., Eveillard, S., Danet, J. L., da Costa, P. I., Ayres, A. J. and Bove, J. 2005. '*Candidatus* Liberibacter americanus,' associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil.*International Journal of Systematic and Evolutionary Microbiology, 55*(5), 1857-1862.

Fraser, L. R. and Singh, D. 1968. Citrus dieback in India the contribution of the greening virus. In Proc. *4th Conf. IOCV. Univ. Florida Press,* 141-144, Gainesville.

Fraser, L.R. and Singh, D. 1966. Greening virus, a threat to the citrus industry. Indian Hort,10: 21-22.

Nariani, T.K. and Rudhuriaycha, S.P. 1968. Occurrence of Tristeza and greening viruses in Bihar, West Bengal, and Sikkim. *Indian Phytopathology,* **21:** 343344.

Graca , J.V . Da. 1991. Citrus greening disease. *Annu Rev Phytopathology*, **29**:109–136.

Su, H. J. and Chang, S. C. 1974. Electron microscopical study on the heat and tetracycline responses and the ultrastructure of the pathogen complex causing citrus Likubin disease. Proceedings of the 8th International Congress Electron Microscopy, 25–31.

MS Received: 02 December 2022
MS Accepted : 28 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 167 -173 (2022)

DOI Number: 10.5958/0974-4541.2022.00056.X

Biocontrol potential of *Bacillus subtilis* Lb22 against fruit rot of King chilli, *Capsicum chinense* Jacq.

BISHAL SAIKIA[1], POPY BORA[2*], TRISHNA TAYE[3], RAJASHREE CHETIA[3], RUTHY TABING[3], TRISHANGI NEOG[4] and SWARUP NAYAK[5]

[1,3]Department of Plant Pathology, Assam Agricultural University, Jorhat-785013, Assam

[2,5]Regional Agricultural Research Station, Titabar, Jorhat, AAU, Assam

[4]Department of Horticulture, Assam Agricultural University, Jorhat-785013, Assam

***E-mail**: pbora.sonitpur10@gmail.com

ABSTRACT: Fruit rot is one of the most destructive diseases of *Capsicum chinense* Jacq., popularly known as *Bhoot jolokia* or King Chilli an export oriented crop in North East India. Morpho-cultural characterization of the isolated fungal pathogen from diseased fruit identified the pathogen as *Colletotrichum gloeosporioides* (Penz.) *Penz*. and *Sacc*. Pathogenicity test further confirmed its association with the disease. A few bacterial and fungal microbial biocontrol agents (MBCAs) were screened *in vitro* against *C. gloeosporioides* as a potential solution in organic and integrated crop management practices. Among all the MBCAs *Bacillus subtilis* LB22 showed highest inhibition (85.00%) on mycelial growth of the pathogen followed by *P. fluorescens* (84.33%) and *B. vallismortis* (83.22%). *Trichoderma pseudokoningii* were recorded the lowest inhibition (53.22%). Our study put forth further exploration *B. subtilis* LB22 (NCBI accession no. ON386193 and NBAIM accession no. NAIMCC-B-03226) *in planta* and their response in disease suppression as well growth promotion in *Bhut jolokia* as a component of sustainable crop health management program.

Keywords: Bioagent, fruit rot, organic production, pathogenicity

INTRODUCTION

Capsicum chinense Jacq., an important cash crop of the Solanaceae family grown extensively in the North Eastern region of India predominantly in the states of Assam, Nagaland, Manipur and Mizoram has huge commercial value (Bora and Bora, 2008). This chilli pepper known by different local names such as Ghost pepper, *Naga chilli* in Nagaland, *Bhoot jolokia* in Assam and *U-Morok* in Manipur (Sanatombi *et al*., 2010 and Verma*et al*., 2013) was ranked as the world's hottest chilli having a rating of 1,013,004 Scoville Heat Units with Guinness World Records (Bosland and Borral, 2007).

King Chilli being one of the hottest chillies with medicinal values has been cultivated commercially in Assam and other NE regions as a major export oriented crop. However, there are various constraints in its cultivation of which die back and fruit rot disease caused by *Colletotrichum gloeosporioides* assumes alarming proportions in the state which cause considerable quantitative and qualitative losses of the produce, warranting effective management measures (Bora and Bora, 2020). In India, a calculated loss of 10-54% has been reported in yield of the crop due to the fruit rot disease (Lakshmesha *et al.,* 2005; Ramachandran and Ramachandran and Rathnamma, 2006). Significant losses have been reported from the other parts of the world as well, with 20-80% loss has been accounted from Vietnam (Don *et al.,* 2007) and about 10% from korea (Byung, 2007). The loss is high owing to the post and pre harvested involvement of the pathogen causing a loss of 10-80% of the marketable yield of chilli fruits (Than *et al.,* 2008). The disease is more conspicuous as it causes severe damage to matured fruits in the field as well as in harvested fruits during transit and storage. Fruit rot is a major constraint in chilli production as even small lesions on the fruit may reduce their market value thereby, affecting profitable yield of the crop (Manandhar *et al*., 1995). Disease management through non chemical methods are the need of the organic growers for export oriented production system considering the NE regions as organic hub of the country. The MBCAs is gaining importance in the light of the hazard caused by chemical pesticides, as a distinct alternative and an eco-friendly approach against many pathogens of horticultural crops (Bora *et al.,* 2020). The presence of naturally occurring microorganisms with antifungal property has been well recognized, documented and these have been tested against an array of *Colletotrichum* spp. infecting many commercially important crop plants (Anand *et al*., 2009 and Ngullie *et al*., 2010).

MATERIALS AND METHODS

Isolation and characterization of pathogen

Diseased fruits of King Chilli(Plate 1)showing typical symptoms of fruit rot disease were collected from the Horticultural Orchard, AAU, Jorhat (26º43′41″ N and 94º12′05″ E).

Plate 1. Typical symptoms of fruit rot of king chilli

The pathogen was isolated by the tissue isolation technique as described by Ricker and Ricker (1936). The infected King Chilli fruits were brought to the laboratory and washed thoroughly with sterile water. A small portion of the progressing diseased tissue with a portion of healthy tissue was cut with the help of sterilized blade. The bits were surface sterilized by dipping in 1 per cent Sodium hypochlorite (NaOCl) solution for 30 seconds. The plant tissues were washed three times with double sterilized distilled water to remove all traces of NaOCl. Excess water was decanted and dried by soaking with sterilized blotting paper. The cut pieces were aseptically transferred to potato dextrose agar (PDA) in petri plate (20 mL/Petri plate) and incubated at 28±1ºC for three days for mycelia formation.

Morpho-cultural identification

The cultural characters of the fungus were studied on PDA medium. The Petri plates having 20 ml sterile media were inoculated aseptically with 5 mm mycelial disc of the fungus from the periphery of actively growing culture and incubated at 28±1°C and cultural features were recorded. The slide culture method was followed for microscopic morphological studies. The fungus was inoculated with a sterile needle on a sterile 2cm² block of PDA and placed over a glass slide present inside the moistened Petri dish. Over the block, one sterilized cover slip was placed and incubation was carried out at 20-25°C for three days (Rosana *et al*., 2014).

Pathogenicity test

The pathogenicity test of the fungus was performed by pin prick injury method (Naik and Rawal, 2002) and Mycelial bit inoculation technique (Rocha *et al.*, 1998). Healthy and uniform sized fruits of King Chilli (10 nos.) were surface sterilized with 4.0 percent sodium hypochlorite and fruits were injured with the help of a sterilized pin. A 5 mm diameter inoculum of mycelial disc was cut using a cork borer from a 7 day old culture in PDA and was transferred on the wounded fruit surface (10 nos. of fruits) using a sterile inoculating needle. Fruits were placed in the Petri plates with moistened blotting paper and incubated at room temperature. A set of another 10 healthy fruits were used as control and were observed for development of symptoms and pathogen was re-isolated.

Source of microbial biocontrol agents (MBCA)

Bacterial and fungal bioagents with NCBI accession number viz**.,** *Trichoderma harzianum* (NCBI-KF439052), *Trichoderma viride* (KF439055)*, T. pseudokoningii* (ON364136)*, Pseudomonas fluorescens* (KT258013), *Bacillus vallismortis* (OM585584), *Bacillus amyloliquiefaciens* (OM232770) and *Bacillus subtilis* LB22 (ON386193) were collected from the author's laboratory (Biocontrol Laboratory, Programme on Biopesticide, DBT-NE Centre of Agricultural Biotechnology, AAU, Jorhat) for the study.

In vitro bioefficacy of MBCAs

*In vitro*bioefficacy of fungal bioagents was performed using dual culture technique (Dennis and Webster, 1971). Briefly, a culture disc (5 mm diam.) of the pathogen was inoculated at one end and culture disc (5 mm diam.) of the fungal bioagent was placed equidistantly exactly at the opposite end of the petri plate. For the bacterial antagonist poisoned food technique of Nene and Thapliyal (2000) was used. A loopful of 48 hrs old bacterial inoculum was aseptically added into molten PDA. The media was poured in 90 mm Petri plates (20 mL per plate) and pathogen was a 5 mm culture disc of the pathogen was placed aseptically at the centre of the plate. Control treatment was maintained with the pathogen alone and plates were incubated in BOD incubator at 28±1° C. The experiment was conducted in CRD with each treatments replicated three times and these till full growth observed in the control.

The per cent inhibition of the mycelial growth was calculated by the following formula Vincent (1927)

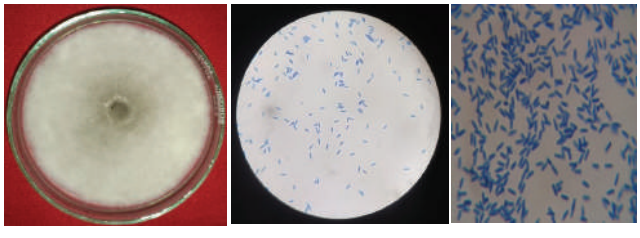
$$I = \left(\frac{C-T}{C}\right) \times 100$$

Where, I= Inhibition of mycelial growth, C= Growth in control, T= Growth in treatment

RESULTS AND DISCUSSION

Isolation and characterization of the pathogen

The pathogen isolated from diseased King chilli were subjected to morpho-cultural characterization and the morphological and cultural characters and microscopic observations are summarized in the Table I and Plate 2. Based on these characters, the pathogen causing fruit rot of *Capsicum chinense* was identified as *Colletotrichum gloeosporioides* in the Mycology laboratory of the Department of Plant Pathology, AAU, Jorhat. Dev *et al*. (2017) isolated *C. gloeosporioides* from symptomatic tissues of anthracnose of pomegranate on PDA producing flat mycelium with regular margin and zonation. Similarly, Papade *et al*. (2019) observed dull white to greyish colour of colony when *C. gloeosporioides* was grown in PDA. However, Kimaru *et al*. (2018) reported that the colony colour of the fungus varied from white to dark grey and growth varied from flat, raised fluffy to sparse in different media. In the present study, the conidial size and morphology of the fungus were also studied. Papade*et al*. (2019) observed cylindrical, hyaline conidia with oil globules at centre where the highest conidial length was of 12.57µm and breadth of conidia ranged between 2.75-7.5µm. On the other hand, Argawy (2012) observed that conidia of *C. gloeosporioides* were mostly monomorphic and exhibited cylindrical, hyaline conidia with size which ranged between 14.5-19.1 µm for length and 4.4-6.5 µm for width. Based on the cultural, morphological and microscopic observations the fungus isolated from the infected fruit of king chilli was identified as *C. gloeosporioides* which was pathogenic and responsible for causing fruit rot disease of the crop.



(A) **(B)** **(C)**

Plate 2 (A-C): Cultural and Morphological characters of *C. Gloeosporioides*

(A) Pure culture of the pathogen, (B) Spore at 10X magnification, (C) Conidia at 40X magnification

Pathogenicity manifestation

C. gloeosporioides produced disease symptoms characteristic to fruit rot with first appearance of symptoms at 5th days after inoculation (Table II and Plate3) while the control fruits inoculated with PDA media bit without pathogen did not develop any symptom. Symptoms appearing at 5 days after inoculation (DAI) progressively developed as angular lesion which enlarged to circular , sunken lesion. The black lesion with concentric ring developed black condia over the lesion. The pathogen was re isolated and checked for morphological details which established C. gleosporioides as the causal agent. The pathogenic nature of the fungus based on pathogenicity test was also supported by earlier reports on *C. chinense* (Sangnunmawia, 2018; Ngullie *et al.,* 2010), on chilli (Manandhar *et al*., 1995; Oanh *et al*., 2004). Talukdar *et al.* (2015) observed the initial symptoms of fruit rot disease of king chilli as shrinkage of the tissue irregularly at different portions of the fruit which were rough and straw coloured and on green fruits, they were pale and dirty green. Both green and ripe fruits were also affected (Kim *et al*., 1999; Sangchote *et al*., 1998).

(A) Healthy B) Control C) Inoculated fruit with symptoms at 5 DAI

Plate 3 (A-C): Pathogenicity test

Efficacy of different biocontrol agents against *C. gloeosporioides*

The antagonistic effects of different biocontrol agents were evaluated against *C.gloeosporioides* in *in vitro* condition.The results presented in Table 3 showed that all the biocontrol agents significantly inhibited the mycelial growth of the pathogen over control with different magnitude. However, among the biocontrol agents treatment *B.Subtilis* LB22 (T2) showed highest inhibition (85.00%) of mycelial growth of the pathogen which was followed by *P. fluorescens*(T5) and *B.vallismortis* (T3) with inhibition of 84.33% and 83.22%, respectively , however which were statistically *at par*. The lowest inhibition (53.22%) on mycelial growth of the pathogen was recorded in *T. pseudokoningii* (T8). Earlier Ashwini and Srividya (2014) recorded that *Bacillus subtilis* isolated from the rhizosphere of Chilli, showed high antagonistic activity against *C. gloeosporioides* OGC1 where the BCA inhibited *C. gloeosporioides* up to 100% in terms of dry weight. *Bacillus amyloliquefaciens* was also found effective against *C. gloeosporioides* isolates infecting fruit crops. *B. amyloliquefaciens* demonstarted 87% inhibition against mango isolate and 67% in case of ornage isolate (Alvindia and Acda, 2015; Arrebola *et al*., 2010) while *B. amyloliquefaciens* in loquat fruit showed 84% reduction of *C. acutatum* (Wang *et al*., 2020). *Bacillus amyloliquefaciens* was reported to produce

Table 1. Morpho - Cultural characters of *Colletotrichum gloeosporioides*

Parameter	Particular
Type of growth	Flate with cottony colonies
Colony colour (top view)	Dull white
Colony colour (Reverse view)	Yellowish
Shape of conidia	Cylindrical
Colour of conidia	Hyaline
Presence of oil globules	Singly at the centre
Size of conidia (Length)*	11.50 µm to 17.45 µm.
Size of conidia (Width)*	2.05 µm to 4.92 µm

Calculated as mean of 10

Table 2. Results of pathogenicity test

Days after inoculation	Symptom development on inoculated fruit
5 Days	Small black circular spots appeared on the skin of the fruit and spread along the long axis of the fruit and thus becoming more or less elliptical
7 Days	Sunken spots with black margin and fruit turning straw colored. Sunken spots are covered with pinkish mass of fungal spores
9 Days	Decaying of the fruits

Table 3. *In vitro* efficacy of different biocontrol agents against *Colletotrichum gloeosporioides*

Treatment	Mycelial growth* (cm)	Per cent inhibition over control
T1 : Control (*Colletotrichum gloeosporioides*)	9.00	0.00
T2 : *C. gloeosporioides* + *Bacillus subtilis* LB22	1.35	85.00 (75.92)**
T3 : *C. gloeosporioides* + *Bacillus vallismortis*	1.51	83.22 (69.25)
T4 : *C. gloeosporioides* + *Bacillus amyloliquefaciens*	1.54	82.88 (70.21)
T5 : *C. gloeosporioides* + *Pseudomonas fluorescens*	1.41	84.33 (73.15)
T6 : *C. gloeosporioides* +*Trichoderma harzianum*	2.00	77.78 (61.89)
T7 : *C. gloeosporioides* + *Trichoderma viride*	2.33	74.11 (59.25)
T8 : *C. gloeosporioides* + *Trichoderma pseudokoningii*	4.21	53.22 (38.35)
SE.(d)	0.150	
$C.D_{(P=0.05)}$	0.321	

** Mean of three replications and ** Data in the parentheses are angular transformed value*

lipopeptideiturin A, which was identified as the main agent producing the antifungal activity (Yan *et al*., 2020). Patel and Joshi (2001), reported maximum per cent inhibition of the colony growth of *C. gloeosporioides* (60.87%) with *T. koningii* . This can be attributed to higher competitive ability of the *Trichoderma* spp. and the antagonism could be due to production of wide range of metabolites as well as parasitism (Bora *et. al.,* 2022). *Trichodermin* and *dermadin* are the major volatile antibiotics produced by *Trichoderma* spp. which suppress several plant pathogens (Dennis and Webster, 1971). Kothikarand Koche (2017) reported that *T. viride* inhibited maximum mycelial growth *i.e*. 80.11 per cent followed by *T. harzianum* which inhibited mycelial growth upto 71.51 per cent. *Trichoderma* species have been reported to effectively control *Colletotrichum* species in chilli with concomitant disease reduction (Boonnratkwang *et al*., 2007). Amongst the antagonists, fungal isolates of *T. viride* and bacterial isolate *Pseudomonas fluorescens* were found effective in inhibiting the growth of *Colletotrichum capsici* (Anand and Bhaskaran, 2009). In contrast, Ngullie *et al*. (2010) reported that the mycelial growth of *C. gloeosporioides* causing anthracnose of Naga chilli fruit rot was inhibited by *P. fluorescens* and *T. viride* to an extent of 67.40 and

63.30 per cent respectively, as against the bavistin (83.40 per cent). This inhibition of mycelial growth of the pathogen might be due to the principle of mycoparasitism with the antagonist for nutrition (carbon source) by secreting cell wall degrading enzymes.

CONCLUSION

Our study demonstrated the *in vitro* bioefficacy of different bacterial and fungal bioagents with different magnitude against *C. gleosporioides,* a major threat to king chilli cultivation in NE India. The most efficient strain *B. subtilis* LB22 in terms of mycelia inhibition needs further screening on *in planta* response against C. *gleosporioides* as well as other pathogens of the crop. Moreover, the genus Bacillus being a endospore former can withstand diverse environmental extremities also known to produce wide range of antimicrobial metabolites. Isolation and purification of such compounds as an alternative to antibiotics may open up a new frontier in plant disease management.

ACKNOWLEDGEMENT

Authors are thankful to Director, DBT-NE Centre of Agricultural Biotechnology, Director of Post Graduate studies and Department of Plant Pathology, Assam Agricultural University, Jorhat for all the support during the study.

REFERENCES

Alvindia, D. G. and Acda, M. A. 2015. The antagonistic effect and mechanisms of *Bacillus amyloliquefaciens* DGA14 against anthracnose in mango cv. 'Carabao'. *Biocontrol Science and Technology,* **25**: 560–572.

Anand, T. and Bhaskaran, R. 2009. Exploitation of plant products and bio-agents for ecofriendly management of chilli fruit rot disease. *Journal of Plant Protection Research*, **49** (2): 195-203.

Anand, T., Chandrasekaran, A., Raguchander, T., Prakasam, T. and Samiyappan R. 2009. Chemical and biological treatments for enhancing resistance in chili against *Collectotrichum capsici* and *Leveillula taurica*. *Archives of Phytopathology and Plant Protection*, **1**: 1-19.

Argawy, E. E. 2012. Characteristics and control of *Colletotrichum gloeosporioides* isolates in El-Behera governorate, Egypt. *Egyptian Journal of Phytopathology*, **40** (1): 41-667.

Arrebola, E., Jacobs, R. and Korsten, L. 2010. Iturin A is the principal inhibitor in the biocontrol activity of *Bacillus amyloliquefaciens* PPCB004 against postharvest fungal pathogens. *Journal of Applied Microbiology*, **108**: 386–395.

Ashwini, N. and Srividya, S. 2014. Potentiality of *Bacillus subtilis* as biocontrol agent for management of anthracnose disease of chilli caused by *Colletotrichumgloeosporioides* OGC1. *Biotech*, **4**: 127–136.

Boonnratkwang, C., Chamswarng, C., Intanoo, W. and Juntharasri, V. 2007. Effect of Secondary Metabolites from *Trichoderma harzianum* Strain Pm9 on Growth Inhibition of *Colletotrichum gloeosporioides* and Chilli Anthracnose Control. Proceeding of the 8th National Plant Protection Conference. Naresuan University, Phisanulok, Thailand, **30**: 31-35.

Bora, L. C. and Bora, P. 2008. Biological control strategies for management of bacterial wilt of brinjal (*S. melongena*). *Journal of Mycology and Plant Pathology*, **38**: 3.

Bora., Popy and Bora L. C. 2020. Disease management in horticulture crops through microbial interventions: An overview. *Indian Journal of Agricultural Sciences*, **90** (8): 1389–96.

Bora., Popy and Bora, L. C. 2021. Microbial antagonists and botanicals mediated disease management in tea, *Camellia sinensis* (L.) O. Kuntze: An overview. *Crop Protection*, **148**: 105711.

