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

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COVER PHOTOS : Coffee white stem borer, *Xylotrechus quadripes* Chev.
(Photo Courtesy: Dr. G.V. Manjunatha Reddy, Coffee Research Sub Station,Chettalli, Karnataka

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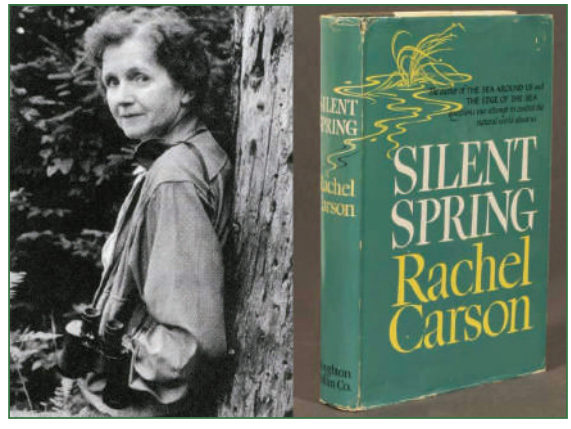
Prologue



Year 2022 has attained a historical significance being the 75th year of Indian independence. While immersed in the joy and pride of this fervor, an idea struck to me to explore any significant event related to plant protection that has reached a milestone this year. The pursuit has led me to know that Rachel Carson's 'Silent Spring', acclaimed as the landmark book of the 20th century on environment has just turned 60 this year. It may not be an exaggeration to say that no other single publication had influenced the course of plant protection research in modern era as much as 'Silent Spring' published in 1962. Hence, I felt it is appropriate to dedicate a column to commemorate the sixty years journey of Silent Spring. This is not a review of the book but an effort to share some documented events attributable to it. Before going further, I acknowledge an article by Krupke *et al*. (2007) published in American Entomologist (Vol. 53 No. 1 pp24-26) which is the source of inspiration and much of information for this column.

Birth of Silent Spring

Rachel Carson, who was working as a field scientist with the U.S. Fish and Wildlife Service from 1936 to 1952 was moved by the alarming level of harm caused by the indiscriminate application of DDT and other synthetic chemicals after World War II to the environment and non target organisms like birds and fish. Being a writer of repute by that time itself, Carson wanted to present the information she gathered on how the poisonous chemicals have been entering the food chain and environment in a semi popular style to have wider reach. The result was 'Silent spring' and the impetus is reflected better in her lines.

"Over increasingly large areas of the United States, spring now comes unheralded by the return of birds, and the early mornings, once filled with the beauty of bird song, are strangely silent."

Silent Spring was initially published as a series of three articles which appeared on 16, 23 and 30 June 1962 in 'The New Yorker' magazine before the book was published by Houghton Mifflin on 27 September 1962.

Noise around Silent Spring

'Silent Spring' Is Now Noisy Summer

Pesticides Industry Up in Arms Over a New Book

Rachel Carson Stirs Conflict—Producers Are Crying 'Foul'

By JOHN M. LEE

The $300,000,000 pesticides industry has been highly irritated by a quiet woman author whose previous works on science have been praised for the beauty and precision of the writing.

The author is Rachel Carson, whose "The Sea Around Us" and "The Edge of the Sea" were best sellers in 1951 and

fending the use of their products. Meetings have been held in Washington and New York. Statements are being drafted and counter-attacks plotted.

A drowsy midsummer has suddenly been enlivened by the greatest uproar in the pesticides industry since the cranberry scare of 1959.

Miss Carson's new book is

Today every one might univocally accept and admire Silent Spring as a land mark book that resulted in reorienting pest management strategies with environmental safety as a major concern. But the situation was different in sixties and it received lot of brickbats besides bouquets. Expectedly the book had evoked sharp criticism from agro chemical industries of that period.

A few of dissenting opinions on Silent Spring …

"Silent Spring is an emotional picture… written with passion and with beauty, but with very little scientific detachment" - Stare (1963).

"If man were to faithfully follow the teachings of Miss Carson, we would return to the Dark Ages and the insects and diseases and vermin would once again inherit the earth". - White-Stevens, Agrochemical industry, US.

- Dr. William J. Darby, Professor at Van derbit University, titled his review of Silent Spring as "Silence, Miss Carson" published in the October 1, 1962, issue of *Chemical & Engineering News* and criticized Carson for not adopting the views of "responsible, broadly knowledgeable scientists" and went on to say "this book should be ignored."

- Even the USDA–ARS opposed Carson for condemning their massive pest eradication programs, particularly the fire ant effort. They felt Carson did not appreciate the "remarkable successes" in agriculture, forestry, and public health attributable to chemical pest control.

Entomologists vs. Silent Spring

Carson's take on economic entomologists that "they operate in the belief that salvation lies at the end of a spray nozzle" was unpalatable to many entomologists. Edward A. Stenhaus, the then president of the Entomological Society of America (ESA), rebutted the charges indirectly saying that 'a true entomologist never focuses only on killing insects with chemicals and the one who does not find insect a thing of beauty is not a true entomologist'. Interestingly, the book Silent Spring was never reviewed by the ESA. Some even commented that economic entomologists of the DDT era viewed Silent Spring as an attack on their professional competence and integrity.

Impact of Silent Spring

Notwithstanding the criticism, Silent Spring created a massive impact and left the legacy in making the administration to wake up and look closely at the effects of insecticides on the environment and the society. The public uproar and debates that followed publication of Silent Spring resulted in implementation of several landmark legislations.

- In 1969, the U.S. Commission on Pesticides and their Relationship to Environmental Health, recommended ban of DDT in agriculture within two years, except when deemed essential for human health and welfare
- In 1970, the U.S. Environmental Protection Agency (EPA) was established to enforce environmental protection standards and conduct environmental research.
- An assessment of the articles appeared over 40 years in the Journal of Economic Entomology (the most widely referred journal in the book), since publication of Silent Spring reflects the conspicuous shifts that took place in the pest control research. There was a significant chronological decrease in papers dealing with chemical control, from 35.2% (1962) to 19.1% (1982) to 2.7% (2002). Correspondingly there was a chronological increase in articles on biological and integrated management (Krupke *et* ., 2007).

The genius of Carson can be understood when she described the accumulation of synthetic chemicals in people including newborn in 1962, more than 40 years ahead of an Environmental Working Group study, that found 287 industrial chemicals in newborns' umbilical cord blood (Grossman, 2012).

Saga of Silent Spring

Silent Spring has been translated into 22 languages and continued to sell more than 20,000 copies annually till recently. It has appeared on lists of the most significant/influential books of the 20th century by various journals, newspapers and organizations. Carson herself was named one of Time magazine's 100 most influential people of the 20th century.

Unfortunately, Rachel Carson, a marine biologist turned conservationist and writer, did not live long to see the success of Silent Spring, her brainchild, in igniting the environmental movement to revive the beauty of bird songs in spring. She passed away on April 14, 1964. Appreciating and supporting any effort, either through research or extension, for the cause of safe pest management would be a befitting tribute to this great soul.

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P. V. RAMI REDDY
Chief Editor

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Genetic characterization and DNA barcoding of the coffee white stem borer, *Xylotrechus quadripes* Chevrolat (Coleoptera: Cerambycidae) infesting Robusta coffee (*Coffea canephora*)

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ABSTRACT: The coffee white stem borer (CWSB), *Xylotrechus quadripes* (Chevrolat) is one of the most destructive pests of Arabica coffee plants (*Coffea arabica*), while, Robusta coffee (*Coffea canefora*) is free from CWSB attack for long time. However in recent years, CWSB infestation is also noticed on Robusta coffee in Polibetta, Mekur, Siddapura and Sunitikoppa in Kodagu district of Karnataka. Hence, the present study was aimed to confirm the infestation of CWSB infesting Robusta and comparing its population infesting Arabica coffee by performing DNA barcoding. The grubs of CWSB were collected by split-opening the infested Arabica and Robusta stems and persevered in 70% ethanol for molecular identification. In addition, infested stems were covered with nylon mosquito net to collect emerging adults and were preserved. The CWSB genomic DNA was extracted and the DNA concentration varied from 615.9 to 1110.6 ng/µL. The CWSB genomic DNA was amplified and was sequenced the mtCOI region. CWSB mtCOI genes were cloned and sequenced and they matched with high similarity in nucleotide Basic Local Alignment Search Tool search and Barcode of life (BOLD) identification. Neighbour joining (NJ) and Maximum likelihood (ML) phylogenetic tree analysis showed that the individuals of the same species clustered together based on the mtCOI sequence similarity, regardless of their collection site and host.

Keywords: COI; DNA barcode, meta barding, maximum likelihood, Robusta Coffee, white stem borer, *Xylotrechus quadripes,*

INTRODUCTION

Coffee is one of the most important plantation crops in India. Arabica (*Coffea arabica*) and Robusta (*Coffea canefora*) are the two commercially grown coffee species in India. Cultivated area of Robusta and Arabica coffee was 226814 (49%) and 233081 hectares (51%), respectively in India (Anon., 2019). During 1951-61 the area under Arabica was 73 per cent as against only 23 per cent area under Robusta in India. The state of Karnataka is the largest Robusta coffee cultivator in India, with over 136 thousand hectares of cultivated area in 2019. Karnataka is the country's largest producer of coffee, accounting for more than 70 per cent (3 lakh tons) of India's production. Coffee production in India is hampered by various insect pests such as white stem borer, shot-hole borer, berry borer and mealy bugs.

The coffee white stem borer (CWSB), *Xylotrechus quadripes* (Chevrolat) is one of the most destructive pests of Arabica coffee plants. While, Robusta coffee is free from CWSB attack for a long time. The CWSB larvae bore into the stem of the plant which resulting in the death of young plants. The older plants though survive, but the yield is drastically reduced and secondarily prone to diseases. The adults are slender, elongate beetles (2-2.5cm in length) with a pair of long antennae. The forewings are black with characteristic white bands. Males are generally smaller than females (Anon., 2014). Stokes (1838) reported the first incidence of CWSB from Karnataka in India. The pest causes crop losses up to 93.6 percent in plantations with poor and untimely management (Basavaraj *et al.*, 2005).

Though the Arabica coffee is the most preferred and principal host, it also rarely attack and breed on other host plants like Robusta coffee, tree coffee, teak, *Olea* sp., etc. The incidence and emergence of adult of CWSB was noticed for the first time on the Robusta coffee

plants in three estates in Coorg region. Even though the infestation was noticed in Robusta, the life cycle was not completed as evidence of no adult emergence in most of the places except Polibetta area of Coorg. In this context, the presence of species complex was suspected in borer infested Robusta coffee in Coorg region. Hence, the present study was conducted to confirm the infestation of CWSB on Robusta and possible variations compared to CWSB infesting Arabica coffee through DNA barcoding as DNA barcoding is generally considered as a reliable, cost-effective and easy molecular identification tool with a wide applicability across metazoan taxa (Hebert *et al.*, 2003; Hajibabaei *et al.*, 2007).

MATERIALS AND METHODS

Collection of CWSB beetles infesting Robusta coffee

During 2019, three adults of *X. quadripes* infesting robusta coffee from Coorg and 15 adults infesting arabica coffee from different locations (eight from Chikkamaglur, three each from Coorg, and Hassan districts of Karnataka, two from Tamilnadu and one from Andrapradesh) were used for DNA analysis. The CWSB infested robusta and arabica stems were collected from field and brought to the laboratory caged under net for emergence of adults (Fig.1-6). The emerged adults were preserved in 70 percent ethyl alcohol until they were used for molecular studies

CWSB genomic DNA isolation

Total genomic DNA was extracted by using the Cetyl Trimethyl Ammonium Bromide (CTAB) method with some modification. The *X. quadripes* specimens were washed with sterile distilled water to remove alcohol prior to homogenization. Rest of the collected specimens were used as voucher specimen and deposited in the Department of Entomology, Regional Coffee Research Station, Balehonnur, Karnataka. The adult beetles

Table 1: Per cent pair wise nucleotide identity of MCoI gene sequences of *X quadripes* infecting coffee with other MCoI gene sequences of stem borers available in the NCBI database

Insect species	Accession numbers	MH758752	MH758751	MH758750	MH758749	MH758748	MH75874	MH758746	MH758745	MH758744	MH758743	MH758742	MH758741	MH758740	MH758739	MH758738
X. quadripes-	KY379322	99.6	996	99.6	99.6	996	996	100	99.6	996	100	90.5	99.6	99.6	99.6	99.6
X. quadripes	KY379321	99.3	99.3	99.3	99.3	99.3	99.3	99.6	99.3	99.3	99.6	90.2	99.3	99.3	99.3	99.3
X. quadripes	KY379320	99.6	99.6	99.6	99.6	99.6	99.6	100	99.6	99.6	100	90.5	99.6	99.6	99.6	99.6
X. quadripes	KU321048	91.0	91.0	91.0	91.0	91.0	91.0	91.3	91.0	91.0	91.3	82.7	91.0	91.0	91.0	91.0
X. quadripes	KU321045	91.0	91.0	91.0	91.0	91.0	91.0	91.3	91.0	91.0	91.3	82.7	91.0	91.0	91.0	91.0
X. quadripe-	MZ379256	95.4	95.4	95.4	95.4	95.4	94.8	95.1	94.8	94.8	95.1	86.7	94.8	94.8	94.8	94.8
X. quadripe	MW008763	99.5	99.5	99.5	99.5	99.5	99.5	99.8	99.5	99.5	99.8	90.4	99.5	99.5	99.5	99.5
X. quadripes	MW008762	99.3	99.3	99.3	99.3	99.3	99.3	99.6	99.3	99.3	99.6	90.2	99.3	99.3	99.3	99.3
X. chinensis	MK098127	87.0	87.0	87.0	87.0	87.0	87.0	87.3	87.0	87.0	87.3	80.3	87.0	87.0	87.0	87.0
X. yanoi	MN905265	79.7	79.7	79.7	79.7	79.7	79.7	80.0	79.7	79.7	80.0	75.1	79.7	79.7	79.7	79.7
X. antilope	KM446368	85.7	85.7	85.7	85.7	85.7	85.7	86.0	85.7	85.7	86.0	78.1	85.7	85.7	85.7	85.7
X. colonus-	MN315194	85.8	85.8	85.8	85.8	85.8	85.8	86.1	85.8	85.8	86.1	79.7	85.8	85.8	85.8	85.8
X. antilope	KM446967	77.9	77.9	77.9	77.9	77.9	77.9	78.2	77.9	77.9	78.2	70.3	77.9	77.9	77.9	77.9
X. grayii	MN905261	85.9	85.9	85.9	85.9	85.9	85.9	86.2	85.9	85.9	86.2	79.7	85.9	85.9	85.9	85.9
X. pantherinu	KJ966163	85.5	85.5	85.5	85.5	85.5	85.5	85.8	85.5	85.5	85.8	80.2	85.5	85.5	85.5	85.5
X. buqueti	KY357555	85.4	85.4	85.4	85.4	85.4	85.4	85.7	85.4	85.4	85.7	79.7	85.4	85.4	85.4	85.4
X. undulatus-	KM845651	84.0	84.0	84.0	84.0	84.0	84.0	84.3	84.0	84.0	84.3	78.2	84.0	84.0	84.0	84.0
X. buqueti-	KY357555	85.4	85.4	85.4	85.4	85.4	85.4	85.7	85.4	85.4	85.7	79.7	85.4	85.4	85.4	85.4
X. arvicola-	KU915557	84.9	84.9	84.9	84.9	84.9	84.9	85.2	84.9	84.9	85.2	78.8	84.9	84.9	84.9	84.9
X. yanoi	MN905265	79.7	79.7	79.7	79.7	79.7	79.7	80.0	79.7	79.7	80.0	75.1	79.7	79.7	79.7	79.7
X. undulatus	KM846304	84.1	84.1	84.1	84.1	84.1	84.1	84.4	84.1	84.1	84.4	78.4	84.1	84.1	84.1	84.1
Rusticoclytus rusticus	MN315200	83.7	83.7	83.7	83.7	83.7	83.7	84.0	83.7	83.7	84.0	78.4	83.7	83.7	83.7	83.7

were grounded in liquid nitrogen using a mortar and pestle. The sample material was collected in 1.5 mL microcentrifuge tube and re-suspended in 300 µl lysis buffer (100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 0.02M ethylene di amine tetra acetic acid, 2X CTAB, 2X PVP and 2 µl β-mercaptoethanol was added to the extraction buffer just prior to use). The homogenised mixture was incubated at 65°C for 15 min. Occasional mixing was done during incubation by inverting the tube. An equal volume of chloroform-isoamyl alcohol (24:1 v/v) was added. Kept the homogenised mixture for 5-10 min at room temperature. The homogenate was centrifuged at 12000 rpm for 15 min. The supernatant was transferred into a new 1.5 mL centrifuge tube, followed by addition of equivalent volume of ice cold isopropanol. The tubes were centrifuged at 13000 rpm for 15 min. The pellet was dried and dissolved in 30µl of TE buffer (1M Tris, 0.5M EDTA pH 8). The quality and quantity of sample genomic DNA was determined by using NanoDrop spectrophotometer and agarose gel electrophoresis.

The DNA obtained from *X. quadripes* beetle samples was used for amplifying a portion of mitochondrial *mtCOI* gene fragment, using forward primer (**F:** 5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer (**CO1 R:**5'-TAAACTTCAGGGTGACCAAAAAATCA-3')(Folmer *et al.,* 1994) .Each reaction mixture of 25 µl consisted of 2.5 µl of 10X PCR buffer, 2.0 µl $MgCl_2$ (2.5 mM), 0.2 µl dNTPs (200 µM), 0.5 µl of *Taq* Polymerase (3U/ µl), 1 µl of each of forward and reverse primer sequences, 1 µl of DNA, and 16.3 µl of distilled water. The amplification was carried out in thermal-cycler (Peqlab, Germany), following PCR conditions of denaturation at 94°C for 60 sec; annealing at 47°C for 45 sec and extension at 72°C for 50 sec (30 cycles, plus an initial denaturation at 94°C for 3 min and a final extension at 72°C for 10 min). The PCR negative control contained the identical amount of PCR mixture with 3 µl distilled water instead of DNA template. A PCR positive control was also included, containing the PCR mixture plus DNA that had been successfully put through the PCR reaction on previous studies in the laboratory. To ensure repeatability, three replications for each of the reactions were conducted. The *X. quadripes* amplified mtCOI products were resolved on 1.5% agarose gel, stained with ethidium bromide (10 µg/ml) to check for successful amplification and visualized in a gel documentation system.

Cloning of CWSB mtCOI PCR fragments and sequencing

The PCR-generated Robusta *X. quadripes* mtCOI amplicons were eluted using NucleoSpin Gel and PCR clean-up kit (Macherey-Nagel, Germany), ligated to pTZ57R vector with 3'-ddT overhangs for TA cloning with blue/white screening according to manufacturer's instructions and cloned in *Escherichia coli* competent cells, strain DH5α (Sambrook and Russell, 2001). Cloning was confirmed by colony PCR, plasmid mobility check and restriction analysis of recombinant plasmid DNA containing *X. quadripes* infesting Robusta coffee mtCOI. Plasmid DNA was isolated from an overnight bacterial culture, using a reliable plasmid miniprep method (Gene JET Plasmid Miniprep Kit, #K0502 - Thermo Fisher Scientific, USA) according to kit guidelines. Selected recombinant plasmids were sequenced using M13F/R primers. At least three independent clones per sample were sequenced.

CWSB mtCOI Sequence analysis

Using DNASTAR software (DNASTAR, Madison, Wisconsin, USA), *X. quadripes* mtCOI, gene chromatograms were tested, assembled and edited. With BIOEDIT version 7.0.9.0, the primer sequences were removed manually. Multiple sequence alignments were performed by CLUSTAL W. In order to ensure the correct target gene fragment, the Basic Local Alignment Search Tool (BLAST) was checked for all sequences in the NCBI database.Using MEGAX and DNA to protein translation (http://insilico.ehu.es/translate/) nucleotide sequences were converted into amino acids. This was done to ensure that stop codons and pseudo genes did not exist (Kumar *et al.*, 2016). *X. quadripes* mtCOI sequences having reference sequences in the NCBI-GenBank alone were used in the homology analysis. The *X. quadripes* mtCOI sequences deposited in the NCBI-GenBank and BOLD were included in the homology analysis.

Homology search

NCBI-Basic Local Alignment Search Tool (BLAST 2.2.20) was performed with mega blast as the default settings against the non-redundant nucleotide collections of NCBI database. All the study sequences were used as queries and the homologous sequences were identified. BLAST was run to determine the identity of the query sequences by comparing with the known DNA sequences in the GenBank records.

Genetic distance and Phylogenetic analysis

Genetic distances of each Robusta and Arabica *X. quadripes* were conducted in MEGAX (Kumar *et al.*, 2016) using Kimura 2 Parameter and *p*- distance model. All the ambiguous positions were removed for each sequence pair. Codon positions, first, second, third and non-coding sequences were included. Phylogenetic analysis was conducted in MEGAX (Kumar *et al.*, 2016) using Neighbour-Joining method (NJ) and Maximum likelihood method (ML) (Saitou and Nei, 1987). Bootstrap replicates were set to 1000 replicates. Codon

Fig. 1-6. Coffee white stem borer *Xylotrechus quadripes* Chevrolat (Coleoptera: Cerambycidae) infested Robusta coffee (*Coffea canephora*) garden Polibetta, Coorg. 1-Collection of adult beetles; 2 & 3- Tunneling behavior of *X. quadripes* infestation in Robusta coffee plant; 4- Adult of *X. quadripes* ready for the emergence. 5 & 6- Adult of *X. quadripes* emerged from Robusta coffee stems characterized by a hole. The collected beetles were used for laboratory studies.

position first, second, third and non-coding sequences were included in the analysis. Gaps were treated through partial deletion. All the *X. quadripes* mtCOI sequences from GenBank, NCBI were extracted in FASTA format. MUSCLE tool was used to align these nucleotide sequences.

RESULTS

Morphological variations in coffee stem borer

The morphological variation was observed in adult CWSB beetles infesting robusta coffee. Coffee stem borer was identified as *X. quadripes* based on the taxonomic keys published by Hiremath (2015). By studying adult beetle morphology, the pest was recognized as *X. quadripes*. The morphology of *X. quadripes* beetles was identical to that of Arabica. The beetles also exhibited characteristically long antennae of the family Cerambycidae. Both male and female beetles were identified. The presence of the ovipositor identified the females (Seetharama *et al.*, 2005). There were no morphological differences in coffee stem borer adults collected from Arabica and Robusta.

CWSB molecular identification and mtCOI sequence analysis

The genomic DNA from the individual specimen of *X. quadripes* was successfully isolated. The genomic DNA concentration of *X. quadripes* ranged from 615.9 to 1110.6 ng/µL. The absorption ratios of 260:280 met pure DNA criteria and ranged from 1.79 to 1.82.

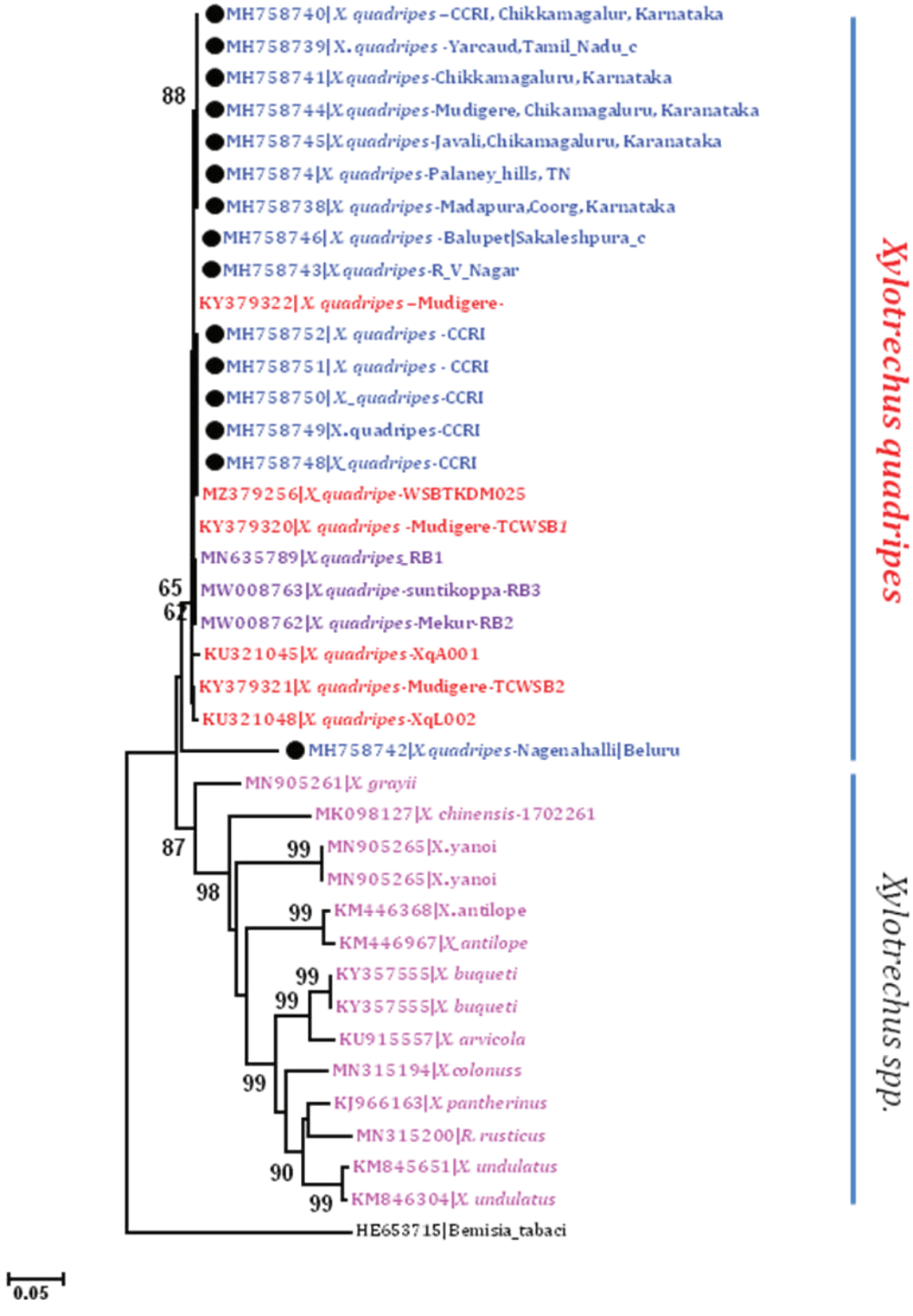

Fig. 7. Cladogram derived from analyses of the mitochondrial cytochrome oxidase subunit I (mtCOI) marker demonstrating the phylogenetic relationships based on neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML), of the combined dataset (658bp) under partition strategies. Evolutionary analyses were conducted in phylogeny.fr. NCBI HE653715 *Bemisia tabaci* served as an out group.

According to the manufacturer's protocol, the PCR products resulting from the amplification of *X. quadripes* mtCOI genes were eluted and cloned to InsTA cloning vector pTZ57R/T (Thermo Scientific, USA). The *X. quadripes* mtCOI, genes were successfully sequenced. The *X. quadripes* mtCOI plasmid comparison of the triplicate sequences for respective *X. quadripes* showed no mismatches, thus no sequencing errors. After alignment and trim, *X. quadripes* mtCOI sequences was 658 in length. Evidence of nuclear copies was not found in any of the sequences subjected to analyses, which was supported by the absence of stop codons and the base composition was similar with no indels. *X. quadripes* nucleotide sequences was deposited in GenBank for the first time. Using MEGAX (Molecular Evolutionary Genetics Analysis), phylogenetic analysis was carried out. The aligned sequences, accessible *X. quadripes* sequences in NCBI GenBank and Barcode of life (BOLD) have been used to build phylogenetic tree. The mtCoI gene sequences of 20 stem borer (*X. quadripes*) samples were characterized in the present study was compared with 19 mtCoI gene sequences of different *Xylotrechus* species retrieved from the NCBI database. The analysis showed that, 20 mtCoI gene sequences of stem borer (*X. quadripes*) collected in different location on coffee showed highest nucleotide identity of 90.1 to 100 % with stem borers infecting robusta and Arabica coffee and other crops reported from different places of south India (Table 2).Whereas different species of stem borer infecting diverse crops are showed very low sequence similarities with coffee infecting stem borers.

The evolutionary relationship of the mtCoI gene sequences of stem borer (*X. quadripes*) characterized in the present study together with 19 mtCoI gene sequences of different *Xylotrechus* species retrieved from the NCBI database were analysis using the neighbor joining method by MEGA X software (Kumar et al., 2016) with 1000 boot strapped replications using *Bemisia tabaci* (HE653715) as out group. The analysis showed that the mtCoI gene sequences of different stem borer samples are formed two major groups with supporting high bootstrap values.

The first group contains only coffee stem borers of Arabica and Robusta and all mtCoI gene sequences characterized in the present study are closed cluster with several stem borers (*X. quadripes*) infecting coffee reported so far in different places of Karnataka. The second group contains stem borers infecting of different crops are formed in to separate group. The analysis also showed that the stem borers (*X. quadripes*) infecting coffee are formed into monophyletic groups. Based on nucleotide identity and phylogentic analysis showed that the stem borers (*X. quadripes*) infecting coffee characterized in the present study have not much variations geographically and different host with respect mtCoI gene sequences. Further the phylogenetic tree showed that Robusta and Arabica CWSB populations are similar in gene sequence, being placed in one branch separated from the out-group (Fig. 7). The phylogenetic tree also emphasizes a very close genetic similarity among populations Robusta and Arabica CWSB.

DISCUSSION

In the present study, based on the fulfillment of the criteria, COI sequences of the study species were assigned to their taxonomic groups. This study contributes to a growing body of work that demonstrates the effectiveness of DNA barcodes in species identifications for members of the Class Robusta CWSB. The approach has now gained preliminary validation in all major lineages of ascidians. The current results extend prior barcoding investigations on ascidians by establishing its effectiveness in a group with an unusually high rate of mitochondrial evolution. Moreover, because of the limited intraspecific variation, an effective identification system can be created by analyzing just a few specimens of each species. However, Robusta coffee plant is resistant for CWSB, the beetles even feed initially but grubs won't develop in to adults but only in cut and dead stems of Robusta the CWSB completes its life cycle (Venkatesha *et al.* 1995). However, Kurian (2000) reported the emergence of adult beetles from Robusta plants from only Coorg. So from the study it is clear that the beetles found in Robusta is also *X. qudripes*. Further the factors responsible for infestation and completion of life cycle in robusta, the host plant intraction may a future line of work can be carried out.

There is no literature on molecular studies of *X. qudripes*, this is the first attempt to study the molecular aspects on this pest. Even there is no sequences available in NCBI data base expect very few from India only by Sridevi *et al.* (2016), and Gowda (2017).

CONCLUSION

According to our findings we can hypothesize and suggest that the phylogenetic tree analysis showed that the individuals of the same species clustered together based on the mtCOI sequence similarity, regardless of their collection host and site. The DNA barcoding for the CWSB identification revealed that identified species remains same for both Robusta and Arabica coffee. This rare incidence on Robusta coffee may be attributed to interplanting of Robusta with Arabica in those estates and also lower shade in Robusta coffee plantations. Further, decrease in shade probably could contribute for the rise in temperatures and ultimately favourable microclimate for the development of CWSB. Hence, in the interplanted Arabica and Robusta estates the more number of adult beetles emerged from Arabica plants and

may be responsible for increased incidence on Robusta plants.

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Effect of bagging time and fruit color on fruit fly attack and its impact on yield of crystal guava in Karanganyar, Indonesia

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ABSTRACT: Crystal guava is a popular fruit in Indonesia. One of the main obstacles in cultivating crystal guava is the fruit fly infestation which reduces productivity up to 100 per cent. The most effective way to control fruit flies is to do fruit bagging since the fruit is small. Studies were conducted to determine the effect of bagging time, fruit diameter, and fruit color on the intensity of fruit fly attacks and their impact on the quality and yield of crystal guava in Karanganyar. The research was carried out at the guava crystal center, Bangsri Karanganyar. The method used was purposive sampling, namely guava fruit that was three weeks old after the flowers bloomed, with variations in bagging time at weeks 3, 4, 5, 6, 7, 8, 9, 10 and 11. Measurements of diameter and color of fruit before bagging, observation of attack intensity and yield and quality of fruit were recorded shortly after harvest. The results showed that bagging time and fruit color affected the intensity of fruit fly attacks. Fruit fly attack significantly affected weight, external appearance and internal appearance.

Keywords: Fruit fly, bagging, crystal guava, fruit quality

INTRODUCTION

Guava is rich in vitamin C content (Tee *et al.*, 1988; Vora *et al.*, 2018) and play a role in maintaining health (Puspitasari and Wulandari, 2017), body immunity to avoid disease attacks. Crystal guava (*Psidium guajava* L.) is a superior variety of guava with a large fruit size and a few seeds (< 3 % of the total fruit mass), soft flesh, sweet taste, and vitamin C content of approximately 87mg in 100g. Guava production in Indonesia, in general, has increased. Based on data from Badan Pusat Statistik (2020), national guava production had significantly increased from 187,418 tons in 2014 to 230,697 tons in 2018. One of the obstacles in the cultivation of crystal guava plants is the attack of pests and diseases, which can reduce productivity and product quality. The fruit fly, *Bactrocera* spp. (Diptera: Tephritidae) is one of the important pests of crystal guava that causes damage, both in terms of quality and quantity of fruit (Taufik *et al.*, 2016). Fruit fly attack on crystal guava causes fruit to rot and fall before harvest time (Taufik *et al.*, 2016). Fruit fly attacks can reduce production by up to 100 percent, especially on star fruit and guava (Kardinan, 2016), so if it is not controlled it will cause crop failure.

Pre-harvest fruit fly management comprises several components including physical (packaging), mechanical, technical culture, biology, quarantine regulations, sterile insect techniques, and chemistry (Hasyim *et al.*, 2020). Physical control of fruit fly pests (packaging) is commonly applied by farmers because it is easy, inexpensive, and can reduce fruit damage by almost 100 percent (Sarwar, 2015). Efforts to control fruit flies on guava plants have been carried out by wrapping the fruit since it is small. However, if *bagging* is done on fruit that is too young, it will be at risk of dropping the fruit. Fruit fly attacks on crystal guava are influenced by many factors such as the shape, color, and texture of the fruit (Hasyim *et al.*, 2020), fruit flies tend to lay their eggs on fruits that are close to ripe or ripe.

MATERIALS AND METHODS

The research was carried out from November 2020 to February 2021 at the Crystal Guava Plantation Center, Bangsri Village, Karangpandan District, Karanganyar Regency. Meanwhile, laboratory activities were carried out at the Surakarta Pest and Disease Observation Laboratory. The research material consisted of 270 samples of crystal guava fruit obtained directly from the garden with fruitage 3 weeks after anthesis (flower blooms).

The research was carried out by purposive sampling, the research material was prepared by selecting fruit that was 3 weeks old after the flowers bloom (anthesis) / ± 20 mm in diameter, and the sample was selected on crystal guava trees that have been producing with a plant age ranging from 2 years with regular spacing. i.e 4 x 3.5 m. The study was arranged randomly with 30 replications, the factor to be studied was the time of fruit packaging, consisting of 8 times, namely the packaging of the 3rd

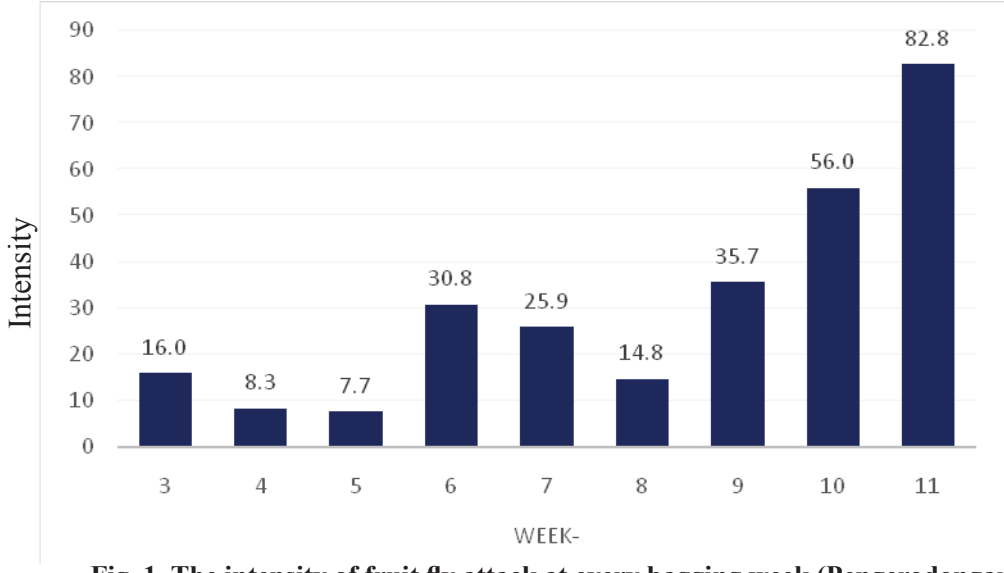

Fig. 1. The intensity of fruit fly attack at every bagging week (Pengerodongan)

week, 4th to the 10th week, and 1 control. All fruit samples were then harvested when 50 per cent of the fruit samples were ready for harvest or were bright green. After harvesting, all samples obtained were observed by splitting and matches to find fruit fly larvae that cause rotten fruit. The observed variables included fruit fly attack intensity, initial diameter, color, weight, external appearance, and internal appearance of the fruit.

Data analysis

The intensity of the attack was calculated in terms of per cent fruit damage. The data obtained during the study were compiled using Microsoft Excel. Statistical data processing was carried out using SPSS and Microsoft Excel software. This study used two statistical tests, *viz.,* Logistics Regression Test and Manova test (Multivariate Anova).

RESULTS AND DISCUSSION

Based on Figure 1, it can be seen that the intensity of the fruit fly attack was highest (82.8%) in the 11th week of grinding, while the lowest attack (7.7 was recorded in the 5th week of grinding.

The highest average initial fruit diameter was at the 9th week of fruit grinding at 67.51 mm while the lowest was 31.49 mm at the 5th week of fruit extraction. The color variables used in this study were CIE L, CIE a, and CIE b. The highest CIE L color at the 11th week of harvesting fruit was 67.68 while the lowest was at the 4th week of harvesting fruit, which was 38.44. In the CIE a color variable, the highest was at the 4th week of harvesting fruit at -17.72 while the lowest was at the 11th fruit-pulling week which was -26.91. For the CIE b color variable, the highest was at the 11th week of harvesting

Table 1. Fruit development parameters in different weeks

Week	Average Initial Diameter (mm)	CIEL	CIE a	CIE b
3	46.91	43.49	-20.25	32.99
4	40.24	38.43	-17.72	32.19
5	31.49	50.75	-21.69	43.41
6	33.40	52.13	-19.99	39.93
7	38,48	55.82	-20.31	41.38
8	61.33	56.24	-18.80	41.75
9	67.51	55.19	-22.09	40.71
10	58.04	67.60	-19.45	45.94
11	37.81	67.68	-26.90	50.88

Table 2. Fruit weight and diameter at different weeks

Week	Average Fruit weight (g)	Fruit diameter (mm)
3	169.08	122.15
4	195.59	71.35
5	152.69	67.23
6	167.42	68.46
7	132.04	63.98
8	139.07	110.43
9	146.57	159.14
10	128.00	64.24
11	86.07	53.87

fruit at 50.88 while the lowest was at the 4th week of grinding fruit, which was 32.19 (Table 1).

Data in table 2 reveal that the highest average fruit weight was in the 4th week of harvesting fruit, which was 195.59 grams, while the lowest was in the 11th week of harvesting at 86.07 g while the highest average final fruit diameter was during the 9th week of harvesting fruit, which was 159.14 cm, while the lowest was at the 11th week of drying, which was 53.87 cm.

Data in table 3 indicate that the variable outer appearance of the fruit with category 1 or the fruit peel is damaged and cannot be consumed. The highest (40%) was at week 10 of fruit extraction and the lowest was at week 5 and 6 of fruit extraction, which is 3, 8 percent respectyively. Meanwhile, for category 4 or suitable for consumption, the highest was in the 3rd week of fruit grinding by 56 percent and the lowest was in the 11th week of fruiting, which was 3.4 percent.

In table 4 it can be seen that the variable appearance in fruit with category 1 or damaged fruit pulp and cannot be consumed is the highest at week 10 of fruit grinding, which is 56 percent and the lowest is at week 5 and 6 of fruit grinding, which is 3, 8 percent, while for category 4 or very good and very fit for consumption, the highest was in the 4th week of fruit grinding at 62.5 percent and the lowest was in the 11th week of fruit grinding, which was 3.4 percent.

Effect of bagging time and fruit color on fruit fly attack

From the results of the logistic regression test, the variables that had a statistically significant effect on fruit fly attack were the week of grinding and fruit color (CIE L). initial diameter and the variables CIE a and CIE b did not have a significant effect. Fruit bagging time has a significant effect on the intensity of fruit fly attacks. Fruit bagging carried out early on will ensure that the fruit is saved from fruit fly attacks and every 1 week of bagging time increases the risk of fruit fly attack by 1.3 times. This means that ripe fruit has a greater chance of being attacked by fruit flies, this is in line with research (Grechi *et al.*, 2021) which states that ripe fruit has a higher chance of being attacked by fruit flies when compared to unripe fruit. Fruit bagging is proven to be able to protect the fruit from the insertion of fruit fly eggs (Abdurahim, 2020; Sharma *et al.*, 2020), so the fruit is relatively safe from fruit fly attacks. The diameter of the fruit does not affect the occurrence of fruit fly attacks, it means that fruit flies can lay their eggs on small or large fruit, size is not a preference for fruit flies in laying their eggs.