Bora., Popy., Bora L.C., Bhuyan, R.P., Hashem, A. and Abd-Alla, E. F. 2022. Bioagent consortia assisted suppression in grey blight disease with enhanced leaf nutrients and biochemical properties of tea (*Camellia sinensis). Biological control,* **170**: 104907.

Bora., Popy., Saikia, K. and Ahmed, S. S. 2020. Pathogenic fungi associated with storage rot of *Colocasia esculenta* and evaluation of bioformulations against the pathogen. *Pest Management in Horticultural Ecosystems*, **26** (1): 134-139.

Bosland, P.W. and Borral, J. B. 2007. 'BhutJolokia'- The World's Hottest Known Chile Pepper is a Putative Naturally Occurring Interspecific Hybrid. *Horticultural Science,* **42**(2): 222-224.

Byung, S. K. 2007. Country report of anthracnose research in Korea. In: First International Symposium on Chili Anthracnose. Seoul: Hoam Faculty House, Seoul National University. 2

Dennis, C. and Webster, J. 1971. Antagonistic properties of species-groups of *Trichoderma*. I. Production of non-volatile antibiotics. *Transactions of British Mycological. Society,* **57**: 25-39.

Dev, D., Somasekhara, Y. M. and Prasannakumar, M. K. 2017. Cultural characterization of *Colletotrichum gloeosporioides* causing anthracnose of pomegranate. *Indian Phytopathology,* **70** (4): 493-495.

Don, L. D., Van, T. T., Phuong, T. T. and Kieu, P.T. 2007. *Colletotrichum* spp. attacking on chilli pepper growing in Vietnam.Country report. In: Abstracts of the First International Symposium on Chilli Anthracnose. Republic of Korea: National Horticultural Research Institute, Rural Development of Administration, 24

Kim, K. D., Oh, B. J. and Yang, J. 1999. Differential interactions of a *Colletotrichum gloeosporioides* isolate with green and red pepper fruits. *Phytoparasitica,* **27**: 1-10.

Kimaru, S. K., Monda, E., Cheruiyot, R. C., Mbaka, J. and Alakonya, A. 2018. Morphological and Molecular Identification of the Causal Agent of Anthracnose Disease of Avocado in Kenya. *Internationl Journal of Microbiology,* **4**: 1-10.

Kothikar, R. and Koche, M. 2017. Screening of fungicides, botanicals and bio control agent against *Colletotrichum dematiumin vitro*. *Journal of Agricultural Science*, **3**:1-6.

Kumar, M. A. and Bhaskaran, R. 2007. Tactics to manage disease problem in chilli: Spice India. 24-27.

Kumar, S., Kumar, R., Singh, M., Rai, A.B. and Rai, M. 2011. Incidences of leaf curl disease on Capsicum germplasm under field conditions. *The Indian Journal of Agriculture Science,* **81**: 187–189.

Lakshmesha, K. K., Lakshmidevi, N., Mallikarjuna, S. A. 2005. Changes in pectinase and cellulase activity of *Colletotrichum capsici* mutants and their effect on anthracnose disease on capsicum fruit. *Archives of Phytopathology and Plant Protection*, **38** (4): 267-279.

Manandhar, J. B., Hartman, G. L. and Wang, T.C. 1995. Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. *Plant Disease,* **79**: 380-383.

Naik, M. K. and Rawal, R. D. 2002. Disease resistance in horticultural crops. In: *Resource Management in Plant Protection*, Ed. SarathBabu., Pl. Prot. Assoc. of India, Hyderabad, 64-84.

Nene, Y. L. and Thapliyal, P. N. 2000. Fungicides in Plant Disease Control. (5th ed.) Oxford and IBH publishing Co. Pvt. Ltd., New Delhi. 325.

Oanh, L. T. K., Korpraditskul, V. and Rattanakreetakul, C. 2004. A pathogenicity of anthracnose fungus, *Colletotrichum capsici* on various Thai chilli varieties. *Kasetsart Journal Natural Science*, **38** (6): 103-108.

Papade, V. V., Potdukhe, S. R., Navsupe, D. R., Guldekar, D. D. and Taral, A. L. 2019. Morphological characters of *Colletotrichum gloeosporioides* from various hosts. *International Journal of Chemical Studies,* **7**(4): 75-78.

Patel, K. D. and Joshi, K. R. 2001. Antagonistic effect of some bio-agents *in vitro* against *Colletotrichum gloeosporioides* Penz. & Sacc., the causal agent of leaf spot of turmeric. *Journal of Mycology and plant Pathology*, **31**: 126.

Ramachandran, N. and Rathnamma, K. 2006. *Colletotrichum acutatum*-A new addition to the species of chilli anthracnose pathogen in India. In: Annual Meeting and Symposium of *Indian Phytopathological Society*. Kasarago: Central Plantation Crops Research Institute, 27-28

Ricker, A. J. and Ricker, R. S. 1936. Introduction of Research on Plant Diseases. John Swift Co., New York, p. 177.

Rosana, Y., Matsuzawa, T., Gonoi, T. and Karuniawati, A. 2014. Modified Slide Culture Method for Faster and Easier Identification of Dermatophytes. *Microbiology Indonesia*, **8** (3): 7.

Rocha, J. R. S., Oliveira, N. T. and Menezes, M. 1998. Comparison of inoculation methods efficiency for evaluation of *Colletotrichum gloeospoiroides* isolates pathogenicity on passion fruits (*Passiflorae dulis*). *Brazilian Archives of Biology and Technology,* **41:** 145-153.

Sanatombi, K., Sen-Mandi, S. and Sharma, G. J. 2010. DNA profiling of Capsicum landraces of Manipur. *Scientia Horticulturae- Amsterdam*, **124** (3): 405-408.

Sangchote, S. and Junbhanich, P. 1984. Seed transmission of *Colletotrichumcapsici* on pepper (*Capsicum* spp.). *Kasetsart Journal Natural Science*, **18** (1): 7-13.

Sharma, P. and Singh, L. 2006. Antagonistic potential of *Trichoderma* and *Aspergilus* sp. on *Sclerotinia sclerotiorum* (Lib.) de Barry causing rots in cabbage and cauliflower.

Talukdar, J., Saikia, A. K. and Borah, P. 2015. Survey and detection of the diseases of Bhut Jolokia (*Capsicum chinense* Jacq.) in Assam. *Journal of Crop and Weed*. **11** (Special Issue): 186-192.

Than, P. P., Jeewon, R., Hyde, K. D., Pongsupasamit, S., Mongkolporn, O. and Taylar, P. W. J. 2008. Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose on chilli (*Capsicum* spp.) in Thailand. *Plant Pathology*, **51**: 562-572.

Verma, P. K., Rawat, K. K., Das, N. and Pradhan, B. 2013. A botanical enigma of India's Hottest chilli 'Bhoot Jolokia' (*Capsicum chinense* Jacq.). *New York Science Journal*, **6** (11): 49-51.

Vincent, J. M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature,* **59**: 850.

Wang, X., Yuan, Z., Shi, Y., Cai, F., Zhao, J., Wang, J. and Wang, Y. 2020. *Bacillus amyloliquefaciens* HG01 induces resistance in loquats against anthracnose rot caused by *Colletotrichum acutatum*. *Postharvest Biology and Technology,* **160**:111034.

Willcott, M. R. 2009. Mest Re Nova. ACS Publications

Yan, F., Li, C., Ye, X., Lian, Y., Wu, Y. and Wang, X. 2020. Antifungal activity of lipopeptides from *Bacillus amyloliquefaciens* MG3 against *Colletotrichum gloeosporioides* in loquat fruits. *Biological Control,* **146**: 104281.

MS Received: 07 November 2022
MS Accepted : 27 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 174 -183 (2022)

DOI Number: 10.5958/0974-4541.2022.00057.1

Etiology of sooty blotch disease of *Aegle marmelos* and its management

SANGEETHA GANESAN[1,3]*, HARI SHANKAR SINGH[2], DEBASISH BISWAL[1], KUNDUN KISHORE[1], DEEPA SAMANT[1], SUPRIYA SAHU[1], MADHURI PATTANAIK[1], PETIKAM SRINIVAS[1]and DULEEP KUMAR SAMUEL[3]

[1]ICAR-IIHR-Central Horticultural Experiment Station, Bhubaneswar-751019, Odisha

[2]ICAR-Central Institute for Subtropical Horticulture, Uttar Pradesh-226101, India

[3]ICAR-Indian Institute of Horticultural Research, Bengaluru-560089

***E-mail:** G.Sangeetha@icar.gov.in, sangeethaau@hotmail.com

ABSTRACT: *Aegle marmelos* (L) Correa. recognized as bael is widely grown in the Eastern and Northern states of India. Incidence of superficial smudgy fungal blotch symptoms were observed on bael fruits in our experimental farm located in the state of Odisha, Eastern India (20°14' N, 85°46' E). The fungal blotch colonies on the fruit skin reduced the visual appeal of the fruits and thereby drastically reduced the market value and saleability of fruits. Etiology of fungi growing on the waxy layer of fruits resulting sooty blotch symptoms were investigated. Among different mycelial types observed on fruits, ramose colony type was observed as predominant (up to 80 %) followed by fuliginous and punctate type of colonies. Representative colonies were subjected to isolation; cultures were purified and analysed based on morphology and sequences generated with nuclear ribosomal genetic marker, the Internal Transcribed Spacer (ITS) nr DNA region and proved for Koch's postulates. The three fungal isolates were identified based on ITS phylogeny *viz*., *Zasmidium* sp. (ISO141), *Passalora* sp. (ISO211) and *Pseudocercospora* sp. (ISO232) however assigned putative status as the cultures were sterile. Among the various pre-harvest management modules evaluated in an effort to produce blemish-free bael fruit, the field spray of 0.3 percent copper oxy chloride (first spray at the lemon stage, second and third spray at 15 and 30 days after the first spray, and the last spray at 30 days before harvest) resulted 95-97 percent blotch control in two varieties of bael CISH-B1 and NB-5 taken for study.

Keywords: Sooty blotch disease, Bael, *Aegle marmelos,* etiology, management, India

INTRODUCTION

Aegle marmelos, commonly known as bael, has also been designated with several appellations like wood apple, Bengal quince, stone apple, holy fruit tree, etc. In India, it is abundantly distributed in Himalayan tracts, Eastern, Central and South India. Almost all parts of the bael tree have immense medicinal and nutritional value (Kumar and Nath 2010). A fully-grown (10-12 years old) grafted bael tree yields normally 150-200 fruits under a good crop care. Bael flowering starts during May, and after fruit set, bael fruit takes 10-11 months for ripening (https://www.krishisewa.com/crop-production/372-bael.html). CISH- B-1, CISH- B-2, Narendra Bael-5, Narendra bael-7, Narendra Bael-9, Pant Shivani, Pant Urvashi, Pant Aparna and Pant Pant Sujata are some of the popular bael varieties released for commercial fruits cultivation by research organizations in India.

Bael is reported to be affected by several diseases like bacterial spot by *Xanthomonas campestris* pv. *bilvae* (Patel *et al*. 1951), shell rot by *Syncephalastrum racemosum* (Mishra *et al*., 2016), black mildew by *Schiffnerula girijae* (Gautam 2014) and leaf spot by *Alternaria alternata* (Maurya *et al*. 2016), etc. Fruits of almost all bael accessions and varietal collection in our experimental farm were found to be covered with smudgy fungal growth wherein fruits became almost black at the of time maturity. The affected fruits lost their visual appeal and therefore, the saleability of fruits was greatly reduced. However, it is pertinent to note that there is no adverse impact on fruit pulp quality. These types of symptoms were reported to occur on pomaceous fruits by a group of epiphytic fungi 'sooty blotch flyspeck' (Sutton and Sutton 1994; Batzer *et al*., 2005). Sooty blotch is more often confused with sooty moulds. Sooty blotch diseases are alike to sooty moulds but are not associated with phloem-feeding insects hence sooty blotch development does not depend upon honeydew excretion of sap sucking insects. Review of literature revealed that sooty blotch diseases are widespread throughout the world occurring on different fruits (Batzer *et al*. 2005 and Gleason *et al*., 2011). Blemishes on apples due to sooty blotch led to a reduction in market value which in turn cause severe economic loss for growers (Johnson *et al*. 1997). Sooty blotch is also reported as an emerging problem on mango in India (IIHR annual report, 2016). Earlier the number of fungi causing sooty blotch was under-estimated but now studies clearly revealed the involvement of a diverse range of pathogens in the sooty blotch fungal complex. Now world-wide the sooty

Table 1. Effect of fungicide spray for the management of blackening caused by sooty blotch disease in Bael var CISH-B1

Treatment	2016-17		2017-18	
	Mean sooty blotch grade (0-5 scale)	Per cent reduction of fruit blackening over control	Mean sooty blotch grade (0-5 scale)	Per cent reduction of fruit blackening over control
Carbendazim (0.2percent) + Captan (0.1percent)	2.2±0.093	54.70	2.18±0.020	58.47
Thiophanate methyl (0.2percent) + Captan (0.1percent)	0.47±0.032	90.23	0.50±0.071	89.51
Copper oxychloride (0.3 percent)	0.17±0.010	96.35	0.12±0.020	97.48
Mancozeb (0.2percent)	3.38±0.171	29.03	3.26±0.103	31.63
Control	4.78±0.080	0.00	5.00±0.000	-
CD (5%)	0.27		0.17	
CV	9.56		5.88	

Note: Values are reported as means ± SE

Replication: 5 trees per treatment; Data were taken randomly on 20 fruits/tree

blotch flyspeck (SBFS) complex has extended to more than hundred named and putative fungal species as per a very recent review (Gleason *et al*. 2019). So far, no studies have yet been carried out on sooty blotch disease in bael and strategies to manage them. Hence, the present investigation was carried out with an aim to determine the causal fungi involved in causing sooty blotch disease of bael in the prevailing climate of eastern coastal regions of India, as well as to develop a strategic management module to reduce the blackening of bael fruits caused by sooty blotch fungal complex.

MATERIALS AND METHODS

This investigation was carried out during 2015-2019 at the experimental farm of IIHR-Central Horticultural Experiment Station (20°14' 17 N, 85°46' E), located in the state of Odisha. This region is characterised by a hot and humid tropical climate with annual rainfall of 1550 mm with relatively long spell of rainfall (June–September).

Symptomatology and mycelial types on fruits

As sooty blotch fungi known to exhibit different mycelial types on fruit surface, the prevalent mycelial type on bael fruit was identified with handheld and or stereo zoom microscope. Mycelial type is a colony morphology on fruit and it is a constant character within every SBFS species. At present, the recognized mycelial types of SBFS include punctate, ramose, fuliginous, speck, discrete, compact speck, ridged honeycomb, flyspeck and fleck (Gleason *et al*. 2011). Data on mycelial types were recorded from 30 fruits collected arbitrarily from trees of bael var. NB-5 (n=10).

Isolation

Fruits of bael cv. NB-5 displaying signs of sooty blotch were collected and washed in tap water gently for 4-5 mins. Surface sterilization of fruit was not useful since these epiphytic fungi were readily killed by surface sterilizing agents. Hence, fruits were gently cleaned with sterile water using sterile cotton swabs under aseptic conditions and fruits were left to dry on sterile filter paper on laminar hood. Then the blotch colonies were marked and every single knot-like fungal structures or mycelial growth (for fuliginous type) was chosen and picked with a sterile scalpel and transferred to half-strength PDA as well as acidified water agar by (adding lactic acid @ 7 drops for 500 ml media) and incubated at 28±2°C for 2 weeks and observed periodically for fungal growth. As the growth of SBFS fungi was very slow, the fast-growing fungal colonies which were saprophytic were not chosen for subculturing. The emerging hypha from acidified water agar as well as half-strength PDA were sub cultured on PDA plates and observed for uniformity. Then the representative fungal isolates exhibiting similar colony morphology were chosen and taken for further studies.

Pathogenicity

Selected seven representative fungal isolates were

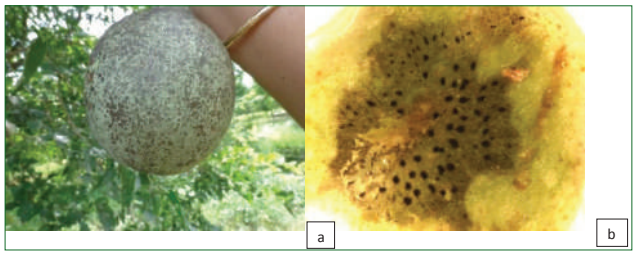


Fig. 1. Sooty blotch on bael fruits (a); Ramose mycelial types of sooty blotch fungi on bael fruits (b) (Bar=1000µm).

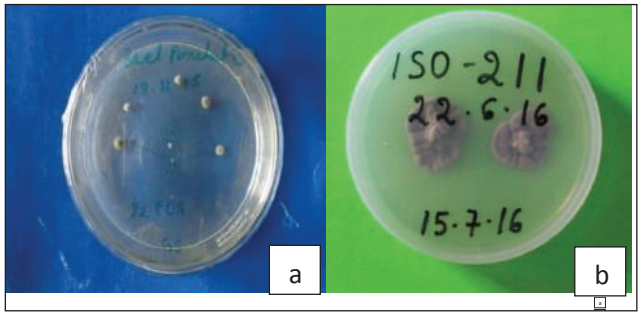


Fig 2. Isolation of blotch fungi from bael on water agar (a); Pure culture *Passalora* sp. ISO 211(b)

subjected to Koch's postulates. Modified Koch's postulates described for epiphytic pathogens by Batzer *et al*., (2015) was adapted in this study. Blemish-free healthy lemon size bael fruits were selected and were surface-disinfested in the orchard using an alcohol spray and washed with sterile distilled water thrice. The fungal propagules prepared from respective pure cultures were swabbed onto the fruit surface with sterile cotton balls, and the inoculated fruits were covered immediately with double-layer polypropylene sleeves followed by polythene covers. Fruits were sprayed with sterile distilled water daily with a small mist spryer to ensure wetness on the fruit surface. The fruits that received no inoculation, served as control. The fruits were observed for signs of sooty blotch at periodical interval. The fruits were inoculated during June month and after three months when the signs of sooty blotch were visible to the naked eye, the fruits were brought to the laboratory, bags were separated, mycelial type appeared was compared with that of the original isolate.

Morphology of sooty blotch fungi *in vitro*

The three fungal isolates which produced sooty blotch symptoms were characterized for morphological parameters. The three isolates were grown on PDA by placing 8 mm diameter mycelial plug of respective cultures from month-old colonies and incubated at 28± 2°C in an alternate cycle of dark and light for 30 days as they were very slow growing in nature. After one month of growth on PDA, the colony texture, colour, growth each fungal colony was documented and observed under microscope (BX 53 Olympus make) for micromorphological characters.

Molecular identification by ITS-rDNA sequence analysis and phylogeny

The three fungal isolates such as ISO141, ISO211and ISO 232 were subject to ITS-rDNA sequence analysis to determine their identity. Mycelial disc from respective pure cultures of the said isolates were aseptically transferred to the potato-dextrose broth and incubated for 20 days at 28±2°C without any disturbance. The mycelial mat was harvested and DNA was extracted by the protocol of Doyle and Doyle 1990. The PCR was carried out using standard universal fungal primer pairs ITS1/ITS4 (White *et al*. 1990). Each PCR reaction consists of total reaction volume of 25µl and the reaction was performed in an Eppendorf Thermal Cycler (made in Germany) with reaction cycles of initial denaturing for 2 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C, and a final extension of 10 min at 72 °C. Amplified products were separated on agarose gel (1.5 % w/v) alongside a 1.0 kb marker (Thermo Scientific, USA) for about 1-1.5 hours. After confirmation, the amplified PCR products were subsequently sent to sequencing (Eurofins Scientific India Pvt Ltd, Bengaluru). Resulted sequences were edited and aligned using Clustal-W (Kumar *et al*. 2016). Then, BLASTn search was performed in the NCBI GenBank database against available nucleotide sequences (https://blast.ncbi.nlm.nih.gov). The preliminary identity of each fungal isolate was decided based on the nearest match of the acquired sequence to the query sequence in the GenBank database (Lim *et al*. 2019) and isolates were given with putative status., as the cultures were observed to be sterile. Putative species is a provisional taxonomic unit, chosen based on several lines of available evidence which has not yet allotted a Latin binomial (Gleason *et al*. 2011). A phylogenetic tree based on the ITS sequences was made using Mega 7 software (Kumar *et al*. 2016).

Scanning electron microscopy

The blotch infected bael fruit peels were subjected to scanning electron microscopy (SEM) studies to assess the damage to the fruit surface and examined under a scanning electron microscope TM 3030 Plus tabletop microscope (Hitachi high Tech corporation).