The color of the fruit has a significant effect on the attack rate of fruit flies, this is indicated by the higher the CIE L (light) value, the higher the attack rate, meaning that the lighter fruit has a higher chance of being

Table 3. Percentage of outer appearance conditions (PL) on every bagging week

Week	External appearance (per cent)			
	1	2	3	4
3	4	16	24	56
4	4,2	4,2	37,5	54,2
5	3,8	19,2	53,8	23,1
6	3,8	3,8	38,5	53,8
7	22,2	11,1	37	29,6
8	11,1	3,7	63	22,2
9	32.1	7.1	32.1	28.6
10	40	32	24	4
11	17.2	48.3	31	3.4

Table 4. Percentage of internal sighting conditions (PD) on every week of bagging

Week	Inner sight (per cent)			
	1	2	3	4
3	4	12	32	52
4	8.3	4.2	25	62.5
5	3.8	11.5	42.3	42.3
6	3.8	3.8	42.3	50
7	22.2	7.4	40,7	29,6
8	11,1	7,4	48,1	33,3
9	32,1	7,1	25	35,7
10	56	4	20	20
11	24,1	48,3	24,1	3,4

attacked by fruit flies. Fruit flies prefer bright green fruit to dark green fruit. Fruit flies are more attracted to bright or yellow-colored objects, this is also in line with the research results (Solihin, 2020; Syofia *et al.*, 2012; Wulan Sari *et al.*., 2017) which state that fruit flies are more attracted to yellow when compared to other colors. Fruit fly attacks are not affected by the CIE a value, which indicates the more positive the color will run from green to red and is also not affected by the CIE b value, which indicates the greater the value, the color will run from blue to yellow.

Impact on the quality of crystal guava fruit

Manova test with a significance level of 90 percent, attack status has a significant effect on weight, external appearance, and internal appearance. Meanwhile, for the final diameter variable, the attack status did not have a statistical effect on the final diameter of the fruit. For the weight variable, if it is not attacked by fruit flies, it will increase the weight of the fruit. Guava fruit that has been attacked by fruit flies will lose weight because the fruit contents will be consumed by fruit fly larvae. This is in line with research (Zulina *et al.*, 2020) which states that fruit grinding can increase fruit weight by 3 times when compared to fruit that is not bagged.

Fruit diameter is not affected by fruit fly attacks, it is suspected that this attack does not affect the development of the volume and size of the fruit so that when viewed from the size of the fruit, the fruit will appear normal like a healthy fruit. For the external appearance variable, if it is not attacked by fruit flies, it will increase the external appearance of the fruit by 1.1 points, so if it is not attacked by fruit flies, the external appearance will be better. Fruit that is attacked by fruit flies will visually have a stab wound or even a bigger hole which is the exit for the 3rd instar larvae that come out of the fruit to continue their life cycle to become pupae in the soil.

For the internal appearance variable, if it is not attacked by fruit flies, it will increase the appearance of fruit by 1.3 points, so if it is not attacked by fruit flies, the internal appearance will be better. If the fruit is split and observed, it will be seen that there are parts of the fruit that are still good so it can still be consumed in part, or the whole fruit becomes damaged due to too many larvae populations or the presence of other pathogenic infections.

CONCLUSION

The results showed that bagging time affected the intensity of fruit fly attacks. The longer the fruit bagging is carried out, the higher the fruit fly attack. Crystal guava fruit that is lighter or lighter in color is preferred by fruit flies. Fruit fly attacks have a significant effect on weight loss and make the fruit unattractive and unfit for the market.

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Diversity of insect-pests of banana in different agro climatic zones of Assam and ecological interaction of major banana insect pests

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ABSTRACT: Roving survey was conducted in 18 districts of Assam to know the status of insect-pests infesting different cultivars of banana. Survey revealed that banana pseudostem weevil (*Odoiporus longicollis*), banana rhizome weevil (*Cosmopolites sordidus*), banana leaf and fruit scarring beetle (*Nodostoma viridepennis*), aphid (*Pentalonia nigronervosa*), slug caterpillar (*Parasa lepida*), banana thrips (*Chaetanaphothrips signipennis*) and leaf eating caterpillar (*Spodoptera litura*) were the commonly occurring pests of banana in Assam, irrespective of cultivars. However, *O. longicollis, C. sordidus* and *N. viridipennis* were considered as economically important one and were observed in all the districts surveyed. Incidence of insect-pest was more in 'Jahaji' cultivar and was less in 'Bhimkol'. Population of banana pseudostem weevil and banana leaf and fruit scarring beetle increased from the month of March- April with the increase in ambient temperature and reached its minimum during winter months. Population of both the pests exhibited positive correlation with maximum and minimum temperature and, rainfall.

Keywords: Assam, banana pests, *Odoiporus longicollis*, *Cosmopolites sordidus*, *Nodostoma viridepennis*, *Pentalonia nigronervosa*, *Parasa lepida*, *Chaetanaphothrips signipennis*, *Spodoptera litura*

INTRODUCTION

The banana is principally a tropical and/or subtropical plant which needs a warm, frost-free climate. The optimum temperature for banana cultivation ranges from 25°C- 30°C, with relative humidity of 75-85 per cent. Having the sub tropical nature of climate, North Eastern (NE) region of India is a hot spot area for banana, harbouring a wide variety of wild germplasm. Maximum genetic variability of *Musa acuminata* and *M. balbisiana* occurs in NE India. Assam and Meghalaya are the two major banana growing states in the NE region, contributing about 4.09 per cent of the total banana pool of India. According to National Horticulture Board (NHB), Goalpara district of Assam and foothills of East Garo Hills district of Meghalaya are among the major potential belts for banana production in India. However, these two states have very low yield of banana than the all-India average (NEDFi, 2005). India is the worlds' largest producer of banana with a production of 30,808 thousand tons from 884 thousand ha area (Anon, 2019a). In Assam, banana is cultivated in an area of 53, 082 ha to produce 9,13,272 MT (Anon, 2019b). The lower yield of banana in the state may be attributed due to traditional cultivation practices with local germplasm in *Bari* system, monoculture in the same place for years, and the incidence of insect pests, nematodes and diseases. Most of the insect pests attack the rhizome, pseudostem, leaves and fruits. It has been reported that banana is attacked by several insect pests during different growth stages of the plants and, more than 470 species of insects and mites have been recorded attacking banana. Of these, 250 are foliage feeders, 10 are pseudostem borers, 70 feed on roots and rhizomes, 130 feed on fruits and flowers, and more than 10 are disease vectors (Shankar *et al.*, 2016). In addition, 58 species of ants have been collected from Central American banana plantations. Earlier, Roy and Sharma (1952) documented several insect pests attacking banana in India. The pests feeding on banana seedling are banana stem borer, banana aphid and banana scale moth. Amongst them, banana stemborer was the most destructive one, causing considerable damage to the commercial production of banana in India. From a survey conducted over 500 banana experts over the world, Pemsl *et al.* (2014) reported that black leaf streak, banana bunchy top disease, Fusarium wilt, bacterial wilts, nematodes and weevils were the most effective limiting factors for successful cultivation of banana. With this background, the present study was undertaken to document the prevailing insect pests in banana plantations of Assam, their percent of incidence under field condition and influence of environmental condition on population build-up of banana pseudostem weevil and banana leaf and fruit scarring beetle.

MATERIALS AND METHODS

Under the aegis of All India Coordinated Research

Table 1. Incidence of insect-pests of banana in different districts of Assam, India

Agroclimatic Zones of Assam	District	Insect pest incidence (%)					
		Odoiporus longicollis	***Cosmopolites sordidus***	***Nodostoma viridepennis***	***Pentalonia nigronervosa***	***Parasa lepida***	***Chaetanopho-thrips signipen-nis***
Lower Brahmaputra Valley Zone	Kamrup	2.0-22.0	2.0-8.60	4.0-26.5	4.0-16.0	4.0-12.0	2.0-8.0
	Nalbari	6.4-36.4	NR	6.2-49.2	2.2-13.6	2.4-9.2	1.5-13.3
	Borpeta	4.6-24.6	3.5-12.9	8.4-51.4	2.8-13.4	2.4-12.0	NR
	Dhuburi	7.7-62.5	NR	5.2-75.0	10.0-44.4	7.6-44.4	3.5-25.2
	Goalpara	5.33-33.04	5.5-35.5	6.33-78.53	2.56-23.57	11.67-32.66	NR
Central Brahmaputra Valley Zone	Nagaon	22.94-38.39	3.0-25.0	10.37-70.39	2.51-19.74	4.40-21.47	NR
	Marigaon	15.67-35.33	NR	15.0-60.0	3.0-10.0	3.0-10.0	NR
Upper Brahmaputra Valley Zone	Jorhat	6.0-30.0	6.0-16.96	5.0-82.0	1.66-18.0	3.36-12.0	3.36-20.58
	Golaghat	8.25-30.0	4.4-13.50	11.11-60.82	1.58-18.3	4.80-23.73	4.45-20.61
	Sibsagar	5.0-35.0	6.0-18.75	11.25-26.25	2.79-18.4	3.74-12.94	2.96-17.78
	Dibrugarh	9.09-28.03	10.57-14.017	13.26-25.19	5.12-14.96	4.95-18.80	1.98-27.35
	Tinsukia	13.80-28.90	10.28-13.72	12.59-55.78	4.92-12.74	5.18-13.08	4.27-16.82
Barak Valley Zone	Cachar	19.46-28.75	9.78-13.87	25.11-28.73	4.66-6.37	3.86-6.46	NR
	Hailakandi	17.17-28.14	NR	11.46-16.14	1.60-3.55	1.11-1.76	NR
	Karimganj	24.11-26.0	NR	14.43-17.38	1.5-4.21	2.37-4.63	NR
Hill zones	KarbiAnglong	11.4-39.6	5.4-10.5	4.2-43.6	3.4-10.8	2.4-13.4	NR
North bank Plains Zone	Darang	4.43-16.0	2.2-16.0	1.43-25.71	4.29-11.43	4.29-20.0	2.0-6.0
	Lakhimpur	5.0-70.0	10.0-30.6	13.3-80.0	4.5-16.0	6.0-25.0	NR

NR: Not recorded during the survey period

Project on Fruits, Jorhat centre; random surveys were conducted in 18 districts of Assam (Kamrup, Nalbari, Barpeta, Goalpara, Dhuburi, Darrang, Nagaon, Marigaon, Jorhat, Golaghat, Sibsagar, Dibrugarh, Tinisukia, Lakhimpur, Cachar, Hailakandi, Karimganj and Karbi-Along) during 2006-2016 to document the diversity of insect pests of banana and their incidence of infestation. In these districts banana was mostly grown in homestead garden and practice of growing ratoon crops was common. The crop was generally poorly managed, except in few cases. The home stead gardens were visited and observed for occurrence of insect-pests damage, and then percent of infestation was recorded. All districts were not surveyed at the same season and year; and therefore, the per cent incidence may vary due to the effect of seasonal variations on pest population. Fixed plot survey was conducted during 2014-15 to see the effect of abiotic factors such as temperature (maximum and minimum), relative humidity and rainfall on population buildup of banana leaf and fruit scarring beetle (*Nodostoma viridepennis*) and banana pseudostem weevil (*Odoiporus longicollis*). Population of both the pests were counted fortnightly from a fixed banana plot grown with three different cultivars of banana (Jahaji, Barjahaji and Chenichampa) at Horticultural Experimental Farm, Assam Agricultural University, Jorhat (24°47′ N-Latitude, 94°12 ′ E-Longitude Altitude 86.8m). Beetle population of *N. viridepennis* was observed from three youngest leaves of randomly selected plants. The beetle populations were recorded by counting the beetles on leaves and inside whorl of crown leaves during morning hours. The average number of insect/plants was worked out by mean number of beetles per plant. In case of *O. longicollis*, beetles were made attracted to the cut

Table 2. Ranges of incidence of different insect –pests in banana at Assam

Banana cultivar (genomic group)	% incidence					
	Odoiporus longicollis	*Cosmopolites sordidus*	*Nodostoma viridepennis*	*Pentalonia nigronervosa*	*Parasa lepida*	*Chaetanophothrips signipennis*
Jahaji (AAA)	7.12-70.0	6.62-13.5	13.62-80.0	4.62-46.6	4.29-13.3	3.4-20.58
Barjahaji (AAA)	5.0-28.9	4.4-11.96	13.64-40.0	6.0-33.3	5.0-12.9	5.71-20.61
Malbhog (AAB)	6.0-24.6	4.0-14.17	12.5-60.0	4.0-25.0	5.36- 13.4	5.36 – 14.9
Manohar (ABB)	6.67 – 35.4	2.0 – 13.33	12.59 -28.5	4.0 – 12.5	3.36 – 13.3	3.36 – 6.90
Chenichampa (AAB)	6.66 – 37.5	4.0 – 18.75	16.0 – 70.0	3.6 – 15.0	3.6 – 12.5	4.0 – 10.40
Bharatmoni (ABB)	5.71 – 21.6	6.0 – 14.73	5.0 – 31.6	2.4 – 12.74	2.2 – 11.57	5.25 – 12.28
Kachkol (ABB)	6.4 – 37.5	10.28 – 13.67	4.2 – 16.78	1.58 – 6.2	2.4 – 13.08	5.0 – 27.35
Bhimkol (BB)	0- 10.0	0 – 6.6	0 – 14.15	0 – 6.6	0 – 23.73	0 – 7.55
Athia kol (BB)	0 – 16.0	0 – 8.26	0 – 14.15	0 – 14	0 – 11.43	0 – 2.0
Kechulepa (AAB)	5.0- 20.13	6.25 – 16.0	14.0 -16.25	4.29 – 6.0	5.0 – 6.0	4.29 – 6.0
Champa (AAB)	24.1 – 28.75	NR	16.1 – 25.6	2.93 – 6.37	1.76 – 8.2	NR
Amrtisagar (AAA)	8.0 – 25.66	NR	25.1 – 80.0	6.0	4.66 – 16.0	NR
Sail (AAB)	17.17 – 26.0	NR	13.9 –17.38	1.5 – 1.6	1.11 – 2.38	NR

NR: Not recorded during the survey period

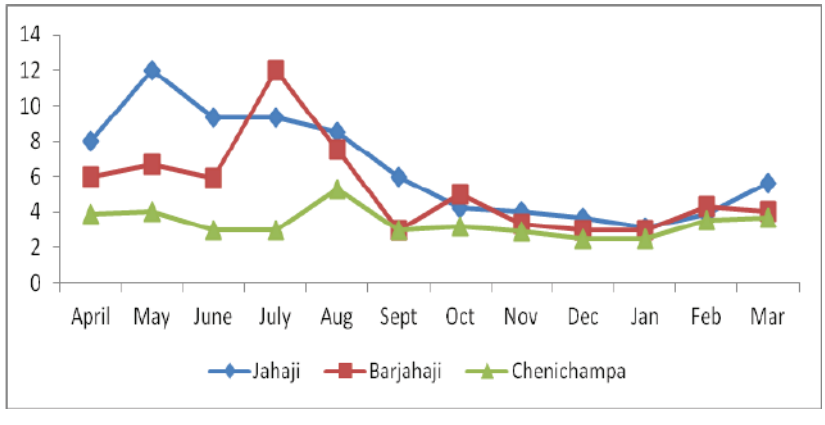

Fig 1. Population fluctuation of *Nodostoma viridepennis* at different months during 2014-15

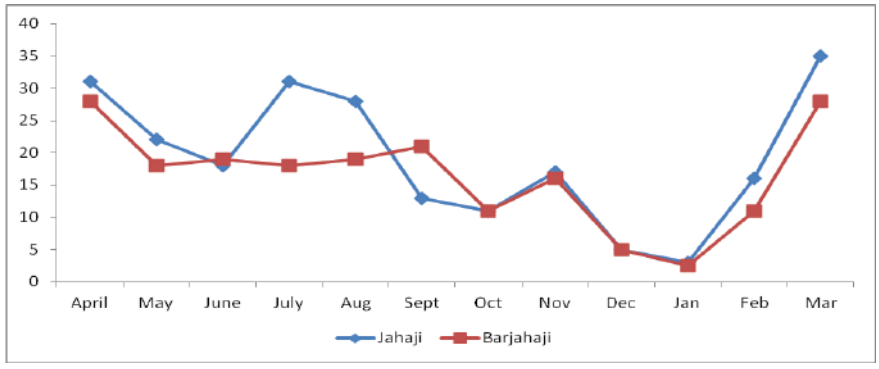

Fig 2. Population fluctuation of *Odoiporus longicollis* at different months during 2014-15

pieces of banana pseudostems that were placed around the fixed plots. Number of beetles attracted to these cut pieces were counted and then average number of insects per plant was worked out. Meteorological information was collected from the Department of Agricultural Meteorology, Assam Agricultural University, Jorhat and were used for correlation analysis.

RESULTS AND DISCUSSION

Survey data revealed that banana pseudostem weevil (*Odoiporus longicollis*), banana rhizome weevil (*Cosmopolites sordidus*), banana leaf and fruit scarring beetle (*Nodostoma viridepennis*), aphid (*Pentalonia nigronervosa*), slug caterpillar (*Parasa lepida*), banana thrips (*Chaetanaphothrips signipennis*) and leaf eating caterpillar (*Spodoptera litura*) were the commonly occurring pests of banana in Assam, irrespective of cultivars. However, *O. longicollis, C. sordidus* and *N. viridipennis* were considered to be the economically important one. Based on the specific symptoms exhibited by these pests on the plants and on their occurrence, percent incidence of these pests at different districts is presented in Table 1. Data revealed that at Upper Brahmaputra Valley Zone of Assam, the incidence of banana pseudostem weevil, rhizome weevil, leaf and fruit scarring beetle, aphids, slug caterpillar, thrips and leaf eating caterpillar was 5.0-35.0, 4.4-18.75, 5.0-82.0, 1.57-18.4, 3.36-23.73, 1.98-27.35 and 16.6 percent, respectively. The percent incidence of pseudostem weevil, rhizome weevil, leaf and fruit scarring beetle, aphids, slug caterpillar and thrips at North Bank Plains Zone was 4.43-70.0, 2.2-30.6, 1.43-80.0, 4.29-16.0, 4.29-25.0 and 2.0-6.0, respectively. Likewise, range of per cent incidence of pseudostem weevil, rhizome weevil, leaf and fruit scarring beetle, aphids, slug caterpillar and thrips at Central Brahmaputra Valley Zone was 22.94-38.39, 3.0-25.0, 10.37-70.39, 2.51-19.74, 4.4-231.47 and 0 (not reported); in Lower Brahmaputra Valley Zone was 2.0-62.5, 2.0-35.5, 4.0-78.53, 2.2-44.4, 2.4-44.4 and 2.0-25.2; in Hill Zone 11.4-39.6, 5.4-10.5, 4.2-43.6, 3.4-10.8, 2.4-13.4 and 0 (not reported), in Barak Valley zone 17.17-28.75, 9.78-13.87, 11.43-28.73, 1.5-6.37, 1.11-6.46 and 0 (not reported), respectively. It is to be mentioned that the insect pest not reported during the survey does not mean that the pest is not present, however, may be missed or escaped during the survey. Roy and Sharma (1952) documented several insect pests attacking banana in India. They reported banana stem borer, banana aphid and banana scale moth from banana seedlings of which, banana stemborer was considered to be the most destructive insect pest causing considerable damage to the commercial production of banana in India. Shankar *et al.* (2016) mentioned that about 470 species of insects and mites attack banana and of these, foliage feeder was 250, pseudostem borer 10, root and rhizome feeder 70 and, fruits and flower feeder was 130. In a survey conducted in Malda district of West Bengal, Chowdhury (1915) considered four insects *viz.,* thrips (*Chaetanaphothrips signipennis*), corm weevil (*Cosmopolites sordidus*), stem weevil (*Odoiporus longicollis*), and aphid (*Pentalonia nigronervosa*) as major insect-pests of banana. Mahanta *et al.* (2018) recorded three insect pests *viz., Nodostoma subcostratum*, *Pentalonia nigronervosa* and *Parasa lepida* to be associated with banana in horticultural orchard of Assam Agricultural University, Jorhat campus with a relative frequency of 1.37, 2.64 and 0.14, respectively.

The incidence of all insect pests in different banana cultivars is presented in Table 2. It was observed that incidence of insect -pests was more in the cultivar Jahaji (AAA), followed by Borjahaji (AAA) and was less in Bhimkol (BB) and Athiakol (BB). Das *et al.* (2016) and Das and Baruah (2018) reported that all most all AAA banana genotypes were found to be susceptible to insect pests and nematodes.

The studies on effect of abiotic factors like ambient temperature, relative humidity and rainfall on population

Table 3. Correlation of population of *Nodostoma viridepennis* and Odoiporus longicollis with abiotic factors

Meteorological parameter	Correlation coefficient (r)	
	Nodostoma viridepennis	***Odoiporus longicollis***
Max. Temp	0.94**	0.68*
Min. Temp	0.94*	0.71*
Rainfall	0.60 *	0.73**
RH	0.35	0.54

behaviour of pseudostem weevil, *O. longicollis* and banana leaf and fruit scarring beetle, *N. viridepennis* revealed that population of both the pests was influenced by the abiotic factors. Population of *N. viridepennis* increased with the rise of atmospheric temperature to reach its peak during July – August. The population of this pest tended to decrease with fall of atmospheric temperature to reach its minimum during December-January (Fig.1). Correlation of population of this pest with abiotic factors revealed that population build up had a positive correlation with temperature (maximum and minimum) and rainfall (Table 3). Similar trend of population was observed in case of banana pseudostem weevil, *Odoiporus longicollis.* Population of this pest tended to increase from the month of March-April with rise of atmospheric temperature and then decreased during the winters (Fig. 2). A positive correlation of this pest was observed with temperature (maximum and minimum) and rainfall (Table 3). Azam *et al.* (2010) observed that population of banana pseudostem weevil remains in the field throughout the year; however, its population increases with the increase in temperature and decreases with decrease in temperature. Tayade *et al.* (2014) observed that there was significant positive correlation between mean numbers of adults of pseudostem weevil and minimum temperature, morning relative humidity, evening relative humidity, average relative humidity, rainfall as well as rainy days; which was in conformity with the present investigation. Similar observation was made by Priyadarshini *et al.* (2014).

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Biorational management and mycosis studies of grape thrips, *Rhipiphorothrips cruentatus* H.

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ABSTRACT: Field experiment was conducted during 2017-18 to test the bioefficacy of certain biorational insecticides against thrips *Rhipiphorothrips cruentatus* Hood on grapevine at the Department of Horticulture, MPKV, Rahuri, India. Among botanical insecticides, neem oil 2% (4.09/shoot) was the most effective treatment followed by, karanj oil (4.51/shoot) and commercial azadirachtin formulation (neemazol) (5.08/shoot). The entomopathogenic fungi, *Lecanicillium lecanii* recorded 4.24/shoot followed by *Metarhizium anisopliae* (4.87/shoot) and *Beauveria bassiana* (5.34/shoot). However chlli methanolic extract (6.29/shoot), garlic methanolic extract (6.78/shoot), chilli water extract (6.85/shoot) and garlic water extract (7.08/shoot) were least effective treatments. Incremental Cost Benefit Ratio (ICBR) in respect of different treatments ranged from 1.30 to 7.92. The highest ICBR of 1:7.92 was recorded with emamectin benzoate 5 SG followed by *L. lecanii* (1:6.34) and *M. anisopliae* (1:5.32). Although neem oil and karanj recorded higher reduction of thrips population, they had lower cost benefit ratio due to high dose and its cost. The pathogenesity of *L. lecanii* and *M. anisopliae* was also confirmed through mycosis test.

Keywords: Botanicals, entomofungi, grapes, *Lecanicillium lecanii*, thrips, mycosis

INTRODUCTION

Grapes (*Vitis vinifera* L.) is an important commercially grown fruit crop of India being cultivated in 1,38,000 ha with annual production of 30 lakh MT. Maharashtra is the leading grape growing state covering an area of about 78000 ha with the production of 1.80 lakh MT (Anonymous, 2018). Thrips, once considered to be the insect pests of minor importance in horticultural crops, have gained the paramount importance due to their ability to cause economic losses, to subsist on new hosts and polyphagous nature (Dahiya *et al.*, 1995). *Ripiphorothrips cruentatus* (H.) and *Scirtothrips dorsalis* (H.) are the species recorded infesting the leaves and berries (Butani., 1979) of grapes. Both nymphs and adults of *R. cruentatus* cause damage by rasping the lower surface of the leaf with their stylets and sucking the oozing cell sap. The injured surface is marked by the number of minute spots thereby producing a speckled silvery effect, which can be detected from a distance. They feed in groups, generally on the undersurface of the leaves. Curling of the leaves is observed in case of severe incidence (Kulakarni *et al.*, 2007).

Large number of chemicals are being used on to manage thrips. Chemical control affects the export value of grapes due to pesticide residues. Also some of the results concluded that 27 chemical pesticides out of 171 chemical pesticides can be found usually in grape samples which indicate that the stability of these pesticides is very high or they retain in the grape fruit for a long time after use of them which affect the export value of grapes (Raikwar *et al.*, 2011). Hence it is essential to find effective biorational pesticides like botanicals and entomofungi to have residue free pest management. With this background, the present study was carried out to evaluate the bioefficacy of biorational insecticides and validation of the entomopathegenic fungi growth on grape vine thrips.

MATERIALS AND METHODS

Bioefficacy studies

A field experiment was carried out in a vineyard under All India Co-ordinated Research Project (AICRP) on Fruits at the Department of Horticulture, MPKV., Rahuri, India after October, 2017 pruning to evaluate the bioefficacy of certain bio-rational insecticides against thrips on grapevine. The grape variety 'Flame Seedless' was chosen for the study. Gardens were selected after ensuring that they were totally unprotected after fruit pruning. The trial was laid out in a Randomized Block Design (RBD) with twelve treatments replicated three times containing two vines each.

Table 1. Bio-efficacy of some biorational insecticides against thrips on grapes (1st spray)

	Treatment	Dose	Number of thrips per shoot						Per cent reduction of thrips over control
			Pre-count	3 DAS	5 DAS	7 DAS	10 DAS	Mean	
T_1	*Beauveria bassiana*	5g/l ($1x10^8$ cfu/g)	8.70 (3.03)	8.13 (2.94)	5.70 (2.49)	3.37 (1.97)	4.47 (2.23)	5.42 (2.43)	38.10
T_2	*Metarhizium anisopliae*	5g/l ($1x10^8$ cfu/g)	8.27 (2.96)	7.43 (2.82)	4.93 (2.33)	3.00 (1.87)	4.20 (2.17)	4.89 (2.32)	44.10
T_3	*Lecanicillium lecanii*	5g/l ($1x10^8$ cfu/g)	8.10 (2.93)	7.10 (2.76)	4.43 (2.22)	2.30 (1.67)	3.90 (2.10)	4.43 (2.22)	49.33
T_4	**Neem oil (2%)**	20 ml/l	8.60 (3.02)	7.03 (2.74)	3.63 (2.03)	2.20 (1.64)	3.97 (2.11)	4.21 (2.17)	51.90
T_5	**Karanj oil (2%)**	20 ml/l	8.50 (3.00)	7.10 (2.76)	3.93 (2.11)	3.07 (1.89)	4.53 (2.24)	4.66 (2.27)	46.76
T_6	**Neemazol (10000ppm)**	3ml/l	8.10 (2.93)	6.93 (2.73)	3.70 (2.05)	2.87 (1.83)	4.23 (2.18)	4.43 (2.22)	49.33
T_7	**Chilli methanolic extract (2%)**	20 ml/l	8.17 (2.94)	7.17 (2.77)	5.37 (2.42)	4.30 (2.19)	8.20 (2.95)	6.26 (2.60)	28.48
T_8	**Garlic methanolic extract (2%)**	20 ml/l	8.27 (2.96)	7.53 (2.83)	5.73 (2.50)	4.90 (2.32)	8.43 (2.99)	6.65 (2.67)	24.00
T_9	**Emamectin benzoate**	0.22g/l	8.30 (2.97)	5.17 (2.38)	2.10 (1.61)	1.73 (1.49)	3.50 (2.00)	3.13 (1.90)	64.29
T_{10}	**Chilli water extract (2%)**	20 ml/l	8.73 (3.04)	8.07 (2.93)	6.47 (2.64)	5.47 (2.44)	8.67 (3.03)	7.17 (2.77)	18.10
T_{11}	**Garlic water extract (2%)**	20 ml/l	8.63 (3.02)	8.00 (2.92)	6.60 (2.66)	5.93 (2.54)	8.60 (3.02)	7.28 (2.79)	16.76
T_{12}	**Untreated Control**	---	8.67 (3.03)	8.83 (3.06)	8.83 (3.06)	8.60 (3.02)	8.73 (3.04)	8.75 (3.04)	0.00
	S. E. m. ±	---	0.117	0.088	0.075	0.065	0.097	0.061	
	CD at 5%	---	NS	0.260	0.220	0.193	0.286	0.181	

* DAS: Days after spraying; NS: Non significant * Figures in the parenthesis indicate √x+0.5 transformed values

Pre-treatment count of thrips, was taken prior to the insecticidal application. Eleven insecticides applications were given in the experimental field with the help of a knapsack sprayer. A total of three sprays were applied at an interval of ten days. The data was recorded on population of thrips by tapping five shoots from each treated vines. Observations on thrips and were taken at 3, 5, 7 and 10 days after spray (DAS), (Duraimurugan and Jagadish, 2004). The insecticidal efficacy was assessed by recording the total number of thrips present on vines as well as bunches on two vines in each treatment. Presence of thrips was recorded on selected shoot and it was expressed as number of thrips per shoot per vine (Kulkarni and Adsule, 2006). On the basis of the absolute counts of the thrips recorded, the population reduction in different treatments over control was calculated by using Modified Abbot's formula given by Fleming and Retnakaran (1985).

Pre count (1 DBS) and post count (mean of 3, 5, 7 and 10 DAS) population and per cent reduction over control were calculated after each spray. Cumulative mean of three sprays is analysed in order to get the best treatment.

$$\text{Per cent reduction over control} = \frac{\text{Population in control - population in treatment}}{\text{Population in control}} \text{ X } 100$$

Incremental cost benefit ratio and yield data

The incremental cost benefit ratio of each insecticide was calculated by taking into account of the prevailing market price of input, produce and labour charges. Grape bunches were harvested from each treatment separately and yield was recorded. Total yield was calculated by adding the yield from different treatments. The per treatment yield was then converted to tonnes per ha.

Mycosis Studies

Three mycoinsecticides like *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii* were studied for mycosis test on grape thrips. The fungal suspension of three mycoinsecticides are prepared separately in beakers by mixing 5 g of each mycoinsecticide in 100 ml of water in beaker. All the three mycoinsecticides suspensions are prepared in three separate beakers. The young grape leaves are collected from field and their surface is cleaned with mercuric chloride by using cotton, in order to remove fungal spores present on the leaves. Later on the leaves are rinsed with the distilled water to remove the chemical on leaves. These grape leaves were smeared with the fungal suspension prepared and placed in the petri plates. For each mycoinsecticide three petri plates were prepared for mycosis test. Thrips nymphs were collected from the field and released into each petri plates in numbers of 10. These petri plates were packed with the polythene stripe in order to avoid the escape of thrips from petri plates. These petri plates were incubated in cool place for seven days to promote the infection of fungus on the thrips (Latha *et al.* 2010). Detailed microscopic examination of thrips samples collected from the petri plates of different treatments were observed after seven days and ten days of treatment under the stereo microscope with various resolutions like 10 and 40X for the growth of different fungus on various body parts of the thrips. These microscopic photographs are clearly mentioned in the results.

RESULTS AND DISCUSSION

Bioefficacy of biorational insecticides against grape thrips

The thrips population recorded, a day before spraying (PTC) varied from 8.10 to 8.73 thrips per shoot, which showed non significant difference among treatments indicating homogenous distribution of thrips population in the experimental area (Table 1). There was significant difference among the treatments after 3, 5, 7 and 10 days of first spraying. Considering the mean population of thrips after first spray, it was found that biorational insecticides neem oil (4.21/shoot) and *L. lecanii* (4.43/shoot) was the most effective treatment with least population of thrips. Whereas, chilli water extract and garlic water extract was least effective with 7.17 and 7.28 thrips per shoot, respectively. However standard check emamectin benzoate 5 SG @ 11 g a.i.ha^{-1} proved to be significantly superior recording minimum thrips population (3.13/shoot). The data also indicated that higher reduction of population over control was observed in plots treated with standard check emamectin benzoate (64.29%). Among biorational insecticides neem oil (51.90%). *Lecanicillium lecanii* and neemazol had same per cent reduction over control *i.e* 49.33%. Next in order of effectiveness were karanj oil (46.76%), *M. anisopliae* (44.10%) and *B. bassiana* (38.10%).

Second Spraying

The results on efficacy of insecticides on population of thrips after second spray were presented in (Table 2).The data on thrips population collected at 10 DAS after I spray was considered as pre count for second spray. Considering the mean population of thrips after second spray, it was found that standard check emamectin benzoate was the most effective treatment with least population of thrips (3.06/shoot). Among biorational insecticides neem oil (4.07/shoot) and *L. lecanii* (4.16/shoot) proved as effective treatments. Whereas, chilli

Table 2. Bio-efficacy of some biorational insecticides against thrips on grapes (2nd spray)

	Treatment	Dose	Number of thrips per shoot					Per cent reduction of thrips over control
			3 DAS	5 DAS	7 DAS	10 DAS	Mean	
T_1	***Beauveria bassiana***	5g/l ($1x10^8$ cfu/g)	8.06 (3.02)	6.00 (2.55)	3.27 (1.94)	4.07 (2.14)	5.48 (2.45)	37.45
T_2	***Metarhizium anisopliae***	5g/l ($1x10^8$ cfu/g)	8.03 (2.92)	5.47 (2.44)	2.80 (1.82)	3.50 (2.00)	4.95 (2.33)	43.54
T_3	***Lecanicillium lecanii***	5g/l ($1x10^8$ cfu/g)	7.20 (2.77)	4.83 (2.31)	1.97 (1.57)	2.63 (1.77)	4.16 (2.16)	52.57
T_4	**Neem oil (2%)**	20 ml/l	6.70 (2.68)	4.67 (2.27)	1.77 (1.51)	3.13 (1.91)	4.07 (2.14)	53.61
T_5	**Karanj oil (2%)**	20 ml/l	5.93 (2.54)	4.87 (2.32)	3.27 (1.94)	3.80 (2.07)	4.47 (2.23)	49.05
T_6	**Neemazol (10000ppm)**	3ml/l	7.23 (2.78)	6.00 (2.55)	5.00 (2.35)	4.80 (2.30)	5.76 (2.50)	34.32
T_7	**Chilli methanolic extract (2%)**	20 ml/l	7.40 (2.81)	6.53 (2.65)	5.03 (2.35)	6.27 (2.60)	6.31 (2.61)	28.04
T_8	**Garlic methanolic extract (2%)**	20 ml/l	8.10 (2.93)	7.07 (2.75)	6.07 (2.56)	6.97 (2.73)	7.05 (2.75)	19.58
T_9	**Emamectin benzoate**	0.22g/l	4.30 (2.19)	3.67 (2.04)	1.63 (1.46)	2.63 (1.77)	3.06 (1.89)	65.11
T_{10}	**Chilli water extract (2%)**	20 ml/l	7.80 (2.88)	6.73 (2.69)	5.67 (2.48)	7.20 (2.77)	6.85 (2.71)	21.86
T_{11}	**Garlic water extract (2%)**	20 ml/l	7.40 (2.81)	7.07 (2.75)	6.33 (2.61)	7.60 (2.85)	7.10 (2.76)	19.01
T_{12}	**Untreated Control**	---	8.63 (3.02)	8.67 (3.03)	8.87 (3.06)	8.90 (3.07)	8.77 (3.04)	0.00
	S. E. m. ±	---	0.100	0.082	0.067	0.074	0.060	
	CD at 5%	---	0.295	0.241	0.197	0.217	0.176	

* DAS: Days after spraying; NS: Non significant * Figures in the parenthesis indicate √x+0.5 transformed values

Table 3. Bio-efficacy of some biorational insecticides against thrips on grapes (3rd spray)

	Treatment	Dose	Number of thrips per shoot					reduction of thrips over control (%)	ICBR
			3 DAS	5 DAS	7 DAS	10 DAS	Mean		
T_1	*Beauveria bassiana*	5g/l ($1x10^8$ cfu/g)	8.20 (2.95)	5.17 (2.38)	3.13 (1.91)	4.03 (2.13)	5.13 (2.37)	39.19	5.13
T_2	*Metarhizium anisopliae*	5g/l ($1x10^8$ cfu/g)	7.63 (2.85)	5.20 (2.39)	2.80 (1.82)	3.40 (1.97)	4.76 (2.29)	43.63	5.32
T_3	*Lecanicillium lecanii*	5g/l ($1x10^8$ cfu/g)	7.17 (2.77)	4.90 (2.32)	2.10 (1.61)	2.30 (1.67)	4.12 (2.15)	51.23	6.34
T_4	**Neem oil (2%)**	20 ml/l	6.40 (2.63)	4.73 (2.29)	1.97 (1.57)	2.90 (1.84)	4.00 (2.12)	52.62	2.81
T_5	**Karanj oil (2%)**	20 ml/l	6.13 (2.58)	4.80 (2.30)	2.93 (1.85)	3.80 (2.07)	4.42 (2.22)	47.68	3.04
T_6	**Neemazol (10000 ppm)**	3ml/l	7.23 (2.78)	4.80 (2.30)	3.10 (1.90)	5.10 (2.37)	5.06 (2.36)	40.08	1.98
T_7	**Chilli methanolic extract (2%)**	20 ml/l	8.10 (2.93)	6.00 (2.55)	4.80 (2.30)	6.27 (2.60)	6.29 (2.61)	25.47	2.19
T_8	**Garlic methanolic extract (2%)**	20 ml/l	8.27 (2.96)	6.23 (2.59)	5.10 (2.37)	6.97 (2.73)	6.64 (2.67)	21.32	1.30
T_9	**Emamectin benzoate**	0.22g/l	4.40 (2.21)	3.77 (2.07)	1.73 (1.49)	2.53 (1.74)	3.11 (1.90)	63.18	7.92
T_{10}	**Chilli water extract (2%)**	20 ml/l	7.60 (2.85)	6.23 (2.59)	5.20 (2.39)	7.10 (2.76)	6.53 (2.65)	22.61	2.19
T_{11}	**Garlic water extract (2%)**	20 ml/l	7.80 (2.98)	6.73 (2.99)	5.67 (2.99)	7.20 (3.00)	6.85 (2.99)	18.85	1.48
T_{12}	**Untreated Control**	---	8.37 (2.88)	8.43 (2.69)	8.47 (2.48)	8.50 (2.77)	8.44 (2.71)	0.00	
	S. E. m. ±	---	0.097	0.084	0.072	0.093	0.063	---	
	CD at 5%	---	0.286	0.249	0.214	0.274	0.186	---	

* DAS: Days after spraying; NS: Non significant * Figures in the parenthesis indicate √x+0.5 transformed values.

water extract and garlic water extract was least effective with 6.85 and 7.10 thrips per shoot, respectively. The cumulative effect of treatments indicated that higher reduction of population over control was observed in plots treated with standard check emamectin benzoate (65.11%). Among biorational imsecticides neem oil (53.61%) emerged as best treatment over control. Next in order of effectiveness were *L. lecanii* (52.57%), karanj oil (49.05%), *M. anisopliae* (43.54%) and *B. bassiana* (37.45%).

Third Spraying

The results with regard to the efficacy of treatments after third spray were presented in (Table 3). Considering the mean population of thrips after third spray, it was found that standard check emamectin benzoate was the most effective treatment with least population of thrips (3.11/ shoot). Among biorational insecticides neem oil (4.00/ shoot), *Lecanicillium lecanii* (4.12/shoot) and karanj oil (4.42/shoot) were the best treatments. Whereas, garlic methanolic extract and garlic water extract was least effective with 6.64 and 6.85 thrips per shoot respectively. The cumulative effect of treatments indicated that higher reduction of population over control was observed in plots treated with standard check emamectin benzoate (63.18%). Among biorational insecticides neem oil is the best treatment with 52.62% reduction over control. Next in order of effectiveness were *L. lecanii* (51.23%), karanj oil (47.68%), *M. anisopilae* (43.63%), neemazol (40.08%) and *B. bassiana* (39.19%).

Pooled data

The data pertaining to efficacy of insecticides against thrips during first, second and third spray are pooled and presented in Fig. 1. It could be seen that all the insecticidal treatments were significantly superior over untreated control. The pooled data of three sprays revealed that standard check emamectin benzoate 5 SG consistently proved to be the most promising by recording the least population (3.10/shoot). Among biorational insecticides neem oil 2% (4.09/shoot), karanj oil (4.51/shoot) and neemazol (5.08/shoot). While entomopathogenic fungi *L. lecanii* recorded less population (4.24/shoot) as compared to the *M. anisopliae* (4.87/shoot) and *B. bassiana* (5.34/shoot). The data also indicated that higher per cent reduction over control of population was observed in plots treated with standard check emamectin benzoate 5 SG (64.21%). Among biorational insecticides neem oil (52.71%) and *L. lecanii* (51.04%). Next in order of effectiveness were karanj oil (47.83%), *M. anisopliae* (43.76%), neemazol (41.26%), *B. bassiana* (38.23%), chlli methanolic extract (27.35%), garlic methanolic extract (21.63%), chilli water extract (20.83%) and garlic water extract (18.20%).

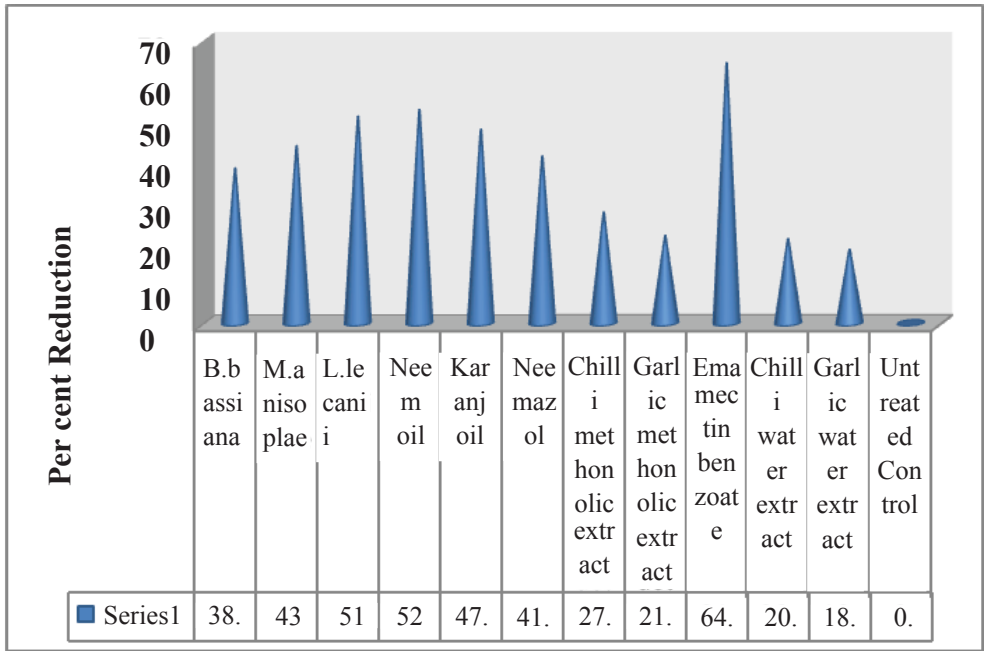

Fig 1. Per cent Reduction over Control of biorational insecticides against thrips on grapes (pooled data)

Cost economics of grapes

The cost effectiveness of the different insecticides used during study was assessed and presented in the (Table 3). The ICBR in respect of different treatments ranged between 1.30 to 7.92. The highest C:B ratio in *L. lecanii* (1:6.34) and *M. anisopliae* (1:5.32). Although neem oil and karanj oil has great reduction of thrips population, but has less cost benefit ratio *i.e* 2.81 and 3.04, respectively due to high cost of the insecticide.

Mycosis test of mycoinsecticides on grape vine thrips

The fungal suspension of three mycoinsecticides *viz. B. bassiana, M. anisopliae* and *L. lecanii* were studied for mycosis test on grape thrips. Detailed microscopic examination of the thrips samples collected from the petri plates showed that all the test entomopathogenic fungi were found growing in the body of the thrips. The moribund adult thrips showed profuse fungal growth in the body cavity. The microscopic photographs in the plates clearly indicated the mycosis by *B. bassiana* was predominant behind compound eyes, prothorax, near fore coxa, stomach portion and between inter segmental spaces. Close up view showed clear growth of fungus in thorax and abdominal portion. In advanced stages after tight filling the body cavity the fungus outgrowth was observed on head, legs and posterior part of abdomen. Highly pronounced mycosis by *M. anisopliae* was observed in the thrips which shrunken and hardened its body. The growth was observed in almost all body parts. The growth was observed along inter segmental joints around genital parts, tergo- sternum joint, head and prothorax and inter wings. *L. lecanii* soften the body of the thrips and growth was observed on antennary tips, around compound eyes, legs and tissues in different part of the body and alimentary canal in mid infestation (Plate 3 – a), in advanced stages whole body was captured by the fungus and growth was also vivid on surface of the body (Plate 1).

Beauveria bassiana ***Metarhizium anisopliae*** ***Lecanicillium lecanii***

Plate 1. Mycosis of thrips by three species of entomofungi

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Biology and demography of cassava red spider mite, *Oligonychus biharensis* (Hirst) (Acari: Tetranychidae) on *Manihot esculenta* L.

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ABSTRACT: Development and population performance of mite, *Oligonychus biharensis* was studied on cassava at three different constant temperatures (28°C, 24°C and 32°C) in the laboratory. The developmental period from egg to adult varied from 11.57 days at 24°C to 5.57 days at 32°C. The average longevity of adult mated female ranged from 9.10 days to 15.72 days, while the oviposition period varied from 8.46 days to 13.66 days. Fecundity ranged from 52.03 to 79.42 eggs/ female. At 24°C, each mated female laid 79.42 eggs over 13.66 days and produced a female-biased progeny of 11.48:1 sex ratio. Also, higher Net Reproduction Rate of 69.96 female off-springs/ female/ generation, Mean Generation Time of 20.15 days, Doubling Time of 3.33 days, Intrinsic rate of natural increase (r_m) of 0.2259 female off-springs/female/day were observed.

Keywords: *Oligonychus biharensis,* cassava, life history, population performance

INTRODUCTION

Cassava (*Manihot esculenta* L.) is a primary food crop of South Indian state of Kerala and also an industrial crop. Because of its starchy roots, ease of establishment and nutritional value, it is mostly farmed. In India, there is a scarcity of detailed information on pest species associated with Cassava. Spider mites are the important pests of agricultural crops, damaging all types of economically important cultivated crops, including cereals, pulses, millets, vegetables, plantations, ornamental and medicinal plants (Vacante, 2015). Among them *Oligonychus biharensis* (Hirst), a mite that has been a significant threat as a sporadic pest of vegetables, rose, camphor, litchi and many other plants of economic importance. The overall development and vigor of the plants are generally reduced when mites attack such plants. *Manihot esculenta* L. was successfully used as a laboratory host for studying detailed biology and demography of this emerging spider mite pest *O. biharensis.* Temperature has been the crucial environmental factor affecting development and reproduction of poikilothermic organisms like spider mites. Hence, we felt it is necessary to study mite's biological characteristics at different temperature conditions for the construction of developmental curves which can be used for the prediction of mite's developmental time as a function of temperature factor. Such predictions may serve as population models to study the population fluctuation of pest organisms. Thus, present study was conducted to generate data on the development and population performance of *O. biharensis* under constant temperature conditions between 24°C and 32°C in the laboratory.

MATERIALS AND METHODS

Life history of mite: Developmental biology of the spider mite *O. biharensis* was studied on Cassava leaf discs separately at three different constant temperature conditions *viz.,* 24±1°C; 75-80% RH, 28±1°C; 70-75% RH and 32±1°C; 75-80% RHwith 14h: 10h L: D conditions in a BOD incubator. Initially, a cohort of 30 to 50 eggs laid on 5cm x 5cm excised Cassava leaf discs were observed periodically for hatching. Soon after hatching, using a fine camel hair brush, the larvae were individually transferred to 50 different 1.5 cm x 1.5 cm fresh leaf discs kept on wet foam in 9" ×6" polyethylene trays (Excised leaf disc technique). Further the larvae were observed every 3 to 6 hours under a stereo binocular microscope and the data in respect of development from larva to adult including the quiescent stages (larvochrysalis, nymphochrysalis and teliochrysalis) were recorded. Period of development was computed for each stage of development, such as larva, quiescent 1 (larvochrysalis), protonymph, quiescent 2 (nymphochrysalis), deutonymph and quiescent 3 (teliochrysalis) including compiled data for the total development from egg hatching to adult emergence. The sex of the emerging adult was also recorded to compute the developmental time of male and female mite, separately.

Reproduction: Female teliochrysalis stages selected from the nucleus mite culture maintained in the laboratory were individually transferred onto 50 (1.5 cm × 1.5 cm) fresh leaf discs. Subsequent to the emergence of female adult from the teliochrysalis stage, two male adults were released onto each leaf disc to ensure mating. Further,

Table 1. Reproduction and demographic parameters of *Oligonychus biharensis* on Cassavaat different constant temperatures in the laboratory

Reproduction attributes	24°C; 75-80% (*n=33*)	28°C; 70-75% (*n=30*)	32°C; 75-80% (*n=30*)
Pre-oviposition period (days)	1.75 ± 0.15[b]	2.36 ± 0.25[b]	0.50 ± 0.15[a]
Oviposition period (days)	13.66 ± 0.58[c]	10.20 ± 0.60[b]	8.46 ± 0.26[a]
Post-oviposition period (days)	0.30 ± 0.11[a]	0.23 ± 0.09[a]	0.13 ± 0.63[a]
Longevity of mated females (days)	15.72 ± 0.56[c]	12.80 ± 0.63[b]	9.10 ± 0.25[a]
Mean no. of eggs/ female	79.42 ± 4.88[b]	70.50 ± 5.11[b]	52.03 ±3.76[a]
Mean no. of female offsprings/female	70.96	60.03	43.73
Mean no. of male offsprings/female	6.18	9.13	8.00
Sex ratio of progeny (♀: ♂)	11.48:1	6.57:1	5.46:1
Demographic parameters			
Mean Generation Time (days)	20.15 ± 0.16[c]	17.02 ± 0.13[b]	12.62 ± 0.12[a]
Doubling Time (DT)	3.33 ± 0.03[c]	2.92 ± 0.02[b]	2.37 ± 0.02[a]
Net Reproduction Rate (No. of female offsprings/ female/generation)	69.96 ± 0.40[c]	59.78 ± 0.31[b]	43.38 ± 0.27[a]
Gross Reproduction Rate (GRR)	93.45 ± 0.30[c]	89.67 ± 0.31[b]	67.60 ± 0.30[a]
Finite Rate of Increase (No. of female offsprings/female/day)	1.2563 ± 0.003[a]	1.2953 ± 0.002[b]	1.3961 ± 0.005[c]
Intrinsic Rate of Natural Increase (No. of female off-springs/female/day)	0.2259 ± 0.002[a]	0.2562 ± 0.002[b]	0.3273 ± 0.004[c]

n: number of mites observed; Mean values* (±*SE obtained by boot strapping method*) *with same alphabetical superscript within the row are not significantly different as per Tukey's HSD test (p<0.05)

observations were made at 24 hours interval to record the pre-oviposition period, eggs laid every day, oviposition period, post-oviposition period *etc.* Observations were recorded from the first day of egg laying until the female completed laying eggs and died naturally. As the life span of male mite was short, it was replaced with fresh male, as and when found dead on the leaf discs. Ovipositing females were carefully transferred to fresh leaf discs everyday and the eggs laid in the previous leaf disc were observed till they developed into adults, simultaneously recording the sex of the emerging adult then. Mite's reproduction attributes *viz.,* pre-oviposition, oviposition, post-oviposition, fecundity and sex ratio (♂: ♀), as proportion of male and female off-springs were recorded across different temperature conditions and compared to know the influence of temperature on the reproduction attributes.

***Population performance or demography*:** Temperature -wise age specific life table of *O. biharensis* was constructed separately. Demographic/Life table parameters such as, Mean Generation Time (T), Net Reproduction Rate (Ro), Gross Reproduction Rate (GRR), Finite Rate of Increase (λ), Intrinsic Rate of Natural Increase (r_m) and Doubling Time (DT) were computed following the procedure suggested by Birch (1948) and Atwal and Bains (1974) as below and the data were analysed (Chidananda, 2016; Pooja, 2018).

Net Reproduction Rate (R_o)= $\Sigma\, l_x m_x$

Mean Generation Time (T)= $\frac{\Sigma\, x^{l}xmx}{Ro}$

Finite Rate of Increase in number (λ)= $\text{anti ln}\left[\frac{\log eRo}{T}\right]$

Intrinsic Rate of Natural Increase$_{(rm)}$=1n(7)

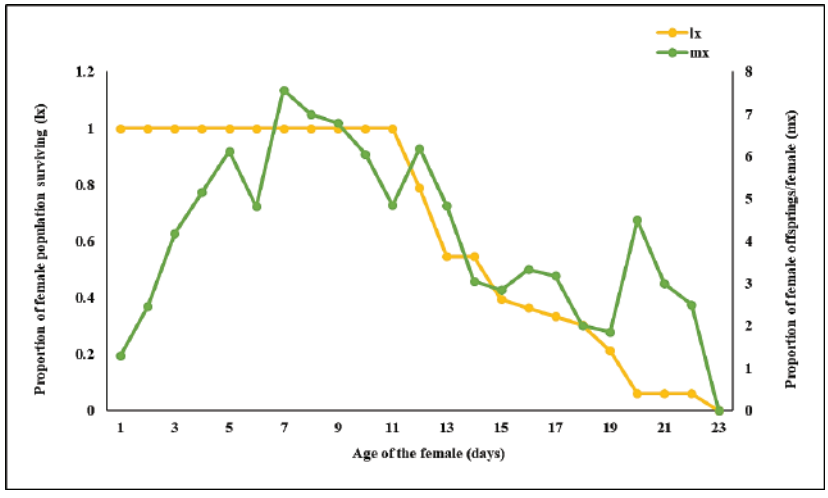
Doubling time, DT = $\frac{1n\ 2}{rm}$

where,

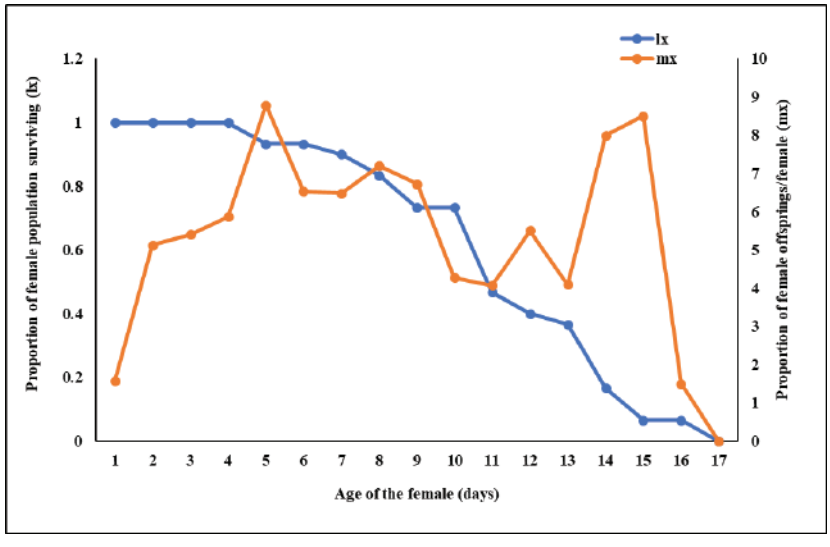
l_x = proportion of females alive at age interval x

m_x = number of female off-springs produced by the surviving female at the age interval x

(a)



(b)



(c)

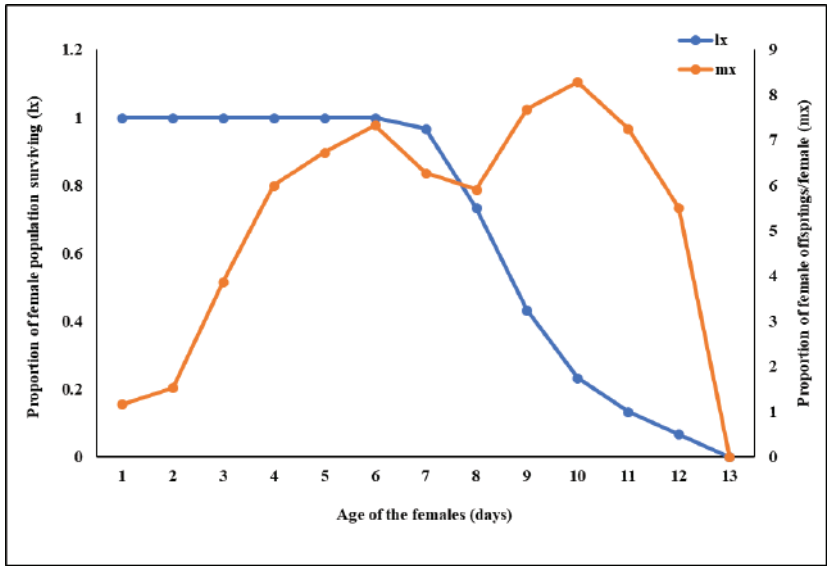


Fig 2. Age specific survival and fecundity of *Oligonychus biharensis* on Cassava at different temperature and humidity conditions: (a) 24°C; 75-80% RH, (b) 28°C; 70-75% RH and (c) 32°C; 75-80% RH

Statistical analysis and interpretation of data

Data in respect of development and different reproduction parameters were expressed as mean ± SE and the mean data were analysed using One-way ANOVA followed by Tukey's HSD test (P=<0.05) using statistical software SPSS 23 to compare the mean values across different temperature conditions. Demographic parameters were computed using the said formulae and expressed as mean±SE (determined by bootstrapping method). The data were subjected to one way ANOVA to compare them across different constant temperature conditions.

RESULTS AND DISCUSSION

As the rearing temperature conditions increased from 24º to 32ºC at the incremental rate of 4ºC, the duration of development at each stage of both the sexes recorded a gradual decrease (fig. 1). Total development of both female and male also decreased reasonably (11.11 to 6.22 days for female & 10.03 to 5.92 days for male). Mean developmental duration of *O. biharensis* (female + male) was lowest, 6.07 days at 32ºC on Cassava. Correspondingly, higher developmental duration was 10.57 days at 24ºC.

It is evident that at 24±1^{O}C and 28±1^{O}C constant temperature conditions the development of both male and female of *O. biharensis* mite was longer whereas, at 32±1^{O}C the development of both male and female was shorter.The developmental biology of *O. biharensis* was studied by Kaimal and Ramani (2011) on Cassava and the duration was 10.1 days at 30 ^{O}C±2°C & 70±5% RH conditions, which is more than 6.07 days at 32^{O}C and near similar of 9.71 days at 28°C recorded in the present study. However, Roknuzzaman *et al.* (2021), who studied *O. biharensis* biology at 25±1^{O}C on country bean (*Lablab purpureus* L.) recorded 10.9 for female & 11.00 days for male, which is similar to development period on Cassava 11.11days for female & 10.03 days for male in our study. Their studies on mung bean (*Vigna radiata* (L. Wilczek)) recorded 12.2 & 12.4 days for female and male, respectively, which is more than the present study data on Cassava host. This variation in the developmental time of *O. biharensis* may be attributed to the difference of host plants used for mite rearing.

Data in respect of longevity of mated females of *O. biharensis* at constant temperatures (24ºC, 28ºC and 32ºC) on Cassava revealed that the females survived for 15.72, 12.80 and 9.10 days, respectively. The mated female laid an average of 79.42, 70.50 and 52.03 eggs over a period of 13.66, 10.20 and 8.46 days (fig. 2). The fecundity of *O. biharensis* female on Cassava, the fecundity was significantly high at 24ºC *i.e*., 79.42 eggs laid over a period of 13.66 days (Table 1).

Kaimal and Ramani (2011) who studied reproduction features of *O. biharensis* on Cassava leaf stated that the mites' pre-oviposition period lasted for one day, oviposition period for 10.9 days and post-oviposition period for 0.8 days, more than the present study data at 32ºC with 0.50 days, 8.46 days and 0.13 days, respectively. However, average fecundity recorded by them, 37.6 eggs was less than in the present study with 52.03 eggs. The sex ratio in the present study was 1: 5.46 which was less than the result of Kaimal and Ramani *i.e.,* 1-2: 10. Kaimal (2013) studied the reproduction of *O. biharensis* mite on cowpea and reported that pre-oviposition, oviposition and post-oviposition periods were 1.5 days, 11.5 days, and 0.9 days, respectively, which is almost similar to the present study data recorded on Cassava at 24ºC. The ovipositional rate was 50.9 eggs for mated females which is less than the present study, on Cassava 79.42 eggs. The male-to-female ratio in that study was 1-2: 10 comparable to the ratio of 1: 11.48 on Cassava in the present study.Chen *et al*. (2005) studied the reproduction of *O. biharensis* on four different cultivars of litchi and reported that the pre-oviposition period ranged from 2.42 to 3.15 days, which is similar to our study at 28ºC. The oviposition varied from 68.80 eggs on Baitangying to 34.00 eggs on Sanyuehong variety, comparable with 70.50 eggs on Cassava in the study. However, the sex ratio which ranged from 1: 2.74 to 1: 5.83, which is less than our present study.

Net reproductive rate (R_0), intrinsic rate of natural increase (r_m), mean generation time (T), finite rate of increase (λ) and gross reproduction rate (GRR) values of *O. biharensis* were significantly affected by the host plant in the laboratory. The chief demographic parameters were, r_m value of 0.2259, 0.2562 & 0.3273, Net Reproduction Rate of 69.96, 59.78 & 43.38 and Mean Generation Time of 20.15, 17.02 & 12.62 days on Cassava(Table 1). Statistically, there was significant difference in r_m value across three different temperatures, and the value was highest at 32ºC *i.e*., 0.3273.It is evident that the mean generation time decreased as the rearing temperature increased from 24°C to 32°C and it was lowest, of 12.62 days at 32°C. The doubling time was significantly lowest, 2.37 days at 32°C. GRR ranged from 67.60 to 93.45. The higher $R_{0,}$ 69.96 females/ female/ generation was observed. According to Chen *et al.*, (2005) R_0 and r_m values ranged from 79.3–473.5 females/female/generation and 0.1349–0.2143 female off-springs/female/day, respectively on different cultivars of litchi at 24°C. R_0 and r_m values of *O. biharensis* on Lab lab was 12.30 and 0.1551and on mungbean, it was 8.19

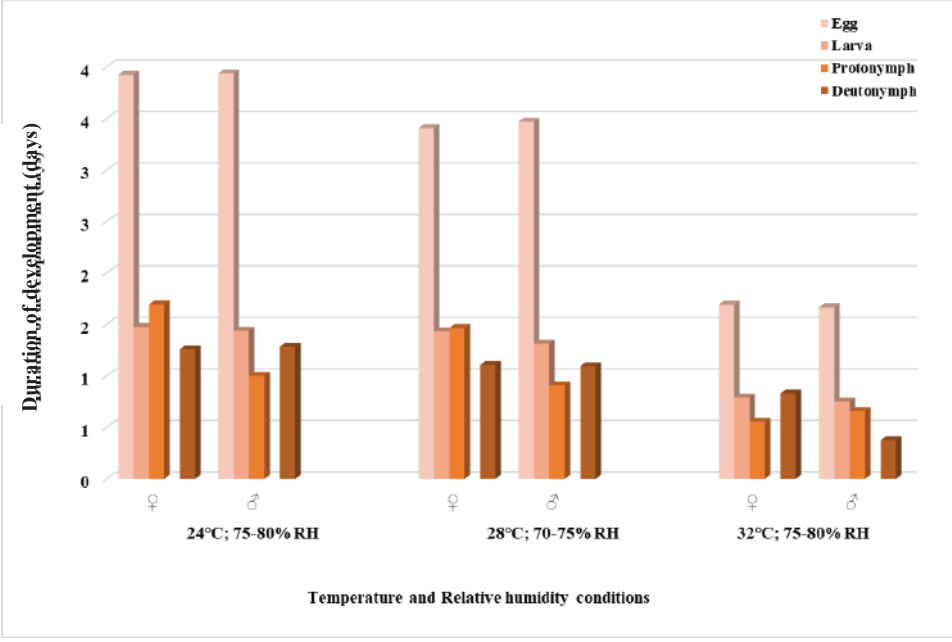


Fig. 1. Development of *Oligonychus biharensis* on Cassava at different constant temperatures in the laboratory

and 0.1254, respectively according to Roknuzzaman *et al.,* (2021).Thus, it is evident that *O.biharensis* showed varying R_0 and r_m values on different host plants like Cassava and litchi.*O. gossypii* had highest r_m value of 0.214 on Cassava was at 31°C (Bonato *et al.*, 1995),while *O. biharensis* had highest r_m value of 0.3069 on loquat leaves was at 35°C (Ji *et al.*, 2008), which indicated the positive influence of temperature of more than 30°C on the reproduction potential of *Oligonychus* mites. As noticed in the present study demography of *O. biharensis* was better for Cassava suggesting it as a more suitable host plant for population performance of the mite, and this mite would be a more potential emerging pest of Cassava.

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New host record of *Thrips parvispinus* (Karny) (Thysanoptera: Thripidae) in India

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ABSTRACT: The invasion and infestation of *Thrips parvispinus* (Karny)(Thysanoptera: Thripidae) on Guava (*Psidium guajava* L.) (Myrtales: Myrtaceae) is reported for the first time in India. Taxonomical and molecular identification of *T. parvispinus*, its nature of damage, symptoms, severity and its impact on guava cultivation and its export is discussed. Larvae and adult were found feeding on flowers. The feeding damage manifested on fruits however, mature fruits were found free of live infestation at the harvesting stage.

Keywords: *Thrips parvispinus,* guava, *Psidium guajava,* India*,* impact, invasive species

INTRODUCTION

Guava, *Psidium guajava* L., is native to Mexico and grows in all the tropical and subtropical areas of the world (Stone, 1970) and is grown commercially in Brazil, China, Columbia, Cuba, Egypt, Hawaii, India, Indonesia, Mexico, New Zealand, Philippines, South Africa, Thailand, United States of America, Venezuela, Vietnam, West Indies and Yemen (Wilson, 1980; Yadava, 1996; Le *et al.*, 1998; Tate, 2000). It is popular due to its all-season availability, rich nutritional and medicinal value, affordable price, suitability for transportation and handling (Nimisha *et al*., 2013). In India, Guava is being infested by many insect pests including two species of thrips *viz.*, *Selenothrips rubrocinctus* (Giard) and *Rhipiphorothrips cruentatus* Hood (Butani, 1979).

Thrips parvispinus commonly known as Tobacco thrips/South-East Asian thrips has gained cosmic importance in recent times due to the severe damage caused in chilli and other crops. Being native to the tropical regions of Asia, its presence had also been documented in Australia, China, France, Greece, Hawaii, India, Indonesia, Malaysia, Mauritius, Netherlands, Philippines, Reunion, Singapore, Solomon Islands, Spain, Taiwan, Tanzania and Thailand (Mound and Collins, 2000; Mound *et al.*,2016). It was first reported by Tyagi *et al.* (2015) in India followed by, Rachana *et al.*(2018), Roselin *et al.* (2021) and Verghese (2021) in papaya, *Dalhia rosea*, *Brugmansia* sp. and chilli respectively. It had also been found on cotton, bitter gourd, chrysanthemum, watermelon, mango, tamarind and marigold (Nagaraj *et al.*, 2021; Rachana *et al.* 2022).

Thrips parvispinus has become a major threat to chilli growing regions in India, due to which chilli growing farmers have incurred a great economic loss during 2021-22. Following that a lot of studies were taken up to find the host range of *T. parvipsinus*, their feeding behaviour, nature of damage and damage symptoms and its management practices. Although *T. parvispinus* is polyphagous by its nature, it has never been reported from Guava in India. The purpose of this article is to document *T. parvipsinus* as a pest of guava in India, and to illustrate the important diagnostic characters of the species collected on that host.

MATERIALS AND METHODS

During regular survey by Regional Central Integrated Pest Management Centre (RCIPMC), Bengaluru during 2020-21 and 2021-2022, infestation by *T. parvispinus* was observed in a five-year-old guava orchard (Variety: Taiwan Pink) at Avalahalli village (15°25'54.75"N, 76°31'53.33"E) in Gangavathi taluk of Koppal district, Karnataka, India. *T. parvispinus* population was determined by documenting the number of thrips/flowers during morning hours using standard beating method and the dislodged thrips were transferred to Eppendorf tubes containing AGA medium (9 parts of 10% ethyl alcohol; 1 part of glacial acetic acid; 1 ml of Triton X-100 in 1,000 ml of the mixture). It was observed that most of the thrips were resting in flowers and population ranged from 4-12 thrips/flower and the presence of thrips was recorded in 46.80%of the flowers sampled (Table 1).

The adult thrips specimens were removed from the preservative medium, kept in 2% NaOH for 30 min,

Table 1. Incidence of *T. parvispinus* in guava flowers and on mature fruits

Tree #	Flowers			Mature fruits*		
	# flowers	# thrips	Per cent incidence	No. of fruits	No. damaged	Per cent damage
1	2	2	100	4	0	0
2	3	1	33.33	2	0	0
3	1	0	0	4	2	50
4	2	0	0	7	2	28.57
5	3	2	66.67	2	0	0
6	3	3	0	0	0	0
7	0	0	0	4	1	25
8	3	3	100	0	0	0
9	3	1	33.33	0	0	0
10	6	0	0	0	0	0
11	3	0	0	0	0	0
12	0	0	0	2	0	0
13	3	1	33.33	0	0	0
14	3	2	66.67	0	0	0
15	3	1	33.33	1	0	0
16	4	4	100	2	0	0
17	2	2	100	3	1	33.33
18	0	0	0	2	1	50
19	1	0	0	0	0	0
20	2	0	0	2	1	50
	47	**22**	**46.80**	**35**	**8**	**22.85**

***No live thrips were found**

transferred to 60% ethyl alcohol and left for a day after which the specimens were dehydrated through a series of 70–100% ethyl alcohol washes. The specimens were cleared in clove oil for 5–10 min before mounting individually on microscope slides in Canada balsam. Finally, the slides were dried at 45°C for 30 min in an oven. Microscope slide-mounted adults were observed under a Nikon Eclipse 80i microscope (4× and 10×) and photomicrographs of habitus, antennae, head, prothorax, pterothorax, forewing, and abdomen of the species were acquired using a Nikon DS-Vi1 camera mounted on this microscope. The plate was formed using Adobe Photoshop CS2 software. Measurements (µm) of the important diagnostic characters were taken for female specimen using an ocular micrometer installed in an Olympus BX51 research microscope.

RESULTS

The species was morphologically identified as *Thrips parvispinus* (Karny) (Thysanoptera: Thripidae) using appropriate keys (Mound and Azidah, 2009). The specimens were deposited in the National Insect Museum at the Indian Council of Agricultural Research – National Bureau of Agricultural Insect Resources in Bengaluru, India.

Females of *T. parvispinus* are brownish (1175µm) (Fig. 1 A); legs yellow; forewing brown with pale base (650µm) (Fig. 1 I). Ocellar setae pair III small and positioned on anterior margins of ocellar triangle (10µm) (Fig. 1 F). Antennae 7 segmented (Fig. 1 B). Metanotum with median reticulations (Fig. 1 C); median setae long and placed behind anterior margin; without campaniform sensilla. Forewing first and second veins with complete setae rows. Abdominal tergite VIII without posteromarginal comb, a few microtrichia laterally present (Fig. 1 G); pleurotergites without discal setae. Abdominal sternite II with two marginal setae pairs, III–VII with three pairs, VII with median setae pair arising in front of posterior margin; II and VII without discal setae, III–VI with about 6–12 discal setae arranged in an irregular row (Fig. 1 D).

Molecular identification of *T. parvispinus*

Genomic DNA was isolated from an individual thrips specimen by using QIAGEN DNeasy® blood and tissue kit, Germany, following the manufacturer's

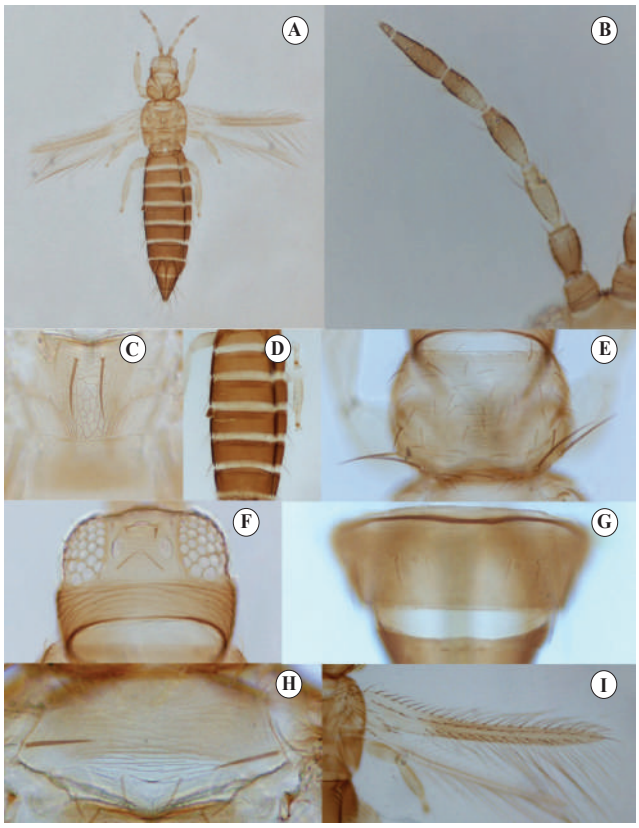

Fig 1. *Thrips parvispinus*. A, Female; B, Antenna; C, Metanotum; D, Discal setae on abdominal sternites III-VI; E, Pronotum; F, Head, dorsal; G, Abdominal tergite VIII; H, Mesonotum; I, Forewing.

protocols. The remaining specimens were kept as voucher specimens at −80 °C in absolute ethanol. The DNA was then subjected to PCR amplification using an iGENE Labserve gradient thermal cycler. The PCR reaction of 50 µL consisted of 5 µL of 10X Genei™ Taq Buffer B (Tris without $MgCl_2$), 8 µL of 10 mM Genei™ dNTP mix, 5 µL of 25 mM Genei™ $MgCl_2$, 1 µL (20 pmol/µL) each of the universal COI primer pair- 5′-ATTCAACCAATCATAAAGATATTGG-3′ and TTCTGGATGTCCAAAAAATCA-3′ (Hebert *et al.*, 2004), 1 µL of Genei™ Taq DNA polymerase (1 U/µL), 4 µL of DNA template (50 ng/µL), and 25 µL of water (Protease, DNase, RNase Free). The conditions for PCR for the study was an initial denaturation of 95 °C for 5 min, which was followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 46°C for 30 seconds, extension at 72 °C for 1 minute. The amplified products were analyzed on 1.5% agarose gel electrophoresis. The PCR products were then bi-directionally sequenced and checked for homology, insertions and deletions, stop codons, and frame shifts by using NCBI-BLAST and ORF finder. The sequence was then deposited in NCBI GenBank and Barcode of Life Database.

Molecular analysis of the thrips specimens collected from Guava corroborated the morphological identification. The sequence showed 100% similarity to *T. parvispinus* (OM453924.1) through BLAST sequence analysis. The sequence was submitted to NCBI and the accession number was retrieved (GenBank Acc. No. ON303614) and DNA barcode was obtained from BOLD systems (BIN No. AAM8085).

Further surveys were conducted to assess the *T. parvispinus* population and intensity of damage on twenty randomly selected five-year-old guava trees in a two-acre farm where the damage was reported.The infestation due to thrips was more during December to March. Larvae and adult thrips were found in groups feeding on the petals of unopened flower buds. They were also seen in small numbers on the calyx region. In opened flower, both larvae and adults were found among anthers and the damage symptoms were documented in early fruit setting stage and medium to large sized fruits and the peculiar symptom caused by thrips i.e. scraping / lacerating the young tissues was found in the early fruit setting stage (10 days old) (Fig. 2), Scarring and/or marking around the calyx of young fruits was also observed. The initial lacerations on developing fruits manifested as brownish corky irregular patches on developed fruit. However, neither larvae nor adult thrips were found associated with the matured fruits (Fig. 2), The extent of damaged fruits in the present study was to the tune of 22.85% (Table 1).

DISCUSSION

Although *T. parvispinus* has already been documented as a pest in *Carica papaya* (Tyagi *et al.,* 2015), *D. rosea* (Rachana *et al.*2018), *Brugmansia* sp. (Roselin *et al.* 2021) and chilli (Verghese 2021) in India it has never been documented as a pest in guava which is an important fruit crop in India. Guava is cultivated covering an area of 2,90,000 ha with a productivity of 4359 MT during 2019-20 (dacnet.nic.in) and have gained an export value amounting 53.26 crores with 1269.75 MT of fresh and dried guavas exported to various countries (agricoop.nic.in), thus *T. parvispinus* as a pest in Guava acquires significance. *T. parvispinus* was found causing scarring symptoms in the fruit whereas lacerating symptom and drying of early fruit forming stage was observed during the study which is distinctive damage symptom caused by Thysanoptera. It is concluded that, the thrips population was seen on most of the flowers and are able to infect the fruits, the damage done by thrips to guava fruit is minimal and the reason behind this may be due to the spraying of pesticides and botanicals by the farmer at regular intervals and also

Fig 2. Thrips infestation and damage symptoms on unopened flowers, calyx and fruits of guava

the inability of the thrips to survive as they are unable to inflict serious damage. Further *T. parvispinus* might have infected the guava plant from the adjacent chilli growing fields, which was seriously affected by the same thrips species during September-October months. The thrips species which completely destroyed chilli crop during 2021 was never found to be a threat in Guava fruit as the damage incurred by them are manageable. As *T. parvispinus* has already been documented as a serious invasive pest in chilli, extensive studies have to be done to document its possible alternate hosts, its damage severity on alternate hosts and predominantly its impact on the economic yield of alternate hosts. Further, campaigns should be organized by the extension workers/scientists/NGOs to educate the farmers about the alarming thrips species, its management strategies and an organized programme to contain its spread and development is timely needed.

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Assessment of yield loss in Vanilla, *Vanilla Planifolia* Andrews (Orchidaceae) caused by the Giant African Snail, *Lissachatina fulica* (Bowdich, 1822)

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ABSTRACT: The impact of Giant African Snail, *Lissachatina* (*Achatina*) *fulica* (Bowdich, 1822) damage on quantitative and qualitative loss of Vanilla (*Vanilla planifolia* Andrews) beans produced in different cropping systems was assessed. Snails were active during night, crawled on the vanilla vines and fed on the column (united stamen and pistil) of pollinated flowers. During day time, snails hid under leaf mulch and in the irrigation channels. Feeding damage to the floral parts resulted in production of inferior quality beans with lower market marketable properties. An economic loss of Rs. 765 to Rs.1020 was recorded per inflorescence. However, the density of snails on plant surface did not indicate the corresponding level of pest damage.

Keywords: Vanilla, beans, column, *Lissachatina fulica,* damage

INTRODUCTION

Vanilla, the most widely used food flavourant is derived from beans (fruits) of the orchid, *Vanilla planifolia* Andrews (Orchidaceae), a native of South-eastern Mexico and the world's second most valuable spice crop next to saffron (Anonymous, 2020). It is used extensively to flavour ice-cream, chocolates, beverages, cakes, custards, pudding and other confectionery, also used in perfumery and to a small extent in pharmaceuticals. Traditionally, vanilla flavourings are extracted from the matured beans of *V. planifolia.* The beans are harvested before they fully ripe. Subsequently, they are fermented and cured (Purse glove, *et al.*, 1988). 'Vanillin' accounts for about 2% of the dry weight of cured vanilla beans and is the chief among about 200 other flavour compounds found in beans responsible for unique vanilla flavour. The flavour of vanilla beans is far superior to that of synthetic vanillin due to the presence of other flavour compounds in the natural product (Sudharshan *et al.*, 2006). The yield of the vanilla flavour from the processed beans is highly influenced by the length of the beans. Longer the beans, higher the price. Primarily the length of beans is an attribute of successful pollination and fertilization of vanilla beans. The main reason for shortness of beans is detachment of 'column' (united stamen and pistil) from the developing beans. *V. planifolia* has been successfully introduced as an inter-crop in arecanut (*Areca catechu* L.), coffee (*Coffea* spp.), black pepper (*Piper nigrum* L.) and cardamom (*Elettaria cardamomum* Maton) plantations in Western hilly areas of Southern India (Sudharshan and John, 2003).

Vanilla is known to plague by many annoying insect pests in India (Prakash *et al.,* 2002; Varadarasan *et al.,* 2002b). Infestation of molluscans from different parts of the world also has been reported on vanilla. Chiders and Cibes (1948) have reported the snail, *Thelidomus lima* Fer., and the slug *Veronicella kraussii* Ferussac damaging vanilla by removing outer portion or entire sections of buds, leaves, shoots and entire beans in Puerto Rico. In Southern India, incidence of *A. fulica* on *V. planifolia* was reported by Varadarasan *et al.,* (2002a).

The Giant African Snail, has been considered the most widely introduced and invasive land snail species in the world and a major agricultural and garden pest (Mead, 1979; Karnatak *et al.,* 1998; Raut and Barker, 2002; Sridhar *et al.,* 2013). In our pest surveillance studies in Karnataka, we observed that the Giant African Snail, *Lissachatina fulica* (Bowdich) was one of the factors behind shorter sized vanilla beans. Earlier reports have considered the direct damage caused by *L. fulica* on vanilla by feeding on the vegetative parts *viz.*, young leaves, tender shoots and younger plants (Varadarasan *et al.*, 2002a). Hitherto, no attempt has been made to estimate the crop loss caused by the snails obstructing the successful fertilization of the beans.

Hence, a study was undertaken to quantify the economic loss caused by the snail, *L. fulica.* In this paper the abundance of snails in different cropping systems associated with vanilla, nature of damage by the snails and the extent of economic loss incurred followed by snails has been documented.

MATERIALS AND METHODS

Study sites

Five fields were selected in two major vanilla growing districts of Karnataka in the hilly zone. Climate of Hilly zone is humid and tropical and receives an annual rainfall ranging from 719 to 5225 mm. Study was conducted during the flowering period from January-April, 2019. The locations and site characteristics are given in Table 1. Of five locations, vanilla was cultivated as an intercrop in three locations. Vines were grown under the natural shade of support trees. In areca based cropping system, vanilla vines were permitted to grow up to 1.2-1.5m on the support trees *viz*., *Erythrina indica* and *Gliricidia sepium,* even on areca itself allowed to hang down from the same. Coffee, Gliricidia and Erythrina trees were allowed to form branches to different directions to have an umbrella shaped appearance about 1.5-2.0m above the ground. At initial stages vanilla vines get attached to these support trees and are allowed to grow later. Support trees were pruned to regulate appropriate shade. In Kesuve, vanilla was cultivated as sole crop under the shade and support of Gliricidia, spacing of 2m between rows and 1.5m within a row was maintained. In Harekoppa, instead of live support trees hardwood posts and nylon ropes were used. Posts were put at 1.2 to 1.5m above the ground, ropes tied to posts and the vines being brought over them and looped up as they grow long. This artificial structure was housed under the shade of perforated agro-shade net. At all the locations mean age of the vines was 3.16±0.76 years and approximately 3000 vines occupied one hectare. Well decomposed organic matter and mulch in the form of dry leaves were placed to a thickness of 10-15cm at the base of the vines. Irrigation was provided using sprinklers. At all the sites, vanilla blooming commenced during January and February, reached its peak during March and ended during April. A trained worker carried out the hand pollination of vanilla flowers throughout the season at the respective locations. Observations were recorded twice in March at second and fourth week after flowering.

Assessment of abundance of snails

The mechanical wooden frames measuring 0.5 m^2 area were prepared and placed between plants/ structures supporting vanilla vines at 17:00 h. One such frame was placed at the centre between four palms/ vines approximately 4.5 m away from each plant base. Abundance of snails was randomly assessed from 25 marked spots in the reported plantations. At 22:00 h the number of snails in such wooden frames (0.5 m^2 areas) was counted and recorded as abundance of snail/0.5 m^2 area. Snail population was not recorded from the plant base, on the roads, near water source, near fence and near compost pits/heaps.

Assessment of abundance of snails on plant surface

At 23:00 h the number of snails that were present on 25 randomly selected vanilla vines was counted and recorded as *abundance of snail/vine.* Among 25, ten vines harboured snails were marked using coloured ribbons for assessing crop loss study. Snails of all sizes were considered for assessing abundance both on ground and plant surface.

Estimation of quantitative loss caused by snails

Assessment of crop loss due to snail damage

Ten racemes (each from a single vine) were randomly selected from the previously marked vines at 23.00h. The total number of beans (TB) and number of beans devoid of column (L1) were counted and recorded at 23:00 h. On the next day before commencement of pollination operation at 08:00 h, all the 10 previously marked racemes were re-examined and total number of beans devoid of column (L2) was counted and recorded. The number of beans devoid of column (L3) following snail infestation was estimated by calculating L3=L2-L1. The economic loss incurred was worked out for each location as Loss of column of one bean = loss of one good grade bean.