Management of sooty blotch disease of bael under field condition

Ten years old bael plants of cv CISH-B1 and NB-5 maintained under uniform cultural practices were selected for the present study. Based on the review of literature,

Table 2. Effect of fungicide spray for the management of blackening caused by sooty blotch disease in bael var. NB-5

Treatment	2016-17		2017-18	
	Mean sooty blotch diseasegrade (0-5 scale)	Percent reduction of fruit blackening over control	Mean sooty blotch disease grade (0-5 scale)	Percent reduction of fruit blackening over control
Carbendazim (0.2percent) + Captan (0.1percent)	2.05±0.022	57.01	2.14±0.051	55.12
Thiophanate methyl (0.2percent) + Captan (0.1percent)	0.40±0.017	91.65	0.46±0.01	90.35
Copper oxychloride (0.3 percent)	0.11±0.013	97.94	0.19±0.079	96.02
Mancozeb (0.2percent)	3.14±0.093	34.06	3.28±0.183	29.95
Control	4.77±0.077	0.00	5.0±0.000	0.00
CD (5%)	0.16		0.29	
CV	5.60		9.70	

Note: Values are reported as means ± SE

*Replication: 5 trees per treatment; *Data were taken on 20 fruits collected randomly per tree*

fungicides shown effectiveness against sooty blotch disease on other crops such as copper oxychloride 50WP, captan 50WP, thiophanate methyl 72WP, mancozeb 75WP, carbendazim 50WP were taken for study. The treatments were T-1: carbendazim (0.2percent) + captan (0.1percent); T-2: thiophanate methyl (0.2percent) + captan (0.1percent); T-3: copper oxychloride (0. 3 percent); T-4: mancozeb (0.2) and T-5: control. At the time of fungicide spraying, fruits were sprayed till run-off to ensure complete coverage with fungicide solution. The first fungicidal spray was done as a preventive spray when fruits were in the lemon stage before the onset of the southwest monsoon. The second and third spray was given 15 and 30 days after the first spray, and the last spray at 30 days before harvest. Each treatment was replicated three times @3 trees per replication. Plants subjected to treatment were observed regularly. At harvest, data were taken randomly on 20 fruits per tree including control and brought to the laboratory for disease assessment. Disease assessment was done visually for individual fruit with a 0-5 scale as described in the above section. The experimental data were subjected to the Kruskal Wallis one-way analysis of variance Test and analysis of variance (ANOVA) using Minitab version 20.1.2.

RESULTS

Symptomatology and mycelial types

Sooty blotch fungi colonize the waxy layer of fruits, which was confirmed by studying the sign associated with the beal fruits. The fungi that cause them, grew on the surface of waxy layer of fruits and did not damage the fruit itself. Initially, the blotch colonies were isolated in nature, but as the disease progressed, the colonies become enlarged with indefinite outlines which coalesced to cover large areas of the fruit and caused complete superficial fruit blackening. The blotches ultimately became duskier and denser and the whole affected fruit area turned black (Fig.1a). The variation in shapes and colour of blotch colonies was attributed to the differences among the sooty blotch fungal complex causing the blackening and environmental conditions. Even though the occurrence of flyspeck was reported to occur on apples and other fruits, flyspeck was not observed on bael fruits during our study.

Among various mycelial types commonly reported to be produced by sooty blotch fungal complex, on bael fruits, ramose colonies were observed as predominant (up to 80 %) followed by punctate and fuliginous type. The ramose mycelial types displayed branching with a strong radial growth pattern (Fig 1b). The fuliginous type produced smudgy colony type covering large area of fruits with or without defined margins and punctate mycelial type formed dark dots/specks interconnected by mycelium.

Isolation of blotch fungi on acidified water agar yielded fair results (Fig 2a) even though routine method of isolation on PDA was not helpful due to the slow growing nature of blotch fungi as they are easily overgrown by saprophytes. However, subsequent sub culturing was done on PDA, but sooty blotch fungi took almost 30 days to grow into an inch colony diameter. The colonies ISO141 on PDA were observed to be very leathery and

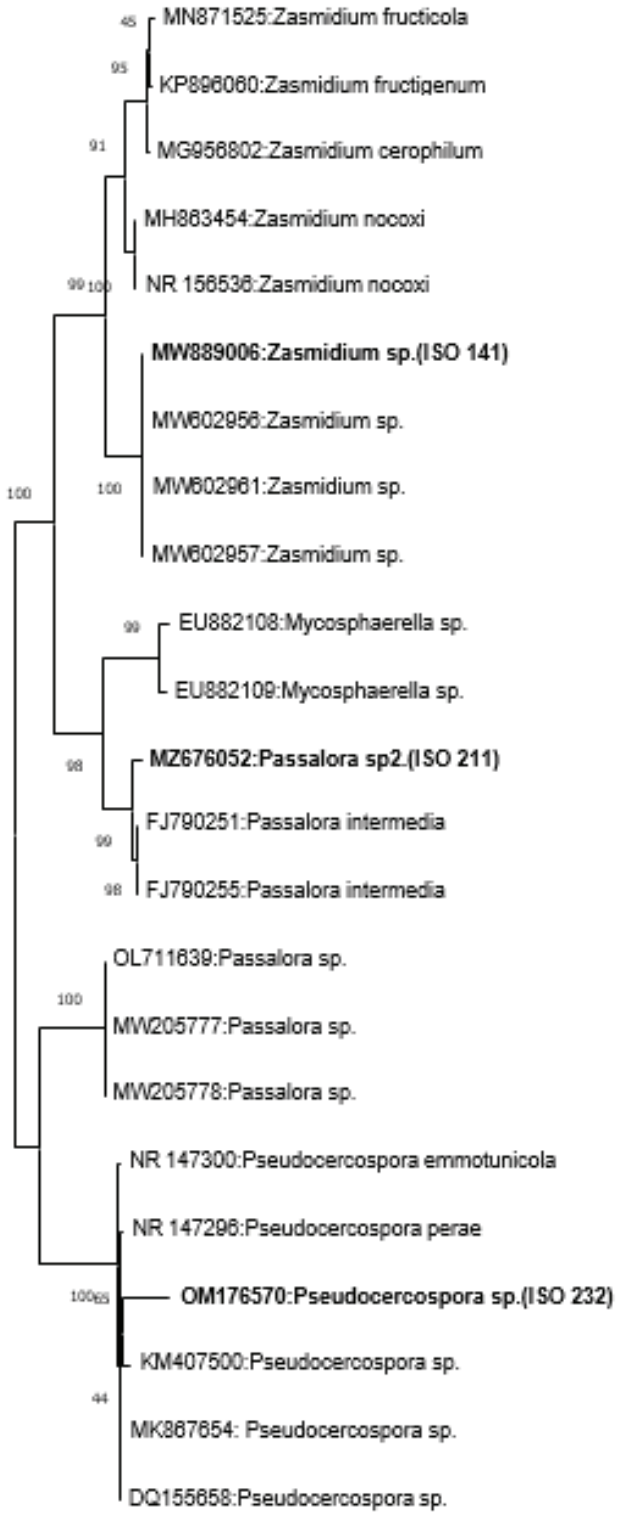


Fig 4. Phylogenetic relationship of fungi associated with sooty blotch complex in Bael such as Isolate ISO 141, ISO211 and ISO 232 and reference isolates retrieved from GenBank, Branch support (bootstrap values) given on the branches.

heaped, varied from dark grey, deep brown to black, slightly elevated at the centre, the surface appeared to be grey to dark grey, in reverse was iron black, wrinkled and crumpled. ISO211 (Fig 2b) and ISO 232 both produced grey leathery colonies. However, none of the three fungi sporulated on cultures and the culture was completely sterile in spite of efforts to induce sporulation in oatmeal agar, cornmeal agar or PDA amended with host extracts. It is in agreement with earlier observations made by Gleason *et al*. (2011) wherein many SBFS fungal species sporulate seldom or not at all on fruit surface as well as on in culture media, which frustrates description of associated fungal species morphologically. The three representative isolates ISO141, ISO211 and ISO 232 were subjected to ITS-rDNA analysis to assign putative status. These three fungal isolates produced similar sooty blotch signs on bael fruits upon artificial inoculation. The representative images of modified Koch's postulates study were depicted in Fig 3a and 3b.

Molecular identification by ITS-rDNA sequence analysis

The partial ITS sequences of three fungi described in this study were deposited in NCBI GenBank and received accession numbers. Based on a mega blast search in GenBank nucleotide database, the nearest hits of ITS sequence of ISO 141-MW889006 shared 98.80 identity with *Zasmidium syzygyi* (GenBank AccessionNo. NR111826) hence given the putative status of *Zasmidium* sp. Likewise, ISO211 (NCBI Accession No.MZ676052 has shown 98.91 percent identity with *Passalora intermedia* GenBank Accession No.FJ790251 and FJ790255) hence assigned the putative status as *Passalora* sp. Similarly, ISO232 (Accession No.OM176570) has shown 99.75% percent similarity with *Pseudocercospora* sp. available in (GenBank Accession No. MH059763) hence assigned the putative status of *Pseudocercospora* sp. As all these three fungi causing the sooty blotch on bael were sterile, Latin binomial could not be assigned hence we have given only putative status. Phylogenetic relationship of fungi associated with sooty blotch complex such as *Zasmidium* sp, (ISO141), *Passalora* sp. (ISO211) and *Pseudocercospora* sp. (ISO 232) in bael and relevant isolates retrieved from GenBank, inferred by the neighbor-joining method using ITS sequences. Branch support (bootstrap values) were given on the branches (Fig.4).

Scanning electron microscopy

The scanning electron microscopy (SEM) images of infected bael fruit's peel revealed that the blotch fungi grow on a waxy layer (Fig 5) but did not appear to

Fig. 3. Pathogenicity evaluation on bael, Control fruits were bagged but not inoculated (a) ISO 211 inouclted on bael fruit, wherein fruits were bagged and inoculated (b). Isolation, identification and pathogenicity test

dissolve the waxy layer. Further, it was also confirmed that during storage it did not result in any kind of skin shrinkage of bael fruit. Hence it is concluded that sooty blotch fungi resulted in superficial colonization of bael fruits.

Management of sooty blotch disease of bael under field condition

Our investigation over a period of four year revealed that sooty blotch infection initiated during onset of southwest monsoon ie., during last week of July and by the end of September, the bael fruits were almost black. Hence prophylactic fungicide spray was initiated when the fruit were in lemon size.

Kruskal Wallis Test revealed significant differences on four levels of treatment with a p-value of 0.0001 and the mean rank was different across all treatments. Among the four fungicides/fungicide combinations evaluated under field condition on bael for managing the blackening caused by sooty blotch fungal complex, four-time field sprays of 0.3 percent copper oxychloride (first spray at the lemon stage, second and third spray at 15 and 30 days after the first spray, and the last spray at 30 days before harvest) provided around 96-97 percent blotch control in both the varieties CISH-B1 and NB-5 which were evaluated for two years 2016-17 and 2017-18 (Table 1&2; Fig. 6&7). It was followed by spray with thiophanate methyl (0.2percent) + captan (0.1percent) which provided89-91 percent blotch control over unsprayed control (Fig 7). However, carbendazim (0.2 percent) + captan (0.1percent) and mancozeb (0.1 percent) spray could not offer a satisfactory level of blotch control.

DISCUSSION

In the current study, it has been established that the blackening in bael was caused by sooty blotch fungal complex and superficial fungal blackening was not found to be associated with phloem-feeding insects. Although these groups of fungi did not affect the development of fruit and did not result in direct yield loss, but severely impact the eye appeal and market value of fruits. In apple, sooty blotch was reported to reduce the market value to 90 percent and above (Williamson and Sutton 2000; Batzer *et al*. 2002). In a study conducted in apple orchards located in North Carolina, United States, Sutton and Sutton (1994) observed that the punctate mycelial type was most predominant in areas where sooty blotch severity was severe, however, the punctate mycelial type increased with cumulative hours of high humidity and the ramose mycelial type increased with increasing rainfall and temperature which is prevalent in the Coastal Plain region. Similarly, ramose mycelial type was observed predominantly on bael fruits in our study as our experimental farm is located in coastal plains. All three fungal pathogens such as *Zasmidium* sp., *Passalora* sp. and *Pseudocercospora* sp. found to be associated with sooty blotch of bael fruits belongs to family *Mycosphaerellaceae* (*Capnodiales, Ascomycota*). In China, *Zasmidium litseae* has been reported to cause SBFS symptoms on the petiole of brown Bolly gum (*Litsea glutinosa*), based on phylogenetic relationship of ITS region and LSU loci along with morphological characterization (Zhao *et al*. 2016). *Zasmidium angulare*, as identified from the blotched fruit surface of *Malus domestica* in the USAc ausing SBFS based on morphology and phylogenetic analyses ITS and LSU loci. *Zasmidium* is presently placed in class Dothideomycetes, order Capnodiales (Hongsanan *et al*., 2020). So far in Myco

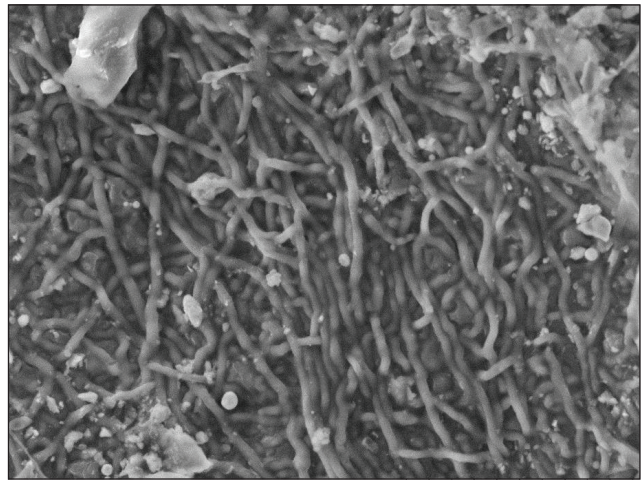


Fig 5. Scanning electron micrograph of sooty blotch fungi grown on bael fruit surface. The bar represents 50 µm.

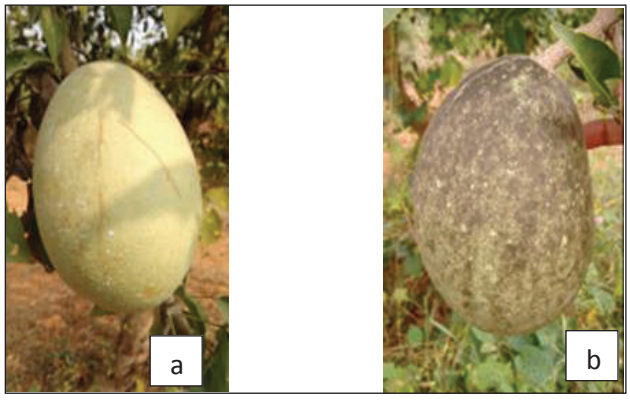


Fig 6. Impact of copper oxychloride (0.3 %) on sooty blotch fungal complex of bael fruit var CISH-B1 (a) Sprayed fruit (b) Unsprayed control fruit

Bank 196 species of the *Zasmidium* genus (*Stenella*-like hyphomycetes) have been documented majorly from plant hosts (Crouset al. 2014). Similarly *Passalora*-like sp. FG3 and *Pseudocercospora* sp. LLS1 2 and *Pseudocercospora* sp. LLS2 has been documented to be associated sooty blotch of apple (Gleason *et al*. 2011).

The appearance of the blotch colonies was closely associated with the weather parameters. It is favoured by abundant rainfall, free water on fruit surfaces, high humidity with warm temperature. Since the growth of sooty blotch fungi was restricted to the fruit's surface, the nutritional requirements of the fungi must be available on the fruit's surface in order to sustain their growth. Earlier studies revealed that cuticular wax has its maximum influence on the regulation of cuticular permeability. Therefore, it is apprehended that the cuticle could play a role in determining the extent of growth of SBFS fungi by its permeability to nutrient (Belding *et al*. 2000). Further works are needed in this area on the permeability of the cuticle and fruit leachates in relation to sooty blotch severity. In a study with apple, Nasu and Kunoh (1987) observed that the flyspeck fungi *Zygophiala jamaicensis* did not penetrate cuticle, but when observed with scanning electron microscopy (SEM), waxy crystals appeared broken and dissolved along the hyphal strands. Mycelia of sooty blotch fungi, *Peltaster fructicola* grew on the waxy layer of apple, but did not seem to degrade it (Belding *et al*. 2000). In our study on growth of blotch fungi on bael fruits, the fungal growth was seen entirely on fruit's waxy layer and could not find evidence of disruption of waxy layer.

The blotch fungi survive on twigs of the bael tree may serve as an immediate source of inoculum for fruit infection evidenced from the greater sooty blotch colonies on the upper side of the fruits near to the peduncle in addition to other reservoir hosts. In our study, we documented the sooty blotch fungal colonies on fruits as well as twigs of mango (*Mangifera indica*), Aou (*Dillenia indica*), Carambola (*Averrhoa carambola*), turkey berry (*Solanum torvum*) anola (*Phyllanthus emblica*) and twigs of jack fruit (*Artocarpus heterophyllus*), sapota (*Achras zapota*), star gooseberry (*Phyllanthus acidus*), acacia (*Acacia nilotica*), Simarouba (*Simarouba glauca*), piasala (*Pterocarpus marsupium*) and Calotrophis (*Calotropis procera*). Layer of free water on surface of fruit is required essentially to initiate the infection. Sooty blotch infection may start at any stage of the fruit development if suitable environmental conditions are available. If favorable conditions continue, the fungal growth continues to cover almost 80-90 percent of the fruit surface and complete fruit becomes black. The study conducted in North Carolina, United States reported that the SBFS incidence reached 100% in the four locations selected for study by the last part of each growing season (Sutton and Sutton, 1994).

In our study, field spray of 0.3 percent copper oxychloride (first spray at the lemon stage, second and third spray at 15 and 30 days after the first spray, and the last spray at 30 days before harvest) provided around 96-97 percent blotch control. With the knowledge of the fact that the inoculum of sooty blotch-causing fungi is already present in the orchard at the time of fruit set, the management schedule needs to be implemented as a prophylactic way. In apple, control of sooty blotch and flyspeck was often achieved by timely fungicide sprays (Rosenberger *et al*.1996). The fungicides used in this trial like thiophanate methyl, carbendazim and captain have been shown to control SBFS in apple orchards (Williamson and Sutton 2000). Factors like inefficient and poor fungicide coverage, weather conditions not allowing fungicide spray, and inaccessible tree height may lead to poor disease management. It is pertinent to emphasize the use of stickers and complete coverage of fruits by the fungicide solution are needed to achieve efficient management of sooty blotch.

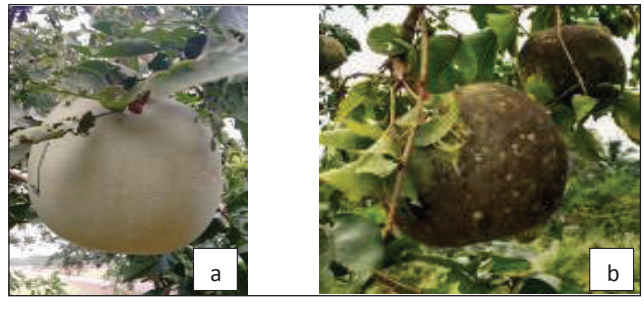


Fig 7. Effect of combined application of thiophanate methyl (0.2)% + captan (0.1 %) on sooty blotch fungal complex of bael fruit var. NB-5. (a) Sprayed fruit; (b) Unsprayed control fruit

ACKNOWLEDGMENT

The authors are grateful to the Director, ICAR-Indian Institute of Horticultural Research, Bengaluru for the facilities provided to carry out this work.

REFERENCES

Batzer, J. C., Gleason, A. J., Harrington, T. C., Mayfield, D. A. and Gleason, M. L. 2012. Temporal patterns in appearance of sooty blotch and flyspeck fungi on apples. *Microbial Ecology,* **64**(4): 928-941.

Batzer, J. C., Gleason, M. L., Weldon, B., Dixon, P. M. and Nutter. J. R. F. W. 2002. Evaluation of postharvest removal of sooty blotch and flyspeck on apples using sodium hypochlorite, hydrogen peroxide with peroxyacetic acid, and soap. *Plant Disease,* **86** (12): 1325-1332.

Batzer, J. C., Gleason, M., Harrington, T. and Tiffany, L. H. 2005. Expansion of the sooty blotch and flyspeck complex on apples based on analysis of ribosomal DNA gene sequences and morphology. *Mycologia,* **97** (6): 1268-1286.

Batzer, J. C., Diaz-Arias, M. M., Harrington, T. C., Gleason, M. L., Groenewald, J. Z.andCrous, P. W. 2008. Four species of *Zygophiala* (Schizothyriaceae, Capnodiales) are associated with the sooty blotch and flyspeck complex on apple. *Mycologia,* **100**: 246-258.

Belding, R. D., Sutton, T. B., Blankenship, S. M. and Young, E. 2000. Relationship between apple fruit epicuticular wax and growth of *Peltaster fructicola* and *Leptodontidium elatius,* two fungi that cause sooty blotch disease. *Plant Disease,* **84**: 767-772.

Bensch, K., Braun, U., Groenewald, J. Z. and Crous, P. W. 2012. The genus Cladosporium. *Studies in Mycology*, **72**: 1- 401.

Braun, U., Crous, P. W., Schubert, K. and Shin, H. D. 2010. Some reallocation of *Stenella* species to *Zasmidium*. *Schlechtendalia,* **20**: 99-104.

Brown, E. M. and Sutton, T. B. 1986. Control of sooty blotch and flyspeck of apple with captan, mancozeb, and mancozeb combined with dinocap in dilute and concentrate applications. *Plant Disease,* **70**: 281-284.

Brown, E. M. and Sutton, T. B. 1995. An empirical model for predicting the first symptoms of sooty blotch and flyspeck of apples. *AGRIS,* **79**(11): 1165-1168.

Crous, P. W., Lopez, G. A., Hawksworth, D. L., Robert, V., Kirk, P. M., Guarro, J., Robbertse, B., Schoch, C. L., Damm, U., Trakunyingcharoen, T.andGreonewald, J. Z. 2014. The genera of fungi: fixing the application of type species of generic names. IMA Fungus.**5**(1): 141-160.

Dhakar, M. K., Das, B., Nath, V., Sakar, P. K.and Singh, A. K. 2019. Genotypic diversity for fruit characteristics in bael [*Aegle marmelos* (L.) Corr.] based on principal component analysis. *Genetic Resources and Crop Evolution,* **66**: 951–964.

Diaz Arias, M. M., Batzer, J. C., Harrington, T. C., Wang, Wong., Bost, S. C., Cooley, D. R., Ellis, M. A., Hartman, J. R., Rosenberger, D. A., Sundin, G. W., Sutton, T. B, Travis, J. W., Wheeler, M. J, Yoder, K. S.and Gleason, M. L. 2010. Diversity and biogeography of sooty blotch and flyspeck fungi on apple in the eastern and midwestern United States. *Phytopathology,* **100**: 345-355.

Doyle, J. J. and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus,***12**: 13-15.

Dutta, A., Lal, N., Naaz, M., Ghosh, A.and Verma, R. 2014. Ethnological and Ethno-medicinal importance of *Aegle marmelos* (L.) Corr (Bael) among indigenous people of India. *American Journal of Ethnomedicine,* **5**: 290–312.

Fries, E. M. 1849. Summa vegetabiliumScandinaviae. Sectio posterior, Uppsala.

Gautam, A. K. 2014. Occurrence of black mildew on *Aegle marmelos* at Himachal Pradesh, India**.** *International Journal of Phytopathology,***03** (03): 161-162.

Gleason, M. A., Zhang, R., Batzer, J. C. and Sun, G. 2019. Stealth pathogens: the soot blotch and flyspeck fungal complex. *Annual Review of Phytopathology,* **57**: 135-164.

Gleason, M. L., Batzer, J. C., Sun, G., Zhang, R., Diaz Arias, M. M., Sutton, T. B., Crous, P.W., Ivanovic, M., Mcmanus, P. S., Cooley, D. R., Mayr, U., Weber, R. W. S., Yoder, K. S., Ponte, E. M.D., Biggs., A. R. and Oertel, B. 2011. A new view of sooty blotch and flyspeck. *The American PhytopathologicalSociety,***95** (4): 368-383.

Hemnani, K., Malley, P. J. O., Tanovic, B., Batzer, J. C.and Gleason, M. L. 2008. First report of seven species of sooty blotch and flyspeck on *Asimina triloba* in Iowa. *Plant Disease,* **92**: 1366.

Hongsanan, S.*et al*. 2020. Refined families of Dothideomycetes: Dothideomycetidae and Pleosporomycetidae. *Mycosphere,***11**(1): 1553–2107.

Hosagoudar, V. B. 2011. The genus Schiffnerula in India. *Plant Pathology and Quarantine,* **1**(2): 131-204.

Johnson, E. M., Sutton, T. B. and Hodges, C. S. 1997. Etiology of apple sooty blotch disease in North Carolina. *Phytopathology,* 87: 88–95.

Kamal. 2010. Cercosporoid Fungi of India. Bishen Singh Mahendra Pal Singh, India.

Kamalakkannan, N. and Prince, P. S. M. 2003. Hypoglycemic effect of water extracts of *Aegle marmelos* fruits in streptozotoc in diabetic rats. *Journal of Ethnopharmacology,* **87** (2-3): 207–210.

Kintzios, S. E. 2006. Terrestrial plant-derived anticancer agents and plant species used in anticancer research. *Critical Reviews in Plant Sciences,* **25**(2): 79–113.

Kumar, D. and Nath, V. 2010. Variability in bael (*Aegle marmelos* Correa) genotypes from Orissa. *Indian Journal of Plant Genetic Resources,* **23**(3): 303-305.

Kumar, S., Stecher, G.and Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution,***33**: 1870–1874.

Li, H. Y., Sun, G.Y., Zhai, X. R., Batzer, J. C., Mayfield, D. A., Crous, P.W., Groenwald, J. Z.and Gleason, M. L. 2012. Dissoconiaceae associated with sooty blotch and flyspeck on fruits in China and the United States. Persoonia. *Molecular Phylogeny and Evolution of Fungi,* **28**: 113-125.

Maurya, S., Kumar, R., Kumari, A.and Choudhary, J. S. 2016. First report of Alternaria leaf blight in bael (*Aegle marmelos* (L.) Corr.) from eastern plateau and hill region of India. *Journal of Agri Search,***3**: 248-250.

Mayr, U. and Spath, S. 2008. Sooty blotch of apple: Efficacy of different application strategies. In: Boos, Markus (Ed.) *Ecofruit - 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: Proceedings to the Conference from 18thFebruary to 20th February 2008 at Weinsberg/Germany*: 82-86.

Mayfield, D. A., Karakaya, A., Batzer, J. C, Blaser, J. M.and Gleason, M. L. 2013. Diversity of sooty blotch and flyspeck fungi apples in north-eastern Turkey. *European Journal of Plant Pathology,***135** (4): 805-815.

Mishra, A. K., Garg, N. and Yadav, K. K. 2016. First report of shell soft rot of bael (*Aegle marmelos*) Caused by *Syncephalastrum racemosum* in North India. *Plant Disease,***8**: 1779.

Mukherjee, P. K, Rai, S., Kumar, V., Mukherjee, K., Hylands, P. J.and Hider, R. C. 2007. Plants of Indian origin for drug discovery. *Expert Opinion on Drug Discovery,***2**(5): 633–657.

Nasu, H. and Kunoh, H. 1987. Scanning electron microscopy of flyspeck of apple, pear, Japanese persimmon, plum, Chinese quince and pawpaw. *Plant Disease,***71**: 361-364.

Patel, M. K., Allayya Navaramath, S. B. and Kulkarni, Y. S. 1953. Bacterial shot-hole and fruit canker of *Aegle marmelos* Correa. *Current Science,* **22** (07): 216–217.

Patel, M. K., Bhatt, V. V. and Kulkarni, Y. S. 1951. Three new bacterial diseases of plants from Bombay. *Current Science,* **20**: 326-327.

Pathirana, C. K., Rnaweera, L. T., Madhujith, T., Ketipearachchi, K. W., Gamlath, K. L.andEeswara, J. P. 2020. Assessment of the elite accessions of bael [*Aegle marmelos* (L) Corr.] in Sri Lanka bases on morphometric, organoleptic, and phylogenetic relationships. *PLoS ONE,* **15**(5): e0233609.

Quadevlieg, W., Binder, M., Groenawald, J. Z., Summerell, B. A., Carnegie, A.J., Burgess, T. I.andCrous, P. W. 2014. Introducing the consolidated species concept to resolve species in the Teratosphaeriaceae. *Persoonia,* **33**: 1-40.

Rosenberger, D. A., Engle, C. A. and Meyer, F. W. 1996. Effects of management practices and fungicides on sooty blotch and flyspeck diseases and productivity of 'Liberty' apples. *Plant Disease,* **80**: 798-803.

Rosenberger, D. A. and Meyr, F. M. 2007. Timing summer fungicides to control flyspeck disease

on apples. *New York State Horticultural,* **15**: 10-13.

Rosil, H., Mayfield, D. A., Batzer, J. C., Dixon, P. M., Zhang, W.and Gleason, M. L. 2017. Evaluating the performance of a relative humidity-based warning system for sooty blotch and flyspeck in Iowa. *Plant Disease,***101**(10): 1721-1728.

Shivas, R. G., Young, A. J.andMcneil, B. D. 2010. *Pseudocercospora microsori* Fungal Planet 68. *Persoonia,***25**: 156-157.

Sutton, A. L.and Sutton, T. B. 1994. The distribution of the mycelial types of *Gloeodes pomigena* on apples in North Carolina and their relationship to environmental conditions. *Plant Disease,* **78**: 668–73.

White, T. J., Bruns, T., Lee, S. J. W. T., Taylor, J., Innis, M. A., Gelfand, D. H. and Sninsky, J. J. 1990. PCR Protocol: a guide to methods and applications. *Academic Press,* **38**: 315-322.

Williamson, S. M.and Sutton, T. B. 2000. Sooty blotch and flyspeck of apple: Etiology, biology, and control. *Plant Disease,* **84** (7): 714-724.

Xu, C., Chen, H., Gleason, M., Xu, J. R., Liu, H., Zhang, R.and Sun, G. 2016. *Peltaster fructicola* genome reveals evolution from an invasive phytopathogen to an ectophytic parasite. *Scientific Reports. 6. 10.1038/srep22926*.

Zhao, W., Hou, Y., Bai, J., Zhang, W., Gao, L., Gleason, R. Z. M. L.and Sun, G. 2016. A new species of *Zasmidium* associated with sooty blotch and flyspeck. *Phytotaxa,* **258** (2): 190-194.

MS Received: 22 November 2022
MS Accepted : 20 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 184 -188 (2022)

DOI Number: 10.5958/0974-4541.2022.00058.3

Reaction of ginger cultivars against *Phyllosticta zingiberi* causing leaf spot and its management

K. T. ARUNAKUMARA* C. SATYANARAYANA and S.V. PATIL

Department of Plant Pathology, College of Horticulture, Bidar - 585 403, Karnataka, India

***E-mail:** arunakumarakt@gmail.com

ABSTRACT: Ten Ginger cultivars were tested, during *kharif* 2018 and 2019 for their reaction to *Phyllosticta zingiberi* under field conditions. Varieties viz., 'Humnabad local' and 'Maran' were found to be moderately resistant and none of them were free from the disease. Among 10 fungicides, 10 botanicals and 6 bio-agents evaluated in *in vitro* condition against *Phyllosticta zingiberi* Ramkr, Propiconazole 25 EC (92.30%) followed by Azoxystrobin 23 EC (91.70%), SAAF 75 WP (Mancozeb and Carbendazim) (89.30%), *Allium sativum* (75.70%) and *Trichoderma harzianum* (78.80%) recorded the highest inhibition of mycelial growth of *Phyllosticta zingiberi.* The field evaluation of different fungicides and botanicals during *Kharif*-2018 indicated that Propiconazole-25EC was recorded minimum PDI of 36.00 and yield of 202q/ha. Azoxystrobin-23EC,Hexaconazole-5EC,SAAF-75WP (Mancozeb-63WP and Carbendazim-12WP),Mancozeb-75WP,Propineb-50WP and *Allium sativum* cloves extract were next best treatments by recording a PDI of 40.00,45.00,46.00,50.00,55.00 and 60.00 and a yield of 185q/ha,180q/ ha,179q/ha,175q/ha,173q/ha and 169q/ha respectively. Again during *kharif* 2019, propiconazole 25 EC was significantly superior over other treatments.

Keywords: Bio-agents, fungicides, ginger, mangement,*Phyllosticta zingiberi*

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is a herbaceous perennial, the rhizomes are used as a spice and it has medicinal value against Diabetes disease in animals (Shanmugam *et al*., 2009). During 2012-13 the country produced 7.45 lakh tonnes of the spice from an area of 1.58 lakh hectares (Anonymous, 2014). India is the largest producer of ginger accounting for about one third of total world output so it is basic need to develop high yielding varieties with better quality to increase the production and productivity of ginger in India (Ravishanker *et al*., 2014). The major constraints that limit production of ginger are soft rot, yellows, rhizome rot complex, leaf spot and storage rots. Leaf spot caused by *Phyllosticta zingiberi* is one of the most threatening foliar disease, first time reported in India by Ramakrishnan (1942). Later, it was reported from Himachal Pradesh (Sohi *et al.,* 1973) and Maharashtra (Kanware, 1974). Leaf spot disease of ginger leads to heavy reduction in rhizome yield through the destruction of chlorophyll tissue (Ramakrishnan, 1942). Symptoms are observed on leaves as oval to elongated spots that later turn to whitish surrounded by dark brown margin with yellow halo. Knowledge on the reaction of various ginger germplasm to leaf spot disease is very scanty. In earlier studies, Nybe and Nair (1979), Premanathan *et al.* (1980) and Dohroo *et al.* (1986) were reported the screening of some cultivars to locate the tolerance and resistant types. In the present studies, an attempt has been made to test ginger cultivars against *Phyllosticta* leaf spot and also to manage the disease. Spraying of broad spectrum fungicides like Mancozeb 75 WP, Bordeaux mixture, Chlorothalonil 75 WP and Captan 50 WP has been recommended for control of leaf spot of ginger by several workers (Sohi *et al*.,1973; Nazareno,1995; Das and Senapati,1998). Control achieved by these chemicals was inadequate. Therefore, it is thought worthwhile to test the efficacy of more promising chemicals like Propineb 50 WP, SAAF 75 WP (Mancozeb 75 WP and Carbendazim 50 WP), Propiconazole 25 EC, Hexaconazole 5 EC, Azoxystrobin 23 EC, Benomyl 50 WP against fungus. Not much light has been shed on biological control, botanicals which are effective against *Phyllosticta zingiberi*. Hence, an attempt has been made to test commonly available botanicals and bio agents against the pathogen.

MATERIALS AND METHODS

Reaction of Ginger cultivars

An experiment was conducted at College of Horticulture, Bidar, Karnataka during *kharif* 2018 and 2019 in Randomized Complete Block Design with a plot size of 3m x 1m. Ten cultivars *viz.,* Humnabad Local, Himagiri, Maran, Suprabha, Himachal, Suruchi, Suravi, ISSR-Vardha, ISSR- Mahima and ISSR-Rejitha were planted at a spacing of 20cm x 30cm (rhizome to rhizome x row to row) and all the recommended agronomic practices were followed to raise crop except fungicidal spray to avoid the killing of fungal pathogen (Anonymous, 2017). As there was heavy incidence of *Phyllosticta* leaf spot during both the years, the cultivars were scored for the

disease incidence under natural field conditions without artificial inoculation. Disease score was measured, on fifteen randomly selected leaves from each plot, by using 1-5 point rate scale (Dohroo *et al.,* 1986).

***In vitro* evaluation of fungicides**

Ten different fungicides with different modes of action were evaluated in the laboratory for their efficacy against *Phyllosticta zingiberi* by the poisoned food technique (Nene and Thapliyal, 1979). The each treatment was replicated 3 times. The molten sterilized PDA was used as nutrient medium and required quantity of each fungicide was added separately so as to get a required concentration of that fungicide. The fungicides were thoroughly mixed by stirring and about 15ml poisoned medium was poured to each of the 90mm petri dishes and allowed for solidification. The actively growing periphery of 9 day old culture of *Phyllosticta zingiberi* was carefully cut by using a gel cutter and transferred aseptically to centre of each petri dish containing the poisoned solid medium. Suitable control was maintained by growing the cultures on PDA without the fungicides. The plates were incubated at 27±1ºC for 9 days and the colony diameter was recorded 9 days after growth (Table 2). The percent inhibition of mycelial growth over control was calculated using the formula of Vincent (1947)

$$I = \frac{C - T}{C}$$

I = per cent inhibition of mycelial growth
C = radial growth of fungus in control
T = radial growth of fungus in treatment

In vitro evaluation of botanicals

Healthy plants were selected from which the fresh leaves and other parts were obtained and thoroughly washed with tap water then air dried. Aqueous plant extract was prepared by grinding 100g leaves/other parts with 100ml distilled water using a blender and filtrate was collected by passing through double layered muslin cloth. The supernatant was taken as standard plant extract solution (100%). All the extracts obtained were passed through filter paper used for assay. The poisoned food technique (Nene and Thapliyal, 1979) was followed to evaluate the efficacy of botanicals in laboratory against *Phyllosticta zingiberi* at 15% concentration (Table-3). The each treatment was replicated 3 times. The method followed for conducting the experiment was same as that used for fungicide evaluation.

***In vitro* evaluation of bio-agents**

Dual culture technique was followed to study interaction of six antagonists in the laboratory. Six bio-agents with a control treatment were used for evaluation (Table 4). Pour 20ml of PDA into 90mm petri dishes and allowed for solidification. Discs measuring 5mm of *Phyllosticta zingiberi* was taken from 9 day old culture and was placed at one end of the petri dish then respective antagonistic organisms were inoculated at the opposite side. A control was maintained by inoculating only *Phyllosticta zingiberi* at one end in case of fungal antagonist. In case of bacterial antagonist *Phyllosticta zingiberi* was placed at both ends of petri plates and bacterial culture was inoculated at centre of the petri plate, control was maintained by inoculating *Phyllosticta zingiberi* at the both the ends of the petri plates. Each treatment was replicated 4 times and incubated for 6 days at 27 ± 1ºC. The activity of antagonistic organisms were recorded by measuring the colony diameter of *Phyllosticta zingiberi* in each treatment and compared with control.

Management of leaf spot, *Phyllosticta zingiberi*

The field experiment was laid out in RCBD with13 treatments and 3 replications during *kharif* 2018 and 2019 at College of Horticulture, Bidar, Karnataka. Healthy Humnabad local Ginger seed rhizome suitable to this area (Shadap *et al.,* 2013) were planted in the field with 30cm X 20cm (row to row X rhizome to rhizome) spacing in plots size of 3m x 1m. All other cultural practices and pest control practices were followed as recommended in package of practices (Anonymous, 2017). The first spray was carried out as soon as first symptom of disease was noticed in the field. 4 sequential sprays of fungicides and botanicals were taken at an interval of 15 days (Table 5). Disease severity was recorded on fifteen randomly selected leaves in each plot, just one day before each spraying and fifteen days after last spraying. Observations on severity of disease on foliage was recorded by using 1- 5 point scale and PDI was worked out as follows.

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of individual rating X 100}}{\text{Number of plants or leaves examined X maximum disease grade}}$$

The rhizome yield in each plot was recorded and computed to hectare basis, the percent increase over control was computed.

RESULTS AND DISCUSSION

Reaction of Ginger cultivars

Ten cultivars were tested during *kharif* 2018 and 2019 none of them were found free from the disease. All the cultivars were categorized into five different groups based on disease severity. The Humnabad local and Maran

Table 1. Performance of ginger cultivars against *Phyllosticta zingiberi* under field conditions

Kharif 2018 Cultivars	Disease severity (%)	Reaction	Yield (q/ ha)	*Kharif 2019* Cultivars	Disease severity (%)	Reaction	Yield (q/ ha)
Humnabad local	20	MR	180	Humnabad local	18	MR	177
Himagiri	35	S	139	Himagiri	32	S	143
Maran	19	MR	230	Maran	20	MR	223
Suprabha	23	S	170	Suprabha	26	S	173
Himachal	37	S	182	Himachal	35	S	189
Suruchi	30	S	123	Suruchi	28	S	129
Suravi	33	S	177	Suravi	35	S	175
ISSR-Vardha	26	S	229	ISSR-Vardha	29	S	233
ISSR-Mahima	28	S	234	ISSR-Mahima	25	S	230
ISSR-Rejitha	30	S	218	ISSR-Rejitha	27	S	223

Note: HR = Highly Resistant, R = Resistant, MR = Moderately resistant, S=Susceptible, HS = Highly susceptible

Table 2. *In vitro* evaluation of fungicides against *Phyllosticta zingiberi*

Treatments	Fungicides	Concentration (%)	Percent inhibition of mycelia growth
T1	Chlorothalonil 75 WP	0.2	51.7^{d}
T2	Propineb 50 WP	0.2	83.0^{b}
T3	Mancozeb 75 WP	0.2	85.7^{b}
T4	Copper oxy chloride 50 WP	0.3	88.0^{ab}
T5	SAAF 75 WP (Mancozeb 75 WP 50 WP and Carbendazim	0.3	89.3^{ab}
T6	Carbendazim 50 WP	0.1	56.0^{cd}
T7	Propiconazole 25 EC	0.1	92.3^{a}
T8	Hexaconazole 5 EC	0.1	85.7^{b}
T9	Azoxystrobin 23 EC	0.1	91.7^{ab}
T10	Benomyl 50 WP	0.1	52.7^{d}

Note: In the vertical columns means followed by same letters are not different statistically by DMRT (P=0.01).

Table 3. *In vitro* evaluation of botanical against *Phyllosticta zingiberi*

Botanicals	Plant Parts used	Concentration (%)	Percent inhibition of mycelia growth
Parthenium hysteroporus	Leaves	15	37.0^{gh}
Eucalyptus globes	Leaves	15	65.0^{c}
Clerodendron inerme	Leaves	15	53.7^{d}
Allium sativum	Cloves	15	75.7^{a}
Zingiber officinales	Rhizomes	15	34.0^{h}
Aloe vera	Leaves	15	67.0^{bc}
Lantana camera	Leaves	15	42.7^{f}
Durantha repens	Leaves	15	43.3^{ef}
Azadirachta indica	Leaves	15	41.7^{f}
Glyricidia maculata	Leaves	15	30.0^{i}

Note: In the vertical columns means followed by same letters are not different statistically by DMRT (P=0.01).

Table 4. Effect of different antagonists on growth of *Phyllosticta zingiberi*

Antagonists	Percent inhibition of mycelia growth
Trichoderma viride	67.0^{b}
Trichoderma harzianum	78.8^{a}
Trichoderma virens	70.0^{ab}
Trichoderma konnigii	66.0^{b}
Pseudomonas fluorescence	18.8^{d}
Bacillus subtilis	32.8^{c}

Note: In the vertical columns means followed by same letters are not different statistically by DMRT (P=0.01).

cultivars were moderately resistant and others were susceptible (Table1). At the time of harvest, rhizome yield was recorded and computed to hectare basis. No correlation between disease severity and rhizome yield among the cultivars was observed, it might be due to genetic potential of cultivars. Nybe and Nair (1979) reported that Tafingiva was the most tolerant cultivar followed by Maran. Premanathan *et al.* (1980) reported Maran and Karakkal as resistant.