Table 1. Locations and site characteristics

District	Location	Latitude	Longitude	Altitude (m)	Main crop	Support tree/structure
Chikkamagalur	Kesuve	13.576438°	75.388038°	734	Vanilla	*Gliricidia sepium*
	Golgar	13.587221°	75.374865°	764	Area & coffee	Areca & coffee
Shivamogga	Araga	13.689496°	75.244989°	631	Areca	Areca
	Devangi	13.629998°	75.283904°	647	Areca & Banana	*Erythrina indica*, *G. sepium*
	Harekoppa	14.156828°	75.092699°	508	Vanilla	Hardwood posts

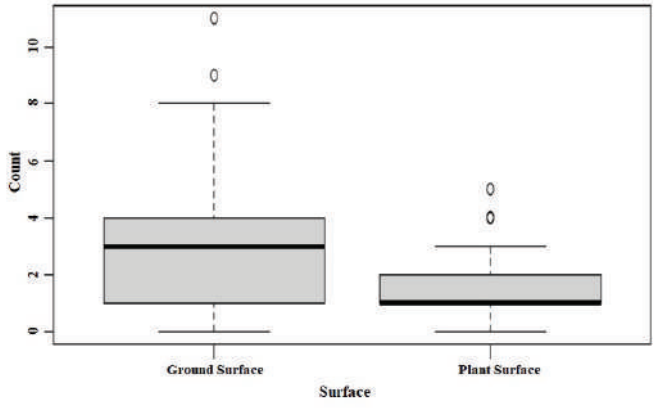

Fig 1. Distribution of snails on ground and plant surfaces

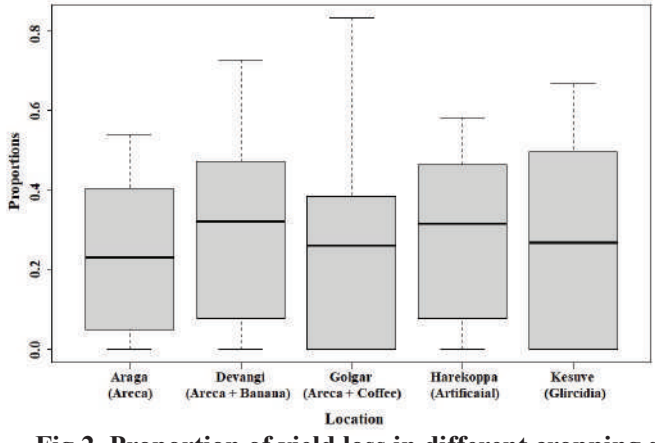

Fig 2. Proportion of yield loss in different cropping systems

Plate 1. a.Snail on vanilla inflorescence
b.Vanilla beans

Estimation of monetary loss

To estimate the monetary loss caused by the snails to vanilla, the price offered by the auctioneer for different grade of beans at auction centre was recorded from 2015 to 2018 (Anonymous, 2018). Further, the number beans that constitute one kg in different grade categories were also estimated.

Estimation of qualitative loss caused by snails

The normal (grade-I), medium quality (grade-II) and dwarf (grade-III) beans were subjected to curing and analysed for chief physical and chemical quality parameters.

Statistical analysis

The data analyses were performed using R software (Version: i386 4.0.3). The differences among different locations, weeks and surfaces were analyzed using three-way ANOVA. Mathematical pitfalls of using independent P-values of t-test to determine the utility of a multiple-variables hypothesis as a whole, *i.e* t-tests applied to three or more groups at the same time, inflates the Type I error rate, thus is considered as dishonest. Hence, Tukey's "Honestly Significant Difference" test was employed to have in-depth comparison (Hu *et al.,* 2021) between different combinations of location (cropping system), surface, and over weeks.

Table 2. Abundance of *Lissachatina fulica* in different cropping systems at study locations

Location	Abundance of snails					
	Snails on ground surface (No. of snails/0.5 m^2 area)			Snails on vine (No. of snails /vanilla vine)		
	II week	IV week	Mean	II week	IV week	Mean
Kesuve (Gliricidia)	2.36±1.46	1.80±1.22	2.08±1.34	1.32±1.08	1.28±0.97	1.30±1.02
Golgar (Areca+coffee)	2.48±1.58	2.30±1.65	2.40±1.61	1.08±0.95	0.80±0.91	0.94±0.93
Araga (Areca)	4.64±1.35	4.44±2.23	4.54±1.79	2.16±1.10	1.48±1.22	1.82±1.16
Devangi (Areca+Banana)	3.64±1.72	2.76±1.78	3.20±1.75	1.48±0.96	1.28±1.08	1.38±1.01
Harekoppa (Artificial)	1.36±1.41	0.92±1.03	1.14±1.22	1.32±0.98	1.64±1.11	1.48±1.04

3 Factor ANOVA (R^2: 0.75)

Week	Surface	Location	Week x Location	Location x Surface	Week x Surface	Location x Week x Surface
5.264*	117.5***	21.8178***	11.1**	38.622***	3.16*	43.7***

Table 3. Results of post-hoc multiple comparison test: Tukeys HSD on abundance of snail over combinations of different weeks/ surface/ cropping systems

Location (Cropping System)	Tukey (HSD)	p-Adjusted
Comparison of over different locations (Cropping System)		
Devangi (Areca+Banana) & Araga (Areca)	-0.89 **	0.00
Golgar (Areca+coffee) & Araga (Areca)	-1.49 **	0.00
Harekoppa (Artificial) & Araga (Areca)	-1.87 **	0.00
Kesuve (Gliricidia) & Araga (Areca)	-1.49 **	0.00
Golgar (Areca+coffee) & Devangi (Areca+Banana)	-0.60 *	0.05
Harekoppa (Artificial) & Devangi (Areca+Banana)	-0.98 **	0.00
Kesuve (Gliricidia) & Devangi (Areca+Banana)	-0.60 *	0.05
Comparison for given week		
Plant surface:W2 & Ground surface:W2	-1.44 **	0.00
Plant surface:W4 & Ground surface:W4	-1.15 **	0.00
Comparison over week & surface		
Plant surface:W4 & Ground surface:W2	-1.62 **	0.00
Ground surface:W4 & Plant surface:W2	0.98 **	0.00
Comparison over week & cropping systems		
W2:Golgar (Areca+coffee) & W2:Araga (Areca)	-1.58 **	0.00
W2:Golgar (Areca+coffee) & W4:Araga (Areca)	-1.14 **	0.01
W2:Harekoppa (Artificial) & W2:Araga (Areca)	-2.06 **	0.00
W2:Harekoppa (Artificial) & W2:Devangi (Areca+Banana)	-1.22 **	0.00

W2:Harekoppa (Artificial) & W4:Araga (Areca)	-1.62 **	0.00
W2:Kesuve (Gliricidia) & W2:Araga (Areca)	-1.56 **	0.00
W2:Kesuve (Gliricidia) & W4:Araga (Areca)	-1.12 **	0.01
W4:Devangi (Areca+Banana) & W2:Araga (Areca)	-1.38 **	0.00
W4:Golgar (Areca+coffee) & W2:Araga (Areca)	-1.84 **	0.00
W4:Golgar (Areca+coffee) & W2:Devangi (Areca+Banana)	-1.00 *	0.05
W4:Golgar (Areca+coffee) & W4:Araga (Areca)	-1.40 **	0.00
W4:Harekoppa (Artificial) & W2:Araga (Areca)	-2.12 **	0.00
W4:Harekoppa (Artificial) & W2:Devangi (Areca+Banana)	-1.28 **	0.00
W4:Harekoppa (Artificial) & W4:Araga (Areca)	-1.68 **	0.00
W4:Kesuve (Gliricidia) & W2:Araga (Areca)	-1.86 **	0.00
W4:Kesuve (Gliricidia) & W2:Devangi (Areca+Banana)	-1.02 **	0.04
W4:Kesuve (Gliricidia) & W4:Araga (Areca)	-1.42 **	0.00
Comparison over different surface and cropping systems		
GS: Devangi (Areca+Banana) & GS: Araga (Areca)	-1.34 **	0.00
GS: Devangi (Areca+Banana) & PS: Araga (Areca)	1.38 **	0.00
GS: Golgar (Areca+coffee) & GS: Araga (Areca)	-2.10 **	0.00
GS: Golgar (Areca+coffee) & PS: Devangi (Areca+Banana)	1.06 **	0.00
GS: Harekoppa (Artificial) & GS: Araga (Areca)	-3.40 **	0.00
GS: Harekoppa (Artificial) & GS: Devangi (Areca+Banana)	-2.06 **	0.00
GS: Harekoppa (Artificial) & GS: Golgar (Areca+coffee)	-1.30 **	0.00
GS: Kesuve (Gliricidia) & GS: Araga (Areca)	-2.46 **	0.00
GS: Kesuve (Gliricidia) & GS: Devangi (Areca+Banana)	-1.12 **	0.00
GS: Kesuve (Gliricidia) & GS: Harekoppa (Artificial)	0.94 *	0.02
GS: Kesuve (Gliricidia) & PS: Golgar (Areca+coffee)	1.14 **	0.00
PS: Araga (Areca) & GS: Araga (Areca)	-2.72 **	0.00
PS: Devangi (Areca+Banana) & GS: Araga (Areca)	-3.16 **	0.00
PS: Devangi (Areca+Banana) & GS: Devangi (Areca+Banana)	-1.82 **	0.00
PS: Golgar (Areca+coffee) & GS: Araga (Areca)	-3.60 **	0.00
PS: Golgar (Areca+coffee) & GS: Devangi (Areca+Banana)	-2.26 **	0.00
PS: Golgar (Areca+coffee) & GS: Golgar (Areca+coffee)	-1.50 **	0.00
PS: Golgar (Areca+coffee) & PS: Araga (Areca)	-0.88 *	0.04
PS: Harekoppa (Artificial) & GS: Araga (Areca)	-3.06 **	0.00
PS: Harekoppa (Artificial) & GS: Devangi (Areca+Banana)	-1.72 **	0.00
PS: Harekoppa (Artificial) & GS: Golgar (Areca+coffee)	-0.96 *	0.01
PS: Kesuve (Gliricidia) & GS: Araga (Areca)	-3.24 **	0.00
PS: Kesuve (Gliricidia) & GS: Devangi (Areca+Banana)	-1.90 **	0.00
PS: Kesuve (Gliricidia) & GS: Golgar (Areca+coffee)	-1.14 **	0.00

RESULTS AND DISCUSSION

Snail's activity commenced at dusk around 08:00 h at all the locations. During day time, snails hid below the mulch and cooler parts in the plantations. Usually, snails took shelter in cooler irrigation channels in the vanilla garden. Snails crawled on the ground surface. Later they marched towards vanilla/areca crops and started climbing the trees. The snails took the vines in 2 to 3 hours. Snail abundance varied across the cropping pattern. In the intercropping system snail abundance was as high as 4.54±1.79 where as sole crop garden recorded 2.08±1.34 snails. Snail abundance was the highest in the areca and area+banana cropping system (Table 2) compared to artificial system.

Although, average number of snails observed was 1 to 2 on plant surface and 2 to 4 on ground surface, the variability was very high between weeks, surface and over different locations. Post-hoc test *viz*., Tukeys HSD test employed in the study captured higher variability present and enhanced the ability to detect the statistical significance. Comparisons between different combinations of locations (cropping system), surface, and over weeks were further tested using Tukeys HSD. The combinations which have exhibited significant difference are enlisted in Table 3. Other combinations not mentioned in the paper exhibit no significance difference with respect to snail infestation.

Snail abundance on vanilla grown as an intercrop in areca (vines trailed on areca at Araga) was found highly significant followed by vines trailed on Areca+Banana (Devangi) cropping system. Other cropping systems *viz.,* vanilla grown as sole crop (vines trailed on Gliricidia), as an intercrop (vines trailed on Areca+coffee) and a sole crop (vines trailed on hardwood posts/ artificial) cropping systems have similar snail density. Banana plants are heavy water feeder which makes a suitable microclimate for the development of moist loving snails (Umashankar *et al.*, 2016). This substantiates that infestation on Areca+Banana was more compared to Areca+coffee cropping system. However, Vanilla trailed on artificial support system had the least snail infestation (Table 3).

Abundance of snails on ground was significantly high compared to plant surface for a given week (Table 3). The week *per se* had no significant influence on number of snails either on ground or plant. However, within 2nd and 4th week, presence of snails on ground contributed to overall significance.

Irrespective of surface, abundance of snail reduced over the second fortnight and the reduction is significant. Vanilla vines trailed on areca at Araga harboured higher number of snails compared to all other cropping systems. This is followed by vines trailed on Areca+ Banana (Devangi) cropping system over both weeks. Abundance on Areca+Coffee cropping system was significantly less compared to Areca+Banana. Intrestingly, artificial support system had the least number of snails over both weeks and the reduction is non-significant (Table 3).

Irrespective of week, pattern of snail abundance was entirely different on plant and ground surfaces. Abundance was significantly least on ground in artificial support system, while on plant surface in Areca+Coffee cropping system (Table 3). While the abundance remained significantly high on both surfaces in Areca and Areca+Banana cropping systems. Snail abundance on vines in artificial support system was on par with vines trailed on Gliricidia and Areca+Banana cropping system.

It was clear that both the abundance of snails and variability in number of snails over ground surface was higher compared to its abundance and variability on plant surface (Fig 1). It is obvious that pest variability should be less for successful control of pests. In actual sense, when abundance of snails was considered, the control measures should be targeted on snails prevailed on ground surface, as the variability in this count is high that forces us to not just rely on one control measure with respect to ground surface. As, the abundance of snails was sparse on plant surface hand-picking and destroying would be more economical while, integrated approach would be highly effective with respect to ground surface.

Assessment of quantitative crop loss due to snail damage

Snails caused damage to column in following ways: (1) They crawled on the distal end of the beans at which point flower is borne; the column drops off by the virtue of snail's body weight (2) Snails landed on the shoot/beans, dragged the column portion of the surrounding beans and later devoured the column along with dried sepals and petals. Damage was limited to newly pollinated beans. An economic loss of Rs. 765 to Rs.1020 was recorded per inflorescence at all the location. The population density on plant surface did not indicate the corresponding level of pest damage and the economic loss caused by snails at the study sites (Table 4).

Proportion of yield loss was calculated by taking snail damage (loss of column) to the total bean observed (L3/Total). 2 Factor ANOVA showed that no significance difference (R^2 =0.053) between weeks, locations and over both week and locations (Table 5). Figure 2 indicates that proportion of loss over different locations have similar

Table 4. Impact of snail damage on vanilla beans

Location	II week after pollination (n=10 inflorescence)	IV week after pollination (n=10 inflorescence)	Total Number of beans damaged by snails	
	Number of beans damaged by snails (L3)	Number of beans damaged by snails (L3)	Mean Number of beans damaged by snails (L3)	Economic loss (Rs.)
Kesuve (Gliricidia)	3.3±2.71	3.0±2.62	6.3±2.21	945±332.03
Golgar (Areca+coffee)	2.3±2.31	2.8±2.52	5.1±3.90	765±585.02
Araga (Areca)	3.4±2.41	2.9±2.64	6.3±3.40	945±515.02
Devangi (Areca+Banana)	3.1±2.37	3.1±2.68	6.2±2.09	930±314.64
Harekoppa (Artificial)	2.7±2.16	4.1±2.76	6.8±2.52	1020±379.47

* The Price of one glade-I bean was Rs.150.

mean and variability though seems to be high, it is not enough to exhibit significance in its contribution towards loss. Albeit, the abundance of snails was the least on ground in artificial trailing system, loss incurred was high in artificial trailing system.

Assessment of qualitative crop loss due to snail damage

At the time of harvest, the beans needed to make one kilogram was assessed for all three grades. On an average 75.51±5.5 fresh beans constituted one kilogram in grade-I. However, more than 150 beans were needed to make one kilogram in grade II (146.9±3.80) & grade-III beans (169.83±6.20). The chief physical and chemical quality parameters of the cured beans varied among three grades. Grade-I beans underwent normal curing process and become fleshy, supple, very dark brown to black in colour, somewhat oily in appearance, strongly aromatic and yielded good quality beans (vanillin: 2.13%; moisture: 26.70%). The grade-II beans became hard, dry, thin, brown or reddish brown and possess a poor aroma and yielded less vanillin (1.58%) and low moisture content (21.30%). The grade-III beans failed completing the curing process and become dry and shrivelled after slow drying. These beans were disposed to prevent the spoilage of other good grade beans in the storage. Although, the intensity of snail's damage was not conspicuous at field level, it caused appreciable loss in the marketable parameters of the vanilla beans due to snail feeding. Vanilla is sold in different forms: beans, extract and essence, powdered and vanilla sugar. The processed vanilla beans are sold as whole pods packed in small plastic/glass containers in specialty grocery stores and health centres, called 'Gourmet quality or for extraction'. Gourmet quality beans ought to be top quality beans that are long, fleshy, supple, very dark brown to black in colour, somewhat oily in appearance. Though there can be about 18-20 racemes in a plant and as much number of flowers, it was suggested to pollinate first 10 or 12 flowers and removing terminal flowers. Similarly, it is advised to maintain only 10-12 racemes per plant in order to get beans with maximum size and of high-quality standards. In this context any kind of loss (quantitative and qualitative loss) incurred to vanilla beans adversely affects the economic value of the end product.

The major reason for shortness of beans is improper fertilization. Impact of failure of pollination is highly conspicuous in the field as it is evident by fall off of the column (united stamen and pistil) from the flower within 24 hours after the pollination. Maximum growth rate (about 80 per cent length) of beans was observed in the first 45 days after pollination. Our study showed that the snails preferred columns of developing vanilla beans of less than 15 days old (Plate 2). This clearly indicated the role of snail's feeding damage and further yield loss. In recent years, rapid spread of *L. fulica* in the vanilla gardens is posing severe threat to the production of quality vanilla beans. Hence, prevention of snail's damage during vanilla flowering period about 150 days (January to May) helps in production of good grade beans. Maintenance of high humidity in the vanilla garden during flowering period is an essential agronomic practice which extends the viability of the flower opening and receptivity time of the stigma. Humidity has been found to be a more reliable predictor of naturalization and activity of *L. fulica* than temperature, and continuous activity is restricted largely to areas with 80% relative humidity (Raut and Barker, 2002). Hence, suppression of snail's population during flowering period in vanilla gardens offers a new challenge.

Table 5. Proportion of yield loss in different cropping systems

Location	W2	W4	Average
Devangi (Areca+Banana)	0.331	0.280	0.306
Golgar (Areca+coffee)	0.281	0.206	0.244
Kesuve (Gliricidia)	0.342	0.214	0.278
Araga (Areca)	0.296	0.194	0.245
Harekoppa (Artificial)	0.273	0.315	0.294
Average	0.305	0.242	0.273
Week	Location	Week * Location	R^2
1.97 ^{NS}	0.32 ^{NS}	0.43 ^{NS}	0.053

In this paper we have documented for the first time the crop loss caused by the *L. fulica* on vanilla by feeding on floral parts under different cropping systems. Though abundance of snail varied across different cropping systems, weeks and locations, the loss incurred is almost same in all. This shows that the density of snail is just a number with less economic significance. It implies that the density of snail (threshold levels) should not be considered while deciding on the plant protection measures. Even lesser numbers can lead to serious concerns. This study suggests a need for further investigation on the management of the snails in the vanilla gardens.

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Feeding and oviposition preference of anthocorid predator, *Blaptostethus pallescens* Poppius to different prey species

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ABSTRACT: Laboratory studies were conducted on the feeding and oviposition preference of anthocorid predator, *Blaptostethus pallescens* Poppius through behavioural bioassay to different prey species. The orientation and predation of *B. pallescens* was maximum on *Corcyra* eggs followed by spider mite, *Tetranychus urticae* Koch. The least preferred prey was whitefly, *Aleurodicus dispersus*. Among host plants tested, the nymphs and adults of *B. pallescens* preferred to orient and predate more preys on brinjal followed by bhendi. The least preferred host was cluster bean. Even though French bean pod was commonly used in mass culturing of *B. pallescens*, green pods of cluster bean were the most preferred substrate for oviposition. And also, the eggs laid on cluster bean pods showed the maximum hatching. The adults of *B. pallescens* provided with honey and water along with the normal diet of *Corcyra* eggs showed increased fecundity.

Keywords: *Blaptostethus pallescens,* biocontrol, feeding, oviposition, alternate host

INTRODUCTION

The use of indigenous natural enemies in biological control of crop pest is an alternative way to enhance the use of exotic natural enemies (Bonte and de Clercq, 2011). Indigenous natural enemies provide an advantage of their ability to readily exploit native or invasive pests as their prey and also persist on alternate prey when the target pests are rare or absent (Symondson *et al.*, 2002; Castane *et al.*, 2014). Anthocorid predators, commonly known as minute flower bugs or minute pirate bugs are recognized as a potential biocontrol agents of crop pests (Barber, 1936). They feed on small lepidopteran larvae, small grubs, psocids, mites, thrips, aphids and storage pests (Tawfik and El-Husseini, 1971). Natural population of anthocorid predators has been successful in maintaining pest populations at low levels (Muraleedharan and Ananthakrishnan, 1974). The majority of anthocorids are predaceous at nymphal and adult stages and few are phytophagous (Oku and Kobayashi, 1966). Anthocorids possess many of the characteristics of an ideal biocontrol agent, such as high searching efficiency and feeding rate, shorter duration of development, density dependent response to the pest population and synchronization of predator and prey population (Ballal *et al.*, 2009).

In countries like France, United Kingdom, Nether lands, Germany etc., several species of anthocorid predators are available commercially and are released in green houses and fields for the management of insect pests, especially sucking pests such as thrips and mites (Ballal *et al.*, 2003). In India, research has generally focused only on identifying indigenous anthocorids on different pests infesting different crops (Ballal and Yamada, 2016). Therefore, it is necessary to test the feeding and oviposition behaviour of anthocorids, before employing it as a general predator in any ecosystems. With this context, the present study has been taken up to identify the alternate natural ovipositional substrate, feeding choice of one such anthocorid predator *Blaptostethus pallescens* Poppius (Heteroptera: Anthocoridae) and also tested with different adult nutrition to enhance the fecundity.

MATERIALS AND METHODS

Mass culturing of predator *B. pallescens*

Mass culturing of *B. pallescens* was carried out as per the method developed by Ballal *et al.* (2003). UV irradiated *Corcyra cephalonica* eggs were sprinkled on cotton pad placed at the bottom of the transparent plastic container (500ml). Nymphs were released into the container along with bean pods which supply the required water for the nymphs. Fresh eggs of *Corcyra* were provided on alternate days till the adults emerge. Freshly emerged adults were shifted to plastic containers with green bean pods for oviposition. The pods with the eggs were removed daily and fresh pods were replaced as an oviposition substrate.

Mass culturing of red spider mite, *Tetranychus urticae* Koch

One month old host plants, brinjal raised in pots

was infested with *T. urticae* by releasing the mite stages using camel hair brush and by placing the infested leaves collected from the field. Further, the uninfested potted plants were placed along the infested potted plants for uniform infestation of mites. The newly uninfested potted plants maintained in the insectary were periodically replaced after the removal of dried plants. Likewise, the mass culturing of *T. urticae* is maintained in the Insectary.

Prey preference of fourth stage nymph of *B. pallescens*

Sucking pests like whitefly (*Aleurodicus dispersus*), mite (*T. urticae*), aphids (*Aphis gossypii*), mealybug (*Phenacoccus solenopsis*) and factitious host *Corcyra* eggs were the prey tested for the preference of fourth instar nymph of *B. pallescens*. Twenty life stages of different prey were kept in moist filter paper placed in iron trough of 28 cm dia. at equidistant and ten fourth stage nymphs of the predator pre starved for two hours were released in the centre. The experiment was carried out with five treatments and four replications. Two hours after the release, the congregation of predator nymphs in different prey arena was recorded. The number of preys fed by the nymphs was also recorded at six and 24 hours after release. The preference of the prey by the fourth instar nymph of *B. pallescens* was studied using six arm olfactometer also. Twenty insects of five different preys were placed in each arm and ten fourth instar nymphs were placed in the centre. The settlement of nymphs in individual prey species two hours after the release of predator was recorded. Besides, the observation on the number of preys fed six and 24 hours after the release of predator also recorded.

Prey preference of adult *B. pallescens*

The preference of different prey species by the adult of *B. pallescens* was studied in two separate experiments using the multiple-choice test in open circular basin and in closed six arm olfactometer as mentioned previously for the fourth instar nymph of the predator.

Influence of prey host plant on feeding of fourth instar nymph of *B. pallescens*

The volatiles of the plant housing the prey shall act as allomones or kairomones for the predators feeding on the prey. The effect of plant hosts housing *T. urticae* on the orientation and feeding of *B.pallescens* was assessed through laboratory experiment with four treatments (brinjal, bhendi, cluster bean and cucurbit) and five replications. Ten nymphs and adults of *T. urticae* were released on the host leaf arena with the camel hair brush. The leaf discs of different host plants *viz*., brinjal, bhendi, cluster bean and cucurbit infested with mites were kept at equidistant in circular zinc tray of 28cm diameter lined with moist filter paper. Later ten fourth instar nymphs of the predator were released in the centre. The preference of predator to mite as influenced by the host was assessed two hours after the release by recording the assemblage of nymphs on different host arena housing the mite. In addition, the number of preys fed by the nymphs was also recorded at six and 24 hrs after the release.

Similar study to assess the influence of host plant on prey feeding was also conducted using six arm olfactometer. The four host plant discs infested with twenty mites kept in the arm of olfactometer were released in the centre with ten fourth instar nymphs of the predator. The number of predator nymphs occupying the different host plants housing mites two hours after release and the number of mites fed by the predator six and 24 hours after release were recorded.

Influence of host plant on prey feeding of adult *B. pallescens*

The effect of plant hosts housing *T. urticae*on the orientation and feeding of *B. pallescens* was assessed through laboratory experiment with four treatments (brinjal, bhendi, cluster bean and cucurbit) and five replications. Ten nymphs and adults of *T. urticae* were released on the host leaf arena with the camel hair brush. After settling of mites, the predator adults (10) were released in circular test arena which contains all the host plant leaves kept at equidistant around the inner periphery of the tray. Observation on the orientation of the predator adults two hours after the release was noted. Further, the number of mites preyed by the adult at six and 24 hours after the release was also recorded. The laboratory experiments were conducted as per the methodology mentioned previously for the fourth instar nymph of the predator.

Preference of plant host for oviposition

Endophytic insect predators generally prefer a host to lay eggs in a substrate which provide the essential nourishment to the newly hatched young ones. In the case of *B. pallescens,* it is mass cultured in the laboratory with the help of green French bean pods as ovipositional substrate which has sufficient moisture retention to support the requirement of hatched out nymphs. In order to find an alternate and cost-effective ovipositional substrate, a laboratory experiment was conducted with six plant substrates which were replicated four times. Each substrate (as fresh green pod) was taken in a plastic container (500ml) which was provided with *Corcyra* eggs and cotton pad at the bottom. Each container with

Table 1. Influence of prey on orientation and predation of fourth instar nymphs of *B. pallescens*

Prey	Orientation of predators* 2HAR Test arena		Number of prey predated*	
	Circular basin	Olfactometer	6HAR	24 HAR
Aphis gossypii	1.75 (1.32) [ab]	1.8 (1.32) [ab]	1.50 (1.22) [b]	1.50 (1.22)[b]
Tetranychus urticae	2.50 (1.58) [a]	2.5 (1.58) [a]	3.25 (1.80) [a]	4.50 (2.12)[a]
Aleurodicus disperses	0.50 (0.71) [c]	0.5 (0.71) [c]	0.25 (0.50) [c]	0.50 (0.71)[b]
Phenacoccus solenopsis	0.75 (0.87)[bc]	0.8 (0.87) [c]	1.00 (1.00)[bc]	1.50 (1.22)[b]
Corcyra eggs	2.00 (1.41) [a]	3.0 (1.73) [a]	4.00 (2.00) [a]	6.50 (1.55)[a]
SE(d)	0.20	0.18	0.23	0.22
CD(0.05)	0.43	0.39	0.48	0.46

*Mean of four replications; HAR- hours after release ; Values in the parentheses are square root transformed values

Table 2. Influence of prey on orientation and predation of adult *B. pallescens*

Prey	Orientation of predators* 2HAR Test arena		Number of preys predated*	
	Circular basin	Olfactometer	6HAR	24 HAR
Aphis gossypii	2.00(1.41)[b]	2.00(1.41)[ab]	1.75(1.32)[b]	1.8(1.32)[b]
Tetranychus urticae	3.50 (1.87)[ab]	3.00 (1.73)[a]	4.00 (2.00)[a]	5.0 (2.24)[a]
Aleurodicus disperses	0.50 (0.71)[c]	0.80 (0.87)[b]	0.25 (0.50)[c]	0.5 (0.71)[c]
Phenacoccus solenopsis	0.80(0.87)[c]	1.30(1.12)[b]	1.25(1.12)[b]	1.8(1.32)[b]
Corcyra eggs	3.80(1.94)[a]	3.80(1.94)[a]	5.00(2.24)[a]	7.0(2.65)[a]
SE(d)	0.20	0.23	0.20	0.22
CD(0.05)	0.42	0.49	0.43	0.47

*Mean of four replications; HAR- hours after release; Values in the parentheses are square root transformed values

substrate was released with mated female and allowed for oviposition upto seven days. Every 24 hours of release, green pod laden with eggs was removed and fresh green pod was supplied to the container. The number of eggs laid on the removed green pod was counted and kept in a separate container for observation on hatching.

Adult nutrition to enhance fecundity

The ongoing protocol of mass culturing of *B. pallescens* under the laboratory conditions uses green French bean pods and *Corcyra* eggs as adult diet. In order to enhance the fecundity of the adult, the mated female can be supplied with sugar rich adult diet. In this regard, the specialized adult diet followed in the culturing of other predators like *Chrysoperla* was tested along with treatment of honey + water. There were three treatments and seven replications. The treatments were *Corcyra* eggs + green French bean pod(T1), honey+ water+ *Corcyra* eggs+ green French bean pod(T2) and specialized diet (yeast + fructose + honey + Proteinex + water @ 1:1:1:1:1) + water + *Corcyra* eggs + green French bean pod (T3). The liquid diet was provided separately through soaked cotton pads stuck to the inner wall of the plastic container along with green French bean pod and cotton pads containing Corcyra eggs at the bottom. Individual mated female was released into the container and observed for seven days to record the number of eggs on the pods.

Statistical analysis

The results are expressed in the form of means ± S.D. Data analysis was done with significance ($p < 0.05$) of treatment effects using one-way ANOVA, followed by *post hoc* comparisons. The significance of the results was determined by Duncan's multiple range test (DMRT) using the Statistical Package for the Social Sciences (SPSS) software (version 20, IBM).

RESULTS AND DISCUSSION

Influence of prey on orientation and predation of fourth instar nymph

The orientation of predator two hours after the release observed on various preys like aphid (*A. gossypii*), mite (*T.urticae*),whitefly (*A. disperses)*, mealybug (*P.solenopsis)* and *Corcyra* eggs indicated maximum aggregation of predator nymph in mite (2.50) followed by *Corcyra* eggs (2.00). The least preferred prey was

whitefly (0.50). The orientation of fourth instar nymph was also observed in olfactometer. The results indicated maximum aggregation of nymph in *Corcyra* eggs (3.00) followed by mite (2.50). The least preferred prey was whitefly (0.80). The prey predation six and 24 hours after the release was high in *Corcyra* eggs (4.00 and 6.50) followed by mite (3.25 and 4.50) respectively. Least predation was noted with whitefly (Table 1).

Influence of prey on orientation and predation of adult *B. pallescens*

The orientation of adult predator two hours after the release on various prey species *viz.,* aphids (*A. gossypii*), mite (*T.urticae*), whitefly (*A. disperses)*, mealybug (*P. solenopsis)* and *Corcyra* eggs was observed. The congregation of adult predator on *Corcyra* eggs was maximum (3.80) followed by mite (3.50). The least preferred prey was whitefly (0.25). The orientation of adult was also observed in olfactometer. The results indicated maximum aggregation of adult in *Corcyra* eggs (3.80) followed by mite (3.00). The least preferred prey was whitefly (0.80). The predation was maximum in *Corcyra* eggs (5.00 and 7.00) followed by mite (4.00 and 5.00) at six and 24 hours after the release respectively (Table 2).

Influence of prey host plants on the orientation and predation of fourth instar nymph

The aggregation of predator nymphs on mite prey provided in the host plant arena of bhendi, cluster bean, brinjal and bitter gourd was observed 2hrs after the release. The assemblage of predator nymphs to mites on host bhendi was maximum (4.8) followed by brinjal (3.0).Similar result was obtained on the orientation of nymph observed with olfactometer. The consumption of mites by the predator nymph six and 24 hours after the release noted in brinjal was high with 4.4 and 7.0 mites respectively. This was followed by predation of mites in bhendi host which recorded 2.6 and 4.0 mites at six and

Table 3. Influence of host plants of prey species on the orientation and predation of fourth instar nymph of *B. pallescens*

Prey host plants	Orientation of predators* 2 HAR Test arena		Number of mites fed*	
	Circular basin	Olfactometer	6 HAR	24 HAR
Bhendi	4.8 (2.19) [a]	3.6 (1.90) [a]	2.6 (1.61) [a]	4.0 (2.00) [b]
Cluster bean	0.8 (0.89) [c]	0.6 (0.77) [b]	0.4 (0.63) [b]	0.6 (0.77) [c]
Brinjal	3.0 (1.73) [b]	3.2 (1.79) [a]	4.4 (2.10) [a]	7.0 (2.65) [a]
Cucurbit	1.4 (1.18) [c]	0.8 (0.89) [b]	1.2 (1.10) [b]	3.2 (1.79) [b]
SE(d)	0.16	0.18	0.22	0.19
CD(0.05)	0.34	0.37	0.46	0.40

*Mean of five replications; HAR- hours after release; Values in the parentheses are square root transformed values

Table 4. Influence of host plants of prey species on the orientation and predation of adult *B. pallescens*

Prey host plants	Orientation of predators* 2 HAR Test arena		Number of mites fed*	
	Circular basin	Olfactometer	6 HAR	24 HAR
Bhendi	5.0 (2.24) [a]	3.8 (1.95) [a]	3.0 (1.73) [a]	6.0 (2.45) [a]
Cluster bean	0.8 (0.89) [c]	1.0 (1.00) [b]	0.4 (0.63) [b]	0.8 (0.89) [c]
Brinjal	2.6 (1.61) [b]	3.4 (1.84) [a]	4.0 (2.00) [a]	7.2 (2.68) [a]
Cucurbit	1.0 (1.00) [c]	1.2 (1.10) [b]	1.4 (1.18) [b]	3.6 (1.90) [b]
SE(d)	0.17	0.20	0.22	0.20
CD(0.05)	0.36	0.43	0.47	0.41

*Mean of five replications; HAR- hours after release; Values in the parentheses are square root transformed values.

24 hours after release respectively. The feeding of mites 0.4 and 0.6 noted in cluster bean was least respectively in six and 24 hours after the release (Table 3).

Influence of prey host plants on the orientation and predation of adult

The orientation of adult predator two hours after the release on various hosts like bhendi, cluster bean, brinjal and bitter gourd showed maximum congregation to bhendi (5.0) followed by brinjal (3.0). Similar result was obtained on the orientation of adult predator observed with olfactometer. The consumption of mites by the predator adult respectively in six and 24 hours after the release was significantly high in brinjal (4.0 and 7.2) and found on par with bhendi (3.0 and 6.0). The feeding of mites respectively in six and 24 hours after the release on cluster bean was least (0.4 and 0.8) (Table 4).

Preference of host plant as substrate for oviposition

The results revealed that the predator oviposited readily in all the substrates provided. The mean number of eggs laid per day on substrates like lablab, beans, cowpea, cluster bean, bhendi and green peas were 4.79, 4.46, 4.21, 8.43, 5.25 and 4.54 respectively. The mean number of eggs laid was high (8.43) in cluster bean as compared to beans (4.46). The hatching of eggs laid on different substrate was also observed. The per cent hatching was high in green peas (96.21) followed by cluster bean (94.64).The hatching of eggs in cowpea and beans were low 86.77 and 89.24 per cent respectively (Table 5).

Adult nutrition on fecundity

The data on egg laying of adult *B. pallescens* provided with different adult nutrition to increase the fecundity indicates that the adult fed on honey +water +*Corcyra* eggs laid a greater number of eggs (10.46) followed by the specialized diet mix (8.37). The number of eggs laid was low (7.20) when normal diet of *Corcyra* eggs was given (Table 6).

The variation of prey preference can be attributed to the nutritional quality of the prey besides the aggregation of prey in the feeding arena. The least preferred whitefly prey noted in the present study can attributed to the succulence of the prey. The nymphs of whitefly are less succulent and scaly which might have contributed to the lesser preference and predation of *B. pallescens*. In addition, the nature of exoskeleton of the prey and its external structure also plays a role in the preference of the prey. Among the prey host plants tested, brinjal followed

Table 5. Preference of *B.pallescens* to different plant substrates for oviposition

Ovipositional substrate	No. of eggs/pods* at different days after the release of mated female							Mean number of eggs/days	Egg hatching (%)
	1	2	3	4	5	6	7		
Lab lab	0.50 (0.71)	2.00 (1.41)	4.0 (2.00) [b]	5.50 (2.35)	7.50 (2.74) [b]	6.50 (2.55) [bc]	7.5 (2.74) [ab]	4.79	90.62
Beans	0.75 (0.87)	5.00 (2.24)	3.5 (1.87) [b]	6.00 (2.45)	5.75 (2.40) [bc]	6.00 (2.45) [bc]	4.25 (2.06) [c]	4.46	89.24
Cowpea	0.50 (0.71)	4.00 (2.00)	5.00 (2.24) [ab]	6.00 (2.45)	3.75 (1.94) [c]	4.25 (2.06) [c]	6.00 (2.45) [bc]	4.21	86.77
Cluster bean	1.25 (1.12)	6.25 (2.50)	7.75 (2.78) [a]	7.50 (2.74)	11.75 (3.43) [a]	13.75 (3.71) [a]	10.75 (3.28) [a]	8.43	94.64
Bhendi	0.50 (0.71)	3.75 (1.94)	5.25 (2.29) [ab]	5.25 (2.29)	6.25 (2.50) [bc]	7.75 (2.78) [bc]	8.00 (2.83) [ab]	5.25	93.48
Green peas	0.25 (0.50)	1.75 (1.32)	4.50 (2.12) [ab]	4.50 (2.12)	6.50 (2.55) [b]	9.75 (3.12) [ab]	4.50 (2.12) [c]	4.54	96.21
SE(d)	0.30	0.49	0.27	0.30	0.28	0.43	0.27	-	-
CD (0.05)	0.63	1.03	0.56	0.64	0.59	0.91	0.56	-	-

*Mean of four replications ; Values in the parentheses are square root transformed values.

Table 6. Effect of adult nutrition on fecundity

Treatment	No. of eggs laid*					Mean
	Day 1	Day 2	Day 3	Day 4	Day 5	
Honey + Water + *Corcyra* eggs	3.00 (1.73)	6.71 (2.59) [a]	9.86 (3.14) [a]	15.00 (3.87) [a]	17.71 (4.21) [a]	10.46
Specialized diet + water + *Corcyra* eggs	1.29 (1.13)	4.86 (2.20) [b]	8.57 (2.93) [ab]	12.00 (3.46) [b]	15.14 (3.89) [b]	8.37
Corcyra eggs + water	1.14 (1.07)	3.86 (1.96) [b]	6.57 (2.56) [b]	11.43 (3.38) [b]	13.00 (3.61) [b]	7.20
SE(d)	0.29	0.14	0.20	0.16	0.15	-
CD(0.05)	0.60	0.29	0.43	0.34	0.31	-

*Mean of seven replications ; Values in the parentheses are square root transformed values

by bhendi showed positive influence on the orientation and predation of *B. pallescens* on mites. Yarahmadi and Rajabpour (2017) also reported similar findings on the variation of predation of *Orius albidipennis* on *Tetranychus turkestani* and *Bemisia tabaci* in sweet pepper and cucumber. They opined those morphological characters like hairiness of leaves of cucumber might have negative impact on the predation as compared to sweet pepper. The volatiles of host plants harbouring the mite prey could also influence the orientation and predation of *B.pallescens*. The results obtained in the present study on preference of predator to brinjal and bhendi hosted mites shall also have attributed to the above factors.

The predator *B. pallescens* preferred to lay more eggs on cluster bean pods and least preference on vegetable cowpea and French bean pod. Regarding the hatching of laid eggs, maximum hatching was noted in green peas followed by cluster bean. The findings of Sobhy *et al.* (2005)who reported the variation among the oviposition substrates (bean pods, geranium leaves, sweet pepper seedling) in the egg laying of *Orius albidipennis* was in line with the present finding. Regarding the hatchability of eggs, Sobhy *et al.* (2005) showed variation among the ovipositional substrates with maximum hatchability in bean pods. The higher preference of cluster bean as an ovipositional substrate by *B. pallescens* might be due to the absence of dense trichomes and hairiness in cluster beanas compared to other substrates besides optimum moisture content retained for longer period. In addition, fungal moulds present on the pods of ovipositional substrate grow faster in other host pods as compared to cluster bean which slow down the mould growth. In addition, the plant volatiles also influence the ovipositional response in predators. The above reasons can also be attributed to more preference of cluster bean as anovi positional substrate to *B. pallescens*.

The reports of Lundgren and Fergen (2006) explains that the predatory bug *Oriusin sidiosus* preferred pole bean for oviposition to green foxtail, orchard grass and soybean well supported to the present results. Coll (1995) also found that *O.insidiosus* does not prefer to lay its eggs on the vegetative structures of *Zea mays* when given a choice among *Phaseolus lunatus*, *Capsicum anuum*, *Lycopersicum esculentum*. This observation was akin to the results obtained in the present study. The results of the study indicated that the mean number of eggs laid by the predator adult was maximum (10.46) when honey is provided along with *Corcyra* eggs and water followed by specialized diet mix (8.37). Heimpel and Jervis (2004) reported that provision of carbohydrates and water as adult food to predators and parasitoids enhance the longevity and fecundity. This report supports the present findings on the enhanced egg laying of predator with the adult food of honey +*Corcyra* eggs + water.The findings of Kiman and Yeargan (1985) who reported that the nymphs reared on pollen alone and along with arthropod preys successfully completed its development and when beans and water alone were given no nymphs completed its development into adults. They also found that the fecundity was significantly higher on diets containing *Heliothis virescens* eggs. This finding was in line with the present finding.