***In vitro* evaluation of fungicides**

The results indicated that significant difference among fungicides in inhibiting the growth of the *Phyllosticta zingiberi.* Among fungicides evaluated, Propiconazole-25EC recorded maximum inhibition of mycelial growth (92.30%) followed by Azoxystrobin 23 EC (91.70%), SAAF 75 WP (89.30%), Copper oxy chloride 50 WP (88.00%) and least inhibition was observed in Chlorothalonil 75 WP (51.70%) (Table-2).

***In vitro* evaluation of botanicals**

The results revealed that effect of plant extracts on the fungal growth was significant. The *Allium sativum* cloves extract was found effective in inhibiting the mycelia growth (75.70%) followed by *Aloe vera* (67.00%), *Eucalyptus globes* (65.00%) and least inhibition was observed in *Glyricidia maculate* (30.00%) (Table-3).

In vitro* evaluation of antagonists against *Phyllosticta zingiberi

All the *Trichoderma* sp inhibited the growth of *Phyllosticta zingiberi* effectively. Among these antagonists *Trichoderma harzianum* showed highest

Table 5. Effect of different fungicides and botanicals on leaf spot of ginger caused by *Phyllosticta zingiberi* during *kharif* -2018 and 2019

Treatment	2018			2019		
	Mean PDI	Rhizome Yield (q/ha)	Percent yield increase over control	Mean PDI	Rhizome Yield (q/ha)	Percent yield increase over control
T1 -Mancozeb 75 WP	50^{fg}	175^{d}	21.53	50^{e}	174^{cd}	20.00
T2-Propineb 50 WP	55^{e}	173de	20.14	51^{e}	176^{c}	21.37
T3-Copper oxy chloride 50 WP	53^{ef}	174de	20.83	54de	175^{c}	20.68
T4- Chlorothalonil 75 WP	57de	170de	18.05	56^{cd}	173^{cd}	19.31
T5-SAAF 75 WP (Mancozeb 75 WP and Carbendazim 50 WP)	46^{g}	179^{cd}	24.30	48^{e}	180^{b}	24.31
T6-Propiconazole 25 EC	36^{i}	202^{a}	40.27	35^{g}	203^{a}	40.00
T7-Hexaconazole 5 EC	45^{h}	180^{c}	25.00	44^{f}	182^{b}	25.51
T8-Azoxystrobin 23 EC	40^{i}	185^{bc}	28.47	42^{f}	183^{b}	26.20
T9-*Eucalyptus globes*	62^{cd}	167^{e}	15.97	64^{b}	170^{d}	17.24
T10-*Allium sativum*	60^{d}	169^{e}	17.36	58^{c}	171^{d}	17.93
T11-*Aloe vera*	61^{cd}	168^{e}	16.66	62^{b}	165^{e}	13.80
T12- *Clerodendron inerme*	65^{bc}	165^{e}	14.58	63^{b}	167^{e}	15.17
T13-Control	79^{a}	144^{f}	-	80^{a}	145^{f}	-

Note: In the vertical columns means followed by same letters are not different statistically by DMRT (P=0.05).

inhibition (78.80%). Both bacterial antagonists used in the study *viz., Bacillus subtilis* (32.80%) and *Pseudomonas fluorescens* (18.80%) were moderate in controlling *Phyllosticta zingiberi* (Table 4).

Management of leaf spot, *Phyllosticta zingiberi*

In subsequent sprays all the fungicides and botanicals treated plots recorded significantly less disease index over control. During *Kharif*-2018, among fungicides 0.1% Propiconazole- 25EC was significantly effective in reducing the disease by recording a PDI of 36.00 and a yield of 202q/ha (Table-5). 0.1% Azoxystrobin-23EC,0.1% Hexaconazole-5EC, 0.2% SAAF-75 WP (Mancozeb-63WP and Carbendazim-12WP), 0.2% Mancozeb-75WP and 0.2% Propineb-50 WP were next best treatments found effective in reducing the disease intensity by recording a PDI of 40.00, 45.00, 46.00, 50.00 and 55.00 and a yield of 185q/ha, 180q/ha, 179q/ha, 175q/ha and 173q/ha, respectively. Among botanicals tested, mimimum PDI of 60.00 and yield of 169q/ha was recorded in *Allium sativum* cloves extract (15%) and maximum PDI of 79.00 and yield of 144q/ha was recorded in the control plot (Table-5). Again during *Kharif* 2019, Propiconazole 25 EC was significantly superior over other treatment and similar trend was observed for other treatments also (Table-5). Sohi et al., 1973 reported that Copper oxy chloride 50 WP and Mancozeb 75 WP were effective against *Phyllosticta zingiberi* and gave maximum yield per ha.

ACKNOWLEDGEMENT

We thank College of Horticulture, Bidar and University of Horticultural Sciences, Bagalkot, Karnataka for their provisions to this study.

REFERENCES

Anonymous. 2014. Ginger (Extension Pamphlet) *Indian Institute of Spice Research*. p. 1.

Anonymous. 2017. *Package of Practices*, University of Horticultural Sciences, Bagalkot, Karnataka, India. pp.186-190.

Das, N. and Senapati, A. K.1998. Chemical control of leaf spot of ginger. *Annals of Plant Protection Sciences,* 6: 207-208.

Dohroo, N. P., Shyam, K. R., Bhardwaj, S. S. and Korla, B. N. 1986. Reaction of ginger germplasms to *Phyllosticta* leaf spot. *Indian Phytopathlogy,* 39: 60S.

Kanware, H. T. 1974. Studies on leaf spot of ginger caused by *Phyllosticta zingiberi*. *M.Sc. thesis,* Punjab rao Krishi Vidyapeeth, Akola, India. 53:

Nazareno, N. R. X .1995. Controle da mancha do amarelao (*Phyllosticta sp.)* do gengibre com fungicida comerciale. *Horticultura- Brasileira.* **13:** 142-146.

Nene,Y. L. and Thapliyal, P. N. 1979. Fungicides in Plant Disease Control. 3rd edition. *Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi.* p.325.

Nybe, E. V. and Nair, P. C. S. 1979. Field tolerance of ginger type to important pest and diseases. *Indian Arecanut. Spices and Cocoa,* **1**: 109-111.

Premanathan, T., Peethambaran, C. K. and Abi Cheeran. 1980. Screening of ginger cultivars against *Phyllosticta* leaf spot. *Proceedings of National Seminar on Ginger and Turmeric,* Calicut. April 8-9, 1980 pp.126-127.

Ramakrishnan, T. S. 1942. A leaf spot disease of *Zingiber officinale* caused by *Phyllosticta zingiberi*. *Proceedings of Indian Academy of Sciences,* **15:** 167-171.

Ravishanker Kumar, S., Chatterjee, A., Baranwal, D. K. and Solankey, S. S. 2014. Genetic variability for yield and quality traits in ginger (Zingiber officinale Roscoe). *The Bioscan.* **8** (4): 1383-1386.

Shadap, A., Hegde, N. K. and Pariari, A. 2013. Performance of ginger var. Humnadad local as influenced by planting Dates under northern dry zone of Karnataka, *The Bioscan*. **8** (1): 131-133.

Shanmugam, K. R., Ramakrishna, C. H., Mallikarjuna, K. and Sathyavelu Reddy, K. 2009. Antihyperglycemic and Antihypolipidemic effects of *Zingiber officinale* Ethanolic extract in Streptozotocin induced diabetic Rats, *The Bioscan.* **4** (2): 241-245.

Sohi, H. S., Sharma, S. L. and Varma, B. R. 1973. Chemical control of *Phyllosticta* leaf spot of ginger *(Zingiber officinales).Pesticides*, **7:** 21-22.

Vincent, J. M.1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature.* 59: 850.

MS Received: 21 November 2022
MS Accepted : 26 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 189 -191 (2022)

DOI Number: 10.5958/0974-4541.2022.00059.5

RESEARCH NOTE

Efficacy of biorational insecticides against aphids, *Aphis craccivora* in amaranthus

B. J. MANJUSHREE*[1], JAYALAXMI NARAYAN HEGDE[2], K. RAJASHEKARAPPA[3], B. GANGADHARA NAIK[4] and NAGARAJAPPA ADIVAPPAR[5]

[1,2,3]Department of Entomology, [4]Department of Plant Pathology, [5]Zonal Agricultural and Horticultural Sciences, College of Agriculture, Navile, Shivamogga- 577 204, Karnataka

***E-mail**: manjushreebj@gmail.com

ABSTRACT: A field experiment was conducted to study the bioefficacy of biorationals against cowpea aphid, *Aphis craccivora* infesting amaranthus during *rabi* 2019-20 at the Zonal Agricultural and Horticultural Research Station (ZAHRS), Shivamogga. Out of six biorationals evaluated, the highest per cent reduction of aphid population was recorded in *Lecanicillium lecanii* 1 × 109 CFU @ 1 g/l (62.87 %), followed by NSKE 5% (53.59%). Concerning the B: C ratio, NSKE 5 % recorded the highest return per rupees invested, *i.e.,* 1: 2.45, followed by azadirachtin 10,000 ppm @ 2 ml/l (1: 2.16) and *Beauveria bassiana* 1×10^9 CFU/g @ 1 g/l (1: 2.12).

Keywords: Amaranthus, Aphids, *Lecanicillium lecanii*, NSKE 5%, Organic management,

Amaranthus is one of the most important leafy vegetables with high nutritional and medicinal properties (Martirosyan *et al.,* 2007). Several insect and non-insect pests attack amaranthus, *viz.*, *Hymenia recurvalis* (Fabricius)*, Spodoptera litura* (Fabricius)*, Erectomocera impectella* (Walker), *Psara basalis* (Walker), *Helicoverpa armigera* (Hub.), *Agrotis segetum, Liriomyza* sp. beet worm moth, *Spoladea recurvalis*, *Hypolixus truncatullus,* leaf beetles, aphids and grasshoppers (Manjula and Kotikal, 2018, Manikandan and Kannan, 2019, Seni, 2018).

The cowpea aphid, *Aphis craccivora* Koch. (Hemiptera: Aphididae) is an important sucking pest that damages different parts of the plants throughout the crop growth and affect the economic yield. To manage the aphid in amaranthus, farmers are indiscriminately spraying different insecticides in unscientific ways without knowing the ill effects of synthetic inorganic insecticides. Since amaranthus is consumed regularly in our daily food, it is necessary to manage the insect pests organically to avoid health hazards and reduce the insecticide residue in the produce. Keeping this in view, a field experiment was conducted on the bioefficacy of organic insecticides against aphids, *A. craccivora* in amaranthus, to know the effective biorationals to manage the aphids in amaranthus.

The present study was carried out at Zonal Agricultural and Horticultural Research Station (ZAHRS), Keladi Shvappa Nayaka University of Agricultural and Horticultural Sciences, Navile, Shivamogga during *Rabi* 2019-20 (13°27' N and 74°37' to 75°52' E, 650 meters above MSL). Six biorationals were evaluated and compared with standard check, malathion 50 EC @ 2 ml/l and untreated control. The experiment was laid out in randomized complete block design with three replications. Six biorationals *viz*., Neem Seed Kernel Extract (NSKE) 5 %, azadirachtin 10,000 ppm @ 2 ml/ l, pongamia oil 5 %, *Beauveria bassiana* 1×10^9 CFU/g @ 1 g/l, garlic extract 2 % and *Lecanicillium lecanii* 1×10^9 CFU/g @ 1 g/l along with a standard check, malathion 50 EC @ 2 ml / and untreated control were evaluated against aphids. Pre-treatment counts were made the day before spraying on 5 cm of the shoot length, and post-treatment observations were recorded at 3, 5, 7 and 10 days after spraying. The yield was also recorded, and the cost-benefit ratio was worked out for each treatment. Per cent reduction over the control was also calculated. The statistical analysis of the data obtained using Web Agri. Stat Package (WASP- 2) developed by the Indian Council of Agricultural Research, Research Complex, Goa.

Fifty grams of well-dried neem seed kernels were powdered using a pestle and mortar and soaked overnight in 500 ml water. The next morning, the solution was stirred with a wooden stick till the solution became milky white. One per cent detergent was added to the solution. Then the solution was filtered through double-layered muslin cloth, and the volume was made to one litre by adding water. To prepare 2 % garlic extract, 20 g of grinded garlic paste was soaked in 20 ml of kerosene overnight. The next morning, the mixture was stirred well, and one per cent of detergent was added to the solution. Then the solution was filtered through muslin cloth, and the volume was made to one litre by adding water.

Table 1. Efficacy of biorationals against aphids, *Aphis craccivora* on amaranthus during *Rabi* 2019-20

Treatment details	Mean number of aphids per 5 cm shoot					Overall Mean	Per cent reduction over control	B: C ratio
	1 DBS	3 DAS	5 DAS	7 DAS	10 DAS			
T_1-NSKE 5%	26.93 (5.23)	11.53 (3.45)^{de}	9.46 (3.15)^{de}	12.43 (3.56)^{c}	14.26 (3.82)^{de}	11.92	53.59	2.45
T_2-Azadirachtin 10,000 ppm 2ml/l	25.13 (5.06)	13.63 (3.75)^{cd}	11.66 (3.48)^{cd}	15.96 (4.05)^{b}	16.76 (4.15)^{cd}	14.50	43.54	2.16
T_3-Pongamia oil 5%	26.13 (5.16)	15.73 (4.02)^{bc}	13.86 (3.78)^{bc}	17.13 (4.19)^{b}	19.33 (4.45)^{bc}	16.51	35.71	1.76
T_4- *Beauveria bassiana* 1×10^9 spores 1g/l	27.06 (5.24)	18.46 (4.35)^{b}	16.86 (4.16)^{b}	19.26 (4.44)^{b}	21.43 (4.68)^{b}	19.00	26.02	2.12
T_5- Garlic extract 2%	25.86 (5.13)	15.56 (4.00)^{bc}	14.36 (3.85)^{bc}	17.46 (4.23)^{b}	19.13 (4.42)^{bc}	16.62	35.27	1.71
T_6- *Lecanicillium lecanii* 1×10^9 spores 1g/l	27.40 (5.28)	9.33 (3.11)^{ef}	7.63 (2.84)^{e}	9.53 (3.16)^{cd}	11.66 (3.48)^{ef}	9.53	62.87	1.88
T_7- Malathion 50 EC 2ml/l	25.86 (5.13)	6.53 (2.64)^{f}	4.46 (2.22)^{f}	8.33 (2.96)^{d}	9.66 (3.16)^{f}	7.24	71.79	3.07
T_8- Control	27.53 (5.29)	25.23 (5.07)^{a}	22.56 (4.80)^{a}	26.53 (5.19)^{a}	28.43 (5.37)^{a}	25.68		1.54
SEm±	0.16	0.15	0.14	0.13	0.13			
CD@(P=0.05)	0.50	0.47	0.44	041	0.40			
CV (%)	NS	7.16	6.45	6.29	5.68			

Figures in parentheses are √x+0.5 transformed values;
Means in the columns followed by the same alphabet do not differ significantly by DMRT (P = 0.05);
DBS- Day before spray;
DAS- Days after spraying

Before the imposition of the treatment, the mean population of aphids ranged from 25.13 to 27.53 per 5 cm of the shoot length and were found to be statistically non-significant in different treatment plots (Table 1).

Three days after spray, there was a significant difference among the treatments with respect to the mean number of aphids per 5 cm of shoot length. Among the organic insecticides evaluated, *L. lecanii* @ 1 g / l was found superior (9.33 mean aphids / 5 cm of shoot length), followed by NSKE 5 % (11.53 mean aphids / 5 cm of shoot length). Standard check malathion 50 EC @ 2 ml / l was found superior of all the treatments, which recorded the lowest mean number of aphids per 5 cm of shoot length (6.53).

There was a significant difference among the treatments five days after the spray. Among the biorationals evaluated, the lowest aphid population per five centimetres of shoot length was recorded with *L. lecanii* @ 1 g / l (7.63), followed by NSKE 5 % (9.46). Standard check malathion 50 EC @ 2 ml/l was found superior among all the treatments, with the lowest mean number of aphids per 5 cm of shoot length (4.46). *Lecanicillium lecanii* @ 1 g / l recorded the least mean aphid population per 5 cm shoot length (9.53), followed by NSKE 5 % (12.43). Standard check malathion 50 EC @ 2 ml / l was found superior, with the lowest mean number of aphids per 5 cm of shoot length (8.33).

Among the biorationals evaluated, *L. lecanii* @ 1 g/l recorded the least number of aphids per plant (11.66 aphids/ 5 cm of shoot length), followed by NSKE 5 % (14.26 aphids/5 cm of shoot length). Standard check malathion 50 EC @ 2 ml/l was found superior among all the treatments, with the lowest mean number of aphids per 5 cm of shoot length (9.66).

Among the biorationals evaluated, the significantly highest per cent reduction of aphid population over the control was recorded in treatment *L. lecanii* @ 1 g /l (62.87). These research findings are in close line with the results of Salam and Hawary (2011), who reported the

virulence of *L. lecanii* in the adults and nymphal stages of aphids. They also observed 100 per cent mortality in adults and nymph over three days of treatment with the concentration of 5×10^6 CFU/ml and 1 × 106 CFU/ml. Salam *et al*. (2012) reported that *V. lecanii* was virulent against bean aphids, *A. craccivora*, and it could reduce the aphid population density by 73.33 per cent. Suresh *et al*. (2012) also reported that *V. lecanii* @ 1×10^9 CFU/ml recorded 71.62 % mortality of aphids. Khade *et al*. (2014) reported that *V. lecanii* @ 4 g showed a 64.84 per cent reduction of aphids. In addition, the present study reported that the application of NSKE 5 % resulted in a 53.59 % reduction in the aphid population. These are in close line with Aziz *et al.* (2014), who evaluated the different neem products against mustard aphids on the Canola crop and reported that NSKE 5 % reduced the pest population by 86.13 %.

Out of the biorationals evaluated against aphids, the benefit-cost (B:C ratio was highest in the case of NSKE 5 %, *i.e*. 2.45 due to a higher leaf yield of 14.54 t/ha, followed by azadirachtin 10,000 ppm @ 2 ml/l which recorded B: C ratio of 2.16 with leaf yield of 13.14 t/ha, *B. bassiana* which recorded B: C ratio of 2.12 with leaf yield of 12.72 t/ha, *L. lecanii*, which recorded B: C ratio of 1.88 with leaf yield of 11.30 t/ha, pongamia oil 5% which recorded B: C ratio of 1: 1.76 with leaf yield of 10.62 t/ha and garlic extract 2 % which recorded B: C ratio of 1.71 with leaf yield of 10.24 t/ha (Table 1). Manjula *et al*. (2015) also reported cost benefits of insecticides and botanicals against defoliators on amaranthus, wherein treatments NSKE 5 % and azadirachtin gave leaf yield of 12.22 t/ha and 13.89 t/ha with 22.00 and 14.28 % increase in the cost-benefit ratio, respectively. However, by standard check, malathion 50 EC @ 2 ml/l recorded the highest B: C ratio (1: 3.07) compared to all other organic insecticides with a leaf yield of 18.60 t/ha. But, since green leafy vegetables are consumed daily, the aphids and other insect pests should be managed organically to avoid pesticide residue in the harvested greens and to avoid health hazards to the consumers. Hence, the organic insecticides NSKE 5 %, followed by azadirachtin 10,000 ppm @ 2ml/l, can be used to manage this aphid.

REFERENCES

Aziz, A. M., Shahzad, R. A., Naeem, M. and Shabbir, G. 2014. Evaluation of different neem products in comparison with imidacloprid against different morphs of mustard aphid (*Lipaphis erysimi* KAL.) on canola crop. *Asian Journal of Agriculure and Biol*ogy, **2** (3): 191-201.

Khade, K. N., Undirwade, B. D., Tembhurne, R. D. and Lande, G. K. 2014. Biorational management of sucking pests of cowpea, *Vigna sinensis* L. *Trends in Biosci*ences, **7** (20): 3212-3217.

Manikandan, P. and Kannan, R., 2019. Efficacy of an insect growth regulator on Amaranthus leaf caterpillar, *Spoladea recurvalis* (Fab.) (Lepidoptera; Crambidae). *Pest Management in Horticultural Ecosystems*, **25**(2): 241-246.

Manjula, K. N., Kotikal, Y. K., Patil, H. B. and Biradar, I. B. 2015. Studies on insect fauna, their natural enemies and pollinators in fenugreek. *Karnataka Journal of Agricultural Sciences*, **28** (2): 279-281.

Manjula, K. N. and Kotikal, Y. K. 2018. Studies on insect fauna of Amaranthus and loss estimation due to major defoliators. *International Journal of Pure and Applied Biosci*ence, **6** (2): 341-351.

Martirosyan, D. M., Lidia, A. M., Svetlana, N. K., Pogojeva, A. V. and Zoloedov, V. A. 2007. Amaranth oil application for coronary heart disease and hypertension. *Lipids in healh and disease*, **6** (1): 1-12.

Salam, A. M. F. and Hawary, F. M. A. 2011. Lethal and pathogenic effects of *Beauveria bassiana* and *Lecanicillium lecanii* on the adul and nymph of *Aphis craccivora* Koch. *Archives of Phytopathology and Plant Prot*ectiton, **44** (1): 56-66.

Salam, A. M. E., Salem, S. A. and Kholy, M. Y. 2012. Efficacy of nimbecidine and certain entomopathogenic fungi formulations against bean aphids, *Aphis craccivora* in broad bean field. *Archives of Phytopathology and Plant Prot*ectiton, **45** (19): 2275-2277.

Seni, A. 2018. Insect pests of Amaranthus and their management. *International Journal of Enviroment, Agriculure and Biotechnology,* **3** (3): 1100-1103.

Suresh, B. C., Khader, K. H. and Prasanna, P. M. 2012. Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* Koch under laboratory and field conditions. *International Journal of Plant Prot*ection, **5** (1): 68-71.

MS Received: 04 August 2022
MS Accepted : 03 October 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 192 - 194 (2022)

DOI Number: 10.5958/0974-4541.2022.00060.1

RESEARCH NOTE

Evaluation of insecticides for management of thrips, *Scirtothrips dorsalis* Hood in cashew

V. K. ZOTE[1]*, A. Y. MUNJ[1], V. S. DESAI[1] and R. C. GAJBHIYE[1]

[1]Regional Fruit Research Station, Vengurle, Dist. Sindhudurg, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (MS) 415 712, India.

***E-mail**: vaishalizote@gmail.com

ABSTRACT: The production and productivity of cashew is influenced by many factors, among which incidence of insect pests is one of the major factor. Cashew thrips is one of the most important pest of cashew. Considering the importance of thrips, experiment was carried out at Regional Fruit Research Station, Vengurla under All India Coordinated Research Project on Cashew for management of cashew thrips during 2015-16 to 2017-18. From the pooled data of three years, it is revealed that the insecticide acetamiprid20SP @ 0.5 g/L was found to be the most effective for management of cashew thrips with the least incidence of thrips (2.29%) after third spray followed by deltamethrin 2.8 EC @ 0.9ml/ lit (3.68%).

Keywords: Cashew, insect pest, management, thrips, *Scirtothrips dorsalis*

Cashew is one of the most important cash crop grown in India. The production and productivity of cashew is influenced by many biotic and abiotic factors; among them, incidence of insect pests is the major constraint (Dumbare *et al.,* 1987; Godase *et al.,*2005; Raviprasad, 2015; Anamika Kar and Poduval, 2016; Zote *et al*., 2017; Gupta, 2020;. Lakshmana *et al.,* 2020; Molly Irine *et al*., 2020). Pillai *et al.* (1976) documented sixty insect species causing regular damage to cashew crop; among which tea mosquito bug (*Helopeltisantonii*), stem and root borer (*Plocaederus ferrugineus*), inflorescence thrips (*Scirtothrips dorsalis*), apple and nut borer (*Nephopteryx sp.*) etc. are the major pests of cashew in India. Patil *et al.,* (1979) reported *Scirtothrips dorsalis* as predominant species of cashew thrips in Konkan region of Maharashtra. Godase *et al.,* (2005) and Navik (2015) reported that, thrips has become a major pest of cashew causing upto 30 per cent reduction in nut weight in Konkan region of Maharashtra. About six species of thrips are known to attack cashew in India; out of which four species *viz*., *Scirtothrips dorsalis* Hood., *Thrips hawaiiensis* Morgan., *Selenothrips rubrocinctus* Giard and *Haplothripstenuipennis* Bagnal have been recorded infesting cashew in Konkan region of Maharashtra (Parab, 2010).

Adults and nymphs are seen in colonies on the lower surface of leaves. Due to sustained feeding by large number of thrips, the terminal leaves curl downward from margin toward mid rib. In due course, the young leaves fall down. Due to feeding by huge number of thrips, the apples and nuts become corky, remain under sized with shabby appearance. Also, the juice content of apple is reduced (Maruthadurai *et al*., 2012 and Navik, 2016). The thrips alone accounts for severe fruit drop (Panda, 2013). Considering the importance of thrips in Konkan region, the present study was conducted to evaluate the efficacy of some insecticides against cashew thrips at Regional Fruit Research Station, Vengur launder All India Coordinated Research Project on Cashew during 2015-16 to 2017-18.

A field trial was conducted in randomized block design with seven treatments (Table 1) and three replications during the years 2015-16, 2016-17 and 2017-18 at Regional Fruit Research Station, Vengur launder AICRP on Cashew to find out the effective insecticide for the management of thrips with following different treatments.

For recording per cent incidence of thrips (corky growth or presence of scabs) hundred nuts as well as apples per tree were selected randomly and thripsdamage score was recorded in 0-4 scale (Table 1) ((Ambika *et al*., 1979, Godase *et al*., 1990).

The data on the percent incidence of thrips recorded during the year 2015-16, 2016-17, 2017-18 along with pooled data of three years is presented in Table 1. All the insecticide treatments were found effective for management of cashew thrips, as these treatments reduced the pest incidence over control significantly. During 2015-16, the treatment T_2 (Acetamiprid20 SP @0.5 g/L) was found to be the most effective treatment which recorded the least incidence of thrips (2.88 %). It was significantly superior over all other treatments.

Table 1. Scoring of thrips damage

Rating	Extent of damage
0	No damage
1	1-25 per cent nut or apple surface damaged (up to 1/4 of the damaged surface area)
2	26-50 per cent nut or apple surface damaged (up to 1/2 of the damaged surface area)
3	51-75 per cent nut or apple surface damaged (up to 3/4 of the damaged surface area)
4	76-100 per cent nut or apple surface damaged (more than 3/4 of the damaged surface area)

The recoded data were converted into percent incidence on the basis of formula given below,

Table 2. Efficacy of insecticides for management of thrips in cashew (2015-16 to 2017-18)

Treatment		Mean per cent incidence of thrips			
		2015-16	2016-17	2017-18	Pooled
T_1	Emamectin Benzoate 5 SG @ 0.2 g/L	6.08 (14.19)	3.20 (10.20)	4.80 (12.56)	4.69 (12.31)
T_2	Acetamiprid 20 SP @ (0.5 g/L)	2.88 (9.66)	1.28 (6.26)	2.72 (9.12)	2.29 (8.34)
T_3	Flonicamid 50WG @ (0.3g/L)	6.08 (14.21)	4.64 (12.42)	4.16 (11.59)	4.96 (12.74)
T_4	Deltamethrin 2.8 EC @ 0.9 ml /L	5.28 (13.20)	2.24 (8.44)	3.52 (10.72)	3.68 (10.78)
T_5	Standard check	3.84 (11.69)	2.88 (9.63)	4.32 (11.95)	3.68 (11.09)
T_6	Buprofezin 25SC @ 2ml/L	8.01 (16.38)	1.79 (7.54)	4.32 (11.95)	4.70 (11.95)
T_7	Untreated control	12.01 (20.21)	8.65 (17.04)	7.05 (15.32)	9.23 (17.52)
	S.Em	0.635	0.583	0.615	0.864
	CD at 5%	1.92	2.80	1.86	2.62

• Figures in parenthesis are arcsine transformed values

During 2016-17, the treatment T_2 (Acetamiprid20SP @ 0.5 g/L) was found to be the best for the management of thrips with the least incidence of thrips (1.28%) but it was at par with the treatment T_5 (Buprofezin25 SC@2ml/L) and T_4 (Deltamethrin 2.8 EC @0.9ml/L). During 2017-18, the treatment T_2 (Acetamiprid20 SP @ 0.5 g/L) was found the most effective treatment for management of thrips with least incidence (2.72%) however, it was at par with the treatment T_4 (Deltamethrin2.8 EC @ 0.9ml/L). From the pooled mean of three years, it is evident that, the treatment T_2 (Acetamiprid 20 SP @0.5 g/L) was found to be the most effective treatment for the management of thrips with least incidence of thrips (2.29%), however, it was at par with the treatment T_4(Deltamethrin2.8 EC @ 0.9ml/L).

The present finding are in close agreement with those of Anamika Kar (2017) who reported acetamiprid as effective insecticide for the management of cashew pests. Samota (2017) reported the efficacy of acetamiprid against *Scriptothrips dorsalis* in chilli. Many earlier research workers have studied the efficacy of different insecticides for management of cashew thrips. Ayyanna *et al*. (1985), reported the efficacy of phosalone and diamethoate against cashew thrips. Mahapatro (2008), Navik *et al*., (2016) and Zote *et al.,* (2017a) reported the efficacy of lambda cyhalothrin against cashew thrips. Godase and Bhole (2002) reported the efficacy of permethrin, cypermethrin and deltamethrin against cashew thrips. Jalgaonkar, *et al.,* (2011) reported the efficacy of lambda cyhalothrin and triazophos against cashew thrips.

ACKNOWLEDGEMENT

The authors are thankful to the Project Coordinator, AICRP, Cashew for providing funds for conducting present research. Also, the authors are thankful to the Director of Research, Dr. B.S. Konkan Krishi Vidyapeeth, Dapoli for providing facilities for conducting the research.

REFERENCES

Ambika, B., Abraham, C. C. and Vidyadharan, K. K. 1979. Relative susceptibility of cashew types to infestation by *Helopeltisantonii* Sign. In: *Proc. PLACROSYM-II*, 28-29 June, 1979, Ootacamund, India, 513-516.

Anamika Kar. 2017. Evaluation of few insecticides against insect pests of cashew. *Journal of Entomology and Zoology Studies,* **5** (2): 1345-1347.

Anamika Kar and Poduval, M. 2016. Seasonal incidence of insect pests in cashew in relation with weather conditions in red and lateritic zone of West Bengal. *The Eco Scan,* **10** (3 and 4): 535-537.

Ayyanna, R., Arjuna Rao, P., Narayana, K. L. and Krishnamurthy Rao, B. H. 1985. Chemical control of cashew leaf thrips. *Acta Horticulture*, **108**: 301-302.

Dumbare, R. B., Godase, S. K. and Dhuri, A. V. 1987. Current status of entomological problems of cashewnut. In: Seminar on "*Strategies in increasing cashew production*" held at Dapoli during 1987, 75-84.

Godase, S. K. and Bhole, S. R. 2002. Evaluation of some synthetic pyrethroids against flower thrips of cashew. *Pestology,* **26** (3): 19-20.

Godase, S. K., Bhole, S. R. and Patil, B.P. 2005. Population fluctuation studies of tea mosquito bug of cashew and its relation with weather parameters. *Journal of Agrometereology*, **7** (1):107-109.

Godase, S. K., Dumbare, R. B. and Kharat, S. B. 1990. Chemical control of flower thrips on cashew. *The cashew*, October-December 1990, 14-15.

Gupta, A. K. 2020. Curative control of cashew stem and root borer, *P. ferrugineus .Journal of Entomology and Zoology Studies,* **8** (4): 1583-1585.

Lakshmana, M. M. and Ranganath, T. K. 2020. Evaluation of insecticides against cashew stem and root borer. *Inter. Journal of Currant Microbiology and Applied Science,* **9** (3): 2149-2152.

Mahapatro, G. K. 2008. Evaluation of insecticidal spray for control of tea mosquito bug and other insect pests in cashew. *Indian Journal of Entomology,* **70** (3): 217-222.

Maruthadurai. R., Desai A. R., Prabhu , H. R. C. and Singh, N. P.2012. Insect pest of cashew and their management. *Technical Bulletin*, 28, ICAR Research Complex for Goa, Old Goa.

Molly Irine, G. S., Vetriselva, V., Thamizhvel, R. and Ganesh, M. 2020. Possible approaches and future thrust area to protect infestation by cashew stem and root borer- review. *Pal Arch's Journal of Archaelogy of Egypt,* **17** (9): 5845-5848.

Navik, O. S. 2015. Seasonal incidence and management of pests of cashew. Ph.D. thesis submitted to Dr. B. S. K. K. V., Dapoli.

Navik, O. S., Godase, S. K. and Turkhade, P. D. 2016. Population fluctuation of cashew thrips under Konkan region of Maharashtra. *Journal of Environment and Ecology*, **4** (2A): 615-618.

Panda, H. 2013. The Complete Book on Cashew (Cultivation, Processing and By- products). *Asia Pacific Business Press Inc,* 85-86

Parab, V.V. 2010. Record of cashew thrips species in the Konkan region of Maharashtra. *Asian Journal of Experimental Biological Science,* Special issue: 124-125.

Patil, P. D. and Dumbre, R.B. 1979. An unusual occurrence of thrips on cashew. In: Proceeding of the *International Cashew Symposium* held at Cochin, pp. 136.

Pillai, G. B., Dubey, O. P. and Singh, V. 1976. Pests of cashew and their control in India- review of current status. *Journal of Plantation Crops,* **4**: 37-50.

Raviprasad, T. N. 2015. Pest and disease management in cashew including biological control. *Journal of Cashew and Cocoa*, **4** (3): 9-17.

Samota, R. G., Jat, B. L. and Chaudhari, M. D. 2017. Efficacy of newer insecticides and biopesticides against thrips, *Scirtothrips dorsalis* Hood in chilli. *Journal of Pharmacognosy and Phytochemistry*, **6** (4): 1458-1462

Zote, V. K., Salvi, S. P., Haldavanekar, P. C. and Narangalkar, A. N. 2017. Influence of biotic factors on the population dynamics of cashew pests in Konkan region of Maharashtra. *Journal of Entomology and Zoology Studies*, **5** (1): 860-863.

Zote, V. K., Munj, A. Y. and Salvi, S. P. 2017a. Bioefficacy of insecticides for the management of cashew thrips (*Scirtothripsdorsalis* Hood) under South Konkan Coastal region of Maharashtra. *Journal of Entomology and Zoology Studies,* **5** (1): 783-785.

MS Received: 16 June 2022

MS Accepted : 05 September 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 195 - 197 (2022)

DOI Number: 10.5958/0974-4541.2022.00061.3

RESEARCH NOTE

Influence of pollination by honey bee (*Apis cerana indica* F.) on the yield parameters of bottle gourd

D. H. PADHIYAR* and S. R. PATEL

Department of Agricultural Entomology, N. M. College of Agriculture, Navsari Agricultural University, Navsari- 396 450, Gujarat, India

***E-mail:** digu.p2014@gmail.com

ABSTRACT: The effect of different modes of pollination on quantitative parameters of bottle gourd was studied during *kharif* 2019 at Navsari Agricultural University, Navsari. The different quantitative parameters like the number of fruits/plant, per cent fruit set, per cent malformed fruit, fruit diameter, fruit weight, yield, and yield/ha were maximum in open pollination (5.38, 59.67%, 25.09%, 6.84cm, 961.24g, 5.17 kg/plant and 25,836 kg/ha, respectively) compared to pollination with *Apis cerana indica* (5.14, 54.03%, 20.43%, 6.76cm, 878.57g, 4.52 kg/plant and 22,607 kg/ha, respectively). The number of fruit drops was maximum in pollination with *A. cerana indica* (4.57), followed by open pollination (3.69). In the treatment of pollination without insects, no fruit formation was observed. The cost-benefit ratio was highest in open pollination (22.49:1) compared to pollination with *A. cerana indica* (4.69: 1).

Keywords: Bottle gourd, *Apis cerana indica*, pollination, quantitative parameters

Bottle gourd, *Lagenaria siceraria* (Molina) Standl. (F: Cucurbitacea) is an important multi-purpose vegetable grown for its leaf, fruit, and seed. In Ayurveda, bottle gourd is advocated for treatment of diabetes mellitus, hypertension, liver diseases, weight loss and other associated benefits (Prajapati *et al*., 2010). Being a monoecious crop, bottle gourd is mostly cross pollinated. There are several studied undertaken to seen the impact of *Apis cerana* on crop yield and productivity which showed that pollination by *A. cerana* increased fruit and seed set, increased the quality of fruit and seeds, and reduced premature fruit drop (Koetz, 2013; Kumar *et al*., 2021). Different types of pollinator fauna are available for the pollination of the bottle gourd. Among available pollinator insects, honey bees are the major pollinator (Ipsita Panigrahi, *et al.,* 2018) and they also contribute in the increasing of yield and quantitative parameters of bottle gourd. But, the detailed information on pollinator complex and role of pollinators on quantitative improvement of the bottle gourd is very scanty. There for present study is directed to access the role of honey bee, *A. cerana indica* F. on quantitative parameters of bottle gourd.

The study was carried out during *kharif* (July-December 2019) at college farm, Navsari Agricultural University, Navsari, Gujarat. Bottle gourd crop (MGH 4- WARAD) was sown in three plot measuring 12m × 12m size comprising three treatments. Planting was done with a spacing of 2×1 m between plants. The cultural operations were done as and when required. In the treatments, treatment number one (T_1) comprises of open pollination means free excess to insect pollinators were made available to pollinate the flowers. In the treatment number second (T_2), plants were covered with mosquito net 10m × 6m × 3m to prevent the entry of insect pollinators. In the third treatment (T_3), plants were covered with mosquito net and bee pollination was done by placing one healthy colony of *Apis cerana indica* F. containing four frames at the 10% flowering. The plots were kept unsprayed throughout the crop season. To study the effect of bee pollination on the yield parameters of bottle gourd in all treatments, in each replication three plants were tagged and observations were recorded on number of flowers per plant, number of fruits, number of fruits drop, per cent fruit set, healthy and malformed fruits per plant, diameter of fruit, weight of fruit, yield per plant, yield per hectare and economics. The collected data were analyzed statistically.

Among the different treatments, (T1) open pollination, (T2) pollination without insect and (T3) pollination with *A. cerana*, the mean number of male and female flowers per plant was observed that 46.76, 46.71, 47.61 and 9.04, 9.47, 9.71 respectively. The analysis of data revealed that there were non-significant differences among different treatments with respect to male and female flowers. The number of fruits per plant as affected by different pollination treatment revealed that maximum number of fruits per plant was recorded in treatment of open pollination (5.38) which was followed by the treatment of pollination with *A. cerana indica* (5.14). There was no fruit set recorded in treatment (T2) pollination without insect (Table 1). The present findings are more or less in

Table 1. Yield parameters of bottle gourd affected by different pollination treatments

Treatment	No. of fruits/ plant	No. of fruit drop	Per cent fruit set	Per cent malformed fruit	Fruit diameter (cm)	Fruit weight (g)	Yield (kg/ plant)	Yield (kg/ha)	BC ratio
T_1 : Open pollination	5.38	3.69*	59.67 (50.58)	25.09 (30.06)	6.84	961.24	5.17	25,836	22.49:1
T_2: Pollination without insect	00.00	00.00*	00.00 (00.00)	00.00 (00.00)	00.00	00.00	00.00	00.00	00:1
T_3: Pollination with *A. cerana indica*	5.14	4.57*	54.03 (47.31)	20.43 (26.87)	6.76	878.57	4.52	22,607	4.69:1
SEm. ±	0.16	0.25	1.15	1.48	0.19	28.44	0.21	1069.08	
CD (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	
CV (%)	7.87	9.30	6.23	13.82	6.39	8.18	11.68	11.68	

Figures in the parentheses are arc sin transformed value
*figure indicate square root transformed value

conformity with reports of Walters and Bradley (2006) observed that addition of honey bee increased the fruit number per hectare in *Cucurbita spp.*

As regard the number of fruit drop affected by different pollination treatments, the highest number of fruits drop were observed in pollination with *A. cerana indica* (4.57) followed by open pollination (3.66). There was no any fruit drop recorded in treatment (T2) pollination without insect. On the basis of number of female flower and number fruit set, the percent fruit set was worked out for each treatment. The analysis of data revealed that maximum per cent fruit set was observed in treatment (T1) open pollination (59.67%) followed by pollination with *A. cerana indica* (54.03%). There was no any fruit set recorded in treatment (T2) pollination without insect (Table 1).

The present results endorsed by the finding of Shwetha *et al.* (2012) reported in cucumber that highest number of fruit set was observed in case of open pollination (94.60%) compared to honey bee pollination. Srikanth (2012) observed in bottle gourd that fruit set was maximum in open pollinated plot with use of attractant compared to open pollination without attractant. However, Alam and Quadir (1986) reported that fruit set of *L. siceraria* pollinated by honey bee (*Apis cerana*) was 15 per cent compared with hand pollinated flowers (8.33%) and isolated plants (3.33-5.00%). Singh (2002) observed that due to bee pollination fruit set increases up to 30 to 100 per cent in muskmelon. Hossain *et al.* (2018) reported highest fruit set in cucumber by hand pollination (70.68%) compared to other mode of pollination.

The number of healthy and malformed fruits was recorded separately for each treatment and per cent malformed fruits were worked out and presented in Table 1. The data on per cent malformed fruits revealed that maximum per cent of malformed fruits were recorded in treatment of (T1) open pollination (25.09%) followed by treatment (T3) pollination with *A. cerana indica* (20.43%). No malformed fruits were recorded in treatment (T2) pollination without insect (00.00%). The present finding is in close agreement with Meena Thakur and Rana (2008) reported in cucumber that maximum per cent of misshapen fruits was in open pollination (20.05%) followed by hand (14.1%) and honey bee (8.05%) pollination. Hossain *et al.* (2018) recorded in cucumber that per cent of misshapen fruits (24.35%) was maximum in without honey bee pollination.