In conclusion, the nymph and adult predator preferred to orient and predate more preys when present on host plant brinjal followed by bhendi. Among the host plants *viz*., brinjal, bhendi, cluster bean and cucurbit, the least preferred host plant was cluster bean. Among the five preys offered to anthocorid nymph, the orientation and predation was maximum on *Corcyra* eggs followed by mite. The least preferred prey was noted as whitefly. The green pod of cluster bean was the most preferred

substrate for egg laying as against the green French bean pod commonly used in mass culturing. The eggs laid on preferred cluster bean pod also showed maximum hatching (94.64%) next to green peas (96.21%).The adults of anthocorid bugs provided with additional nutrition of honey and water along with the normal diet of *Corcyra* eggs laid more eggs.

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Population dynamics of whitefly, *Bemisia tabaci* Gennadius in tomato and its management using novel insecticides under polyhouse condition

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ABSTRACT: The present study was conducted to assess the effect of environmental factors on population build up of whitefly, *Bemisia tabaci* and management using novel insecticides under polyhouse conditions.The investigation was carried out at Hi-Tech Horticulture of Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur), Bihar during *rabi* season of 2019-20. The whitefly population was highest in 46th SMW and was positively correlated with maximum temperature and the coefficient of determination (R^2) was worked out as 40%. Among insecticides, chlorantraniliprole + thiamethoxam @ 150 g a.i. ha^{-1}gave the maximum per cent reduction over control in all the three sprays**.**

Keywords: Whitefly, temperature, population dynamics, polyhouse, bioefficacy, insecticides

INTRODUCTION

Tomato, *Solanum lycopersicum* L. (Solanaceae) is one among the most popular vegetable crops grown in India. The protected cultivation is the most intensive method of crop production to provide protection to the plants from adverse environmental conditions while, it provides favourable microclimatic condition for growth and development of insect pests which hampers successful propagation of crops under polyhouse condition. In tropical and sub-tropical regions, whitefly causes a huge economic damage (Block, 1982). In tomato it affects the seedling, vegetative and flowering stage. The damage caused by whitefly can cause qualitative and quantitative losses to the tomato production. Different methodologies are recommended for the management of these pests. Among them the most popular one is the chemical management due to their quick results and high efficiency. Several chemicals belonging to organochlorines, carbamates and organophosphates have been used to manage the insect pests in tomato but they leave toxic residues on the fruit and also continuous application of these chemicals leads eventually the development of resistance and resurgence of insect pests. Hence, presently farmers have switched on to the use of novel insecticides with lesser doses of few grams per hectare for the effective management of the pests. The present study has been conducted because of the scarcity of data on the population dynamics of whitefly and its management using newer insecticides under poyhouse in Bihar conditions.

MATERIALS AND METHODS

The investigation was carried out at Hi-Tech Horticulture of Dr. Rajendra Prasad Central Agricultural University, Pusa (Samastipur), Bihar during *rabi* season of 2019-20. Randomized Block Design (RBD) was employed for the purpose of the experiment. There were eight treatments, each having three replications. Tomato variety, Hem shikhar was used and was sown on with a spacing of 100 x 50 cm by adopting standard agronomic practices without plant protection measures.

For the population dynamics experiment, five plants of tomato were chosen randomly and kept free from insecticidal application. The population of whiteflies (nymphs and adults) was recorded from 3 leaves i.e., one each from the upper, middle and lower position. The population was recorded during morning hours 6.30AM to 8.30AM in each plot using a hand lens of 10x magnification. Major abiotic factors, *viz.*, maximum and minimum temperature (ºC) and relative humidity (%) were also recorded during the crop period. The data was utilized to work out simple correlation co-efficient between insect pest population and various abiotic factors and was statistically analysed. Simultaneously, weather parameters were also recorded during the entire experimental period.

In the management study, seven novel insecticides *viz.,* chlorantraniliprole + thiamethoxam (T_1)@ 150 g a.i. ha^{-1}novaluron + indoxacarb (T_2)@ 80 g a.i. ha^{-1}, chlorantraniliprole (T_3) @30 g a.i. ha^{-1}, spinosad (T_4)@ 80 g a.i. ha^{-1}, novaluron (T_5) @ 75 g a.i ha^{-1}, thiamethoxam(T_6) @ 50 g a.i. ha^{-1}, indoxacarb (T_7) @ 75 g a.i. ha^{-1} were evaluated for their field efficacy against the whitefly. There was also an untreated control. These eight treatments were replicated thrice. All the treatments were applied thrice at 15 days interval.

Table 1. Population dynamics of whitefly on tomato during crop season in relation to abiotic factors

Months	Standard weeks	Temperature		Relative humidity (%)		No. / 3 leaves
		Max.	Min.	07 00 h	14 00 h	
September (2019)	36	35.0	29.0	82	70	2.31
	37	35.5	27.0	88	80	6.15
	38	36.5	27.1	87	73	10.5
	39	33.2	27.0	92	86	11.2
October (2019)	40	31.8	25.5	90	79	12.45
	41	32.0	25.3	88	80	15.12
	42	33.0	23.2	87	79	17.99
	43	30.1	22.0	84	73	13.67
	44	32.2	24.7	91	76	17.53
November (2019)	45	32.5	22.3	89	60	21.5
	46	31.6	19.5	90	62	24.49
	47	29.5	16.5	87	60	19.49
	48	30.2	16.9	90	66	15.13
December (2019)	49	28	14.5	75	67	12.11
	50	26.0	13.0	90	75	9.12
	51	20.5	12	88	81	6.72
	52	18.1	8.9	95	77	5.22
January (2020)	1	20	9.0	94	65	5.15
	2	20.0	6.0	85	55	4.54
	3	23.0	12.0	93	74	3.21

Table 2. Correlation coefficient and regression equation between weather parameters (X) and mean number of whitefly per three leaves per plant (Y)

Weather parameters	Correlation coefficient(r)	Regression coefficient(b)
X1- Maximum Temperature (ºC)	0.468*	1.52
X2- Minimum temperature (ºC)	0.324	-0.733
X3- Relative Humidity 07 00 hrs (%)	-0.039	0.362
X4- Relative Humidity 14 00 hrs (%)	-0.222	-0.157

Benefit cost ratio was calculated to understand the economics of insecticidal management of whitefly. After picking plot wise total weight of fruits was noted. Gross benefit was computed for every treatment after removing cost of insecticides and cost of labour. Extra profit by use of insecticides for each treatment was computed by subtracting value realized in control treatment from gross benefit. The per cent increase over control was also computed based on extra profit divided by value realized in control and multiplied in hundred. The additional income obtained over control plot was divided with the additional cost made for controlling the pest to obtain the benefit-cost ratio**.**

RESULTS AND DISCUSSION

The data on whitefly population were recorded on tomato in *rabi* season 2019-20 at 7 days interval from 30 days after transplanting up to the crop was harvested. The data represented in Table 1. shows that all around the vegetative stage the whitefly population was seen. Results revealed that the whitefly population appeared 30 days after crop transplantation and was present throughout the vegetative stage of the crop initially recording a population density of 2.31 per three leaves reaching 17.99 on 42nd SMW. The population showed an increase with the advancement of crop growth and peak population of 24.49 per three leaves on 46th SMW. When the vegetative stage was ending the population decreased and reached up to 3.21 per three leaves. The decline in whitefly population after 48th SMW might be due to the crop reaching its maturity. The lowest population was marked at third SMW.

An attempt was made to study influence of weather parameters on whitefly. The data represented that maximum temperature influenced the whitefly population. The results revealed maximum temperature was positively correlated and statistically significant. Minimum temperature was negatively correlated

Table 3. Effect of insecticides on whitefly (*Bemisia tabaci*) population in tomato after first, second and third spray

Treatment	Dose (g a.i. ha^{-1})	1day before spray	First spray				Second spray				Third spray			
			3rd DAS	7th DAS	14th DAS	Reduction over control (%)	3rd DAS	7th DAS	14th DAS	Reduction over control (%)	3rd DAS	7th DAS	14th DAS	Reduction over control (%)
T_1	150	18.2 (4.25)	8.22 (2.86)^{d}	7.3 (2.69)^{d}	6.46 (2.53)^{d}	61.18	4.58 (2.13)^{e}	4.13 (2.03)^{d}	3.88 (1.97)^{d}	79.3	2.26 (1.50)^{e}	2.31 (1.52)^{e}	2.17 (1.47)^{d}	90.2
T_2	80	19.12 (4.36)	10.23 (3.19)^{bcd}	10.69 (3.26)^{bcd}	11.12 (3.32)^{bc}	43.37	10.21 (3.18)^{bcd}	10.32 (3.20)^{bc}	10.85 (3.28)^{bc}	48.52	7.21 (2.67)^{cd}	8.13 (2.83)^{cd}	8.26 (2.86)^{c}	65.76
T_3	30	18.67 (4.29)	13.43 (3.65)^{b}	13.21 (3.62)^{b}	14.97 (3.86)^{ab}	26.45	12.97 (3.59)^{b}	12.23 (3.49)^{b}	12.68 (3.56)^{b}	37.84	11.92 (3.44)^{b}	11.5 (3.38)^{b}	11.78 (3.42)^{b}	50.21
T_4	80	18.43 (4.27)	10.34 (3.20)^{bcd}	9.23 (3.02)^{cd}	10.56 (3.24)^{bc}	46.76	9.23 (3.02)^{cd}	10.54 (3.23)^{bc}	10.39 (3.21)^{bc}	50.54	6.58 (2.55)^{d}	7.12 (2.66)^{d}	7.36 (2.70)^{c}	69.94
T_5	75	19.32 (4.39)	11.23 (3.34)^{bcd}	11.45 (3.38)^{bc}	11.95 (3.45)^{bc}	38.81	10.57 (3.24)^{bcd}	10.63 (3.25)^{bc}	11.32 (3.35)^{bc}	46.65	9.13 (3.02)^{bcd}	9.23 (3.03)^{bcd}	9.26 (3.04)^{bc}	59.93
T_6	50	18.28 (4.27)	9.23 (3.04)^{cd}	8.29 (2.88)^{cd}	8.45 (2.90)^{cd}	54.13	8.25 (2.87)^{d}	7.92 (2.81)^{c}	8.01 (2.83)^{c}	60.33	6.14 (2.47)^{d}	6.47 (2.54)^{d}	7.13 (2.66)^{c}	71.34
T_7	75	18.23 (4.27)	11.97 (3.45)^{bc}	11.81 (3.42)^{bc}	12.34 (3.50)^{bc}	36.16	12.32 (3.50)^{bc}	11.75 (3.42)^{b}	12.49 (3.52)^{b}	40.05	10.07 (3.15)^{bc}	11.24 (3.33)^{bc}	11.85 (3.42)^{b}	51.87
T_8		18.4 (4.29)	18.45 (4.29)^{a}	18.59 (4.30)^{a}	19.56 (4.39)^{a}		19.56 (4.42)^{a}	20.28 (4.49)^{a}	21.12 (4.58)^{a}		22.21 (4.70)^{a}	22.91 (4.78)^{a}	23.76 (4.87)^{a}	
SEm (±)			0.18	0.19	0.22		0.17	0.18	0.20		0.18	0.18	0.17	
CD (p=0.05)		NS	0.54	0.58	0.67		0.52	0.56	0.61		0.55	0.55	0.53	
CV (%)			9.09	9.95	11.23		9.20	9.84	10.62		10.63	10.38	9.85	

T1- Chlorantraniliprole + Thiamethoxam; T2- Novaluron + Indoxacarb; T3- Chlorantraniliprole; T4- Spinosad; T5- Novaluron; T6- Thiamethoxam; T7- Indoxacarb; T8- Control.

Values in the parenthesis are the square root transformed values of mean; DAS- Days after spraying.

and statistically non-significant while morning and evening relative humidity was non-significant and negatively correlated. The correlation coefficient (r) was worked out as 0.324, 0.468, -0.222 and -0.39 for minimum temperature, maximum temperature, evening and morning relative humidity, respectively. The coefficient value of determination (R^2) computed as 40 %indicating 40% variation in whitefly attributed by weather parameters (R^2 = 0.40). This is in partial conformity with the results of Sharma *et al.* (2017) who found a positive correlation of whitefly population with maximum and minimum temperature and negative correlation with relative humidity, results of Subba *et al.* (2017) whose correlation studies between whitefly population and environmental parameter revealed that whitefly population had a significant positive correlation with temperature difference while significant negative correlation with relative humidity (maximum, minimum and average) and Nissar *et al.* (2019) who found a positive significant correlation between whitefly population (nymph and adult) and weather parameters viz. temperature (maximum and minimum) whereas relative humidity (maximum) exhibited positive non-significant whereas relative humidity (minimum) showed a negative non significant correlation with population.

The mean per cent reduction in whitefly population over control after third, seventh and fourteenth day of 1st spray was maximum in chlorantraniliprole + thiamethoxam @150 gm a.i. ha^{-1} (61.18%) after third, seventh and fourteenth day of spray and was efficient than all other insecticidal treatments i.e., novaluron + indoxacarb @ 150 gm a.i. ha^{-1} (43.37%), chlorantraniliprole @ 30 gm a.i. ha^{-1} (26.45%), spinosad @ 80 gm a.i. ha^{-1} (46.76 %), novaluron 75 g a.i. ha^{-1} (38.81%), thiamethoxam @ 50 gm a.i. ha^{-1} (54.13 %), indoxacarb 75 gm a.i. ha^{-1} (36.16%) whereas chlorantraniliprole @30 gm a.i. ha^{-1}(26.45%) was least effective.The mean per cent reduction in whitefly populationovercontrol after third, seventh and fourteenth day of 2nd spray was maximum in chlorantraniliprole + thiamethoxam @ 150 gma.i.ha^{-1} (79.30%). Chlorantraniliprole @ 30 gm a.i.ha^{-1} (37.84%) was least effective among all insecticidal treatments. The mean per cent reduction in whitefly population over control after third, seventh and fourteenth day of 3rd spray was maximum in chlorantraniliprole + thiamethoxam @ 150 gma.i.ha^{-1}(90.20%), whereas chlorantraniliprole@ 30 gma.i.ha^{-1} (50.21%) was least effective. These results are in accordance with literature reports of Patra et al. (2016), Kalyan *et al.* (2012), Patil *et al.* (2014), Gopalaswamy *et al.* (2012), Wagh et al. (2017) and Rajawat *et al.* (2017). Patra *et al.* also reported an excellent control of whitefly population by usingchlorantraniliprole + thiamethoxam @ 150 gma.i.ha^{-1} which completely agree to our present observation.

The maximum percent reduction over control was shown by chlorantraniliprole + thiamethoxam which is a broadspectrum insecticide with trans laminar as well as ovicidal and larvicidal activity and a greater persistence and higher dose. It was followed by thiamethoxam which is systemic and very effective against sucking pest complex. The least per cent reduction over control was shown by chlorantraniliprole which is effective for borer pest complex and didn't produce any significant reduction in whitefly population. Rest all insecticides were ranked in the middle based on their efficacies.

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Seasonal incidence of leaf roller, *Psorosticha zizyphi* (Stainton) (Lepidoptera: Oecophoridae) on curry leaf, *Murraya koenigii* (L.) Sprengel and its management

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ABSTRACT: The seasonal incidence of leaf roller, *Psorosticha zizyphi* Stainton on curry leaf, *Murraya koenigii* (L.) Sprengel was studied at University of Agricultural Sciences, Dharwad, India. The maximum activity of the leaf roller was recorded from 31st SMW to 36th SMW and the population ranged from 15.40 to 23.60 larvae/plant. The peak population of 23.60 larvae/plant was recorded at 36th SMW (1st week of September). There existed a positive and highly significant correlation between larval population and minimum temperature, morning and evening relative humidity. Efficacy of different biorationals was tested against the leaf roller larvae under field conditions. The treatment with spinosad 45 SC proved to be effective by recording the lowest larval population of 2.96 and 3.97/plant in both the sprays at 5 days after spray. The next best treatment in recording lower insect population was novaluron 10 EC.

Keywords: Biorationals, curry leaf, leaf roller, *Psorosticha zizyphi,* spinosad, novaluron, weather parameters

INTRODUCTION

Psorosticha zizyphi (Stainton) (Lepidoptera: Oecophoridae) has a wide host range including ber (*Zizyphus mauritiana* Lamarck), bael (*Aegle marmelos* L.), mandarins, lemons, oranges and *Murraya koenigii* (L.) Sprengel (Sharma and Batra, 1987). Tara *et al*. (2011) reported the severe incidence of *P. zizyphi* on curry leaf in Jammu and Kashmir. Similarly, Devaki *et al.* (2012) reported the incidence of *P. zizyphi* on *M. koenigii* in Andhra Pradesh. The curry leaf cultivation has become an important agricultural practice along with other agricultural activities to the growers in and around Dharwad either as sole crop or intercropped with many other crops. Among all the insect pests, leaf roller, *P. zizyphi* is causing severe damage to curry leaves in the field with the arrival of a new flush. The larvae webs young developing leaves also encloses and damages the bud and thus solemnly hinders new growth of the plant. However, as such the scientific information on incidence of *P. zizyphi* on curry leaf plant and its management is lacking. Further, curry leaf being an important ingredient of day to day dishes thus become part and parcel of every home in South India. Hence, the present study was undertaken to understand the pest menace and for developing suitable eco-friendly practices to manage this pest.

MATERIALS AND METHODS

The experiment was conducted at curry leaf garden, at the Department of Horticulture, University of Agricultural Sciences, Dharwad, India. Incidence of leaf roller on curry leaf (Var. Suvasini) was closely monitored at weekly intervals starting from January to December, 2018. The observations on larval population were recorded from randomly selected five plants and expressed per plant basis. The entire experimental field was kept unsprayed during the experimental period. The data thus collected were correlated with the meteorological parameters.

In order to evaluate the efficacy of biorationals (Table 1) against leaf roller, *Psorosticha zizyphi,* a field experiment was carried out with seven treatments and three replications. Each treatment plot of size 22.5m^2 comprising 12 curry leaf plants was envisaged. Four plants were selected randomly in each treatment plot for recording observations. The treatments were imposed with a knapsack sprayer twice and the interval between two sprays was about one month. The first spray was given on the appearance of sufficient leaf roller in the month of August 2018 and the second spray was given in September 2018. The pre-count on number of leaf roller larvae was counted a day before spray and at 1, 5, 10 and 15 days after treatment imposition. The reduction in insect pest population in relation to the initial population was worked out for different time intervals. The data so obtained were subjected to ANOVA (Randomized Block Design) following Square root transformation ($\sqrt{x+0.5}$ values) for leaf roller larval counts. The treatments were differentiated for the significant differences existing among them following Duncan's New Multiple Range Test (DNMRT).

Table 1. Seasonal incidence of leaf roller on curry leaf during January to December 2018

Month	*SMW	No. of leaf roller larvae/ plant	Mean maximum temperature (°C)	Mean minimum temperature (°C)	Relative humidity (%)		Rainfall (mm)
					Max	Min	
	1	0.00	28.93	13.37	84.71	66.86	0.00
January	2	0.00	29.81	15.20	75.86	54.86	0.00
	3	0.00	30.31	13.93	71.00	57.71	0.00
	4	0.00	30.07	13.66	74.86	54.57	0.00
	5	0.00	30.97	12.61	49.43	43.00	0.00
	6	0.00	30.50	16.29	57.57	46.29	1.00
February	7	0.00	31.87	16.14	62.43	45.14	0.00
	8	0.00	33.33	16.69	58.57	32.71	0.00
	9	0.00	34.73	16.36	40.14	23.29	0.00
	10	0.00	35.26	17.96	68.14	16.29	0.00
March	11	0.00	33.51	20.14	67.00	32.14	26.80
	12	0.00	34.46	19.47	56.14	29.00	45.60
	13	0.00	36.43	20.60	80.71	33.43	0.00
	14	0.00	35.71	20.81	98.43	37.57	5.20
April	15	0.00	35.07	21.01	46.89	37.34	12.40
	16	0.00	35.80	21.90	54.53	49.11	15.20
	17	0.00	37.83	20.84	76.43	26.86	0.00
	18	1.25	37.53	21.90	75.00	40.14	0.20
	19	1.80	35.79	21.83	75.86	51.14	92.00
May	20	2.20	33.43	20.23	80.57	61.00	81.80
	21	3.00	33.56	20.94	77.00	61.29	63.80
	22	3.80	32.81	21.79	86.00	63.71	52.20
	23	4.50	29.73	21.19	89.43	74.43	12.40
June	24	4.20	28.84	21.50	83.71	73.14	39.20
	25	5.40	27.07	20.21	90.43	76.00	21.20
	26	4.40	27.67	20.94	86.71	75.71	3.00
	27	5.60	27.19	20.46	87.29	81.57	11.20
July	28	6.60	25.46	20.51	92.86	86.29	45.60
	29	7.80	25.03	20.79	91.00	87.86	54.40
	30	9.20	25.16	20.73	90.29	87.14	17.20
	31	15.40	26.69	20.59	89.00	83.00	6.00
	32	16.20	25.97	20.34	89.29	86.14	18.60
August	33	18.40	24.77	20.36	91.57	88.43	32.80
	34	19.00	25.91	19.96	89.43	80.00	9.00
	35	19.60	26.73	19.94	89.43	82.43	12.60
	36	23.60	27.76	18.70	86.29	74.86	2.20
September	37	21.80	30.60	17.83	82.43	52.86	0.00
	38	19.40	29.80	19.37	81.43	68.14	24.20
	39	14.40	30.87	19.41	83.29	70.43	36.80
	40	6.40	32.54	19.80	77.29	60.00	13.80
	41	4.40	32.44	19.56	83.00	49.43	0.40
October	42	4.20	30.13	19.31	82.71	71.71	62.60
	43	4.20	32.06	16.80	60.00	55.86	0.00
	44	4.00	30.40	15.93	60.57	43.57	0.00
	45	4.20	31.87	17.10	63.29	34.71	0.00
	46	3.80	31.21	15.56	59.00	32.29	0.00
November	47	3.00	29.87	17.87	79.43	56.71	34.40
	48	2.25	29.21	12.94	60.00	40.00	0.00
	49	1.75	29.67	17.19	77.43	54.86	38.40
	50	1.50	29.41	15.09	76.57	54.14	0.00
December	51	1.25	27.11	13.21	71.00	51.86	0.00
	52	0.50	28.71	13.33	60.14	43.43	0.00

*SMW= Standard meteorological week

RESULTS AND DISCUSSION

The data on the incidence of leaf roller are presented in Table 1. The incidence of *P. zizyphi* commenced from 18th SMW (1.25/plant) and slowly increased then afterwards. Their population from 18 to 25th SMW ranged from 1.25 to 5.40 per plant. The present findings are in line with Batra and Sandhu (1979) who reported that population of *P. zizyphi* remained higher during May to June in Punjab and during June in Jammu and Kashmir (Tara *et al.*, 2011). Devaki *et al*. (2012) reported that incidence in June after the receipt of monsoon showers with the formation of new flush in curry leaf. The increasing population trend was recorded from 27th to 36th SMW (5.60 to 23.60/ plant). The peak population of 23.60/plant was recorded in 36th SMW. Further decrease in population was noticed from 37th to 39th SMW (21.80 to 14.40/plant). Thus, the pest was found to be higher after receipt of monsoon showers with the formation of a new flush. Further, the pest infestation was higher in young tender shoots than in older matured one. The present findings are in line with Gupta (1954) who reported that in Madhya Pradesh the incidence of citrus leaf roller, *Tonica zizyphi* Stainton was high during the rainy season from July to September. Patel and Valand (1994) reported the higher activity of citrus leaf roller in July, during the fifth week of August and in the third week of September. The pest has been recorded to remain at the highest level during monsoon supported the present investigation. The present finding of higher infestation on young tender shoots during July to September is in line with Tara *et al.*, (2011) and Devaki *et al*. (2012) who reported the severe infestation by the pest on young shoots during July to August on curry leaf. The decreasing trend of larval population ranging from 6.40 to 0.50 per plant was recorded in 40th to 52nd SMW. The prevalence of low temperature and higher humidity might have prolonged the generation until the end of December. The reported leaf roller activity gets reduced to a minimum in October-November by Sharma and Batra (1989) and the reported re-infestation during October 2009 to January 2010 by Devaki *et al.* (2012) are in line with the present finding. Most of the earlier findings support the present investigation and little variation could be due to the local acclimatization of leaf roller across the geographical area.

The pest population exhibited a significant and positive correlation between leaf roller and morning and evening relative humidity ($r = 0.520^{**}$, 0.631^{**}) (Table 2) indicating that an increase in above weather parameters tends to increase the infestation of the pest significantly and vice versa. Whereas, it was a highly significant and negative correlation with maximum temperature ($r = -0.55^{**}$) indicating that with an increase in maximum temperature the pest incidence decreases significantly and vice versa. The correlation with rainfall was non-significant indicating an insignificant effect of rainfall on the population fluctuation of the pest. A very little work has been done on the correlation of curry leaf roller and weather parameters elsewhere as evident from the review of literature. Sharma and Batra (1989) reported a positive correlation between mean temperature and citrus leaf roller population. Whereas, relative humidity had no significant effect on the pest population. During the present investigation, a positive correlation between the pest and relative humidity has been observed. Thus, the present results differed from the above report might be due to different interaction between weather parameters and pest population at different places. However, Patel and Valand (1994) reported citrus leaf roller population found to have a significant and positive correlation with minimum temperature and relative humidity which is in agreement with the present findings.

Efficacy of biorationals against leaf roller in curry leaf

The data on leaf roller population are presented in Table 3 revealed that all the insecticidal treatments were significantly superior over untreated control in recording lowest larval population. The number of larval population ranged from 14.03 to 15.82 per plant across the treatments a day before the imposition of the first spray without any statistical difference. At one after the spray, the lower larval population of 3.10 per plant recorded in spinosad 45 SC was significantly superior to rest of the treatments followed by novaluron 10 EC (8.95/plant) which was at par with azadirachtin 1000 ppm @ 2 ml/l (11.75/plant) and azadirachtin 300 ppm (12.54/plant). However, the higher larval population was recorded in azadirachtin 1000 ppm @ 1.5 ml/l (13.66/plant), buprofezin 25 SC (14.86/plant) and untreated check (13.92/plant). The higher larval population of 13.31 per plant recorded in buprofezin 25 SC after 10 days of spray was at par with azadirachtin 1000 ppm @ 1.5 ml/l (12.62/plant). Azadirachtin 300 ppm recorded 10.70/plant larval population which was at par with azadirachtin 1000 ppm @ 2 ml/l (9.83/plant) and novaluron 10 EC (9.26/plant). The lower larval population recorded in spinosad 45 SC (6.36/plant) was at par with novaluron 10 EC (9.26/plant) and differed significantly with the rest of the treatments. There was no statistical difference between treatments 15 days of treatment imposition. The lower larval population of 4.12 per plant recorded in spinosad 45 SC was significantly superior to the rest of the treatments (Table 5) after second spray. This was followed by novaluron 10 EC (9.99/plant) and which was at par with azadirachtin 1000 ppm @ 2 ml/l (15.16/plant). However, the higher larval population was recorded in azadirachtin

Table 2. Correlation between insect pest population and weather parameters (January – December, 2018)

Insect pest	Temperature (^{0}C)		Relative humidity (%)		Rainfall
	Maximum	Minimum	Morning	Evening	(mm)
No. of leaf roller larvae/plant	-0.551**	0.285	0.520**	0.631**	0.050

*Correlation significant at the 0.05 level
**Correlation significant at the 0.01 level

Table 3. Field evaluation of biorationals against leaf roller (I spray)

Treatment	Dosage (ml/l)	No. of larvae/plant				
		1 DBS	1 DAS	5 DAS	10 DAS	15 DAS
Spinosad 45 SC	0.25 ml	14.65 (3.88)^a	3.10 (1.90)^a	2.96 (1.86)^a	6.36 (2.60)^a	11.91 (3.48)^a
Novaluron 10 EC	1.0 ml	14.03 (3.80)^a	8.95 (3.07)^b	5.61 (2.47)^b	9.26 (3.11)^{ab}	13.13 (3.69)^{ab}
Buprofezin 25 SC	1.0 ml	15.52 (4.00)^a	14.86 (3.92)^c	10.79 (3.36)^{cd}	13.31 (3.71)^{bc}	17.37 (4.23)^{ab}
Azadirachtin 300 ppm	5 ml	14.35 (3.83)^a	12.54 (3.61)^{bc}	6.63 (2.67)^b	10.70 (3.34)^b	15.72 (4.03)^{ab}
Azadirachtin 1000 ppm	1.5 ml	14.57 (3.87)^a	13.66 (3.75)^c	8.29 (2.95)^{bc}	12.62 (3.61)^{bc}	16.93 (4.16)^{ab}
Azadirachtin 1000 ppm	2.0 ml	15.82 (4.02)^a	11.75 (3.47)^{bc}	5.99 (2.53)^b	9.83 (3.21)^{ab}	14.62 (3.87)^{ab}
Untreated check	-	14.08 (3.80)^a	13.92 (3.78)^c	14.32 (3.83)^d	15.74 (4.01)^c	17.92 (4.28)^b
S.Em±		0.22	0.20	0.16	0.19	0.22
CD (p=0.05)		NS	0.61	0.50	0.60	0.68
CV (%)		9.61	10.29	9.90	9.96	9.69

Table 4. Field evaluation of biorationals against leaf roller (II spray)

Treatment	Dosage (ml/l)	No. of larvae/plant				
		1 DBS	1 DAS	5 DAS	10 DAS	15 DAS
Spinosad 45 SC	0.25 ml	15.71 (4.00)^a	4.12 (2.12)^a	3.97 (2.10)^a	7.76 (2.87)^a	12.15 (3.54)^a
Novaluron 10 EC	1.0 ml	17.01 (4.15)^a	9.99 (3.22)^b	6.17 (2.57)^{ab}	10.18 (3.24)^{ab}	15.22 (3.95)^{ab}
Buprofezin 25 SC	1.0 ml	20.58 (4.58)^a	19.10 (4.42)^c	14.80 (3.90)^{cd}	17.99 (4.29)^{cd}	22.34 (4.77)^c
Azadirachtin 300 ppm	5 ml	19.41 (4.44)^a	17.17 (4.18)^c	8.89 (3.04)^b	13.58 (3.66)^{bc}	20.18 (4.54)^{bc}
Azadirachtin 1000 ppm	1.5 ml	20.63 (4.59)^a	18.90 (4.39)^c	9.88 (3.21)^{bc}	14.76 (3.63)^{bc}	21.89 (4.70)^{bc}
Azadirachtin 1000 ppm	2.0 ml	19.65 (4.47)^a	15.16 (3.95)^{bc}	6.70 (2.68)^{ab}	11.07 (3.44)^{ab}	17.08 (4.19)^{abc}
Untreated check	-	20.13 (4.54)^a	18.92 (4.49)^c	20.29 (4.55)^d	21.55 (4.69)^d	23.10 (4.86)^c
S.Em±		0.28	0.24	0.18	0.21	0.24
CD (p=0.05)		NS	0.73	0.57	0.64	0.74
CV (%)		11.20	10.69	10.14	9.62	9.54

DBS – Days before spray **DAS –** Days after spray **NS** – Non significant
NB: Figures in parenthesis are $\sqrt{x} + 0.50$ transformed values.
In column means followed by the same letter do not differ significantly by DNMRT (p = 0.05).

300 ppm (17.17/plant), azadirachtin 1000 ppm @ 1.5 ml/l (18.90/plant), buprofezin 25 SC (19.10/plant) and untreated check (18.92/plant).

Recovery of the larval population was noticed after ten days of spray. The larval population ranged from 7.76 to 21.55 per plant. The recovery of the higher larval population was recorded in buprofezin 25 SC (17.99/ plant) which was at par with an untreated check (21.55/ plant). This was followed by azadirachtin 1000 ppm at 1.5 ml/l (14.76/plant), and azadirachtin 300 ppm (13.58/ plant) and which were at par with azadirachtin 1000 ppm at 2 ml/l (11.07/plant) and novaluron 10 EC (10.18/plant). The minimum population was recovered in spinosad 45 SC (7.76/plant) which was significantly superior to rest of the treatments. The higher larval population was recorded in buprofezin 25 SC (22.34/plant), azadirachtin 1000 ppm @ 1.5 ml/l (21.89/plant), azadirachtin 300 ppm (20.18/plant) and azadirachtin 1000 ppm @ 2 ml/l (17.08/plant) after 15 days of spray. The minimum population was recovered in novaluron 10 EC (15.22/ plant) was at par with spinosad 45 SC (12.15/plant) and differed significantly with the rest of the treatments. The effectiveness of spinosad against many lepidopteran caterpillar pests has already been reported elsewhere in different crop ecosystem (Karthikeyan *et al*., 2008 and Jahnavi and Rao, 2016) is also holds good in the present investigation. Since the usage of pesticides leaves residue on Curry leaf which is the edible part of the plant. Thus emphasis should be given on the eco-friendly tools that could be successfully employed in the management of insect pests in curry leaf, so that hazardous impact of chemicals on the environment and human health can be minimized.

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Activity of defensive enzymes in chilli germplasm in relation to their reaction to chilli thrips, *Scirtothrips dorsalis* Hood

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ABSTRACT: Activity of defensive enzymes in the leaves of seven chilli germplasm collections of whicch two were resistant and five were moderately resistant to chilli thrips, *Scirtothrips dorsalis* Hood was studied in comparison to susceptible germplasm at Bhubaneswar, Odisha, India. The resistant chilli germplasm *viz*., BC-7-2-1, BC-25 and moderately resistant chilli germplam *viz*., BC-27-2-2, BC-21, BC-79-1, Utkal Abha and BC-406 had 5.84-8.23 µM min^{-1} g^{-1} peroxidase, 0.1-0.2 µM min^{-1} g^{-1} poly phenol oxidase and 7.39-14.16 µM min^{-1} g^{-1} catalase in the leaf sample respectively, as against 1.42-4.42 µM min^{-1} g^{-1}, 0.03-0.08 µM min^{-1} g^{-1} and 3.18-6.36 µM min^{-1} g^{-1} protein in the leaves of susceptible chilli germplasm(LCA-620,BC-78-1-2 and BC-24-1) and highly susceptible check chilli germplasm (Byadagi kaddi), respectively. A significantly inverse relation existed between the activity of defensive enzymes *viz.,* peroxidase (-0.984**), polyphenol oxidase (-0.965**), catalase (-0.965**) and the incidence of *S. dorsalis*. The multiple linear regression analysis revealed that all these defensive enzymes together influenced the population of *S. dorsalis* to an extent of 97.81 per cent.

INTRODUCTION

Chilli, *Capsicum annum* L. (Family: Solanaceae) is the most common and extensively cultivated spice cum vegetable crop in the tropics and subtropics. In addition to its food value, chili also have important role in the pharmaceutical or medical field, particularly because of the capsaisinoid content in fruit which has been used for the treatment of pain and inflammation associated with various diseases such as rheumatoid arthritis, diabetic neuropathy, postmasectomy syndrome pain, cluster headaches, herpes zoster, and others (Lim, 2013; Srivastava, 2013). Nutritionally, chilli fruits are the rich source of vitamin- A, B, C and E. Capsaicin an alkaloid responsible for pungency in chillies has medicinal properties and it prevent heart attack by dilating the blood vessels (Gill, 1988) and anticancer properties (USDA, 2016).

In spite of concerted efforts at various levels, the productivity of chilli in India is stagnant over several years although, the crop has got great export potential besides huge domestic requirement. The attack of an array of insect pests to the crop right from the nursery stage till harvesting is considered as one of the major biotic constraints especially in tropical and sub-tropical countries due to conducive climate. Among several insect pests, chilli thrips or yellow tea thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), a highly polyphagous pest native to either Southeast Asia (Seal *et al.*, 2006) or in the Indian sub-continent (Kumar *et al.*, 2013) poses a considerable threat to production of chilli in southern and eastern Asia, Africa, and Oceania (Butani, 1976, Ananthakrishnan, 1993 and Kumar *et al.,* 2011).

The biochemical constituents of any crop or variety exert a definite influence over the pest species either by luring or deterring or by supporting or inhibiting growth and development through synergism or antibiosis (Ananthakrishnan, 1996). Biochemical parameters such as sugars, phenols, tannins, proteins, amino acids and several secondary plant metabolites imparting either a resistance or susceptible reaction in the host has been reported in many crops. Thus, better understanding of the biochemical basis of plant defence mechanisms in chilli resistant germplasm is highly imperative. One of the prominent plant responses to insect herbivore attack is the induction and accumulation of oxidative enzymes *viz.*, catalase, peroxidase, phenylalanine ammonia-lyase and polyphenol oxidase which are the important biochemical markers in pest resistant plants (Green and Ryan, 1972; War *et al.*, 2012 and Sha *et al.*, 2015). Comparison of enzymatic responses of resistant vs. susceptible chilli germplasm with a purpose to decipher mechanisms that will facilitate the breeding programme formed the major aspect of the present investigation.

MATERIALS AND METHODS

Studies on enzymatic activities in resistant and susceptible chilli germplasm to *S.dorsalis* were carried out in the Department of Entomology, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha during 2019-20.

Incidence of *S. dorsalis* in selected germplasm: Twelve selected chilli germplasm *viz.,* BC-25, BC-79-1, BC-27-2-2, Utkal Abha, BC-21, BC-406, BC-28, LCA-620, BC-78-1-2, BC-24-1 along with resistant check BC-7-2-1 and susceptible check Byadagi kaddi were raised under insect free conditions in pot tray and transplanted at six weeks after germination. Three plants per genotype were planted in 10 x 12 inches poly bag with three replications in a randomized block design. Plants were spaced 60 cm between rows and 45 cm between plants in a row during summer 2019-20. Observations on population of nymphs and adults of *S. dorsalis* were recorded on three leaves of chilli at top, middle and bottom canopy from three plants at 14DAT, 21 DAT, 28 DAT, 35 DAT, 42 DAT, 56 DAT, 63 DAT, 70 DAT, 77 DAT, 84 DAT, 91 DAT and 98 DAT (days after transplanting). The population was counted visually by using a magnifying lens in early morning hours (Bhede *et al.*, 2008).

Enzyme activity assay in selected chilli germplasm to *S. dorsalis*

Preparation of enzymatic extract: Enzymatic activity of peroxidase, polyphenol oxidase and catalase of twelve selected germplasm was assessed during 2019-20 in order to ascertain the bases of resistance. The standard laboratory procedures adopted are briefly described in the following paragraphs. In this study for preparing the enzyme extract, leaf samples were collected from pot culture experiment at 60 DAT. Enzyme extract for peroxidase, polyphenol oxidase and catalase was prepared by the weighed amount of 2g of sample homogenized at 0-4^0 by using pre chilled mortar and pestle with 10ml extraction buffer (0.1 M phosphate buffer pH 7.0) containing 1mM ascorbic acid and 0.5% polyvinyl pyrollidone. The homogenate was filtrated through three layers of cheese cloth and filtrate was centrifuged at 10000 rpm for 20 minutes. The supernatant was used for enzymatic assay (Malick and Singh, 1980).

Peroxidase: Activity of peroxidase was assessed by following the procedure of Castillo *et al.*, (1984).

Polyphenol oxidase: Activity of polyphenol oxidase was assessed as per the procedure suggested by Augustin *et al.* (1985).

Catalase : Activity of catalase was assessed following the procedure of Barber (1980).

RESULTS AND DISCUSSION

Population of *S. dorsalis* in selected chilli germplasm: The results of analysis of mean pool data of 14DAT, 21 DAT, 28 DAT, 35 DAT, 42 DAT, 56 DAT, 63 DAT, 70 DAT, 77 DAT, 84 DAT, 91 DAT and 98 DAT on population of *S. dorsalis* in different chilli germplasm revealed significantly lowest mean population of *S. dorsalis* in the resistant germplasm BC-7-2-1(resistant check) (0.70) which was at par with other resistant germplasm BC-25 (0.72). Lower population of *S. dorsalis* ranging from 1.18 to 1.31 per leaf was observed in the five moderately resistant germplasm *viz.,* BC-27-2-2 (1.18), BC-21 (1.22), BC-79-1 (1.25), Utkal Abha (1.29) and BC-406 (1.31). The susceptible check Byadagi kaddi recorded the highest population of *S. dorsalis* (2.46/leaf) which was at par with the other susceptible germplasm BC-24-1 (2.38/leaf) (Table 1).

Enzyme activity in resistant and susceptible chilli germplasm

Peroxidase (POD): The activity of peroxidase was highest in the resistant check germplasm BC-7-2-1 (8.23 µM min^{-1} g^{-1} protein) which was closely followed by the other resistant germplasm BC-25 (7.19 µM min^{-1} g^{-1} protein) (Table 1). The moderately resistant germplasm *viz.,* BC-27-2-2, BC-21, BC-79-1, Utkal Abha and BC-406 showed higher peroxidase activity values between 5.84 µM min^{-1} g^{-1} protein (BC-406) and 6.82 µM min^{-1}g^{-1}protein (BC-27-2-2). The activity of peroxidase was lowest in the susceptible check germplasm Byadagi kaddi (1.42 µM min^{-1} g^{-1} protein) which was closely followed by the highly susceptible germplasm BC-24-1 (2.27 µM min^{-1} g^{-1} protein). In other susceptible germplasm the activity of peroxidase ranged between 3.32 µM min^{-1} g^{-1} protein (BC-78-1-2) to 4.42 µM min^{-1} g^{-1} protein (BC-28).

Peroxidases are the glycoproteins with ubiquitous distribution in the plant kingdom. These enzymes are involved in various physiological functions *viz.*, lignification, suberization, phenol oxidation, wound healing, protection against insect attack and regulation of cell elongation (Bruce and West, 1989). Its activity is known to increase with herbivore damage in many crop plants (Chaman *et al.*, 2001 and Allison and Schultz, 2004). Enhanced peroxidase activity allows the plant to detoxicate the peroxides which reduce the tissue damage (Hildebrand *et al.*, 1986). Information on relationship of peroxidase activity with the incidence of *S. dorsalis* in chilli is rather scarce in published literature, except

Table 1. Estimation of enzymes in leaves of selected chilli germplasm along with the incidence of *S. dorsalis* during 2019-20

Germplasm	Mean population of *S. dorsalis* (Nos./leaf)	Peroxidase (µM $min^{-1}g^{-1}$ protein)	Poly phenol oxidase (µM $min^{-1}g^{-1}$ protein)	Catalase (µM $min^{-1}g^{-1}$ protein)	Category
BC-25	0.72 (0.85)	7.19	0.19	13.51	R
BC-27-2-2	1.18 (1.09)	6.82	0.16	11.55	MR
BC-21	1.22 (1.11)	6.39	0.15	10.24	MR
BC-79-1	1.25 (1.12)	6.25	0.13	9.74	MR
Utkal Abha	1.29 (1.14)	6.18	0.11	8.62	MR
BC-406	1.31 (1.15)	5.84	0.10	7.39	MR
BC-28	1.86 (1.36)	4.42	0.08	6.36	S
LCA-620	1.90 (1.38)	3.83	0.06	5.53	S
BC-78-1-2	1.93 (1.39)	3.32	0.07	4.4	S
BC-24-1	2.38 (1.54)	2.27	0.04	3.33	HS
BC-7-2-1(RC)	0.70 (0.84)	8.23	0.20	14.16	R
Byadagi kaddi (SC)	2.46 (1.57)	1.42	0.03	3.18	HS
SE(m) ±	**0.102**	**0.058**	**0.005**	**0.101**	
CD (5%)	**0.30**	**0.17**	**0.02**	**0.29**	

Table 2. Correlation coefficient (r) of incidence of *S. dorsalis* with enzyme activity of chilli germplasm

Incidence of *S. dorsalis*	Activity of enzymes		
	Peroxidase (µM min^{-1} g^{-1} protein)	**Poly phenol oxidase** (µM min^{-1} g^{-1} protein)	**Catalase** (µM min^{-1} g^{-1} protein)
Population of *S. dorsalis* (No./leaf)	-0.984**	-0.965**	-0.965**

** Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level

Table 3. Multiple linear regression equations depicting the influence of enzyme activity on incidence of *S. dorsalis* in chilli germplasm

Incidence of *S. dorsalis*	Regression Models	Coefficient of determination (R^2)
Population of *S. dorsalis* (No./leaf)	Y1= 8.6710-0.0709*X1-1.0071*X2-0.0179*X3	97.81

Where, Y1 = Population of *S. dorsalis*, **X1=**Peroxidase, **X2=**Poly phenol oxidase, **X3=**Catalase

the report of Meena *et al.* (2008) who found a higher intensity of peroxidase activity in diseased (leaf curl virus) chilli leaf as compared to that of healthy leaf. Increased peroxidase activity might be due to increased phenol concentration which acts as a cofactor of peroxidase, thus influenced the resistance in chilli. The present results get ample support from the findings of earlier researchers *viz.,* Dowd and Lagrimini (2006) and Gulsen *et al.* (2010) who reported the higher activity of peroxidase an important defensive enzyme in plants implicating a broad range resistance mechanism to various insect pests.

Poly phenol oxidase (PPO): The activity of poly phenol oxidase in the leaves of the selected chilli germplasm varied from 0.03 to 0.20 µM min^{-1} g^{-1} protein, the lowest activity being in susceptible check germplasm Byadagi kaddi and highest in the resistant check germplasm BC-7-2-1 (Table 1). Higher poly phenol oxidase activity values (0.10 µM min^{-1} g^{-1} protein to 0.20 µM min^{-1} g^{-1} protein) was registered in the resistant and moderately resistant germplasm *viz.,*BC-7-2-1, BC-25, BC-27-2-2, BC-21, BC-79-1, Utkal Abha and BC-406. The susceptible and highly susceptible germplasm exhibited lower poly phenol oxidase values between 0.03 µM min^{-1} g^{-1} protein (Byadagi kaddi) (susceptible check) to 0.08 µM min^{-1} g^{-1} protein (BC-28).

Poly phenol oxidases are the heme-containing monomeric glycoproteins located in the chloroplasts which are involved in the plant defence system (Saiedian *et al.*, 2007). This enzyme was responsible for phenol buildup because it oxidised O-dihydroxy phenol by which it lowers the availability of proteins, control feeding and growth of pests (Meena *et al.*, 2008; Zhang *et al.*, 2008 and He *et al.*, 2011). The activity of polyphenol oxidase as an important anti-herbivore factors was significantly increased resulting in a substantial decrease in the abundant herbivores, including insects (Thaler *et al.,* 2001). The present findings on activity of polyphenol oxidase in the leaves of selected chilli germplasm are in accordance to the report of Mondal *et al.* (2013) who found that the resistant chilli genotype CUCH-4 recorded the greatest oxidase enzyme activity than the sensitive genotype CUCH-23. A positive correlation between host plant resistance and the amount of phenols and increased activity of peroxidase and polyphenoloxidase has earlier been recorded in chilli (Jabeen *et al.,* 2009 and Chandan *et al.*, 2016).

Catalase (CAT): The activity of catalase in the leaves of twelve test chilli germplasm ranged from 3.18 µM min^{-1} g^{-1} protein to 14.16 µM min^{-1} g^{-1} protein (Table 1). The resistant check germplasm BC-7-2-1 had the highest catalase activity of 14.16 µM min^{-1} g^{-1} protein which was closely followed by the other resistant germplasm BC-25 (13.51 µM min^{-1} g^{-1} protein). The moderately resistant germplasm *viz.,* BC-27-2-2, BC-21, BC-79-1, Utkal Abha and BC-406 had comparatively higher catalase activity value of 11.55, 10.24, 9.74, 8.62 and 7.39 µM min^{-1} g^{-1} protein respectively, than susceptible and highly susceptible germplasm where activity of catalase ranged between 3.18 µM min^{-1} g^{-1}protein to 6.36 µM min^{-1} g^{-1} protein. Lowest activity of catalase was recorded in the susceptible check Byadagi kaddi (3.18 µM min^{-1} g^{-1} protein) followed by highly susceptible germplasm BC-24-1 (6.36 µM min^{-1} g^{-1} protein).

Catalase, a major H_2O_2-scavenging anti oxidant enzyme is involved in the cell wall resistance of plants and it also acts as a signal for the induction of defence genes (Chen *et al.*, 1993). Involvement of catalase activity in the plant defence against the sucking insects has been reported by Hanaka *et al.* (2018). Information on relationship of catalase activity with the incidence of *S. dorsalis* in chilli could not be traced out in literature. However, the fluctuation in catalase activity due to insect feeding has been documented in other crops (Heng-Moss *et al.*, 2004 and Khattab, 2007). Dillwith *et al.* (1991) reported higher activity of catalase in the alfa alfa plants resistant to spotted alfa alfa aphid than the susceptible plants. Black gram genotypes resistant to white fly, *Bemisia tabaci* (Gennadius) exhibited high activities of peroxidase and catalase (Taggar *et al.,* 2012). The results of the present studies are consistent with the findings of those previous workers.

Relationship of enzyme activity of chilli germplasm with incidence of *S. dorsalis*

Results on correlation studies between population of *S. dorsalis* and various biochemical parameters of chilli germplasm revealed that the population of *S. dorsalis* showed significant negative correlation with activity of enzymes *viz.,* peroxidase (-0.984**), polyphenol oxidase (-0.965**), catalase (-0.965**) and the population of *S. dorsalis* (Table 2). The multiple linear regression analysis indicated that various defensive enzymes of chilli germplasm *viz.,* peroxidase (X1=0.0709), polyphenol oxidase (X2=1.0071) and catalase (X3=0.0179) together influenced the population of *S. dorsalis* to an extent of 97.81 per cent (Table 3).

CONCLUSION

The results of the study on induced mechanism of defense in chilli revealed that the resistant and moderately resistant germplasm exhibited higher activity of defense related enzymes *viz.,* peroxidase, polyphenol oxidase and

catalase in the leaf sample. The resistant germplasm *viz*., BC-7-2-1, BC-25 and moderately resistant germplasm *viz*., BC-27-2-2, BC-21, BC-79-1, Utkal Abha and BC-406 had 5.84-8.23 µM min^{-1} g^{-1} peroxidase, 0.1-0.2 µM min^{-1} g^{-1} poly phenol oxidase and 7.39-14.16 µM min^{-1} g^{-1} catalase in the leaf sample respectively, as against 1.42-4.42 µM min^{-1} g^{-1}, 0.03-0.08 µM min^{-1} g^{-1} and 3.18-6.36 µM min^{-1} g^{-1} protein in the leaves of susceptible and highly susceptible check germplasm, respectively. However, the defense mechanisms by which these enzymes are accumulated in chilli remain to be explored in future studies.

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Field evaluation of bitter gourd (*Momordica charantia* L.) genotypes for resistance against melon fly, *Zeugodacus cucurbitae* (Coquillett)

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ABSTRACT: Fifteen bitter gourd genotypes have been evaluated for bio-physical basis of plant resistance against melon fly, *Zeugodacus cucurbitae* (Coquillett) under field conditions during 2019. Among 15 bitter gourd genotypes, six genotypes *viz.,* IC622912, IC599423, IC622913, IC622906, IC599434 and IC622908 showed moderately resistant reaction with significantly lower fruit infestation ranging from 37.78 to 48.44 per cent with larval density vary from 3.37 to 8.69 larvae per fruit. Remaining nine genotypes *viz*., IC616045, IC599421, IC611325, IC599420, IC622909, IC599424, IC599401, IC616046 and Pusa Do Mausami were found susceptible with significantly higher fruit infestation (51.44-74.44%) and larval population (4.83-10.13/fruit). Significant and positive correlation was observed between per cent fruit infestation and larval population per fruit. Fruit infestation was positively correlated with biophysical traits of bitter gourd fruits *viz.,* fruit length, fruit diameter and flesh thickness and negatively with number of ridges/cm2 (middle part) of fruit.

Keywords: Melon fly, bitter gourd, fruit infestation, larval population, plant resistance

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is one of the most significantly growing vegetables in India with an annual production of 1.21 MT from 1.01 lakh ha during 2019-20 (Indiastat, 2019-20). Among all the insect pests infesting bitter guard, melon fly, *Zeugodacus cucurbitae* (Coquillett) is the most serious one. It attacks 61 plant species and 28 of them are cucurbits. Economic impacts are due to its quarantine status (Dey Mayer., 2015). In India, melon fly infests the crop from flowering stage up to harvesting of the fruits and extent of crop losses by the pest varies from 30 to 100 per cent (Shooker *et al.,* 2006). It damages the crop by ovipositional injury by female adults, internal feeding on ovaries and fruit pulp by maggots, and rottening of fly-damaged fruits (Viraktamath *et* al., 2003).

Host plant resistance is one of the important IPM strategies where the morphological and biochemical traits of plant significantly influence the degree of damage by pest. Host plant resistance is an interaction between plant and insect pest which results in undesirable host plant for feeding or oviposition of insect pest. Jaiswal *et al.* (1990) reported that the insects feeding and oviposition was interfered by morphological traits of host plant. Lack of knowledge on the availability of resistance sources limits the development and cultivation of fruit fly resistant bitter gourd varieties in IPM program (Dhillon *et al.*, 2005). Though, chemical management is one of the effective tools for managing this pest, the use of broad-spectrum insecticides may leave residues on fruits which results in health hazards. Thus it is necessary to identify the genotypes and biophysical traits of fruits importing resistance against oviposition and larval feeding activities of the melon fly.

MATERIALS AND METHODS

Field trail was conducted in a pendal system at Horticulture garden, S.V. Agricultural College, Tirupati, Andhra Pradesh during *Kharif*, 2019-2020. Fifteen bitter gourd genotypes including check (Pusa Do Mausami) were collected from the Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi.

Raising of seedlings

Seeds were subjected to hot water treatment at 50°C for 30-40 min followed by soaking of seeds in 0.2 per cent KNO_3 solution for 4 hrs to improve the germination. Seeds were sowed in protrays provided with coco-peat, a growing media under greenhouse condition at Insectary, Department of Entomology. Bitter gourd seedlings of 3-4 leaf stage were transplanted to main field. Each genotype was planted in a single row of 7 m length with spacing of 35 cm within the row and 1m between the rows. The crop was raised by following package of agronomic practices recommended by Dr. YSR Horticultural University, Andhra Pradesh except crop protection practices.

Table 1. Incidence of fruit infestation and larval population of melon fly, *Z. cucurbitae* on bitter gourd genotypes under field conditions

1st Picking		2nd Picking		3rd Picking		Pooled Mean	
Fruit infestation (%)*	Maggot population/ fruit**	Fruit infestation (%)*	Maggot population/ fruit**	Fruit infestation (%)*	Maggot population/ fruit**	Fruit infestation (%)*	Maggot population/ fruit**
43.56^{ab}	3.22^{a}	33.09^{a}	3.56^{a}	36.71^{ab}	4.40^{a}	37.78^{a}	3.73^{a}
(41.30)	(2.05)	(35.12)	(2.13)	(37.29)	(2.32)	(37.90)	(2.17)
47.77^{bc}	5.67^{bcd}	38.89^{ab}	6.08^{bcd}	34.31^{a}	7.15^{abcd}	40.32^{ab}	6.30^{cd}
(43.72)	(2.58)	(38.58)	(2.66)	(35.74)	(2.85)	(39.39)	(2.69)
48.55^{bc}	4.88^{abc}	47.11^{cd}	5.33^{abc}	35.19^{ab}	4.67^{ab}	43.61^{abc}	4.96^{c}
(44.17)	(2.42)	(43.34)	(2.51)	(36.30)	(2.38)	(41.30)	(2.53)
38.33^{a}	8.01^{def}	43.06^{bc}	10.67^{f}	51.08^{bcd}	7.41^{abcd}	44.16^{abc}	8.69^{ef}
(38.25)	(2.99)	(41.01)	(3.41)	(45.62)	(2.87)	(41.63)	(3.10)
50.13^{bcd}	5.58^{bcd}	43.20^{bc}	5.08^{abc}	44.76^{abc}	5.37^{ab}	46.03^{abc}	5.35^{bc}
(45.07)	(2.57)	(41.09)	(2.46)	(41.99)	(2.51)	(42.72)	(2.52)
50.95^{bcd}	6.08^{bcd}	51.69^{d}	6.60^{cde}	42.67^{abc}	6.85^{abcd}	48.44^{abc}	6.51^{cd}
(45.55)	(2.64)	(45.97)	(2.76)	(40.78)	(2.80)	(44.01)	(2.74)
45.15^{abc}	5.13^{abc}	52.35^{d}	4.12^{ab}	56.81^{cd}	4.75^{a}	51.44^{bcd}	4.67^{ab}
(42.22)	(2.46)	(46.35)	(2.26)	(49.01)	(2.36)	(45.83)	(2.38)
56.77^{de}	6.70^{bcde}	48.33^{cd}	7.15^{cde}	53.37^{cd}	6.30^{abc}	52.82^{bcd}	6.71^{cd}
(48.89)	(2.77)	(44.04)	(2.85)	(46.93)	(2.70)	(46.62)	(2.77)
50.39^{bcd}	8.67^{ef}	52.29^{d}	7.21^{cde}	61.33^{de}	6.01^{abc}	54.67^{cd}	7.29^{de}
(45.22)	(3.09)	(46.31)	(2.85)	(51.55)	(2.63)	(47.69)	(2.87)
44.30^{abc}	6.67^{bcde}	63.61^{e}	6.63^{cde}	58.53^{cd}	7.11^{abcd}	55.48^{cd}	6.80^{d}
(41.73)	(2.76)	(52.90)	(2.75)	(50.01)	(2.83)	(48.18)	(2.79)
48.49^{bc}	4.62^{ab}	54.62^{d}	5.33^{abc}	64.44^{de}	4.55^{a}	55.85^{cd}	4.83^{ab}
(44.14)	(2.36)	(47.65)	(2.51)	(53.41)	(2.31)	(48.39)	(2.41)
62.12^{e}	8.76^{ef}	63.35^{e}	8.67^{def}	63.23^{de}	10.33^{d}	62.90^{de}	9.25^{f}
(52.02)	(3.12)	(52.74)	(3.10)	(52.84)	(3.36)	(52.48)	(3.20)
51.67^{cd}	7.25^{cde}	62.29^{e}	7.40^{cde}	75.43^{e}	8.08^{bcd}	63.13^{de}	7.58^{de}
(45.96)	(2.87)	(52.11)	(2.89)	(60.58)	(3.01)	(52.79)	(2.92)
62.39^{e}	8.17^{def}	76.06^{f}	11.18^{f}	74.93^{e}	9.02^{cd}	71.13^{e}	9.45^{f}
(52.17)	(3.02)	(60.71)	(3.49)	(59.97)	(3.16)	(57.61)	(3.22)
69.19^{f}	10.61^{f}	78.73^{f}	9.33^{ef}	75.40^{e}	10.46^{d}	74.44^{e}	10.13^{f}
(56.28)	(3.40)	(62.54)	(3.21)	(60.27)	(3.38)	(59.69)	(3.33)
6.45	0.40	4.30	0.42	8.47	0.56	6.62	0.24
2.21	0.14	1.48	0.14	2.91	0.19	2.27	0.08
7.48	8.62	5.41	8.96	10.46	11.98	8.37	5.23

Within a column, means followed by the same letter are not significantly different by DMRT (P = 0.05; LSD).
Figures in the parentheses are retransformed values (*Arc sign transformation; **Square root transformation).
Fruit infestation is expressed in %; larval population as number of larvae/fruits.

Observations recorded

Observations on per cent fruit infestation and larval population per fruit were recorded by picking marketable size fruits (at 60 days after sowing) from randomly selected five plants from each genotype. Total of three pickings were done at 6-days intervals (Dhillon *et al.*, 2005).

Fruit infestation

Marketable size fruits irrespective of healthy and infested fruits are harvested at 6-days intervals from randomly selected five plants from each genotype and the per cent fruit infestation was worked out.

Larval population

The infested fruits from each genotype were brought to the laboratory for recording observation on larval population per fruit. Five randomly selected fruits from each genotype were cut opened to count the number of larvae per fruit (Gogi *et al.,* 2009).

Biophysical traits of bitter gourd fruits

Biophysical characteristics of fruit *viz.,* fruit length, fruit diameter, flesh thickness and number of ridges per cm^2 of fruit were recorded and correlated with per cent fruit infestation and larval population. Five marketable size fruits were randomly selected from each genotype for recording the biophysical traits (Chillar, 2007).

Fruit length (cm): Length of the fruits was measured with the help of digital vernier calliper.

Fruit diameter (cm): Diameter was measured from the centre of the fruit at two different points with the help of vernier callipers.

Flesh thickness (mm): Flesh thickness was measured at two opposite points with Vernier callipers.

Number of ridges per cm^2: Number of ridges per cm^2 area of each fruit (centre part of the fruit) was recorded.

Categorization of bitter gourd genotypes

The genotypes were grouped into different levels of resistance based on per cent fruit infestation by following rating system given by Nath (1966) (Table 1).

Statistical analysis

Data was subjected to angular and square root transformation in order to achieve normality in the data before analysis. The data on per cent fruit infestation, larval population per fruit and biophysical fruit traits were analysed through one-way ANOVA using SPSS 16 software. The mean values of all the parameters were compared using Tukey's HSD tests at probability level of 5 per cent. Correlations between per cent fruit infestation, larval population per fruit and biophysical fruit traits were determined using correlation analysis at the 95% significance level.

RESULTS

Reactions of bitter gourd genotypes to melon fly infestation

During first picking of fruits (60 DAS; preferably marble sized fruits), highest fruit infestation of 62.39 per cent was recorded in IC616045 followed by IC599421 (62.12%) and IC622909 (56.77%) which were statistically on par with larval population of 8.17, 8.76 and 6.70 per fruit, respectively. Whereas, significantly lowest fruit infestation of 38.33 per cent was recorded in genotype IC622906 with larval population of 8.01 per fruit compared to Pusa Do Mausami (69.19% and 10.61 larvae/fruit of marble size) (Table 1). In second picking (67 DAS), the genotypes IC622912 and IC599423 were recorded with lowest fruit infestation of 33.09 and 38.89 per cent and larval population of 3.56 and 6.08 per fruit, respectively which were statistically on par. Highest fruit infestation of 78.73 per cent was recorded in Pusa Do Mausami (Check) with larval population of 9.33/fruit, followed by IC616045 (76.06%). Genotypes; IC599401, IC599421 and IC611325 were recorded with fruit infestation of 62.29-63.61% with larval density of 6.63-8.74 per fruit, which are statistically at par. In remaining genotypes, fruit infestation was ranging from 43.06 to 54.62 per cent with larval density ranging from 4.12 to 7.21 per fruit (Table 2). During the final third picking of fruit (73 DAS), the genotypes IC599423, IC622913, IC622912, IC622908 and IC599434 were recorded with significantly lowest fruit infestation ranging from 34.31 to 44.76 per cent with larval density ranging from 4.40 to 7.15 per fruit compared to Pusa Do Mausami (75.40% and 10.46 larvae/fruit). Highest fruit infestation of 75.43 per cent and larval density of 8.08 larvae per fruit was recorded in IC611325 followed by IC616045, IC599424 and IC599421 which were statistically on par with fruit infestation of 74.93, 64.44 and 63.23 per cent and 9.02, 4.55 and 10.33 larval population per fruit, respectively.

Overall, mean fruit infestation (average of three pickings) is ranged from 37.78 to 74.44 per cent with mean larval population of 3.73 to 10.13 per fruit. Significantly higher fruit infestation was recorded in IC616045 (71.13%) with larval population of 9.45 per fruit followed by IC599421 (63.13% and 7.58/fruit) and IC611325 (62.90% and 9.25/fruit) which were

Table 2. Grouping of bitter gourd genotypes by following the rating system of Nath (1966)

Category	Type of infestation	Level of infestation (%)	List of Genotypes
Immune (0)	No damage	0	Nil
Highly resistant (HR)	Slight damage	1-10	Nil
Resistant (R)	Slight medium damage	11-20	Nil
Moderately resistant (MR)	Medium damage	21-50	IC622912, IC599423, IC622913, IC622906, IC599434, IC622908
Susceptible (S)	Damage	51-75	IC599424, IC611325, IC616045, IC616046, IC599420, IC622909, IC599421, IC599401, Pusa Do Mausami
Highly susceptible (HS)	Severe damage	76-100	Nil

Table 3. Bio-physical traits of bitter gourd genotypes screened against *Z. cucurbitae* under field conditions

Genotype	Fruit length (cm)	Fruit diameter (cm)	Number of ridges per cm²	Rind thickness (mm)
IC622912	10.20^{b}	3.30^{bc}	30.33^{g}	4.41^{ab}
IC599423	12.30^{cd}	3.15^{bc}	24.73^{cde}	5.20^{cd}
IC622913	9.22^{b}	3.12^{bc}	29.47^{fg}	5.64^{de}
IC622906	9.02^{b}	3.08^{b}	27.60^{efg}	5.99^{ef}
IC599434	12.10^{cd}	3.40^{bc}	24.40^{cd}	4.42^{ab}
IC622908	12.39^{cd}	3.26^{bc}	22.13^{c}	4.88^{bc}
IC616046	4.35^{a}	2.52^{a}	42.53^{h}	6.46^{f}
IC599401	9.96^{b}	3.34^{bc}	27.47^{defg}	4.17^{a}
IC599424	13.55^{de}	4.53^{e}	25.60^{de}	6.41^{f}
IC622909	12.36^{cd}	3.39^{bc}	27.61^{efg}	5.09^{cd}
IC599420	11.73^{c}	3.60^{cd}	27.20^{def}	5.50^{cde}
IC611325	5.07^{a}	3.48^{bcd}	26.13^{de}	5.59^{de}
IC599421	14.51^{e}	3.92^{d}	24.80^{cde}	5.86^{ef}
IC616045	9.57^{b}	3.89^{d}	8.01^{a}	5.85^{ef}
Pusa Do Mausami	13.50^{de}	3.28^{bc}	19.07^{b}	4.91^{bc}
C.D.	1.42	0.43	2.78	0.56
SE(m)	0.48	0.15	0.95	0.19
C.V.	7.94	7.65	6.40	6.26

Within a column, means followed by the same letter are not significantly different by DMRT (P = 0.05; LSD).

statistically on par. Significantly lower fruit infestation was observed in genotype IC622912 with larval density of 3.73 per fruit followed by IC599423 (6.30/fruit), IC622913 (4.96/fruit), IC622906 (8.69/fruit), IC599434 (5.35/fruit) and IC622908 (6.51/fruit) were statistically on par. Fruit infestations of melon fly on remaining genotypes were varied from 51.44 to 55.85 per cent with larval population 4.67 to 7.29 per fruit. The susceptible check Pusa Do Mausami was recorded with highest fruit infestation of 73.54 per cent with larval population of 18.78 per fruit.

Grouping of bitter gourd genotypes based on fruit infestation

Fifteen genotypes of bitter gourd were grouped based on per cent fruit infestation by following the Nath's system of classification (Table 2). Out of fifteen genotypes, none of the genotype was found resistant to melon fly. However, six genotypes *viz.,* IC622912, IC599423, IC622913, IC622906, IC599434 and IC622908 were categorized as moderately resistant as the percentage of fruit infestation ranged between 37.78 to 48.44% with larval density varied from 3.37 to 8.69 per fruit. Whereas, the remaining nine genotypes *viz.,* Pusa Do Mausami, IC616045, IC599421, IC611325, IC599420, IC622909, IC599424, IC599401 and IC616046 were found susceptible with fruit infestation of 74.44, 71.13, 63.13, 62.90, 55.85, 55.48, 54.67, 52.82 and 51.44 per cent, respectively with larval population ranged from 4.83 to 10.13 per fruit (Table 1).

Average larval population per fruit was significantly lower (3.73-8.69/fruit) in moderately resistant genotypes compared to susceptible genotypes (4.67-10.13/fruit) (Table 1). This indicated that larval population was significantly and positively correlated (r=0.737) with fruit infestation. Observation on melon fly infestation on bitter gourd genotypes at different intervals reveals that fruit infestation was decreased with increase of crop maturity in moderately resistant genotypes. While in susceptible genotypes the per cent fruit infestation was increased as the crop reaches maturity phase.

Influence of biophysical traits of bitter gourd fruits on fruit infestation and larval population of melon fly in bitter gourd genotypes

Observations on biophysical traits of fruit *viz.,* fruit length, fruit diameter, number of ridges per cm^2 and flesh thickness of all the fifteen genotypes were recorded and presented in Table 4. Influence of biophysical traits of bitter gourd fruits are correlated with fruit infestation and larval population/fruit (Table 4).

Fruit length (cm)

Significant differences were found among the genotypes with respect to fruit length where, fruit length of moderately resistance genotypes is varied from 9.02 (IC622906) to 12.39 cm (IC622908). Whereas in case of susceptible genotypes, fruit length is ranged from 4.35 (IC616046) to 14.51 cm (IC599421). Per cent fruit infestation (r=0.097) and larval population per fruit (r=0.079) are positively correlated with fruit length (Table 4).

Fruit diameter (cm)

Moderately resistant genotypes showed varied fruit diameter varying from 3.08 (IC622906) to 3.40 cm (IC599434) and in susceptible genotypes it is varied from 2.52 (IC616046) to 4.53 cm (IC599424). Per cent fruit infestation (r=0.391) and larval population per fruit (r=0.319) was positively correlated with fruit diameter (Table 4).

Number of ridges per cm^2

Ridge density of fruit showed negative correlation with fruit infestation (r=-0.533) and larval population (r=-0.635). In which significantly higher fruit infestation (71.13%) and larval population (9.45/fruit) was recorded in IC616045 with ridge density of 8.01 per cm^2 whereas the genotype IC622912 with 30.33 ridges per cm^2 is recorded with significantly lower fruit infestation (37.78%) and larval population (3.73/fruit) (Table 5).

Rind thickness (mm)

Rind thickness was found comparatively higher in susceptible genotypes ranging from 4.17 (IC599401) to 6.41mm (IC599424) than moderately resistance genotypes which varied from 4.41 (IC622912) to 5.99 mm (IC622906). Per cent fruit infestation (r=0.220) and larval population per fruit (r=0.204) was positively correlation with rind thickness (Table 4).

DISCUSSION

Field screening of 15 bitter gourd genotypes for sources of resistance against melon fly was conducted under field condition, all the genotypes showed varied reactions to melon fly infestation. Among 15 genotypes, none of the genotypes shown resistance to melon fly. While, six genotypes *viz*., IC622912, IC599423, IC622913, IC622906, IC599434, and IC622908 were categorized as moderately resistant with significantly lower per cent fruit infestation and larval population. The nine genotypes *viz*., IC616045, IC599421, IC611325, IC599420, IC622909, IC599424, IC599401, IC616046 and Pusa Do Mausami (check) showed susceptible reaction with highest per cent fruit infestation. Larval

Table 4. Correlation coefficient (r) between percent fruit infestation and larval population per fruit with different bio-physical traits of fruits of different bitter gourd genotypes

Fruit Parameter	r value with Fruit infestation	r value with Larval population
Fruit length	0.097^{NS}	0.079^{NS}
Fruit diameter	0.391^{NS}	0.319^{NS}
Ridges cm^{-2}	-0.533^{*}	-0.635^{*}
Rind thickness	0.220^{NS}	0.204^{NS}

population showed significant and positive correlation with fruit infestation whereas larval population was significantly higher in susceptible genotypes compared to moderately resistant genotypes. Highest larval population in susceptible genotypes could be due to increased length of the fruits vary from 12.66 to 13.55 cm.

These results were similar to the findings of Dhillon *et al.* (2005) who found that the bitter gourd genotypes *viz.,* IC-256185, IC-248256, IC-213311, IC-248282, IC-256110 and IC-248281 as resistant sources to melon fly with less percentage of fruit damage (8.3-12.6%) and less larval population (3.8-5.10 larvae/fruit). Similarly, Chillar (2007) found six resistant bitter gourd genotypes *viz.,* IC-213311, IC-256185, IC-248256, IC-248282, HK-127 and MC-58 with fruit infestation 11.05 to 21.40 per cent. Singh *et al.* (1977) also reported the lowest per cent of fruit infestation by melon fly in bitter gourd cultivar BG-12 (29.4%) and highest incidence (48.7%) in BG-9 and BG-11.

The results of present investigation are corroborated with the findings of following Authors; Gogi *et al.* (2009), grouped the genotypes; Col-II and FSD-long as resistant with fruit infestation of < 20 per cent and 1-3 maggots per fruit. Moderately resistance genotypes; Col-Nakana sahib, Col-I and GS-21 with 20-50 per cent of fruit infestation and 3 to 6 maggots per fruit. Eight genotypes *viz.,* Col-III, Col-Multan, Col-Vehari, Chaman, Sunder-F1, Janpuri, F1-484 and F1-485 were found susceptible with 50-80 per cent of fruit damage and 6-10 maggots/ fruit. Virendra *et al.* (2010) reported that bitter gourd genotypes *viz.,* IC-213311, IC-256185, IC-248256, IC-248282, MC-58 and HK-127 as resistant (1-10% fruit infestation) and the genotype IC-85619-A as highly susceptible (76-100%). Also, Panday *et al.* (2012) found that bitter gourd genotype IC 248282 as resistant with 13.64 per cent of fruit infestation followed by Kerala collection - 1 (15.68%) and IC 68314 (18.1%). Katiyar *et al.* (2014) reported the highly resistant genotypes (IC 68314 and IC 248256) with lowest fruit infestation (8.09% and 9.01%, respectively) and Arka Harit was grouped as highly susceptible with 78.20 per cent of fruit infestation. Recently, Koushik *et al.* (2019) reported that the genotype US-6214 and Meghnad-2 showed resistant reaction melon fruit fly with fruit damage of 14.50 and 16.50 per cent and larval density of 3.63 and 4.08/fruit, respectively.

Biophysical traits of bitter gourd fruits *viz.,* fruit length, fruit diameter and flesh thickness has non-significant and positive correlation with fruit infestation and larval population per fruit. Whereas, number of ridges per cm^2 of fruit was found to be negatively correlated with fruit infestation and larval population per fruit. Dhillon *et al.* (2005) reported six resistant wild accessions *viz*., IC 256185, IC 248256, IC 213311, IC 248282, IC 256110 and IC 248281 with fruit infestation of 7.26 to 15.20 per cent and 3.8 to 5.7 of larvae per fruit. Two susceptible cultivar Arka Harit and Pusa Do Mausami were recorded with fruit infestation and larval population of 65.5 per cent and 8.0/fruit, 69.5 per cent and 7.8/fruit, respectively. They also found that larval density per fruit was found positively and significantly correlated (P=0.001) with per cent fruit infestation. Both per cent fruit infestation and larval density per fruit were found positively and significantly correlated (P=0.001) with fruit length (r=0.62 and r=0.72, respectively), fruit diameter (r=0.65 and r=0.63, respectively) and flesh thickness (r=0.92 and r=0.77, respectively); negatively and significantly correlated (P=0.00) with number of ribs per cm square. Present findings are on par with findings of Tewatia (1994) that, out of fifty-five genotypes of bitter gourd screened against melon fly, two genotypes, Faizabad Collection-17 and Kerala Collection-1 were found resistant with 12.19 and 15.90 per cent fruit

infestation, respectively and two genotypes, Arka Harit (84.46%) and Pusa Do Mausami (87.00%) were found highly susceptible. Maximum fruit length and diameter was recorded in susceptible genotype Pusa Do Mausami (12.76 cm) and Arka Harit (3.75 cm), respectively. Whereas, in resistant genotype Faizabad Collection-17; fruit length and diameter are 8.60 cm and 2.78 cm, respectively. The maximum flesh thickness of 6.60 mm was recorded in highly susceptible genotype Pusa Do Mausami and lower flesh thickness in resistant genotype Faizabad Collection-17 (5.73 mm) and Kerala Collection-1 (5.13 mm). Similarly, Devaraj *et al.* (2018) classified genotypes; UHSBRG-5, UHSBRG-15, UHSBRG-12, UHSBRG-18, UHSBRG-19, UHSBRG-17, UHSBRG-9, UHSBRG-1, UHSBRG-6, UHSBRG-16 and UHSBRG-13 under resistant category with 11 to 20 per cent of fruit infestation. Boller and Prokopy (1976) reported the potential regulatory action of morphological factors of host plant *viz.,* hairiness, color, smell, fruit structure *etc*., on host preference of fruit fly in cucurbit. Thakur *et al.* (1996) reported similar findings that, the per cent fruit infestation and larval population per fruit were significantly and positively correlated with each other and further significant and positive correlation of these two were observed with fruit length, fruit diameter and flesh thickness. Combined effects of flesh thickness and fruit diameter results in 93 per cent of variation in fruit fly infestation and larval population per fruit followed by flesh thickness and fruit length (76.3%). Similarly, Chillar (2007) found IC-213311, IC-256185, IC-248256, IC-248282, HK-127 and MC-58 as resistant sources with 11.05 to 21.40 per cent of fruit infestation and 2.55 to 4.27 larvae per fruit, where the fruit infestation was positively correlated (P=0.01) with number of larvae per fruit (r=0.96). The fruit infestation was positively correlated (P=0.05) with fruit length (r=0.53), fruit diameter (r=0.64), flesh thickness (r=0.44) and negatively correlated with number of ridges per cm^2 (r=-0.46). Lasker and Chatterjee (2013) reported the effect of morphological traits on fruit infestation and larval density of melon fly on ten bitter gourd cultivars and recorded the significant positive correlation (r=0.48) of maggot density per fruit with per cent fruit infestation. The fruit weight (r=0.76 and 0.75), fruit length (r=0.0.71 and 0.72) and fruit diameter (r=0.68 and 0.60) was positively correlated fruit infestation and larval density per fruit, where ribs density (r=-0.78 and -0.73), ribs depth (r=0.-24 and -0.18) and skin toughness (r=-0.80 and -0.84) are negatively correlated. Fruit fly ovipositor penetration was inhibited due to hard rind of the fruits (Gichimu *et al.,* 2008)

CONCLUSION

Genotypes such as IC622912, IC599423, IC622913, IC622906, IC599434 and IC622908 can be exploited as the sources of host plant resistance against melon fly. Biophysical traits of bitter gourd fruits (fruit length, fruit diameter and flesh thickness) are positively correlated with fruit infestation by melon fly. Whereas number of ridges/cm^2 of fruit is negatively correlated with melon fly infestation, which can be exploited in resistance breeding programmes.

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Translocation of *Bacillus thuringiensis kurstaki* strain HD-1in brinjal seedling by different inoculation methods

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ABSTRACT: In the present study, *Btk* strain HD1 was inoculated at a concentration of 10^8 cfu/ml to brinjal in three methods under pot culture studies. The methods include seed treatment, soil application and foliar spray. *Btk* strain HD1 from leaves of seedlings was recovered in all the application methods. In seed treatment,*Btk* HD1 was detected in leaves after 30th day after inoculation while in soil application and foliar spray the *Btk* HD1 was detected upto 10th DAI. Further the colonies recovered from leaf tissues collected from three inoculation methods were examined for morphological, spore crystal formation and*cry*1 gene amplification. This is the first kind of report regarding the endophytic ability of *Btk* strain HD1 in brinjal by which the approach could be a possible way for the development of insect resistant crop for sustainable agriculture.

Keywords: *Bacillus thuringiensis*, endophytic ability, brinjal

INTRODUCTION

Brinjal or Eggplant (*Solanum melongena* L.) often called as king of vegetables belongs to family Solanaceae and is originated in India. India holds second rank next to china in brinjal production. Brinjal shoot and fruit borer, *Leucinodes orbonalis* is the serious pest and the incidence is still in alar`ming stage despite the continuous use of many synthetic chemicals. As an alternative to synthetic chemicals, microbial pesticides based on fungi, bacteria, viruses and nematodes have been used against various major field pests due to their target specificity and safety to non-target organisms and environment. *Bacillus thuringiensis* (*Bt*) is a well known commercial entomopathogen with more than 240 holotypes of cry toxins that are active against Lepidoptera, Diptera, Coleoptera and Hymenoptera. *Bacillus thuringiensis* with its specific crystal tox in production has been occupied 90% share in biopesticide market. Furthermore, the efficacy of *B. thuringiensis* strains against insects from Lepidoptera, Coleoptera, Diptera and Hemiptera onvarious crops in India have already been documented (Dharavath *et al.*, 2016; Kumar *et al.*, 2019; Rajashekhar *et al.*, 2018a & 2018b and Tripathi *et al.*, 2016). However, the beneficial features of *Bt* are not limited to its role as an insecticide; it is also used as plant growth promoter by encompassingin nutrient uptake (Azizoglu, 2019; Lee *et al.*, 2009; Qi *et al.*, 2016 and Raddadi *et al.*, 2007).Certain entomopathogenic *Bt* strains were reportedto colonize various host plants keeping their efficacy against target pests either by toxin production or altering the plant physiology besides showing plant growth promotion and extra cellular enzymes production. Additionally, *B. thuringiensis* which is a well-known entomopathogen has been reported as an endophytic bacterium in plants such as cotton, soybean, maize, sugarcane, and cabbage (Subrahmanyan *et al.*, 1983; Suzuki *et al.*, 2008; Praca *et al.*, 2012).

The ability of *B.thuringiensis* to colonize endophytically has also been identified as an important feature to actas a biological control agent against pests. Several authors have reported the different delivery methods of endophytic *Bacillus* spp. to host plants (Algam *et al.*, 2005; Bressan and Borges, 2004; Jahanian *et al.*, 2012; Siddiqui and Shaukat, 2002 and Zhang *et al.*, 2009) as well as colonization of *Bacillus* spp. in the target host plants (Fan *et al.*, 2011; Nair *et al.*, 2002; Liu *et al.*, 2006 and Yan *et al.*, 2003) and biocontrol of pests (Disi *et al.*, 2018; Gadhave and Gange,2016 and Zehnder *et al.*,1997).Additionally, tracking the presence of inoculated bacterial strain in the interior plant parts (root,stem and leaf) among the overcrowded microbes by a selective detection methods such as reportergene technology which is found to be an important approach that is used to monitor micro organisms in various environmental samples (Ramos *et al.*, 2000).Hence, with this background the present study on endophytic ability of *Btk* strain HD1 brinjal was investigated.

MATERIALS AND METHODS

Preparation of *Bacillus thuringiensiskurstaki* strain HD-1

For seed treatment and other inoculation methods, bacterial inoculum was prepared with a 24h old bacterial

culture grown on LB agar. The fresh actively grown cell culture then transferred to sterile falcon tubes and centrifuged 8000 rpm for 10 min at 30°C by using sterile oak ridge tubes, pellets were prepared and stored at 0°C for future use. Subsequently, the supernatant was discarded and the pellet was re suspended in 10ml sterile distilled water. The amount of inoculum used for the experiment was OD 600 = 0.8 (10^8 cfu /ml).The surface sterilized seeds were allowed to soak in the bacterial suspension (10^8 cfu/ml) for 3h and dried on sterilized tissue paper before sowing in the potted soil. For control, seeds were soaked in sdw and sown in pots. In soil application method, *Btk* HD1 @ 5ml (10^8cfu /ml) was given near the root zone of each seedling of one month old seedlings. In case of control, sdw @ 5ml was given to each seedling. One month old seedlings were given foliar spray at a concentration of 1x10^8cfu /ml until the leaves become wet.

Lay out of Pot Experiment

The potting mixture was prepared with soil, vermiculite and coco peat in 1:1:1 ratio and autoclaved for three times at 24 h interval. Pots were filled with material. Seeds of brinjal (variety: Bhagyamati) were washed first with tap water thrice, followed by 70% ethanol for 5 min and 2% sodium hypochlorite for 5min and then 3 times washing with sterile distilled water. To ensure that the seed samples had been completely surface disinfected, a volume of 100 µl of last wash water was plated on LBAgar plate and incubated at 30 °C for 48 h; if colonies were found the samples were discarded. Seeds were sown in the 15cm x 10cm size pots and two seedlings were maintained per pot during the experiment. The seedlings were maintained in plant growth chamber under 12 h dark +12 h light period with 30°C temperature and 65-70% relative humidity and watered regularly.

Reisolation and morphological confirmation of *B. thuringiensis* colonies recovered from leaves

Leaves were collected randomly from brinjal seedlings on 3DAI and 10th DAI in soil application and foliar spray treatments while in seed treatment the leaves from seedlings were collected on 15th DAI and 30th DAI. Leaves were macerated and diluted solutions were plated on selective antibiotic plates. Colonies that grew on selective LBA plates similar to the colonies on the reference plates were examined for colony morphology (Algam *et al.*, 2005 Bressan and Borges, 2004 and Tanuja *et al.*, 2013). After confirmation on the plates the recovered colonies of each *Bt* strain were further inoculated into LB broth with selective antibiotic markers and kept for incubation at 30 °C for 48h to 72h and examined vegetative cells as well as crystal and spores in cells under phase contrast microscope in order to confirm the reisolated colonies were similar to inoculated *Btk* strain HD1.