The diameter of fruit was measured by vernier caliper and analyzed statistically. The data on diameter of fruits revealed that maximum average fruit diameter was recorded in treatment (T1) open pollination (6.84cm) followed by (T3) pollination with *A. cerana indica* (6.76cm). The present results are more or less in conformity with Hossain *et al.* (2018) observed that in cucumber fruit diameter (27.1cm) was highest in hand pollination compared to other mode of pollination. As regard the weight of fruit affected by different pollination treatment, the maximum average fruit weight was recorded in the treatment (T1) open pollination (961.24g) followed by pollination with *A. cerana indica* (878.57g). The present findings are in line with the reports of Shwetha *et al.* (2012) found in cucumber that fruit weight was maximum in open pollination (1619.09g) compared to honey bee pollination and among different bee species, the fruit weight (1510.68g) was maximum in pollination with *A. cerana indica.* Srikanth (2012) observed in bottle gourd that fruit weight were maximum in open pollinated plots with attractant compared to open pollination without attractant. However, Meena Thakur and Rana (2008) stated that in cucumber weight of fruits (1184.5g) was highest in honey bee pollination as compared to other mode of pollination.

The data on average fruit yield of bottle gourd revealed that highest average fruit yield per plant was recorded in treatment of open pollination (5.17 kg/plant) followed by treatment of pollination with *A. cerana indica* (4.52 kg/plant). There was no fruit set observed from treatment (T3) pollination without insect. The present findings are more or less in conformity with Singh (2002) reported that *A. mellifera* plays a key role in pollination of muskmelon and improve the fruit yield in protected condition. Motzke *et al.* (2015) reported that flower visiting bees were responsible for 75 per cent increased in yield. The benefit cost ratio was highest in open pollination (22.49:1) followed by pollination with *A. cerana indica* (4.69:1). In case of pollination without insect, no any income obtained due to absent of fruit per plant (Table 4).

From above results it can be concluded that, the availability of pollinators fauna is very essential for the fruit formation in bottle gourd, without pollinators there were no fruit formation in bottle gourd and ultimately no yield. There was no any difference between different quantitative parametric value and it was almost same in both, open pollination and honey bee pollination but cultivation in protected condition increase the cost of cultivation ultimately lead to lower profit.

ACKNOWLEDGEMENT

The authors are grateful to the Professor and Head, Department of Entomology and Principal, N. M. College of Agriculture, NAU, Navsari providing necessary facilities and guidance during the course of research study.

REFERENCES

Alam, M. Z. and Quadir, M. A. 1986. Role of honeybee in fruit and seed setting of bottle gourd, *Lagenaria siceraria* (Mol.) Standl. *Punjab Vegetable Grower*, **21**:32-34.

Hossain, M. S., Yeasmin, F., Rahman, M. M., Akhtar, S. and Hasnat, M. A. 2018. Role of insect visits on cucumber (*Cucumis sativus* L.) yield. *Journal of Biodiversity Conservation Bioresource Management*, **4**(2):81-88.

Ipsita Panigrahi, Duhan, D. S., Panghal, V. P. S., Tehlan, S. K. and Yadav, A. C. 2018. Correlation coefficient analysis between yield defining traits of cultivated genotypes of bottle gourd (*Lagenaria siceraria* (Mol.) Stdl.). *Journal of Pharmacognosy and Phytochemistry*, **7**(2):1378-1380.

Koetz, A. H. 2013. Ecology, behavior and control of *Apis cerana* with a focus on relevance to the Australian incursion. *Insects,* **4**:558-592.

Kumar, S., Keerthi, M.C., Singh, T., Kumar, V. and Yadav, V.K. 2021. Effect of Indigenous Bee Attractants on Qualitative and Quantitative Parameters of Egyptian clover, *Trifolium Alexandrinum* L. *Legume Research*, **1**: 1-6.

Motzke, I., Tscharntke, T., Wanger, T. C. and Klein, A. 2015. Pollination mitigates cucumber yield gaps more than pesticide and fertilizer use in tropical smallholder gardens. *Journal of Applied Ecology*, **52**:261-269.

Prajapati, R.P., Kalariya, M., Parmar, S.K. and Sheth, N.R., 2010. Phytochemical and pharmacological review of *Lagenaria sicereria*. *Journal of Ayurveda and Integrative Medicine*, **1**(4): 266-272.

Shwetha, B. V., Janana Bharathi, Rubina, K., Kuberappa, G. C. and Reddy, M. S. 2012. Insect pollinators diversity, abundance with special reference to role of honeybees in increasing production ofcucumber, *Cucumis sativus* L. *Korean Journal of Apiculture,* **27**(1):9-14.

Singh, B. 2002. Effectiveness of different pollinators on yield and quality of greenhouse grown tomatoes and melon. *Haryana Journal of Horticulture Science*, **31**:245-250.

Srikanth, C. D. and Kuberappa, G. C. 2012. Insect Pollinators Diversity with special reference to the role of attractants in insect pollination for increasing productivity of Bottle gourd (Lagenaria *siceraria* L.). M. Sc. (Agri.) Thesis submitted to University of Agricultural Science, Bangalore.

Thakur, M. and Rana, R. S. 2008. Studies on the role of insect pollination on cucumber yield. *Pest Technology*, **2**(2):130-133.

Walters, S. A. and Bradley, H. T. 2006. Effect of honey bee pollination on pumpkin fruit and seed yield. *Horticultural Science*, **41**(2):370-373.

MS Received: 27 November 2022
MS Accepted : 24 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 198 -200(2022)

DOI Number: 10.5958/0974-4541.2022.00062.5

RESEARCH NOTE

Evaluation of different insecticides against leaf roller, *Pyrausta coclesalis* Walker (Lepidoptera: Pyraustidae) in bamboo

C. MOHAN[1]*, RAMKISHORE MOHABE[2], RAMBHAJAN SINGH[3], NEELU SINGH[4] and FATIMA SHIRIN[5]

[1-4]Forest Protection Division, [5]Division of Genetics and Tree improvement
Tropical Forest Research Institute, Jabalpur – 482021, Madhya Pradesh, India- 482 021

***E-mail**: mohanentomology@gmail.com

ABSTRACT: Studies were carried out during 2021-2022 to evaluate toxicity levels of different insecticides against larvae of bamboo leaf roller *Pyrausta coclesalis* (Lepidoptera: Pyraustidae) are one of the most important group of leaf feeders on bamboo (Family: Poaceae) in Central India. Spinosad 45 SC (0.03%), profenofos 50 EC (0.04%), Cypermethrin 25 EC (0.03%), fenvalerate 20 EC (0.03%), chlorantraniliprole 18.5 SC (0.03%) and bio pesticide Azadirachtin 10000 PPM (0.05%) at the recommended concentration was evaluated against of bamboo leaf roller at the Division of Forest Protection, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India. The study revealed that spinosad 45 SC (0.03%), profenofos 50 EC (0.03%) and cypermethrin 25 EC (0.03%) were effective with the maximum larval mortality of 91.66, 83.33 and 75 percent at 72 hours after treatment followed by fenvalerate 20 EC (0.03%) and Chlorantraniliprole 18.5 SC (0.03%) which recorded similar mortality of 66.66 were at par to each other at the same period of observation. Among bio pesticide azadirachtin (0.05%) treatment recorded least larval mortality of leaf roller 58.33 percent at 72 hours after treatment.

Key words: Bamboo, bioassay, insecticides, leaf roller, *Pyrausta coclesalis*

Bamboo, belonging to the family Poaceae, has always been a material of immense importance to the culture of south-east Asia. Bamboo plays an important role in the life of human beings. One third of the human race at least uses bamboo for several purpose. Very few plant species have inspired such a wide variety of uses as bamboo. Bamboo has vast spectrum of uses. Bamboo is the fastest growing species. It is a favoured species in the national afforestation programmes being a marvellous substitute of timber towards meeting the industrial and rural requirements, checking erosion, conserving soil and moisture. Bamboo has an important industrial role in paper pulp manufacture, especially in China and India. Some of the species of bamboo are edible. Some species yield medicine (Dutta and Tomar, 1964; Tewari, 1992). Bamboo has a rich complex of insect fauna and suffers assiduously from insect damage (Singh and Bhandari, 1988; Tewari, 1992). There is a dearth of literature on bamboo entomology, especially impact of insects on growth and quantification of economic losses. About 170 species of insects have been reported to be associated with different species of bamboos in India and adjacent countries. However, defoliators are the main enemies of bamboo and among all; leaf rollers cause severs epidemic defoliation in bamboo (Mathur, 1943). The most devastating bamboo leaf rollers that occur in Indian sub-continent are: *Pyrausta bambucivora* Moor and *Crypsiptya coclesalis* Walker (syn. *Pyrausta coclesalis*) (Lepidoptera: Pyralidae: Pyraustinae) causing serious defoliation, resulting in reduced vigour and even the death of culms. Damage is found to be more severe in nurseries and plantations than in natural stands and individual plantings (Tewari, 1992; Pal *et al*., 1996). Among the various methods of insect pest management, the use of insecticides forms the first line of defence against the insect pests. Newer insecticide molecules may be a better alternative than the application of conventional synthetic insecticides in the context of environmentally benign management tactics so also in order to mitigate the adverse effect on the total environment. In many cases, alternate or eco-friendly method of insect pest management offers adequate level of pest control with less hazards and safe to non-target organisms (Chavan *et al*., 2012). The present investigation was planned to evaluate such conventional and new insecticides profenofos 50 EC (0.03%), cypermethrin 25 EC (0.03%), fenvalerate 20 EC (0.03%), chlorantraniliprole 18.5 SC (0.03%) and bio pesticide spinosad 45 SC (0.03%), Azadirachtin 10000 ppm (0.05%) against *P. coclesalis* under laboratory conditions.

Table 1. Bioassay of different insecticides against bamboo leaf roller *Pyrausta coclesalis* at laboratory condition.

Treatments	Concentration	Larval mortality % (HAT*)		
		24 (HAT)	48 (HAT)	72 (HAT)
		75.0*	83.33	91.66
Spinosad 45 SC	0.03%	(60.0)	(65.90)	(73.21)
		66.66	75.0	83.33
Profenofos 50 EC	0.03%	(54.73)	(60)	(65.90)
		58.33	66.66	75.0
Cypermethrin 25 EC	0.03%	(49.79)	(54.73)	(60)
		50.0	58.33	66.66
Fenvalerate 20 EC	0.03%	(45)	(49.79)	(54.73)
		41.66	58.33	66.66
Chlorantraniliprole 18.5 SC	0.03%	(40.19)	(49.79)	(54.73)
		33.33	50.0	58.33
Azadirahctin 10000 ppm	0.05%	(35.26)	(45.0)	(49.79)
				8.33
Control	--	0	0	(16.77)
Standard deviation		0.911	0.863	0.94
Standard error		0.178	0.163	0.178
F value		1.258	0.253	0.895
Sig. Value		0.318	0.952	0.517

*Figures in parenthesis are arc sin √n values of percentages; *HAT: Hours After Treatment

Toxicity studies of different insecticides were carried out in Entomology Laboratory of Forest Protection Division, Tropical Forest Research Institute, Jabalpur, M.P. In this investigation, spinosad 45 SC (0.03%), profenofos (50 EC 0.03%), cypermethrin (25 EC 0.03%), fenvalerate (20 EC 0.03%), chlorantraniliprole (18.5 SC 0.03%) and azadirachtin (10000 ppm 0.05%) and water (control) were tested under laboratory conditions using Completely Randomized Design (CRD) with three replications of each on third instar larvae of *P. coclesalis*. Third instar larvae were collected from Bamboo multiplication garden in TFRI, Jabalpur and used for toxicity test.

To find out the toxicity of each insecticide on the larvae *P. coclesalis*, the stock solutions of above said insecticide were prepared in water and fresh leaves of bamboo were dipped in different insecticide solution for one minute. The leaves treated with treatment solutions were shade dried on a filter paper in open air and ten larvae were released on the treated leaves kept inside the plastic container. Small pin holes were made on top of the container for ventilation. Totally three replications were maintained for each treatment. Based on the mobility of body parts and change in the colour of the body the mortality of larvae was confirmed and the data recorded at 24, 48 and 72 hours after treatment (HAT) and percent mortality were worked out. The data collected under laboratory experiments in Completely Randomized Design were analysed using analysis of variance (ANOVA) using AGRES 3.01 and AGDATA software. Data in the form of percentages were transformed to arcsine values and those in numbers were transformed to √□+0.5 and analysed. The mean values of the treatments were compared using DMRT at 5 per cent level of significance.

The data on per cent mortality of the larvae of the bamboo leaf roller mentioned in Table1 indicated that all the treatments during different observation period showed significant results over control. Spinosad (45 SC), cypermethrin (25EC), fenvalerate (20 EC), profenofos (50 EC), chlorantraniliprole (18.5 SC) and azadiractin (0.15 EC) were evaluated against the control of bamboo leaf roller when applied at different concentrations. All insecticide treatments showed significant differences with the control (mortality observed in control where no insecticide was applied). The highest mortality @ 0.03% with (91.66%) was shown by (Spinosad) followed by (profenofos) @ 0.03% with (83.33%), (cypermethrin)

@ 0.04% with (75%), (fenvalerate) @ 0.03% with (66.66%), (chlorantraniliprole) @ 0.03% with (66.66%), (azadirachtin) @ 0.05% with (58.33%) and control with (8.33%) percent larval mortality of bamboo leaf roller at 24, 48 and 72 hours after treatment respectively (Fig.1). Afzal *et al.* (2002) stated that the reduction in pest population was the greatest in the treatment with karate 2.5 EC (96%) followed by sevin 10 SP (85%). The findings are in agreement with that of Kalia and Joshi (1995) who reported for foliar spraying of 1% *Bacillus thurunginsis* var *kurstaki* against bamboo leaf roller larvae. On the basis of LC_{50} value of a biopesticide Ivermectin, it is recommended to use 0.1% as foliar spray for control of *P. coclesalis* larvae (Roychoudhury, 2012). Lalitha *et al*. (2012) stated that the *Bacillus thuringiensis* against *Helicoverpa armigera* larvae (second instar and third instar) were found treated with *B.t.* strains and recorded mortality in the range of 94.44 and 83.33 %. All the earlier findings are in conformity with the present findings. Radha (2013) Reported cowpea aphid *Aphis craccivora* was effectively controlled using spinosad followed by neem seed kernel extract. These results also confirm the result obtained in the present study. Patidar and Kumar (2018) recorded that Chlorantraniliprole 18.5% SC and Flubendiamide 39.35% SC found most effectives on larval population of *S. oblique.* Present finding are in line with the findings of above workers.

CONCLUSION

The data on percent mortality after 72 hours of exposure showed that the chemical pesticide showed not only significant but superior results over control. Therefore it is suggested that the population of bamboo leaf roller larvae can be checked by spraying chemical insecticides *viz.,* spinosad 45 SC (0.03%), profenofos 50 EC (0.03%) and cypermethrin 25 EC (0.03%) are effective pesticides against bamboo leaf roller and can act as better tools in insect pest management for leaf roller menace in bamboo nursery and young plantations.

ACKNOWLEDGEMENT

This research was supported by Compensatory Afforestation Fund Management and Planning Authority (CAMPA), MoEF & CC, Govt. of India under the scheme 'Strengthening Forestry Research for Ecological Sustainability and Productivity Enhancement'. We are thankful to Director and Group Coordinator (Research) of Tropical Forest Research Institute (TFRI), Jabalpur for their valuable guidance and continuous support. Thanks are also due to the technical staffs of Forest Entomology Discipline of TFRI, Jabalpur for regular collection of data and mass culturing of insect pests in laboratories.

REFERENCES

Afzal, M., Aleem, M. and Basit, M. 2002. Comparative efficacy of different insecticides against sesame leaf webber/pod borer, *Antigastra catalaunalis* (Dup.). *Annals of Plant Protection Sciences*, **24** (2): 149-152.

Dutta, J.J. and M.S. Tomar (1964). Bamboo Forest of Madhya Pradesh. Forest Bulletin No.8, Government Regional Press of Madhya Pradesh, Rewa.71pp.

Kalia, S. and Joshi, K.C. (1995). Efficacy of three commercially available products of *Bacillus thuringiensis* Berliner against bamboo leaf roller, *Crypsiptya coclesalis* Walker. *Myforest*, **31** (4): 1-3.

Lalitha, C., Muralikrishna, T., Sravani, S. and Devaki, K. 2012. Laboratory evaluation of native *Bacillus thuringiensis* isolates against second and third instar *Helicoverpa armigera* (Hubner) larvae. *Journal of Biopesticide*, **5** (1): 4-9.

Mathur, R.N. (1943). Bamboo defoliators. *Indian Entomological Science* (1): 117-128.

Radha R. Comparative studies on the Effectiveness of Pesticides for Aphid control in Cowpea. Research Journal of Agriculture and Forestry Science. 2013; **1** (6): 7-18.

MS Received: 25 November 2022
MS Accepted : 26 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 201 -202 (2022)

DOI Number: 10.5958/0974-4541.2022.00063.7

RESEARCH NOTE

Parasitisation efficiency of *Tetrastichus howardi* Olliff (Hymenoptera: Eulophidae) on *Diaphania pulverulentalis* Hampson (Lepidoptera: Pyralidae) pupae at different depths of soil

C. HARSHITHA*, B. SANNAPPA, S. MAHIBA HELEN and J. B. NARENDRA KUMAR

Department of Sericulture Science, University of Mysore, Manasagangotri, Mysuru - 570006

Central Sericultural Research & Training Institute, Mysore – 570008

Research Extension Centre, Central Silk Board, Madivala, Kurugal Post, Kolar District -563 157

***E-mail:** harshitha.shekar14@gmail.com

ABSTRACT: A laboratory experiment was carried out to determine the parasitization efficiency of *Tetrastichus howardi* (Hymenoptera: Eulophidae) on the pupae of *Diaphania pulverulentalis* Hampson (Lepidoptera: Pyralidae) at various soil depths. The results showed that the parasitoid successfully parasitized the host pupa at various depths, but the emergence rate was highest (96%) when the pupae were on the top layer of soil, followed by 2cm (90%), 4cm (84%), and 6cm (86%).

Keywords: *Tetrastichus howardi, Diaphania pulverulentalis,* parasitoid, mulberry

The leaf roller, *Diaphania pulverulentalis* Hampson (Lepidoptera: Pyralidae), is a serious pest of mulberry (*Morus* spp.), the exclusive food plant of the silkworm, *Bombyx mori* L. In the early stages, larvae of *D. pulverulentalis* inhabit the apical succulent portion of the shoot, leading to its destruction, resulting in stunted growth and affecting a considerable decline in leaf yield of about 12.8% with an average incidence of 21.77 % (Rajadurai *et al*., 2000). Considering silkworms' economic importance and susceptibility to insecticide residue, biologically managing mulberry pests is a sustainable approach. *Tetrastichus howardi* Olliff (Hymenoptera: Eulophidae) is a gregarious endo-pupal parasitoid of lepidopteran pests. The parasitoid exhibited a broad host spectrum with a distinct preference for lepidopteran insects (Baitha *et al*., 2004, Kfir *et al*., 1993, Moore and Kfir, 1995).

Tetrastichus howardi parasitizes the larvae, pupae and adults of the sugarcane borer, *Diatraea saccharalis* (Fabricius), with the highest parasitism on the pupae (Pereira, 2015). Further, given its potential to suppress the field populations of several insect pests, economic entomologists have attempted to exploit this parasitoid for the biological management of the lepidopteran pests associated with agricultural crops (Gangadhar, 2009). Apart from *D. pulverulentalis*, a few more lepidopteran pests that account for considerable damage to mulberry include *Spodoptera litura* Fabricius and *Spilosoma obliqua* Walker. Interestingly, all these lepidopteran pests of the mulberry are known to pupate in the soil; hence, it is essential to know to what extent those pupae at various depths would be searched parasitized by *T. howardi.*

The experiment was carried out in the insectary of the Pest Management Laboratory, CSRTI, Mysore, at a temperature of 25 ± 2ºC and relative humidity of 55-75 % during December 2021. The artificial bed was prepared in a rectangular plastic rearing tray (57×38.5×6 cm) using soil and dried leaves. Four treatments with five replications were maintained. The soil was filled to a depth of 6 cm, and *D. pulverulentalis* pupae were placed at depths of 2, 4, and 6 cm and the top layer. In each treatment, 50 pupae of *D. pulverulentalis* were used, and 150 adults of *T. howardi* were released onto the tray, covered with nylon net and allowed up to four days for parasitisation. A cotton swab dipped in 50% honey was provided to serve as food for the parasitoid.

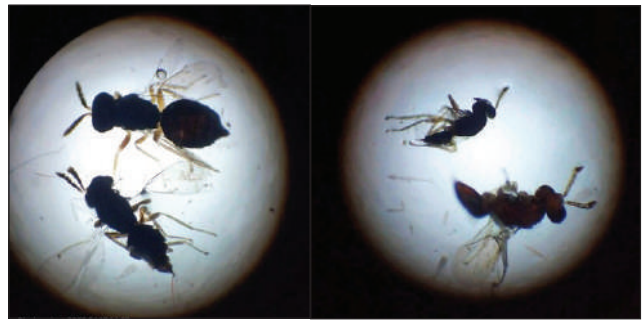


Fig 1. *Tetrastichus howardi* adult female (Left) and male (Right)

Fig 2. Parasitization efficiency of *Tetrastichus howardi* (Hymenoptera: Eulophidae) on *D. pulverulentalis* pupae in different depths of soil

After four days of exposure, *D. pulverulentalis* pupae were collected from the soil at different depths of 2, 4, and 6 cm and the top layer. The pupae were kept in a rearing container till adult *T. howardi* emergence and observation were taken. A comparison of the percentage of *D. pulverulentalis* pupae parasitized by *T. howardi* between treatment groups was made using one-way ANOVA after applying arcsine transformation.

The results (Table 1) revealed that the parasitoid successfully parasitized the host pupa at all depths without any significant difference. The emergence rate or parasitism recorded was 96% at (top layer), 90% at (2cm depth of soil), 84% (4cm depth of soil) and 86% in (6cm depth of soil). According to our findings, *T. howardi* is confirmed to be an effective parasitoid of *D. pulverulentalis* that can efficiently search the host pupae that are available beneath the soil. So, the parasitoid can be used as a biocontrol component under the IPM of the mulberry leaf roller.

Table 1. Results of comparison of percentage of *D. pulverulentalis* pupae parasitized by *T. howardi*

Soil depth	Mean Parasitism %	Std. Deviation
Top layer	96.00	5.477
2 cm depth	90.00	7.071
4 cm depth	84.00	18.166
6 cm depth	86.00	11.402
F-value (P-value) = 1.255^{ns} (0.323)		

REFERENCES

Arun Baitha., Jalali, S. K., Rabindra, R. J., Venkatesan, T. and Rao, N. S. 2004. Parasitizing efficiency of the pupal parasitoid, *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae) on *Chilo partellus* (Swinhoe) at different exposure periods. *Journal of Biological Control*, **18** (1): 65-68.

Cruz, I., Redoan, A. C., de Silva, R. B., de Figueiredo, M. L. C. and Penteado-Dias, A.M. 2011. New record of *Tetrastichus howardi* (Olliff) as a parasitoid of *Diatraea saccharalis* (Fabr.) on maize. *Science. Agriculture*, **68** (2): 252-254.

Gangadhar, B. 2009. Biology and evaluation of *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae) with reference to some insect hosts. *Ph. D. Thesis*, University of Mysore, p. 251.

Kfir, R., Gouws, J. and Moore, S. D. 1993. Biology of *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae): A facultative hyperparasitoid of stem borers. *Biocontrol Science Technology*, **3** (2): 149-159.

Moore, S. D. and Kfir, R. 1995. Host preference of the facultative hyperparasitoid, *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae). *Entomophaga*, **40**: 69-76.

Pereira, F. F. 2015. Parasitism and emergence of *Tetrastichus howardi* (Hymenoptera: sand adults. *Florida Entomologist*, **98** (1): 377-380.

Rajadurai, S., Sen, A. K., Manjunath, D. and Datta, R. K. 2000. Natural enemy fauna of mulberry leaf roller *Diaphania pulverulentalis* (Hampson) (Lepidoptera: Pyralidae) and it's Psyche potential," in Proceedings of the Seminar on Sericulture Technology: An Appraisal, Central Sericultural Research and Training Institute, Karnataka, India, p. 37.

MS Received: 26 November 2022
MS Accepted : 28 December 2022

Pest Management in Horticultural Ecosystems
Vol. 28, No.2 pp 203 -206 (2022)

DOI Number: 10.5958/0974-4541.2022.00064.9

RESEARCH NOTE

Efficacy of a new fungicidal molecule for the management of *Phytophthora capsici* in Capsicum

K. AJITHKUMAR, [1*], A. S[2] SAVITHA, G. RAMESH, [3], M. RENUKA, [1] and M. Y. AJAYAKUMAR[1]

[1]Main Agricultural Research Station, [2]Department of Plant Pathology, College of Agriculture
University of Agricultural Sciences, Raichur – 584 104, Karnataka, India.

[3]Department of Horticulture, College of Agricultural Engineering, University of Agricultural Sciences, Raichur – 584 104, Karnataka, India

***E-mail**: ajithk.path@gmail.com

ABSTRACT: Capsicum, *Capsicum annuum* var. *grossum* Sendt is an important spice and annual herbaceous vegetable crop in India. Phytophthora blight is a devastating disease that virtually infects every plant part resulting in root and crown rot, leaf blight, stem blight, and fruit rot. Hence the present investigation is carried out to test the bio-efficacy of novel fungicide molecules against leaf blight, stem blight, and fruit rot of capsicum. The results revealed that among the implemented treatments, valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha was very effective in reducing leaf blight, stem blight, and fruit rot diseases of capsicum with a maximum fruit yield of 31.20 t/ha with B: C ratio of 3.84. Further phytotoxicity was tested, which revealed that there were no visual phytotoxic symptoms observed during the experimentation.

Keywords: Capsicum, fungicide, fruit rot, leaf blight, phytotoxicity and stem blight.

Capsicum (*Capsicum annuum* var. *grossum* Sendt), popularly known as bell pepper or sweet pepper, is an important spice and annual herbaceous vegetable crop grown across India (Herath *et al*., 2020). Capsicum production is very low in India, mainly due to the infectious diseases caused by fungi, bacteria, viruses, and mycoplasmas, which drastically decline yield (Chadha, 2003). Among the fungal infections, the *Phytophthora* blight is a devastating disease of bell pepper caused by the oomycete pathogen, *Phytophthora capsica*. It infects every part resulting in root and crown rot, on aerial parts, it causes leaf blight, stem blight and fruit rot (Madhura et al., 2015; Weber, 1932).

Effective management strategies are required to mitigate the *Phytophthora* leaf blight, which includes cultural measures such as proper drainage facility, raised beds used for transplanting, drip irrigation, straw mulching, crop rotation for at least three years with non-host plants, soil solarization, use of resistant varieties, botanicals and bio-control agents (Ristaino and Johnston, 1999; Savitha and Sriram, 2015). Nevertheless, these cultural measures could have managed the disease to the maximum extent. The utility of fungicides at optimum concentration with timely application marks the ultimate remedy for controlling *Phytophthora*. The present investigation tests the bio-efficacy of one novel combi-fungicide valifenalate 6 % + mancozeb 60 % WG against leaf blight, stem blight, and fruit rot of capsicum.

The field experiment was conducted during 2020-21 and 2021-2022 in a randomized complete block design with nine treatments replicated thrice using the popular hybrid Green Indra with a spacing of 60 × 45 cm. The details of the treatments and the dosage of the chemicals were followed as per the protocol. The first foliar spray of recommended fungicide was given as per the respective treatments before the disease infection period when conditions were favorable for the disease infection. The observations on disease incidence and severity of *Phytophthora capsici* are to be recorded before application and 10 days after each spray. The disease severity to be recorded on leaves, stem, and disease incidence to be recorded on fruit using the 0 to 5 scale adopted by Inglis *et al*. (1988) given below (Table 1).

To know the crop tolerance/safety, the plants were observed at 1, 3, 5, 7, and 10 days after each application for phytotoxic symptoms like leaf injury, wilting, vein clearing, necrosis, yellowing, stunting, epinasty, and hyponasty. The first picking of matured capsicum fruits was started approximately 50-55 days after transplanting. The capsicum harvested throughout the cropping period was noted, and the plot yield was computed later for hectare.

Table 1. Disease scale followed for rating of disease intensity of *Phytophthora* leaf blight, stem blight and fruit rot in capsicum

Disease Scale	Plant parts affected
0	No disease
1	1-10 per cent area with lesions
2	11-25 per cent area with lesions
3	26-50 per cent area with lesions and limited chlorosis
4	50-75 per cent area with lesions and extensive necrosis
5	>75 per cent area with lesions and extensive necrosis

The data were computed to per cent disease index (PDI) using following formula given by Wheeler (1969):

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Number of leaves observed}} \times \frac{100}{\text{Maximum disease rating value}}$$

The combi-fungicide molecule at the different concentrations tested against leaf blight, stem blight, and fruit rot disease of capsicum during 2020-21 and 2021-22, and the pooled results revealed that the treatment plots sprayed with three applications of valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha (T5) with 10 days interval has recorded minimum severity of leaf blight (5.50 %), stem blight (2.67 %) and fruit rot (1.84 %) at 10 days after third spray which was followed by valifenalate 6 % + mancozeb 60 % WG at 2500 g/ha (T4) and 2000 g/ha (T3) with 6.50, 3.17 and 2.34 per cent leaf blight, stem blight and fruit rot, respectively and 8.67, 5.00 and 4.17 per cent leaf blight, stem blight and fruit rot disease, respectively which are on par with each other and are significantly superior over remaining treatments including untreated control (39.33 % leaf blight, 21.34 % stem blight and 17.67 % fruit rot) (Table 2).

Among all the tested combinations, the maximum percentage of reduction over untreated control of leaf blight, stem blight, and fruit rot diseases at 10 days after the third spray was recorded with valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha (T5) (86.00 %, 87.56 %, and 89.52 %) which was found comparable with valifenalate 6 % + mancozeb 60 % WG at 2500 g/ha (T4) (83.50 %, 85.16 %, and 86.77 %) and 2000 g/ha (T3) (78.16 %, 76.87 %, and 77.48 %) (Table 2). The results of the experiment revealed that among the implemented treatments, valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha (T5) recorded a maximum fruit yield of 31.20 t/ha, which was followed by valifenalate 6 % + mancozeb 60 % WG at 2500 g/ha (T4) and 2000 g/ha (T3) with 31.03 t/ha and 30.59 t/ha respectively, which are on par with each other and are significantly superior over remaining treatments including the untreated control (T1 -15.99 t/ha) (Table 2).

The cost-benefit analysis of different treatments revealed that the maximum BC ratio was recorded by valifenalate 6 % + mancozeb 60 % WG at 2500 g/ha (T4) with 3.85 followed by valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha (T5) and 2500 g/ha (T3) with 3.84 and 3.79, respectively. However, the minimum BC ratio (1.20) was recorded by valifenalate 10 % WG at 1500 g/ha (T6). The phytotoxicity of combi-fungicide was tested at X (at 2000 and 2500 g/ha) and 2X (at 4000 and 5000 g/ha) doses on capsicum crop, and the observations revealed that there were no visual phytotoxic symptoms such as leaf injury, wilting, vein clearing, necrosis, epinasty, hyponasty, yellowing, and stunting observed during the experimentation period.

The obtained results agree with Matheron and Porchas (2000), who found that among five fungicides tested against the root, crown, and fruit rot of chile pepper, mefenoxam was the most effective compound for inhibiting the lesion development on stem and fruits at 1200 µg/ml. Verma *et al*. (2006) observed that among various fungicides spray applied on the fruit surface of capsicum, ridomil-MZ effectively managed the fruit rot by up to 86 per cent compared with untreated control. Keinath (2007) conducted a study to determine whether the isolates of *P. capsici* in South Carolina were sensitive to mefenoxam. Out of 120 *P. capsici* collected, 60 isolates were susceptible to mefenoxam at 100 mg/l under *in vitro* conditions. Sumbula and Mathew (2015) observed that foliar spray with cymoxanil + mancozeb at 2 g/l has resulted in 23.33 per cent *Phytophthora* leaf fall disease severity in nutmeg. Ghatak *et al*. (2015) found that combining mancozeb with cymoxanil and mancozeb with phenamidone rendered the fruit rot incidence between 8 and 9.33 per cent, respectively. Mohammad and Jose (2018) recorded that the incidence of fruit rot caused by *P. capsici* was 48 per cent in the control plot, whereas 7, 27, and 13 per cent were obtained in the plots sprayed with cyazofamid, dimethomorph, and mandipropamid, respectively. A field experiment was conducted to manage the foliar blight of bell pepper

Table 2. Bioefficacy of new fungicidal molecule against leaf blight, stem blight and fruit rot of capsicum.

Tr. No.	Treatment Details	g. *a.i.*/ ha	Formulated product (ml or g/ha)	Disease intensity at Pre-application (%)			Disease intensity 10 days after second spray (%)			ROC at 10 days after third spray (%)			Yield (t/ha)	Benefit cost ratio
				Leaf blight	Stem blight	Fruit rot	Leaf blight	Stem blight	Fruit rot	Leaf blight	Stem blight	Fruit rot		
T_1	Untreated control	-	-	6.00 (14.96)	2.67 (9.41)	0	39.33 (38.86)	21.34 (27.52)	17.67 (24.87)	-	-	-	15.99	-
T_2	Valifenalate 6% + Mancozeb 60% WG	990	1500	5.34 (13.36)	1.33 (6.63)	0	22.67 (28.45)	13.00 (21.15)	10.67 (19.08)	42.21	38.63	39.07	23.31	1.93
T_3	Valifenalate 6% + Mancozeb 60% WG	1320	2000	4.67 (12.48)	1.33 (6.63)	0	8.67 (17.13)	5.00 (12.93)	4.17 (11.78)	78.16	76.87	77.48	30.59	3.79
T_4	Valifenalate 6% + Mancozeb 60% WG	1650	2500	5.17 (13.14)	2.67 (9.41)	0	6.50 (14.78)	3.17 (10.25)	2.34 (8.79)	83.50	85.16	86.77	31.03	3.85
T_5	Valifenalate 6% + Mancozeb 60% WG	1980	3000	5.17 (13.14)	2.67 (9.41)	0	5.50 (13.57)	2.67 (9.40)	1.84 (7.79)	86.00	87.56	89.52	31.20	3.84
T_6	Valifenalate 10% WG	150	1500	5.50 (13.57)	3.17 (10.25)	0	22.84 (28.56)	13.50 (21.57)	11.67 (19.98)	42.07	36.82	33.88	20.37	1.20
T_7	Mancozeb 75% WP	1500	2000	5.17 (13.14)	1.67 (7.42)	0	20.67 (27.05)	11.84 (20.13)	11.50 (19.83)	47.67	44.56	35.28	24.26	2.25
T_8	Azoxystrobin 11% + Tebuconazole 18.3% w/w SC	192	600	5.00 (12.93)	2.50 (9.10)	0	9.67 (18.12)	10.50 (18.92)	9.33 (17.79)	63.05	51.62	47.70	26.67	2.84
T_9	Carbendazim 12% + Mancozeb 63% WP	563	750	5.17 (13.14)	2.17 (8.48)	0	22.50 (28.33)	12.50 (20.72)	10.50 (18.92)	43.40	41.92	41.08	20.69	1.28
S. Em.				1.42	1.21		2.08	1.73	1.76	-	-	-	0.87	
C. D. at 5%				NS	NS		6.24	5.28	5.26	-	-	-	2.62	

*Figures in the parenthesis are arc sine transformed values; NS- Non significant; ROC-Reduction Over untreated control

(*Phytophthora* spp). Among different fungicides tested, foliar spray of moximate (cymoxanil 8 % + mancozeb 64 %) at 2 g/l scored minimum disease severity (2.67 %) and maximum fruit yield/plant (520.50 g) (Chaudhary *et al*., 2021).

The results of the evaluation of the bio-efficacy of a new advanced fungicidal molecule revealed that among the treatments, valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha (T5) was very effective in reducing leaf blight, stem blight, and fruit rot diseases of capsicum with maximum fruit yield of 31.20 t/ha. However, the maximum BC ratio (3.85) was recorded by valifenalate 6 % + mancozeb 60 % WG at 2500 g/ha (T4). Hence, considering the economic point of view, valifenalate 6 % + mancozeb 60 % WG at 2500 g/ha (3300 g. a.i. /ha) can be recommended for the management of capsicum diseases like leaf blight, stem blight, and fruit rot.

ACKNOWLEDGEMENT

The authors are highly grateful to the Professor and Head, Department of Plant Pathology, College of Agriculture and the Director of Research, University of Agricultural Sciences, Raichur for their technical support and for providing all the facilities required for conducting the field experiment.

REFERENCES

Chadha, K. L. 2003. Capsicum. *Hand Book of Horticulture*. Indian Council of Agricultural Research, New Delhi, **3**: 368-371.

Chaudhary, S., Ghimire, M., Adhikari, N., Sah, A., Yadav, S. K. and Swar, P. 2021. Effect of fungicides on foliar blight of bell pepper (*Capsicum annum* L.) in Morang, Nepal. *International Journal of Agriculture and Environmental Research*, **7**(4): 701-711.

Ghatak, A., Ansar, M., Ghatak, L. V. and Balodi, R. 2015. Elucidation of relationship between *Phytophthora* leaf blight and fruit rot in tomato. *Journal of Postharvest Technology*, **3**(2): 50-57.

Herath, H. M. S. N., Rafil, M. Y., Ismail, S. I., Nakasha, J. J. and Ramlee, S. I. 2020. Improvement of important eoconomic traits in chilli through heterosis breeding: A review. *Journal of Horticultural Sciences and Biotechnology*, **96**(1): 14-23.

Inglis, D. A., Hagedorn, D. J. and Rand, R. E. 1988. Use of dry inoculum to evaluate beans for resistance to anthracnose and angular leaf spot. *Plant Disease*, **72**: 771-774.

Keinath, A. P. 2007. Sensitivity of populations of *Phytophthora capsici* from South Carolina to mefenoxam, dimethomorph, zoxamide and cyamoxanil. *Plant Disease*, **91**: 743-748.

Madhura, S., Chowdappa, P. and Pavani, K.V., 2015. Aggressiveness of Phytophthora boehmeriae and P. capsici isolates from India on hot pepper (*Capsicum annum* L.). *Pest Management in Horticultural Ecosystems*, **21**(2): 203-209.

Matheron, M. E. and Porchas, M. 2000. Camparison of five fungicides on the development of root, crown and fruit rot of chile pepper and recovery of *Phytophthora capsici* from soil. *Plant Disease*, **84**: 1038-1043.

Mc Kinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *Journal of Agricultural Research*, **26**: 195-217.

Mohammad, B. and Jose, R. D. S. 2018. Chemical management of *Phytophthora capsici* in pumpkin in Illinois. *Modern Concepts and Developments in Agronomy*, **1**(4): 78-82.

Ristaino, J. B. and Johnston, S. A. 1999. Ecologically based approaches to management of *Phytophthora* blight on bell pepper. *Plant Disease*, **83**(12): 1080-1089.

Savitha, M.J. and Sriram, S., 2015. Morphological and molecular identification of Trichoderma isolates with biocontrol potential against Phytophthora blight in red pepper. *Pest Management in Horticultural Ecosystems*, **21**(2): 194-202.

Sumbula, V. and Mathew, S. K. 2015. Management of *Phytophthora* leaf fall disease of nutmeg (*Myristica fragrans* Houtt). *Journal of Tropical Agriculture*, **53**(2): 180-186.

Verma, A., Shyam, K. R. and Gupta, A. K. 2006. Fungicidal management of *Phytophthora* leaf blight and fruit rot of bell pepper (*Capsicum annuum* L.). *Plant Disease Research*, **21**(2): 128-131.

Weber, G. F. 1932. Blight of peppers in Florida caused by *Phytophthora capsici*. *Phytopathology*, **22**: 775-780.

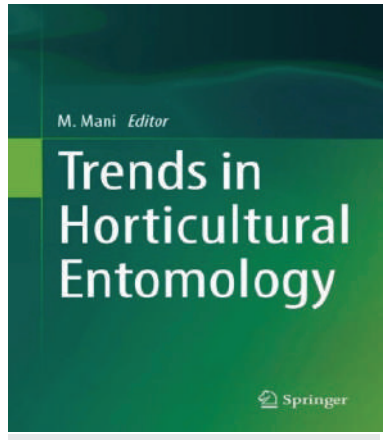
Wheeler, B. E. J. 1969. An introduction to plant diseases, John Wiley Publications, London, p. 301.

MS Received: 18 October 2022
MS Accepted : 27 November 2022

BOOK REVIEW

Trends in Horticultural Entomology (Vol. I and II)

(Editor : M. Mani)



Publishers: Springer Nature Singapore
Year: 2022
ISBN: 978-981-19-0342-7
ISBN : (Online) 978-981-19-0343-4
Volumes: 2, Chapters: 64
Pages : 1553

Dr. M. Mani is an eminent entomologist with over 40 years of experience in pest management research especially biological control. He worked as Principal Scientist and Head, Division of Entomology at ICAR-Indian Institute of Horticultural Research (IIHR) and later as an Emeritus Scientist at IACR-IIHR and ICAR-National Bureau of Agricultural Insect Resource (ICAR-NBAIR), Bengaluru. Having been associated with horticultural entomology throughout his career, Dr. Mani had come out with an exhaustive publication titled "Trends in Horticultural Entomology", which has been published in two volumes by the reputed Springer Publishers.

This is a timely publication to understand the changing pest scenario and emerging areas in pest management in horticultural crops.

Volume I titled 'Recent Advances in Horticultural Entomology' highlights the latest information on molecular identification of horticultural crop pests, changing the pest scenario in relation to climate change, ecological engineering , biotechnological approaches for the pest management, nanotechnology, recent trends in biological control, pest management in protected cultivation, organic pest management, semio chemicals, novel insecticides and the insecticide resistance and its management in horticultural crop pests. The book also covers insect pollinators which play important role for fruits in horticultural crop production.

Volume II titled 'Pest management in different Horticultural crops', diversity and pest management in different horticultural crops viz., fruit crops, vegetables, tuber crops, plantation crops, spices, ornamentals and medicinal plants including under-utilized crops.

'Trends in Horticultural Entomology' is an exhaustive book covering all aspects of the latest developments in the field of Horticultural Entomology. It serves as a valuable reference material for researchers, scholars, faculty, extension personnel and progressive farmers. It deserves to be in every library of agri and horticultural Universities and ICAR Institutes.

I compliment Dr. Mani for jis painstaking efforts in bringing out this valuable publication.

P.V. RAMI REDDY
Chief Editor

CONTENTS *Continued from back cover*

CONTENTS 28 (2)

Continued on back inside cover

Mail your articles to : editorpmhe@gmail.com

Printed at : Shreya Printers & Publishers, Mobile: 9538186800