Molecular confirmation of *Btk*HD-1 colonies recovered from leaves

Confirmation of recovered *Bt* colony identity by PCR method was done as per Algam *et al.*, 2005 and Maduell *et al.*, 2007 with little modification. To ensure the confirmation of the recovered *Btk* strain HD1 corresponded to the inoculated *Btk* strain HD1, *cry*1 gene primers (FP-CATGATTCATGCGGCAGATAAAC;RP-TTGTGACACTTCTGCTTCCCATT) were used. A loopful cells was transferred to 100 µl of sterile distilled water, and the mixture was frozen for 20 min at -80°C and then transferred to boiling water for 10 min to lyse the cells. The resulting cell lysate was centrifuged at 10,000 rpm for 30 s and supernatant was used as a DNA template in the PCR. DNA template (10 µl) was mixed with reaction mixture of 4.3 µl consisting of Taq assay buffer (10x) with $MgCl_2$ (15 mM),1µl dNTPs (10 mM), 1 µl of each primer (10 pM), 0.2 µl Taq DNA polymerase (5 U/µl) and 7.5 µl nuclease free water. The reactions were placed in a thermocycler (Flexigene Techne, UK) programmed as initial denaturation at 94 °C for 5 m followed by 35 cycles of denaturation at 94 °C for 1 m, annealing at 50 °C for 1 m, extension at 72 °C for 2 min and a final extension at 72°C for 5 m. After amplification, 2 µl of loading buffer (0.5% bromophenol blue in glycerol 50%) was added to 5µl of each amplified PCR product and were electrophoresed (at 70 volts for 10 min, followed by 90 volts for 45 min) on a 1x Tris-acetate-EDTA (TAE with ethidium bromide) buffer in 1.2% agarose gel. Gels were visualized in a gel documentation system (AlphaimagerTM) and analyzed with Alpha EaseFC.

RESULTS AND DISCUSSION

Morphological confirmation of *Btk*-HD1 colonies recovered from leaves

The colonies observed on antibiotic amended nutrient agar plates were found to be similar to that of original colonies of *Btk* strain on the basis of colour, elevation and margin of the colony. *Btk*-HD1 colonies were confirmed by white, small and rough margins.No *B.thuringiensis* colonies were appeared on control plates. Further confirmation of colony as *B. thuringiensis* was done by examination of cells, spores and crystal inclusions for *B. thuringiensis* strain under phase contrast microscope (Plate 1). Recovery of *B. thuringiensis* from leaf tissues of brinjal seedlings from seed treatment, soil application and foliar spray confirmed that *Btk*-HD1 was able to colonize leaf tissues of brinjal seedling.

Confirmation of *cry* gene in colonies of *Btk*-HD1 recovered from leaves through PCR

PCR studies showed that the *Btk*-HD1 reisolated from leaves collected from plants inoculated by seed treatment on 15th day after inoculation (DAI) soil application on 3rdDAI and foliar spray on 3rdDAIamplified expected amplicon size of 276 bp of *cry*1 gene. Thus, proved that the *Bt* strain *Btk* -HD1 was successfully colonized the seedlings of brinjal and translocated to leaves from seed, soil and phylloplane of leaves. Further, *Bt* strain reisolated on 30th DAI of seed treatment, 10th DAI of soil application and foliar spray also amplified 276 bp band of *cry* 1 gene (Fig.1) indicated that the *Bt* strains could persist in the leaves in all the inoculation methods.

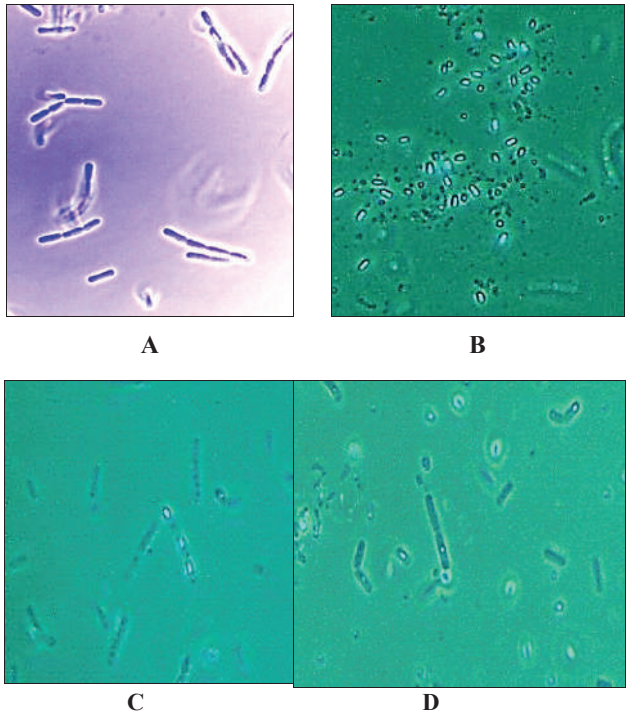

Plate 1. Phase contrast microscopic images of cells and spore crystals of *Bacillus thuringiensis* sub species *kurstaki* strain HD-1recovered from leaves after seed treatment (B), soil application(C) and foliar spray (D). Original culture of *Btk*-HD1 (A)

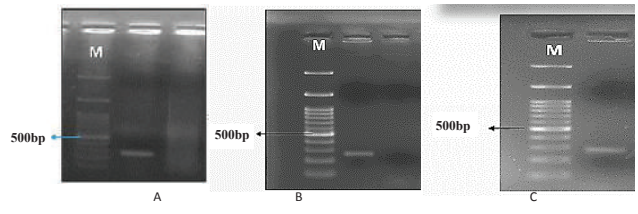

Fig. 1 Amplification of *cry1* gene (276 bp) in colonies of *Bacillus thuringiensis* re isolated from leaf samples of brinjal seedlings from seed (A&B) and soil treatment (C); Lane M: Marker

Only a few studies are reported so far related to colonization of potential entomopathogenic bacteria (*B.thuringiensis*) as endophytes. In our study the main emphasis has been given on detection of *Bt* inside the brinjal leaf tissue rather than root and stem tissues as the neonates of brinjal shoot and fruit borer tend to migrate towards leaf veins and more specifically leaf midribs immediately after hatching (Hanur *et al.*, 2014).Based on the results it can be understood that the *Btk*-HD1 find some favorable site inside the seed tissues by which proliferation could be possible. Earlier, similar findings have been reported by Tanuja *et al.* (2013), showed that *Bt* strains were able to colonize different host plants such as ricebean, soybean, gahat and lentil seedlings and were recovered from leaf sections after 45 days of sowing. In another report, three *Bt* strains possessing high activity against lepidopteran pests exhibited endophytic ability in cabbage seedlings after seed treatment (Praca *et al.*, 2012). In soil application *Btk*-HD1 was detected in leaf tissues of brinjal seedlings and it has been evident that the *Bt* might have entered into root system initially from soil followed by ascending migration into aerial parts probably via xylem vessels. *Bt* strains may get entry into root tissue via germinating radicles (Huang, 1986) or natural wounds due to root growth or through root hairs and at epidermalconjunctions.Hallmann *et al.*, 1997; McCully 2001 reported that bacteria may get entry into leaf tissues during foliar spray due to the possible entry via stomata, wounds and even by means of ability of production of hydrolytic enzymes.

The present investigation clearly demonstrated that *Btk* strain HD1 has the potential to be associated with brinjal as an endophyte as it gain access into the interior of brinjal root, stem and leaf. It was evident that *Btk* strain HD1 showed colonization inside the brinjal seedling as it migrated endophytically upward into stem and leaves of the plants. The new findings indicated that thorough attention need to be paid on beneficial plant bacterium interactions and its potential application for the sustainable management of major pest brinjal shoot and fruit borer, *L.orbonalis*.

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Efficacy of biorational insecticides and entomopathogenic nematodes (EPNs) against brinjal ash weevil, *Myllocerus subfasciatus* Guerin-Meneville (Coleoptera: Curculionidae)

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ABSTRACT: Field experiments were conducted during 2020-21 to evaluate different biorational insecticides and entomopathogenic nematodes against ash weevil, *Myllocerus subfasciatus* Guerin-Meneville. The results revealed that the IPM Module 3 [comprised of soil application of *Metarhizium anisopliae* @ 5 kg/ha followed by soil drenching with EPN *Heterorhabditis indica* @ 20 kg/ha at 30 DAT and foliar spray with *M. anisopliae* @ 5kg/ha at 45 DAT] was found superior with the lowest number of *M. subfasciatus* adults per plant (mean ± SE) (2.06 ± 0.07) and the lowest leaf damage (4.35 ± 0.59 per leaf per plant), followed by the IPM Module 2 [comprised soil application of neem cake followed by application of entomopathogenic nematode *H. indica* @ 20 kg/ha at 30 DAT and foliar application of *B. bassiana* @ 5 kg/ha at 45 DAT] which was also found significant in reducing the number of *M. subfasciatus* and leaf damage by ash weevil (2.30 ± 0.15; 4.85 ± 0.69 respectively), compared to untreated check (2.91 ± 0.13, 5.63 ± 0.34 respectively).

Keywords: Ash weevil, *Myllocerus subfasciatus*, EPNs, *Heterorhabditis indica*

INTRODUCTION

Brinjal or aubergine (*Solanum melongena* L.) is the most widely used vegetable across many countries *viz*., Central, South and Southeast Asia, some parts of Africa and Central America (Harish *et al.*, 2011). It is native to India and grown in almost all parts of India. In India, brinjal is cultivated in 7.6 lakh hectares with a production of 12695 MT (average productivity of 17.5 t/ha). Ash weevil, *Myllocerus subfasciatus* Guerin-Meneville is the most widely distributed across India and attained major pest status in brinjal crop. Adults lay eggs in the soil and grubs are exclusively root feeders resulting in stunting and wilting of the plant. Adults feed on the foliage of brinjal and other hosts by making characteristic notches along the leaf margins. Since, the grubs have subterranean nature of feeding there is difficulty in managing this notorious pest. Nagesh *et al.* (2016) reported that *M. subfasciatus* is considered as quarantine pest as the immature stages can be easily disseminated through the transportation of planting material. Regular surveys and interactions with farmers revealed that the ash weevil is a major pest after the shoot and fruit borer, *Luecinodes orbonalis* Guenee and under favourable conditions, ash weevils have the potential to cause 100% yield loss in brinjal crop (Shanmugam *et al.*, 2018; 2021).

The farmers depend on soil and foliar application of insecticides to manage grubs and adults in brinjal. As the control options for this pest mainly rely on the use of chemical pesticides, concerns about environmental safety, insecticide resistance and effects on non-target organisms are often raised. This alarming situation has increased the pressure to shift from chemical-intensive management to alternative eco-friendly management strategies**.** Entomopathogenic nematodes (EPNs) in the genus *Steinernema* and *Heterorhabditis* and their associated bacteria (*Xenorhabdus* spp.) have been successfully commercialized as potent biological control agents for a variety of curculionid species. These EPNs: can kill hosts rapidly, are easy to apply, and are exempt from federal and local registration requirements in most countries because of their safety for mammals and plants (Georgis *et al.*, 1991). Therefore, certain biorational insecticides (neem cake, NSKE 5%, neem oil 3%, *Metarhizium anisopliae, Beaveria bassiana*) and EPNs are exploited for the management of the ash weevil damage in the brinjal crop.

MATERIALS AND METHODS

This study was conducted for two seasons in *rabi,* 2020 and *kharif,* 2021 at an experimental block of ICAR-Indian Institute of Horticultural Research, Bengaluru, India [13° 8’ 18’’ N and 77° 28’ 40’’ E 890m amsl]. The seeds of brinjal *cv*. Arka Harshitha were sown in portrays and thirty days old seedlings were transplanted in an area of 45m x 1m. The experiment was laid out in

Table 1. Treatment details

IPM Module		Details
M1	Module 1	➢ Soil application of neem cake @250kg/ha before transplanting ➢ Soil drenching of NSKE 5% @25kg/ha at 30 DAT (days after transplanting) ➢ Foliar spray with neem oil 3% at 45 DAT
M2	Module 2	➢ Soil application of neem cake @250kg/ha before transplanting ➢ Application of EPN *Heterorhabdidtis indica* @ 20kg/ha at 30 DAT ➢ Foliar spray with *Beaveria bassiana* 1x10^8 CFU/g/mL @ 5kg/ha at 45 DAT
M3	Module 3	➢ Soil drenching with *Metarhizium anisopliae* 1x10^8 CFU/g/mL @ 5kg/ha ➢ Soil application of EPN *H. indica* @ 20kg/ha at 30 DAT ➢ Foliar spray with *M. anisopliae* 1x108 CFU/g/ml @ 5 kg/ha at 45 DAT
M4	Module 4	➢ Farmers practice Soil application of Carbofuran 3G@15kg/ha ➢ Soil drenching with Chlor pyriphos 20EC @ 2.5 L/ha at 30 DAT ➢ Foliar spray with Fipronil 5SC @ 750 mL/ha at 45 DAT
M5	Module 5	➢ Untreated check

randomized block design (RBD). All the recommended package of practices were followed. For managing shoot and fruit borer, pheromone traps and tricho cards of egg parasitoid, *Trichogramma chilonis* were used. No other plant protection measures were implemented other than the treatments. A total of five integrated pest management (IPM) modules were evaluated against ash weevil, *M. subfasciatus*. Each module was replicated three times. The treatment details are as follows.

Observations were recorded during *rabi,* 2020 and *kharif,* 2021, on the ash weevil feeding damage based on the visual scoring of the leaf damage on 0.00 -10.00 scale, where 0.00 = no damage; 1.00 = 10%; 2.00 = 20% 3.00=30%; 4.00=40%; 5.00=50%; 6.00=60%; 7.00=70%; 8=80%; 9.00=90%; 10.00 = 100% leaf damage. Data was recorded randomly on 10 plants per replication per module and in each plant, leaves were scored randomly (n = 10) for leaf damage by ash weevil. The number of adults present per plant (n=10) were also recorded.

Statistical analysis

The data on the mean leaf damage and mean number of ash weevil present in each module for each season were subjected to one-way ANOVA. The statistical analysis was performed using GraphPad Prism (v 9.3.1) software. The means were compared using the Tukey's multiple comparison test.

RESULTS AND DISCUSSION

Attempts to identify alternate IPM module has been planned to combat the ash weevil menace with selected biorational insecticides and EPNs. Different IPM modules were evaluated and the results on the mean leaf damage and mean number of *M. subfasciatus* analyzed across the seasons *rabi* and *kharif* during 2020-2021 are given below.

***Rabi*, 2020**

Leaf damage: Data revealed that there was a statistically significant difference in the mean leaf damage among the IPM modules evaluated ($F_{4,15} = 40.5$, $P < 0.0001$). All the modules were significantly superior over untreated check, Module 5 (Leaf damage, mean ± SE; 9.25 ± 0.24). The Module 1 (6.03 ± 0.05) and Module 3 (4.58 ± 0.35) ($P = 0.019$); Module 1 (6.06 ± 0.05) and Module 5 (9.25 ± 0.24) ($P < 0.0001$); Module 2 (5.13 ± 0.41) and Module 5 (9.25 ± 0.24) ($P < 0.0001$); Module 3 (4.58±0.35) and Module 4 (5.69 ± 0.21) ($P<0.0001$) were significantly different with each other with respect to leaf damage by ash weevil.

Leaf damage caused by ash weevil was not significantly different between Module 1 (6.03 ± 0.05) and Module 2 (5.13 ± 0.41) ($P = 0.22$); Module 1 (6.03 ± 0.05) and Module 4 (5.69 ± 0.21) ($P = 0.91$); Module 2 (5.13 ± 0.41) and Module 3 (4.58 ± 0.35) ($P = 0.65$); Module 3 (4.58 ± 0.35) and Module 4 (5.69 ± 0.21) ($P = 0.09$) (Fig.1A).

Ash weevil adults: No significant difference was observed in the mean number of *M. subfasciatus* adults in different modules ($F_{4,15} = 7.929$, $P = 0.143$). The mean number of *M. subfasciatus* adults were not significantly different between Module 1 (2.11±0.05) and Module 2

(2.29 ± 0.22) (*P = 0.772*); Module 1 (2.11 ± 0.05) and Module 3 (1.93 ± 0.02) (*P = 0.98*); Module 1 (2.11 ± 0.05) and Module 4 (2.36 ± 0.06) (*P = 0.512*); Module 2 (2.29 ± 0.22) and Module 3 (1.93 ± 0.02) (*P = 0.305)*; Module 3 (1.93 ± 0.02) and Module 4 (2.36 ± 0.06) (*P = 0.151*); Module 4 (2.36 ± 0.06) and Module 5 (2.79 ± 0.02) (*P = 0.091)*. Whereas, significant differences in the mean number of ash weevils were observed between the Module 1 (2.11 ± 0.05) and Module 5 (2.79 ± 0.02) (*P = 0.0043*); Module 2 (2.29 ± 0.22) and Module 5 (2.79 ± 0.02) (*P = 0.039*); Module 3 (1.93 ± 0.02) and Module 5 (2.79 ± 0.02) (*P = 0.0008*) (Fig.1B).

***Kharif,* 2021**

Leaf damage: Statistically significant difference was observed in mean leaf damage among the IPM modules ($F_{4,15}$ *= 101.4, P < 0.0001*). All the modules were significantly superior over untreated check, Module 5 (9.16 ± 0.29). Statistically significant difference was observed between Module 1 (6.07 ± 0.06) and Module 2 (4.48 ± 0.19) (*P = 0.0004*); Module 2 (4.48 ± 0.19) and Module 4 (5.56 ± 0.14) (*P = 0.*011); Module 2 (4.48 ± 0.19) and Module 5 (9.16 ± 0.29) (*P <0.0001*); Module 3 (4.12 ± 0.21) and Module 4 (5.56 ± 0.14) (*P < 0.*0001). But there was no significant difference between Module 1 (M1) (6.07 ± 0.06) and Module 3 (4.12 ± 0.21) (*P =0.39*); Module 2 (4.48 ± 0.19) and Module 5 (9.16 ± 0.29) (*P = 0.69*) (Fig.1C).

Ash weevil adults: There was no significant difference in the mean number of *M. subfacsiatus* adults in different IPM modules evaluated ($F_{4,15}$ *= 2.019, P =0.143*) (Fig. 1D).

Pooled analysis (*Rabi,* 2020 and *Kharif,* 2021)

Leaf damage: Significant differences in the mean leaf damage was observed among the IPM modules evaluated ($F_{4,15}$ *= 101.4, P < 0.0001*). All the modules were significantly superior over untreated check, Module 5. All the modules showed significant difference with each other except Module 1 (6.05 ± 0.04) and Module 4 (5.63 ± 0.12) (*P = 0.436*); Module 2 (4.83 ± 0.24) and Module 3 (4.35 ± 0.20) (*P = 0.362*).

However, significant differences were observed between the Module 1 (6.05 ± 0.04) and Module 2 (4.83 ± 0.24); Module 3 (4.35 ± 0.20) and Module 4 (5.63 ± 0.12); Module 1 (6.05±0.04) and Module 3 (4.35±0.20) (*P < 0.0001*); Module 3 (4.35 ± 0.20) and Module 4 (5.63 ± 0.12) (*P < 0.0001*); Module 2 (4.83 ± 0.24) and Module 4 (5.63 ± 0.12) (*P =0.017*) (Fig.1E).

Ash weevil adults: The pooled data analysis revealed significant differences in the mean number of *M. subfasciatus* adults in different modules ($F_{4,15}$ *= 5.33, P = 0.0018*). The number of ash weevils per plant (mean ± SE) was significantly different between Module 2 (2.39 ± 0.18) and Module 5 (2.91 ± 0.13) (*P = 0.022*); Module 3 (2.06 ± 0.07) and Module 5 (2.91 ± 0.13) (*P =0.0007*). There was no significant difference observed between Module 1 (2.39±0.18) and Module 2 (2.30 ± 0.15) (*P = 0.99*); Module 1 (2.39 ± 0.18) and Module 3 (2.06 ± 0.07) (*P = 0.43*); Module 1 (2.39 ± 0.18) and Module 4 (2.43 ± 0.09) (*P = 0.99*); Module 1 (2.39 ± 0.18) and Module 5 (2.91 ± 0.13) (*P = 0.43*); Module 2 (2.30 ± 0.15) and Module 3 (2.06 ± 0.07) (*P = 0.71*); Module 2 (2.30 ± 0.15) and Module 4 (2.43 ± 0.09) (*P = 0.96*); Module 3 (2.06± 0.07) and Module 4 (2.43 ± 0.09) (*P = 0.325*) ; Module 4 (2.43 ± 0.09) and Module 5 (2.91 ± 0.13) (*P = 0.102*) (Fig. 1F).

On the whole, the Module 3 that comprised soil application of *M. anisopliae* @ 5kg/ha followed by soil drenching with EPN *H. indica* @ 20kg/ha at 30 DAT and foliar spray with *M. anisopliae* @ 5kg/ha at 45 DAT was found to be superior and recorded lower leaf damage and the lowest number of *M. subfasciatus* adults (4.35±0.59, 2.06 ±0.07 respectively). The Module 2 that comprised soil application of neem cake followed by application of entomopathogenic nematode *H. indica* @ 20kg/ha at 30 DAT and foliar application of *B. bassiana* @ 5kg/ha at 45 DAT was also found on par with Module 3 in reducing the leaf damage and number of *M. subfasciatus* adults (4.83 ± 0.24, 2.30 ±0.15 respectively). Interestingly, the Module 3 and Module 2 were also statistically at par with Module 4, a synthetic insecticide intensive farmers practice that comprised soil application of carbofuran 3G@15 kg/ha followed by soil drenching with chlorpyriphos 20EC @ 2.5 L/ha at 30DAT and foliar spray with fipronil 5SC @ 750 mL/ha at 45 DAT (5.63±0.34, 2.43 ± 0.09 respectively).

In conclusion, among the IPM modules, Module 3 [integrated with soil application of *M. anisopliae* @ 5kg/ha followed by soil drenching with EPN *H. indica* @ 20kg/ha at 30DAT and foliar spray with *M. anisopliae* @ 5kg/ha at 45 DAT] and the Module 2 [comprised of soil application of neem cake followed by application of EPN, *H. indica* @ 20kg/ha at 30 DAT and foliar application of *B. bassiana* @ 5kg/ha at 45 DAT] which are mainly comprised of biorational insecticides and EPNs were found effective in managing *M. subfasciatus*. The results indicate the efficacy of tested biorational insecticides in reducing the incidence of *M. subfasciatus* and leaf damage and also found at par with the chemical insecticide application (Module 4). Hence, the present study affirms that the soil application of EPNs and the use of biorational insecticides are effective in managing

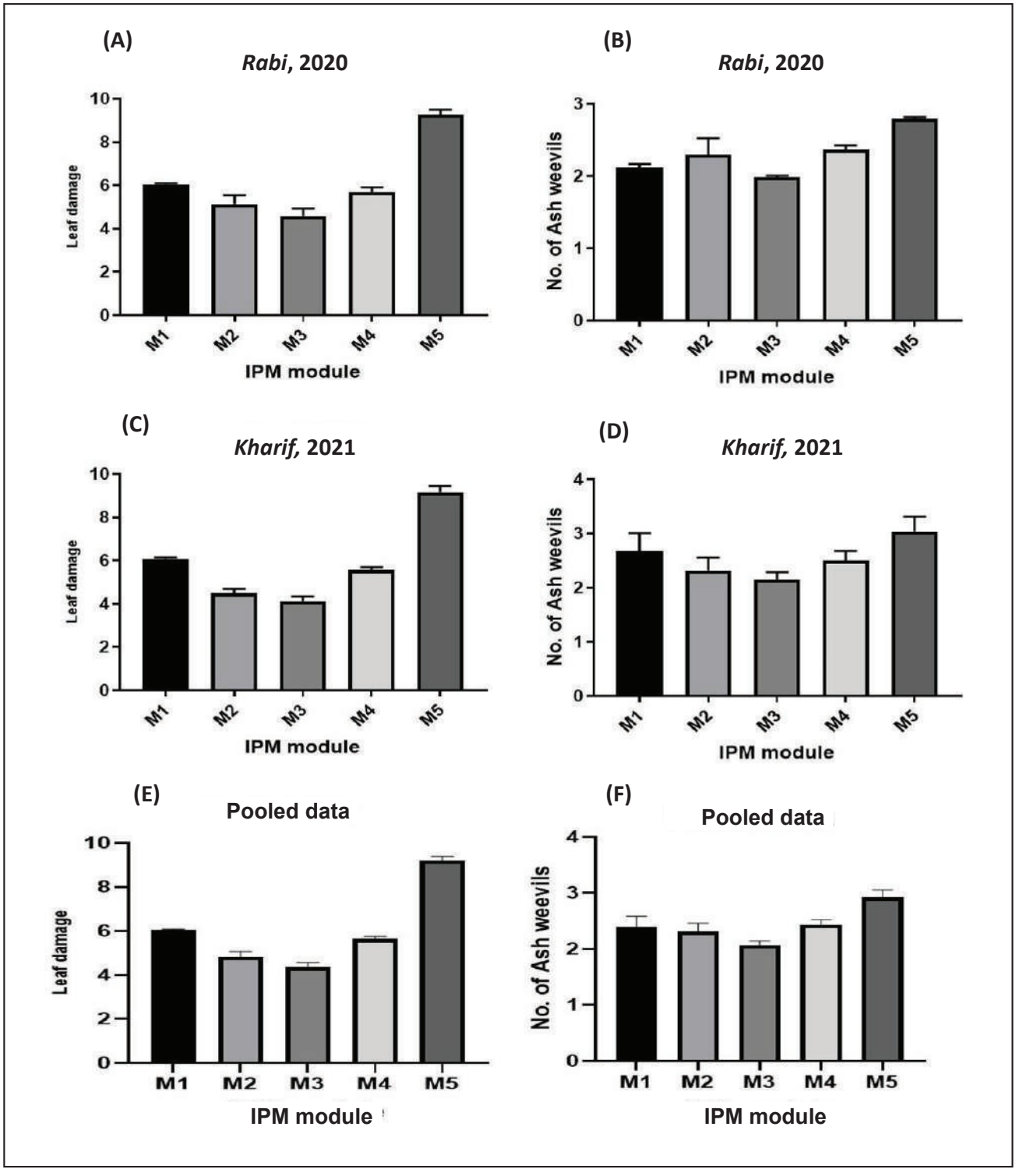

Fig 1. Mean leaf damage and adult ash weevils recorded in different IPM modules during 2020-2021. (M1: Soil application of neem cake @250kg/ha before transplanting-Soil drenching of NSKE 5% @25kg/ha at 30 DAT-Foliar spray with neem oil 3% at 45 DAT; **M2:** Soil application of neem cake @250kg/ha before transplanting -Application of EPN *Heterorhabdidtis indica* @ 20kg/ha at 30 DAT - Foliar spray with *Beaveria bassiana* 1x10^8 CFU/g/mL @ 5kg/ha at 45 DAT; **M3:** Soil drenching with *Metarhizium anisopliae* 1x10^8 CFU/g/mL @ 5kg/ha Soil application of EPN *H. indica* @ 20kg/ha at 30 DAT -Foliar spray with *M. anisopliae* 1x108 CFU/g/ml @ 5 kg/ha at 45 DAT ; **M4:** Farmers practice Soil application of Carbofuran G@15kg/ha - Soil drenching with Chlor pyriphos 20EC @ 2.5 L/ha at 30 DAT - Foliar spray with Fipronil 5SC @ 750 mL/ha at 45 DAT; **M5:** Untreated check).

the subterranean population of ash weevils. These biointensive modules also restrict the usage of synthetic insecticides in managing ash weevils.

Similar findings were reported by Shanmugam *et al.* (2021), where mulching along with EPN, *H. indica* at 2.5 kg/ha recorded zero incidence of *M. subfasciatus* up to 30 DAP (days after planting) and 2.5-7.5 per cent damage up to 150 DAP. The application of EPNs *Steinernema carpocapsae, Steinernema glaseri* and *H. indica*, against *M. subfasciatus* were aslo evaluted by several researchers (Manjunatha *et al.*, 2016; Nisthiskarani *et al.,* 2019), where, Manjunatha *et al.* (2016) reported that *S. carpocapsae* caused greater mortality (20-100 %) than *H. indica* (16-92 %) against pre-pupal stages and *S. carpocapsae* caused 16-92 % mortality in the third instar larvae, while *H. indica* caused 12-80 % mortality. Nisthiskarani *et al.* (2019) found that the application of EPN, *S. glaseri* was effective at the third instar stage of *M. subfasciatus*. Similarly, the studies of Nagesh *et al.* (2016) revealed that seven strains of EPNs, (*Heterorhabdidtis bacteriophora* NBAIIHb105, *H. indica* NBAIIHi101, *H. indica* NBAII Himah, *Steinernema abbasi* NBAIISA01, *Steinernema abbasi* NBAIISA04, *S. carpocapsae* NBAIISc04, *S. glaseri* NBAII Sg01) caused > 80% mortality at 40 IJ/cm^2 in *M. subfasciatus* larvae. Our findings also fall in line with Umamaheshwari *et al.* (2021) who revealed the strength of native strains of EPNs *H. indica, S. carpocapsae, S. glaseri* in combination with *B. bassiana and Bacillus subtilis* against ash weevil, *M. subfacsiatus* under field conditions. Their study revealed that the combination of *H. indica* and *B. subtilis* reduced the leaf damage by ash weevil in brinjal to the tune of 76% .

The present findings affirm that incorporating the EPNs in the IPM modules resulted in greater efficay against *M. subfasciatus*. In general, the use of EPNs for insect pest control has many benefits, including minimal harm to natural enemies, lack of environmental pollution, and end-user safety. Hence, the application of EPNs might play a key role in targetting the ash weevils in brinjal as ensures environmental sustainability through biointensive IPM. Reduced chemical input costs, diminished on and off-farm environmental effects, more efficient and su stainable pest management are some advantages of applying biointensive IPM.

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Management of fruit flies infesting muskmelon through eco-friendly modules

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ABSTRACT: Three different fruit fly management modules were evaluated in muskmelon during the *kharif,* 2018 and *rabi,* 2018-19 at College of Horticulture, Anantharajupeta. The eco-friendly module II (IPM) which is the integration of different aspects *viz*., maintenance of field sanitation, poison baiting with rotten banana, bait spray of deltamethrin mixed with jaggery at flowering, installation of cue lure traps from fruit set onwards, spray of 5% NSKE and malathion at 40 and 60 DAS respectively is the most effective and economical method for the management of fruit flies in muskmelon crop with lower fruit fly infestation (18.58%) and higher returns (BC ratio 2.8). Further, quality analysis results indicated that, average fruit weight, TSS were highest (1.27 Kg, 11.39º) in module II. Finally from the above results it can be concluded that the effectiveness of the three fruit fly management modules lies in the order of IPM > Organic IPM > Farmers practice.

Keywords: muskmelon, fruit fly, *Zeugodacus cucurbitae*, management, eco-friendly module

INTRODUCTION

Muskmelon (*Cucumis melo* L.) is an important horticultural crop in India and worldwide, and plays an important role in international trade. In India, muskmelon is grown in 60,000 hectares with a production of 1312 thousand MT with a productivity of 21.9 tonnes/ha (Anonymous, 2020-21). In Andhra Pradesh muskmelon is grown in an area of 2466 ha with a production of 600 thousand MT (Anonymous, 2019-20). Melon fruit fly, *Zeugodacus cucurbitae* damages over 81 plant species, but plants belonging to the family cucurbitaceae are the most preferred hosts (Allwood *et al.*, 1999). Depending on the environmental conditions and susceptibility of the crop species, the extent of yield loss varies from 30 % to 100 % (Shooker *et al.*, 2006). The pest was reported to cause damage to an extent of 28.55% in watermelon, 77.03 % in bitter gourd, 75.65 % in ridge gourd, 73.83 % in cucumber and 63.31 % in pickling cucumber and 100% damage in cucumber (Krishna Kumar *et al.*, 2006 ; Vignesh and Shashidhar, 2015). This pest has been a major limiting factor in obtaining good quality fruits and high yield of cucurbitaceous vegetables. Female flies prefer to oviposit on soft tender, physiologically ripen fruit tissues puncturing with it's ovipositor, watery fluid oozes out from the punctures, which later transform into dry resinous deposit. The pseudopunctures (punctures without eggs) also reduce the market value. The maggots feed on mature fruits internally but sometimes also feed on young fruits and flowers. The affected fruits are softened, discolored and drop off prematurely, finally losing market value. The maggots of the pest remain inside the infested fruits and the adults are free living. They visit fruits only at the time of oviposition and left immediately after egg deposition. So the control of the pest can hardly be assured. Management of this pest using chemical insecticides is not possible and only integrated pest management is the best available alternative. The present study was aimed to evaluate various management modules against fruit flies in muskmelon.

MATERIALS AND METHODS

Field experiments were conducted during the two seasons; *kharif,* 2018 and *rabi,* 2018-19 at experimental farm, College of Horticulture, Anantharajupeta, Andhra Pradesh, India to evaluate the effectiveness of three different management modules against fruit flies in muskmelon. All the recommended agronomical practices were followed to raise good crop. Three different modules as given under

Module-I (Farmer's practices)

a. Spray of imidacloprid 17.8 SL @ 0.5 ml/l of water at 30 days after sowing (DAS).
b. Spray of dichlorvos 76 EC @ 1 ml/l of water at 45 DAS.
c. Spray of malathion 50 EC @ 2 ml/l of water at 60 DAS.
d. Installation of cuelure traps (4/acre) from fruit set onwards.

Module-II (IPM)

a. Field sanitation (Removal of affected fruits, dried and damaged plant parts, regular soil raking, weed free maintenance etc.).
b. Poison bait consisting of rotten banana/pumpkin pulp 1000g + Yeast 10g + malathion 10ml + citric acid 3g

Table 1. Bio efficacy of different fruit fly modules against fruit fly, *Z. cucurbitae* infesting muskmelon during the two the seasons

Module	Fruit infestation (%)			No. of maggots/fruit			Yield (q/ha)			Economics	
	kharif 2018	*rabi* 2018-19	Pooled	*kharif* 2018	*rabi* 2018-19	Pooled	*kharif* 2018	*rabi* 2018-19	Pooled	Net Returns (Rs)	B:C ratio
Module I (Farmers practice)	38.21 (38.15)^{c}	33.53 (35.38)^{b}	35.87 (36.74)	13.93^{b}	15.25^{c}	14.59^{c}	112.80^{a}	127.68^{a}	120.2^{a}	109,179	1.8
Module II (IPM)	20.73 (27.06)^{a}	16.43 (23.56)^{a}	18.58 (25.42)	8.92^{a}	4.64^{a}	6.63^{a}	168.20^{c}	165.03^{c}	166.23^{c}	172,171	2.8
Module III (Organic IPM)	24.88 (29.88)^{b}	17.04 (24.05)^{a}	20.96 (27.22)	7.84^{a}	7.00^{b}	7.42^{b}	145.60^{b}	147.72^{b}	146.66^{b}	147,103	2.5
S Em ±	0.86	1.17	0.77	0.71	1.09	0.73	4.99	5.27	5.14		
CD (p=0.05)	2.64	3.60	2.36	2.18	3.34	2.26	15.34	16.28	15.81		

Figures in the parenthesis are arc sin transformed values

kept in an earthen pot to attract the adult flies from fruit set onwards.

c. Bait spray consisting of deltamethrin 2.8 EC @ 1ml/l mixed with jaggery 15g from flowering onwards.
d. Installation of cuelure traps (6/acre) from fruit set onwards.
e. Spray of NSKE 5% at 40 DAS.
f. Spray of malathion 50EC @ 2 ml per litre of water at 60 DAS

Module-III (Organic IPM)

a. Seed treatment with beejamrutham.
b. Growing of jowar as border crop (4 rows, sown 15 days before the main crop)
c. Field sanitation (Removal of affected fruits, dried and damaged plant parts, regular soil raking, weed free maintenance etc.).
d. Spray of NSKE 5% at 20 DAS.
e. Spray with jeevamrutham at 35 DAS.
f. Spraying with pongamia soap/neem soap @ 10g/l at 45 DAS.
g. Spray of spinosad 45 SC @ 0.375 ml/l at 60 DAS.

Method of observations recording

All the recommended agronomic practices (e.g. weeding, fertilization, hoeing, etc.) were performed equally in each experimental bed. Adequate distance between two plots was maintained. The muskmelon plot allotted to module-1and II was kept 100 m away from module III. Three harvestings of the fruits were done in each growing season of muskmelon. For recording data on fruit fly infestation, randomly 20 fruits were selected in each module. The infested fruits were sorted and the percent fruit infestation and number of maggots/ fruit were calculated. The total treatmental yield, total marketable yield of all the replications were calculated from different pickings and final cost benefit ratio for the three management modules was worked out. The retail price of each insecticide, labour wages were taken into consideration for computing total cost of cultivation in different management modules. Quality parameters like average fruit weight, total soluble solids and acidity were also worked out for comparison in different modules. The per cent fruit infestation and number of larvae/ fruit (Level of incidence/ larval density per fruit) were calculated using the following formulae.

Final fruit yield was calculated by adding the total harvest attained from all the harvests in individual plot and converted into per hectare yield. The benefit cost ratio was calculated on the basis of net returns from each module and the total cost incurred towards different components of each modules.

Benefit-Cost ratio

The cost of the individual components of the modules *viz*., field sanitation, pheromone traps setting, poison baiting, border crop, organic solutions preparation and pesticides spray and cost for their timely implementation were worked out separately and returns per hectare were worked for different modules. The data were computed

Table 2. Effect of fruit fly management modules in muskmelon on various quality parameters of the fruits

Module	Average fruit weight (Kg)			Total soluble solids (°B)			Acidity (%)		
	kharif, 2018-19	*rabi*, 2018-19	Pooled	*kharif*, 2018-19	*rabi*, 2018-19	Pooled	*kharif*, 2018-19	*rabi*, 2018-19	Pooled
Module I (Farmers practice)	0.93	0.93	1.23	9.50	9.80	9.65	0.59	0.65	0.62
Module II (IPM)	1.35	1.20	1.27	10.56	12.21	11.39	0.65	0.60	0.63
Module III (Organic IPM)	1.20	0.84	1.00	11.14	11.27	11.20	0.66	0.61	0.64
SEm ±	**0.27**	**0.12**	**0.10**	**1.150**	**1.39**	**1.05**	**NS**	**NS**	**NS**
CD (p=0.05)	**0.09**	**0.06**	**0.03**	**0.37**	**0.45**	**0.33**	**0.05**	**0.03**	**0.04**

NS - Non significant

through one-way ANOVA using SPSS 16 software. The means of significant parameters among the tested modules were compared using critical difference (CD) for paired comparisons at 95% probability level.

RESULTS AND DISCUSSION

Fruit fly infestation

kharif, 2018

The fruit infestation showed significant difference among the three tested modules during the *kharif*, 2018. The module II (IPM) was found to be the best module with lowest per cent (20.73%) fruit fly damage followed by module III (Organic IPM) (24.88%). Significantly highest infestation was recorded module I (38.21%). Similarly larval density was found to be less in module III (7.84) and module II (8.92) compared to the module I (13.93) in the first season (Table 1).

The total treatment fruit yield was found highest (141.60 kg) in module II (IPM). Relatively higher treatment yield (126.40 kg) was recorded in module I (Farmers practice) than in Organic IPM module (121.88 kg). However, marketable fruit yield was highest (117.62 kg) in IPM module followed by Organic IPM module (101.83 kg). In farmers practice module, lowest marketable yield of 78.88 kg was obtained. Highest total yield of 168.20 q/ha was recorded in module II (IPM) and lowest of 112.80 q/ha in module I (Farmers practice).

Rabi, 2018-19

During the second season (*rabi,* 2018-19) of evaluation of different management modules, significant difference was noticed both in fruit infestation and maggot density among the three modules. The per cent fruit infestation was lowest in module II (16.43%) and was statistically at par with the module III (17.04%), while larval density was lowest in module II (4.64). Highest fruit infestation and maggot density were recorded in module I (33.53%, 15.25) (Table 1).

Pooled results of the two seasons

The pooled analysis (Table 1) of the two seasons indicated that, among the three tested modules, IPM module was significantly differed with the other two modules with lowest fruit infestation (18.58%). However, both in module II and III was the larval density (6.63 and 7.42) was found to be statistically at par with each other but differing significantly with the module I (14.59 larvae/fruit).

Yield

kharif, 2018

The fruit yield was found to be highest (168.20 Q/ha) in module II (IPM) followed module III (145.60 Q/ha), whereas, lowest yield was recorded in module I (112.80 Q/ha). Similarly, marketable fruit yield was highest (117.62 kg) in module II followed by module III (101.83 kg). In farmers practice module, lowest marketable yield of 78.88 kg was obtained during the first season (*kharif*, 2018 (Table 1).

Rabi, 2018-19

During the second season, highest fruit yield was recorded in module II (165.03 q/ha) followed by in module III (147.72 q/ha) and it was lowest in module III (127.68 Q/ha). The highest marketable fruit yield of 115.41 Kg was recorded in module II. The lowest

marketable yield was recorded in module I (89.27 Kg) (Table 1).

Pooled results of the two seasons

The pooled analysis (Table 3) of the two seasons indicated that, among the three tested modules, highest total and marketable yields (166.23 Q/ha, 113.83 Kg) were recorded in module II. And are in the order of II > III > I (IPM > Organic IPM > Farmers practice).

Assessment of quality parameters

The quality parameters *viz*., average fruit weight, total soluble solids and acidity of the fruits recorded in three different modules were analyzed statistically and the results were presented in the table- 2. The quality analysis results indicated that, all the three modules differed significantly from each other. Average fruit weight was found to be highest (1.27 Kg) in module II (IPM) and was lowest in module III (Farmers practice) (1.0 Kg). TSS was also found to be highest in module II (11.39º) and was at par with module III (11.20º). Lowest TSS was recorded in fruits of module I (9.65º). Significant difference was not found in case of acidity in both the seasons between the modules. The above results indicated that, different fruit fly management modules not only affect the fruit yield and returns but also influence their quality parameters.

Economics of different eco-friendly modules

The data on economics of the different eco-friendly modules applied against the melon fruit fly, *B. cucurbitae* in muskmelon was calculated with net cost benefit ratio (NCBR) are present in Table 1.

The gross returns and net returns worked out on the basis of average fruit yield per hectare. Highest per hectare gross and net returns (Rs.232,722, Rs. 172,171) were obtained in the module II (IPM module). In module III (organic module) Rs.205,324, Rs.147,103 were the gross and net returns recorded. In module I (Farmers practice) lowest net and gross returns of Rs.168,280, Rs.109,179 were obtained. Highest benefit cost ratio (2.8) was recorded in IPM module followed by Organic IPM module (2.5). In Farmers practice module, lowest benefit cost ratio (1.8) was obtained. The present results clearly indicated that, module II (IPM) which is the integration of different aspects *viz*., maintenance of field sanitation, poison baiting with rotten banana, bait spray of deltamethrin mixed with jaggery at flowering, installation of cue lure traps from fruit set onwards, spray of 5% NSKE and malathion at 40 and 60 DAS respectively is the most effective and economical method for the management of fruit flies in muskmelon crop with lower fruit fly infestation (18.58%) and higher returns (BC ratio 2.8). The results obtained are in coordination with the findings of Mandal (2012) who reported that mean per cent fruit damage was lowest in an IPM package consisting of installation of sex pheromone traps and spinosad spray along with discarding of infested/damaged fruits at each harvesting. This was also supported by Shravan *et al.* (2014) who have reported that, an organic IPM module comprising of growing resistant genotype (RM-50), spray of neem oil at 20 DAS, installation of pheromone trap (10/ hectare) at 42 DAS, spray of tumba fruit extract (TFE 5%) at 50 DAS and spray of spinosad 46 SC at 60 DAS was most effective with BC ratio of 8.84. Ajanta *et al.* (2015) developed a successful for the management of cucurbit fruit fly The module comprising of installation of cue-lure baited traps @ 50 traps/ha for mass trapping, weekly clipping of infested fruits, foliar spray of aqueous leaf extracts of *Morinda citrifolia* @ 100g/l and foliar spray of spinosad 45SC or imidacloprid 17.8SL @ 0.3ml/l alternately at 15 days interval and was found effective with respect to less fruit damage due to fruit fly (9 %, 5.9%), maximum fruit yield (10.75,7.59 t/ ha) and higher cost benefit ratio (1:3.35, 1:1.99) in bitter gourd and ridge gourd respectively. Sunil *et al.* (2016) revealed that deltamethrin 2.8 EC + jaggery bait (0.0028 + 0.015 %) was the most effective treatment resulting in minimum fruit infestation (13.15%,8.61%) of melon fruit fly, *Bactrocera cucurbitae* in bitter gourd, as well as lowest number of maggots per fruit (12.58, 9.58) in both *kharif* and *rabi* seasons. Similarly, the present findings are in consent with the findings of Bharadiya *et al.* (2018), who have reported module-3 [destruction of infested fruits during each picking + poison bait @ 50 l/ha (jaggery 50 g/l + abamectin 1.9% EC 0.0025%) + application of *Azadirechtin* 0.003% at 15 days interval starting from flower initiation stage] as effective treatment against fruit flies in sponge gourd. The reason is that the fruit flies being internal feeders, very difficult to manage with the external spraying of pesticides. Unless the different management aspects combined together in a strategic way starting from the beginning of crop cultivation itself, pest infestation cannot be maintained to below economic damage level.

CONCLUSIONS

From the experimental results, it can be concluded that fruit fly infestation in muskmelon affects both quantity and quality parameters of the fruits. An integrated approach including cultural practices such as collection and deep burying of infested and fallen fruits, tillage around the trees/in the fields along with the timely bait sprays, poison baiting and adult trapping methods are effective and eco-friendly fruit fly management strategies

need to be adopted in order to manage the pest below economic threshold level and to reduce adverse effects on pollinators and natural enemies. In the present study, among the three tested fruit fly management modules, module II (IPM), an integration of different aspects *viz*., maintenance of field sanitation, poison baiting with rotten banana, bait spray of deltamethrin mixed with jaggery at flowering, installation of cue lure traps from fruit set onwards, spray of 5% NSKE and malathion at 40 and 60 DAS respectively is the most effective and economical method for the management of fruit flies in muskmelon crop with lower fruit fly infestation (18.58%) and higher returns (BC ratio 2.8). Further, it was again proved that, fruit fly management is possible only when various management aspects are integrated in a strategic manner.

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Efficacy of certain biopesticides against whitefly, *Bemesia tabaci* (Gennadius) on okra and its correlation with abiotic factors

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ABSTRACT: A field experiment was carried out at the Assam Agricultural University, Jorhat, Assam, India during 2018-2019 to evaluate the efficacy of different biopesticides against whitefly, *Bemesia tabaci* (Gennadius) on okra. The treatments *viz.,* neem oil @ 5%, karanj oil @ 5%, *Melia azedarach* leaf extract @ 5%, garlic extract @5%, chilli fruit extract @5%, *Beauveria bassiana* @ 5%, *Lecanicillium lecanii* @ 5%, deltamethrin @ 10 g *a.i.*/ha were applied at 15 days interval starting from seedling stage when whitefly infestation started. Results revealed that overall best performance of insecticides against whitefly recorded in deltamethrin treated plots with lowest mean population followed by neem oil, *Verticillim lecanii*. And observations for whiteflies were recorded at a weekly interval from three leaves per plant, starting from 14th Standard Meteorological Week (SMW). The maximum population of whiteflies (14.91 whiteflies/3 leaves) was observed during the 18th SMW. While the minimum population of whiteflies (3.04 whiteflies/3 leaves) was observed during 31st SMW. Whitefly population was significantly and negatively correlated with maximum temperature and minimum temperature and positively correlated with the bright sunshine hours and the correlation of whiteflies with all other weather parameters was negative and non-significant.

Keywords: Whitefly, neem oil, *Lecanicillium lecanii*, abiotic factors, correlation, okra.

INTRODUCTION

Okra (*Abelmoschus esculentus*), also called Lady's finger/ bhendi, belongs to the family Malvaceae. It is a short duration crop and grown throughout the year. In India, okra is cultivated over an area of 509 (000'Ha) with production of 6095 (000' MT) and productivity of 12 MT/ha. Andhra Pradesh, Arunachal Pradesh, Bihar, Assam, Chhattisgarh, Gujarat, Haryana and Himachal Pradesh are major okra growing states. In Assam, it is cultivated in an area of 12,110 hectares with a production of 191.70 thousand tonnes (Anon., 2017). Throughout its growth period, okra is devastated by around 72 species of insect pests and mites (Rao and Rajendran, 2003). This is the major constraint for the low productivity of okra in India. The intensity of damage caused by pests also varied from one region to another. Among insect pests of okra, leafhopper, *Amrasca biguttula biguttula* (Ishida), whitefly, *Bemesia tabaci* (Gennadius) and shoot and fruit borer *Earias* spp. are the prominent insect pests (Singh *et al.*, 1993). The sucking pest complex comprising whiteflies, thrips, leafhoppers and mites are major pests and causing17.46 percent yield loss in okra (Sarkar *et al.,* 1996). Whitefly, *B. tabaci* has become a crucial pest on vegetables, field crops, ornamental plants and fruits all over the world and attacks 176 plant species with ample damage (Oliveira *et al.,* 2001). The pest also transmits serious disease like yellow vein mosaic, influencing the quality of the produce. At present, schedule based application of various insecticides is recommended for the management of different insect pests. But, the injudicious use of synthetic chemicals to manage these pests has resulted in resistance, resurgence, secondary pest outbreak, toxicity to beneficial organisms (Mandal *et al.* 2006). Botanical and biological agents have a vital role to control pest damage. Abiotic parameters play an essential role to accelerate the population of insect pests. To avoid the reduction in yield caused by the sucking pests, all efforts are needed to manage these pests by gathering the information of appearing of the pests and the influence of various abiotic factors on them. Moreover, correlation study helps in allocating either positive or negative association of pest population with abiotic factors. Thus, in this experiment, efforts have been made to examine the population fluctuation of the whitefly of okra and its correlation with abiotic parameters.

MATERIALS AND METHODS

Experimental site

Field experiment was carried out at the Experimental farm, Department of Horticulture, Assam Agricultural University, Jorhat, Assam. The experiment was conducted with okra (Arka Anamika) in Randomized Block Design with nine treatments including control and three replications. The net area for the experiment was 230sq.m. The area was divided into three blocks and each block was further divided into nine equal plots (2.7m×2.1m) each, respectively. Interspacing between blocks was 0.60m and plots were 0.45m.

Extraction of botanicals

Preparation of *Melia azedarach* L. leaf extract

The fresh leaves of naturally grown mature plants were collected and washed thoroughly and then were dried under shade. After, drying the plant material was grounded to a fine powder and sieved separately through an 80 mesh nylon cloth and soaked in distilled water at room temperature between 24 to 48 hours. The ratio of plant material to water was 1:20 (w:v), which was necessary to make a 5% solution. After soaking, the plant materials were squeezed manually. The solution was then filtered through a fine-mesh nylon cloth to obtain an extract, free of plant residue and detritus.

Preparation of garlic extract

The outer layers of matured garlic were peeled off, after that grounded to paste. 50 gm of paste was mixed with one litre of distilled water at room temperature for 24 to 48 hours to give a 5% solution. The solution was filtered through a fine-mesh nylon cloth to obtain an extract free of any residue and detritus.

Preparation of chilli fruit extract

The chilli fruits were collected and dried under shade and grounded to a fine powder. 50 gm of chilli powder was mixed with one litre of water to make a 5% concentration. After that soaked in distilled water at room temperature for 24 to 48 hours. After soaking the solution was filtered through a fine mesh nylon cloth to obtain an extract, free of residue and detritus.

Preparation of fungal bio-formulations

The fungal bio-agents *viz*., *Beauveria bassiana* and *Lecanicillium lecanii* were collected from the Department of Plant Pathology, Assam Agricultural University. 5 ml of the fungal formulation was mixed with 1000ml of water.

Recording observations

At the time of appearance of the pest, the crop was sprayed with these treatments as mentioned above. The treatments were imposed by using a knapsack sprayer @ 400-500 litres of spray solution/ha depending on the stage of the crop. The crop received a total of 3 sprays. The spray application was given at the time of incidence noticed and second, the spray was given at an interval of 15 days thereafter. For recording the number of whiteflies, five plants were selected randomly in each plot and were tagged. Observations were recorded on three leaves; each at the top, middle and bottom of five tagged plants in each plot. The first observation was recorded 1 day before treatment as a pre-treatment count and post-treatment observations were recorded at the 3, 7 and 10 days after each spraying. Data thus obtained were analysed statistically and presented.

To know the correlation of the whitefly population with the abiotic factors, observations were recorded on 10 tagged plants once in a standard week, which started from the first appearance of the pest and was continued till their availability or maturity of the crop, whichever was earlier. Simultaneously, a corresponding weekly record of meteorological data was recorded. Standard week average of all the data collected *viz.,* minimum and maximum temperature, morning and evening percent relative humidity, total rainfall per week and bright sunshine hours were maintained. These meteorological factors were calculated for statistical analysis. All the possible correlations and multiple regression were worked out. The influence of different weather parameters on whitefly population was studied.

RESULTS AND DISCUSSION

Results revealed that there was no significant difference in the whitefly population among the treatments before spraying. During the first spray (Table 1), the lowest mean population of whiteflies was observed in the deltamethrin treated plots (1.23 whiteflies/3 leaves), followed by neem oil (1.74 whiteflies/3 leaves) and the next best treatment was *Lecanicillium lecanii* (2.03 whiteflies/3 leaves). The other treatments recorded the pest count in the range of 2.63 to 3.82 whiteflies/3 leaves. The data showed that the treatment of deltamethrin @ 10 gm *a.i.*/ha recorded the highest percent reduction (70.78%) of whitefly population followed by neem oil @ 5% (62.74%), *L. lecanii* @ 5ml/l (57.62%), karanj oil @ 5% (50.10%), *B. bassiana* @ 5ml/l (44.75%) and *M. azedarach* leaf extract @ 5% (42.38%). After the second spray (Table 2), results revealed that the deltamethrin recorded the minimum population of 2.18 whiteflies/3 leaves. Followed by neem oil @ 5% (3.01 whiteflies/3 leaves), *L. lecanii* @ 5ml/l (3.15 whiteflies/ 3 leaves). Garlic extract and chilli fruit extract were found to be less effective in reducing the whitefly population but were superior over control. A similar trend was observed in the percent reduction of whitefly population over control as in the first spray. After the final spray (Table 3), the lowest mean population of whitefly was observed in deltamethrin treated plots with 2.58 whiteflies/3 leaves followed by neem oil @ 5% was found best with 3.21 whiteflies/3 leaves, *L. lecanii* @ 5ml/l (3.35 whiteflies/ 3 leaves). The data showed that the treatment of deltamethrin 10 gm *a.i.*/ha recorded the highest percent reduction (71.21%) of whitefly population followed by neem oil @ 5% (63.36%), *L. lecanii* @ 5ml/l (55.75%) and karanj oil @ 5% (44.16%). The minimum percent reduction in the whitefly population was observed in plots

Table 1. Efficacy of different treatments against whitefly, *Bemisia tabaci* population on okra during 1st spray

Treatment	Dose	Number of Whiteflies/3 leaves I spray					Per cent reduction in population
		1 DBS	3 DAS	7 DAS	10 DAS	Mean	
Neem oil	5%	4.67	1.85	1.42	1.96	1.74	62.74
Karanj oil	5%	4.87	2.43	1.65	3.21	2.43	50.10
Melia azedarach leaf extract	5%	5.12	2.92	1.98	3.96	2.95	42.38
Garlic extract	5%	5.23	3.26	3.33	3.35	3.31	26.96
Chilli fruit extract	5%	4.87	3.21	2.92	3.01	3.04	27.93
Beauveria bassiana	5ml/l	4.76	2.81	1.86	3.23	2.63	44.75
Lecanicillium lecanii	5ml/l	4.79	2.16	1.57	2.37	2.03	57.62
Deltamethrin	10g *a.i*/ha	4.21	1.54	0.98	1.18	1.23	70.78
Control	-	5.02	4.34	3.89	6.70	4.98	0.80
S.Ed±	-	0.39	0.43	0.28	0.33	-	-
CD(P=0.05)	-	NS	0.92	0.63	0.71	-	-

NS=Non significant DBS=Day before spray DAS=Days after spray Data are mean of 3 replications

Table 2. Efficacy of different treatments against whitefly, *Bemisia tabaci* population on okra during 2nd spray

Treatment	Dose	Number of Whiteflies/3 leaves					Per cent reduction in population
		1 DBS	3 DAS	7 DAS	10 DAS	Mean	
Neem oil	5%	6.23	3.86	2.33	2.83	3.01	51.68
Karanj oil	5%	5.65	3.55	2.87	3.34	3.25	42.48
Melia azedarach leaf extract	5%	5.52	3.86	3.70	5.99	4.52	18.12
Garlic extract	5%	6.12	5.19	4.87	4.96	5.01	18.14
Chilli fruit extract	5%	5.53	5.52	4.11	5.12	4.92	11.03
Beauveria bassiana	5ml/l	6.39	4.22	3.81	4.61	4.21	34.12
Lecanicillium lecanii	5ml/l	5.92	3.97	2.16	3.33	3.15	46.79
Deltamethrin	10g *a.i*/ha	6.53	2.56	2.15	1.82	2.18	66.62
Control	-	6.91	6.46	6.23	6.73	6.47	6.37
S.Ed±	-	0.40	0.41	0.29	0.39	-	-
CD(P=0.05)	-	0.85	0.88	0.62	0.82	-	-

DBS=Day before spray DAS=Days after spray Data are mean of 3 replications

Table 3. Efficacy of different treatments against whitefly, *Bemisia tabaci* population on okra during 3rd spray

Treatment	Dose	Number of Whiteflies/3 leaves					Per cent reduction in population
		III spray					
		1 DBS	3 DAS	7 DAS	10 DAS	Mean	
Neem oil	5%	8.76	3.45	2.98	3.21	3.21	63.36
Karanj oil	5%	7.54	4.43	3.65	4.54	4.21	44.16
Melia azedarach leaf extract	5%	8.12	4.98	4.55	6.12	5.22	35.71
Garlic extract	5%	7.92	5.76	4.98	6.61	5.78	27.02
Chilli fruit extract	5%	8.58	5.98	4.93	6.35	5.75	32.98
Beauveria bassiana	5ml/l	7.22	4.98	4.12	5.40	4.83	33.10
Lecanicillium lecanii	5ml/l	7.57	3.25	3.01	3.79	3.35	55.75
Deltamethrin	10g *a.i*/ha	8.96	2.65	2.45	2.65	2.58	71.21
Control	-	8.91	8.40	8.78	8.57	8.58	3.70
S.Ed±	-	0.51	0.53	0.43	0.36	-	-
CD(P=0.05)	-	1.08	1.12	0.92	0.77	-	-

DBS=Day before spray DAS=Days after spray Data are mean of 3 replications

Table 4. Correlation coefficient (r) of whitefly on okra with meteorological parameters

Insect Pests	Temperature (ºC)		Relative Humidity (%)		Rain fall (mm)	BSSH
	Max.	Min.	RH I	RH II		
Whitefly	-0.698**	-0.790**	-0.370^{NS}	-0.439^{NS}	-0.236^{NS}	0.224^{NS}

** Correlation is significant at P=0.01 level

treated with garlic extract @ 5% (27.02%). In control plots, there was 3.70 percent reduction in the population of whiteflies over the pre-treatment count.

The mean data of three sprays imposed in okra, targeting whiteflies indicated that among biopesticides, neem oil @ 5% was the most superior treatment (2.65 whiteflies/3 leaves) followed by *L. lecanii* @ 5ml/l (2.84 whiteflies/3 leaves), karanj oil @ 5% (3.30 whiteflies/3 leaves). The reduction of whitefly population in different treatments was in order of deltamethrin > neem oil > *L. lecanii* > karanj oil > *Beauveria bassiana* > *Melia azedarach* leaf extract > chilli fruit extract > garlic extract. The higher effect of neem oil against sucking pests may be due to feeding deterrence in addition to mortality. As back as 1962, the antifeedant property of neem has been discovered by Pradhan *et al.* the neem seeds contain azadirachtin which possesses antifeedant, repellents as well as insecticidal properties. The findings on the higher efficacy of neem oil against whitefly are in line with Rosaiah (2001) who reported that neem oil @ 0.5% was significantly superior in reducing the population of whitefly *B. tabaci* on okra. The effectiveness of *L. lecanii* is in agreement with Quinden (1984), Meade and Bruce (1991) and Nier *et al.* (1993) and according to them *L. lecanii* was found effective against whitefly, *B. tabaci*. According to Mallappanavar (2002) higher concentration of *L. lecanii* @ 1.33×10^7 spores/ml was found most effective against spiralling whitefly. According to Abdel-Raheem, M.A. and Lamya Ahmed Al-Keridis (2017), *L. lecanii* and *B. bassiana* isolates are promising agents for whitefly control in the field. The effectiveness of *M. azedarach* is in line with Abou – Fakhr *et al.* (2000) who reported that aqueous extracts of *M. azedarach* fruit were detrimental to first and second instars of *B. tabaci*, causing significant mortality of 30.3% compared with the control. During the present investigation, chilli fruit extract and garlic bulb extract were found effective against whitefly. Similar to the present finding Nayem and Rokib (2013) also reported garlic bulb extract to be effective against whitefly.

Correlation of whitefly population with abiotic factors

The results presented in (Table 4), revealed that the population of whitefly ranging from 3.04 to 14.91 whiteflies/3 leaves. The whitefly incidence was started from the 14th SMW (First week of April) at 31.5 ^{0}C maximum temperature, 18.4 ^{0}C minimum temperature, 92% and 56% morning and evening relative humidity, respectively. During the experiment, peak population whitefly (14.91 whiteflies/3 leaves) was observed at 18th SMW (First week of May) with 28.6 ^{0}C maximum temperature, 20.5 ^{0}C minimum temperature, 93% and 72% morning and evening relative humidity, 39.7mm rainfall. After the peak point, the population declined gradually during the successive weeks (Fig.1). The present observations are in corrobortion with the findings of Anitha (2007), who reported as a peak incidence of whitefly attack during the last week of April. Earlier, Hasan *et al.* (2008) reported peak whitefly population when the crop was 60 days old and these results are similar to the present observations. A study on the effect of weather parameters on the whitefly population on okra (Table 5), indicated that maximum temperature (r=-0.698) and minimum temperatures (r=-0.790) were negative and significant. While morning and evening relative humidity was found to have a non-significantly negative correlation with the whitefly population (r= -0.370 and -0.439 respectively). The correlation of whiteflies population with rainfall was also noted to be negative and non-significant (r=-0.236). While with bright sunshine hours it was positive and non-significant (r=0.224). The present findings are in line with Singh *et al.* (2013) who reported that the whitefly population responded negatively with maximum and minimum temperatures. Further, a non-significant negative correlation was obtained by Sharma and Rishi (2005) in relation to rainfall and the whitefly population incidence. Chandrakumar *et al.* (2008) found a negative correlation with maximum temperature and rainfall.

Yield

The yield of okra was significantly different among treatments. The highest fruit yield of okra was recorded in deltamethrin treated plots followed by neem oil, karanj oil. Whereas, the yield obtained from untreated control plots was 22.05q/ha.

CONCLUSION

The present study on evaluation of different biopesticides for eco-friendly management of whitefly population of okra revealed that among the biopesticides used, neem oil and *Lecanicillium lecanii* were found very effective against the target pest. Therefore, neem oil and *L. lecanii* can be an alternative eco-friendly management option for the whitefly of okra. And from the present study, we can conclude that maximum population of whitefly was observed at last week of April and first week may. During the study of whitefly population, significant negative correlation between whitefly population and the abiotic factors like maximum temperature, minimum temperature. However, parameters like morning and evening RH, rainfall and BSSH showed non-significant negative correlation during experimentation.

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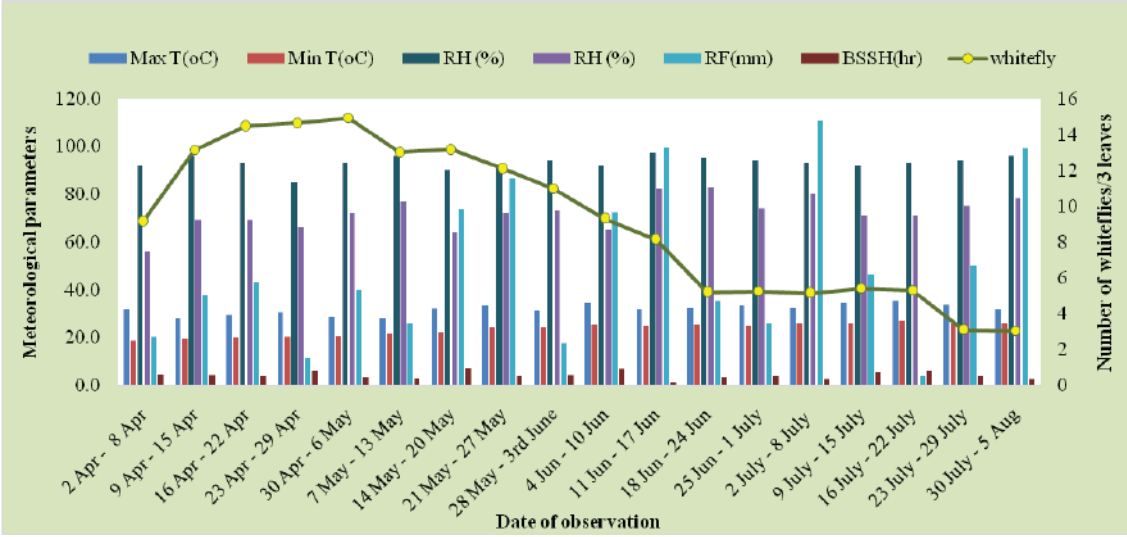

Fig. 1. Population build-up of whitefly, *Bemisia tabaci* in relation to meteorological parameters during April, 2018 to August, 2018

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Bioefficacy of newer insecticide molecules against sucking insect pest complex in okra and their effect on predators

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ABSTRACT: Field experiments were conducted to evaluate the bioefficacy of newer insecticide molecules against okra pest complex and their effect on natural enemies during *kharif* 2019-20. It was found that acetamiprid 20% SP registered significantly lower population of thrips (8.87/3 leaves), leafhopper (6.46/3 leaves), aphid (8.40/3 leaves) and whiteflies (8.46/3 leaves). Further, acetamiprid 20% SP recorded highest reduction in sucking insect pest population compared to untreated control. Imidacloprid 17.8 SL was next best option and registered significantly lower thrips (10.67/3 leaves), leafhoppers (8.53/3 leaves), aphids (10.60/3 leaves) and whiteflies (10.53/3 leaves) population. All the other treatments were significantly superior over untreated control. Acetamiprid 20% SP was also found to be relatively safer and did not have significant deleterious effect on natural enemies. Among all treatments, acetamiprid 20% SP resulted in significantly highest fruit yield (13.45 t/ha).

Keywords: Acetamiprid, okra, thrips, leafhoppers, aphids, whiteflies

INTRODUCTION

Okra (*Abelmoschus esculentus*), popularly known as lady's finger is an important dietary component having very high therapeutic value and health benefits (Anonymous, 2020). Besides, it is also a good source of various vitamins like A, B, C and is also rich in protein, carbohydrates, fats, iron and iodine etc. The okra is one of the important vegetables grown throughout the tropical and sub-tropical regions and also in the warmer parts of the temperate regions. In India okra, is cultivated in 5.46 lakh hectares of area with the production of 54.52 lakh tonnes and productivity of 10 t/ha (Anonymous, 2017). Like other crops, okra also suffers from several biotic and abiotic factors, including insect pests. However, insect pests are major production constraints in okra cultivation and the crop is ravaged by numerous insect pests *viz.,* aphids, leafhoppers, whiteflies and thrips right from sowing till harvesting. These pests cause damage to the crop directly by sucking the sap or indirectly by transmitting a large number of viral diseases. Due to desapping and injection of toxic saliva into plants by sucking pests, leaves turn brownish and may eventually fall down (Rudra and Saikia, 2020). Of late the conventional insecticides are reported to be less effective. There is a need to evaluate new molecules with different mode of action. Hence, present study was conducted to evaluate the bioefficacy of newer insecticide molecules on okra pest complex and their effect on natural enemies.

MATERIALS AND METHODS

A field experiment was conducted at College of Horticulture, Bidar, University of Horticultural Sciences, Bagalkot, during *Kharif* 2019-20 and 2020-21 in Randomized Block Design (RBD), with seven treatments viz., dimethoate 30 EC @ 250 g.a.i/ha, imidacloprid 17.8 SL @ 71.8 g.a.i/ha, acetamiprid 20% SP @ 75 g.a.i/ha, thiamethoxam 25% WG @ 100 g.a.i/ha, profenofos 50 EC @ 250 g.a.i/ha, clothianidin 50 WG @ 25 g.a.i/ha and untreated control replicated thrice. Treatments were imposed on Arka Anamika cultivar of okra which was sown with spacing of 60cm between lines and 30cm from plant to plant. The crop was raised by following package of practices of University of Horticultural Sciences, Bagalkot (Anonymous, 2017a) except plant protection measures. When the pest population crossed the Economic Threshold Level (ETL) treatments were imposed with 1000 litres of spray fluid per hectare using Knapsack Sprayer. The untreated control plot was not sprayed with any chemical.

Observations on thrips, leafhoppers, aphids and whiteflies were recorded on ten randomly selected tagged plants in each plot. Three leaves representing top, middle and bottom portion were selected for recording observations in each plant. The total number of nymphs and adults on each leaf were counted and expressed in terms of numbers per three leaves per plant. The pre-treatment counts were made a day before spray and post treatment counts were made 3, and 7 days after spray. The observations on predators *viz*., Green lace wings and coccinellids were recorded from five randomly selected plants a day before and 3 and 7 days after spray and expressed in terms of numbers per plant. The data recorded on the population of thrips, leafhoppers, aphids and whiteflies were square root transformed. At harvest, total fruit yield per plot was recorded and was computed to hectare basis. The data was subjected single factor Analysis of Variance (ANOVA).

Table 1. Effect of different insecticides on sucking insect pests in okra crop during 2019-20

Treatment	No.thrips / 3 leaves				No.leafhoppers / 3 leaves				No. aphids / 3 leaves			Mean	No.whiteflies / 3 leaves			
	1DBS	3DAS	7DAS	Mean	1DBS	3DAS	7DAS	Mean	1DBS	3DAS	7DAS		1DBS	3DAS	7DAS	Mean
Dimethoate 30 EC @ 250 g a.i./ha	22.8 (4.83)	17.5 (4.24)	14.73 (3.90)	16.12	16.20 (4.09)	15.27 (3.97)	11.73 (3.50)	13.5	18.46 (4.35)	15.51 (4.00)	13.95 (3.80)	14.73	20.20 (4.55)	16.73 (4.15)	14.73 (3.90)	15.73
Imidacloprid 17.8 SL @ 71.8 g a.i./ha	22.73 (4.82)	14.07 (3.82)	10.67 (3.34)	12.37	16.20 (4.09)	12.37 (3.59)	8.53 (3.00)	10.45	18.46 (4.35)	12.06 (3.54)	10.60 (3.33)	11.33	20.20 (4.55)	12.47 (3.60)	10.53 (3.32)	11.15
Acetamiprid 20% SP @ 75 g a.i./ha	22.73 (4.82)	12.27 (3.57)	8.87 (3.06)	10.57	16.00 (4.06)	10.9 (3.38)	6.46 (2.64)	8.68	18.33 (4.34)	10.8 (3.36)	8.40 (2.98)	9.60	20.00 (4.53)	10.40 (3.30)	8.46 (2.99)	9.43
Thiamethoxam 25% WG @ 100 g a.i./ha	22.67 (4.81)	15.85 (4.04)	12.67 (3.63)	14.26	16.27 (4.10)	14.05 (3.81)	10.66 (3.34)	12.36	18.8 (4.39)	14.13 (3.82)	12.6 (3.62)	13.37	20.27 (4.56)	14.67 (3.89)	12.66 (3.63)	13.66
Profenofos 50 EC @ 250 g a.i./ha	22.93 (4.84)	16.33 (4.56)	17.25 (4.21)	16.79	16.27 (4.10)	16.05 (4.07)	12.86 (3.66)	14.46	18.86 (4.40)	17.06 (4.19)	15.86 (4.04)	16.46	20.27 (4.56)	19.67 (4.49)	17.86 (4.28)	18.76
Clothianidin 50 WG @ 25 g a.i./ha	22.67 (4.81)	20.33 (4.39)	16.67 (4.14)	18.50	16.13 (4.08)	16 (4.06)	12.8 (3.65)	14.4	18.26 (4.33)	16.13 (4.08)	14.93 (3.93)	15.53	20.13 (4.54)	18.57 (4.37)	16.8 (4.16)	17.68
Untreated Control	22.87 (4.83)	24.4 (4.99)	26.8 (5.22)	25.6	16.27 (4.10)	19.27 (4.45)	21.4 (4.67)	20.33	18.26 (4.33)	20.06 (4.53)	24.13 (4.96)	22.10	20.27 (4.56)	23.27 (4.88)	25.4 (5.09)	24.34
S Em +	0.3	0.06	0.08		0.18	0.05	0.03		0.02	0.06	0.05		0.18	0.07	0.09	
CD (P=0.05)	NS	0.19	0.26		NS	0.15	0.11		NS	0.18	0.17		NS	0.21	0.27	
CV %	15.23	16.25	13.55		12.1	13.1	19.58		13.2	18.4	19.4		12.1	13.1	16.58	

DBS- Day before spray

DAS-Days after spray

NS- Non-significant

Figures in the parenthesis are √0.5+x transferred values

RESULTS AND DISCUSSION

Thrips population

A day before the imposition of the treatments, population of thrips was uniform and there was no significant difference among the treatments during 2019-20 (Table 1). The observations recorded 3DAS and 7DAS clearly revealed that there was a significant difference among the treatments and all the treatments were superior over the untreated control. Acetamiprid 20 SP registered lowest thrips population (12.27 and 8.87/3leaves at 3DAS and 7DAS, respectively), with mean population of 10.57 thrips/3 leaves (Table-1) which was 76 per cent reduction over the untreated control during 2019-20 (Fig.1). The Imidacloprid 17.8 SL which recorded 14.07 and 10.67 thrips/3 leaves at 3DAS and 7DAS respectively and with mean population of 12.37 thrips/3 leaves was next best treatment. Acetamiprid belongs to new class of insecticide 'neonicotinoids' and precise structure of the acetamiprid is chloronicotinyl compound. It has been shown to be a potent against the nicotinic acetylcholine receptors in insects causing quick knock down of the insects compared to other class of insecticides (Wallace, 2014). Imidacloprid acts on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system (Bunckingham *et al.*, 1997; Matsuda and Sattelle 2005). In insects these receptors are located only within the central nervous system. Following binding to the nicotinic receptors, nerve impulses are spontaneously discharged at this first, followed by, failure of neuron to propagate any signal. Sustained activation of the receptors results from the inability of the acetylcholinisterases to breakdown the pesticide. This binding process is irreversible and brings about the insect death.

Thiamethoxam 25 WG, dimethoate 30EC and clothianidin 50 WG recoded higher mean population of 14.26, 16.12 and 18.50 thrips per 3 leaves, respectively. Untreated control recorded highest population of thrips (25.6/3 leaves). Present findings are in agreement with Udikeri *et al.* (2009; Duraimurugan and Alivelu (2017) who reported the effective control of thrips by newer insecticide acetamiprid 20 SP in cotton and castor crop system, respectively. Similar trend was observed during 2020-21 also wherein acetamiprid was found to be superior over other control by registering highest reduction over untreated control (78%) (Table 2).

Leafhopper population

There was no significant difference among the treatments with respect to leafhopper population a before spray (1DBS) and the population ranged between 16.00 to 16.27 per three leaves during 2019-20 (Table1).

Observations on leafhoppers 3DAS and 7DAS during 2019-20 clearly revealed that acetamiprid 20% SP was significantly superior over other treatments by recording lowest leafhopper population (10.90/3 leaves and 6.46/3 leaves, respectively) with mean population of 8.68 leafhoppers/3 leaves (Table-1) and 85 per cent reduction over control (Fig.1) during 2019-20. Imidacloprid 17.8 SL with 12.37 and 8.53 leafhoppers per plant and thiamethoxam 25% WG with 14.05 and 10.66 leafhoppers per plant 3 DAS and 7 DAS respectively, were next best treatments in row (Table 1). Untreated control recorded significantly highest leafhopper (19.27 and 21.40 leafhoppers/3 leaves, 3 DAS and 7DAS respectively and with highest mean leafhopper population (20.33/3 leaves). Present findings are in accordance with results of Udikeri *et al.* (2009); Duraimurugan and Alivelu (2017) who reported the effective control of leafhoppers in cotton and castor crop, respectively by acetamiprid and imidacloprid. Further, they inferred that neonicotinoid group of insecticides are more effective in controlling the herbivores compared to conventional insecticides mainly because there was no pesticide resistance due to their recent development. Simon Delso (2015) through series of experiments found out that when these neonicotinoid insecticides are translocated through the plant system their physicochemical properties such as high persistence they cause higher mortality in insects compared to previous generation insecticides. The observations made during 2020-21 again revealed that acetamiprid was best among the different treatments with 89 per cent reduction of leafhopper population over control. Similar trend for the performance of other treatments (Table 2).

Aphid population

Aphid population pre count made a before the spray depicted the uniform distribution among the different treatments and there was no significant difference (Table 1). The observations made on aphid population at 3DAS and 7DAS revealed that there was a significant difference among the treatments and all the treatments were superior over untreated control. However, among different treatments, acetamiprid 20% SP was the best by recording lowest aphid population (10.80 and 8.40 per 3 leaves at 3DAS and 7 DAS, respectively with mean population of 9.60 aphids per 3 leaves during 2019-20 (Table 1). Further, the superiority of the acetamiprid was also evidenced by the highest reduction over untreated control (99%) (Fig.1). As observed in the management of other sucking pests imidacloprid 17.8 SL and thiamethoxam 25% WG showed consistent performance against aphids also with 11.33 and 13.37 mean population of aphids. Among all the treatments untreated control was significantly inferior and recorded highest aphid

Table 2: Effect of different insecticides on sucking insect pests in okra crop during 2020-21

Treatment	No. thrips / 3 leaves			Mean	No.leafhoppers / 3 leaves			Mean	No.aphids / 3 leaves			Mean	No. whiteflies / 3 leaves			Mean
	1DBS	3DAS	7DAS		1DBS	3DAS	7DAS		1DBS	3DAS	7DAS		1DBS	3DAS	7DAS	
Dimethoate 30 EC @ 250 g a.i./ha	21.8 (4.72)	16.5 (4.12)	13.73 (3.77)	15.12	15.20 (3.96)	14.27 (3.84)	10.73 (3.35)	12.50	17.46 (4.24)	14.51 (3.87)	12.95 (3.67)	13.73	19.2 (4.44)	15.73 (4.03)	13.73 (3.77)	14.73
Imidacloprid 17.8 SL @ 71.8 g a.i./ha	21.73 (4.71)	13.07 (3.68)	9.67 (3.19)	11.37	15.20 (3.96)	11.37 (3.45)	7.53 (2.83)	9.45	17.46 (4.24)	11.06 (3.40)	9.60 (3.18)	10.33	19.2 (4.44)	11.47 3.46)	9.53 (3.17)	10.5
Acetamiprid 20% SP @ 75 g a.i./ha	21.73 (4.71)	11.27 (3.43)	7.87 (2.89)	9.57	15.00 (3.94)	9.90 (3.22)	5.46 (2.44)	7.68	17.33 (4.22)	9.80 (3.21)	7.40 (2.81)	8.60	19 (4.42)	9.4 (3.15)	7.46 (2.82)	8.43
Thiamethoxam 25% WG @ 100 g a.i./ha	21.67 (4.71)	14.85 (3.92)	11.67 (3.49)	13.26	15.27 (3.97)	13.05 (3.68)	9.66 (3.19)	11.36	17.8 (4.28)	13.13 (3.69)	11.6 (3.48)	12.37	19.27 (4.45)	13.67 (3.76)	11.66 (3.49)	12.67
Profenofos 50 EC @ 250 g a.i./ha	21.93 (4.74)	19.33 (4.45)	16.25 (4.09)	17.79	15.27 (3.97)	15.05 (3.94)	11.86 (3.52)	13.46	17.86 (4.28)	16.06 (4.07)	14.86 (3.92)	15.46	19.27 (4.45)	18.67 (4.38)	16.86 (4.17)	17.77
Clothianidin 50 WG @ 25 g a.i./ha	21.67 (4.71)	17.77 (4.27)	15.67 (4.02)	16.72	15.13 (3.95)	15.00 (3.94)	11.80 (3.51)	13.40	17.26 (4.21)	15.13 (3.95)	13.93 (3.80)	14.53	19.13 (4.43)	17.57 (4.25)	15.8 (4.04)	16.69
Untreated Control	21.87 (4.73)	23.4 (4.89)	25.8 (5.13)	24.6	15.27 (3.97)	18.27 (4.33)	19.93 (4.51)	19.10	17.26 (4.21)	19.06 (4.42)	17.13 (4.20)	18.10	19.27 (4.45)	22.27 (4.77)	24.4 (4.99)	23.34
S Em +	0.3	0.05	0.07		0.18	0.04	0.04		0.02	0.05	0.04		0.18	0.06	0.08	
CD (P=0.05)	NS	0.18	0.24		NS	0.13	0.12		NS	0.16	0.15		NS	0.19	0.26	
CV %	14.2	16.5	18.3		15.1	14.15	14.58		16.28	20.4	20.4		14.5	16.1	16.58	

DBS- Day before spray; DAS-Days after spray; NS- Non-significant

Figures in the parenthesis are √0.5+x transferred values

Table 3: Impact of different insecticides on natural enemies and yield of okra

Treatment	2019-20							2020-21						
	Chrysoperla (No./plant)			Coccinellids (No./plant)			Yield	*Chrysoperla* (No./plant)			Coccinellids (No./plant)			Yield
	1DBS	3DAS	7 DAS	1DBS	3DAS	7 DAS	(t/ha)	1DBS	3DAS	7 DAS	1DBS	3DAS	7 DAS	(t/ha)
Dimethoate 30 EC @ 250 g a.i./ha	3.63 (2.14)	2.20 (1.78)	1.25 (1.50)	1.20 (1.30)	1.02 (1.23)	0.98 (1.21)	9.45	3.13 (2.03)	1.70 (1.64)	0.75 (1.32)	0.70 (1.30)	0.52 (1.23)	0.42 (1.19)	9.25
Imidacloprid 17.8 SL @ 71.8 g a.i./ha	3.40 (2.09)	2.10 (1.76)	1.22 (1.49)	1.14 (1.28)	0.90 (1.18)	0.93 (1.19)	12.20	2.90 (2.21)	1.60 (1.61)	0.72 (1.31)	0.64 (1.28)	0.40 (1.18)	0.43 (1.20)	11.70
Acetamiprid 20% SP @ 75 g a.i./ha	2.89 (1.97)	1.90 (1.70)	1.55 (1.59)	1.15 (1.28)	1.03 (1.23)	0.87 (1.17)	13.45	2.39 (1.84)	1.40 (1.55)	1.05 (1.43)	0.65 (1.28)	0.53 (1.24)	0.37 (1.17)	12.95
Thiamethoxam 25% WG @ 100 g a.i./ha	3.41 (2.10)	2.01 (1.73)	1.96 (1.71)	1.17 (1.29)	1.04 (1.24)	0.84 (1.15)	10.65	2.91 (1.98)	1.51 (1.58)	1.46 (1.57)	0.67 (1.29)	0.54 (1.24)	0.34 (1.16)	10.40
Profenofos 50 EC @ 250 g a.i./ha	2.78 (1.94)	2.20 (1.78)	1.88 (1.69)	1.16 (1.28)	1.00 (1.22)	0.62 (1.05)	6.90	2.28 (1.81)	1.70 (1.64)	1.38 (1.54)	0.66 (1.28)	0.50 (1.22)	0.12 (1.06)	6.80
Clothianidin 50 WG @ 25 g a.i./ha	3.12 (2.02)	2.33 (1.82)	1.25 (1.50)	1.15 (1.28)	1.03 (1.23)	0.93 (1.19)	8.20	2.62 (1.90)	1.83 (1.68)	0.75 (1.32)	0.65 (1.28)	0.53 (1.24)	0.43 (1.20)	8.05
Untreated Control	3.40 (2.09)	2.20 (1.78)	2.01 (1.73)	1.20 (1.30)	1.02 (1.23)	1.04 (1.21)	4.30	2.90 (2.21)	1.70 (1.64)	1.51 (1.58)	0.70 (1.30)	0.52 (1.23)	0.54 (1.24)	3.65
S Em ±	0.07	0.05	0.06	0.08	0.07	0.06	0.36	0.05	0.02	0.04	0.06	0.05	0.04	0.31
CD (P=0.05)	NS	NS	NS	NS	0.21	0.18	1.15	NS	NS	NS	NS	0.20	0.14	1.10
CV %	6.26	5.79	6.69	7.25	8.54	8.35	12.43	5.61	6.28	5.55	6.15	7.35	7.30	8.32

DBS- Day before spray; DAS-Days after spray;

Figures in the parenthesis are √0.5+x transferred values; NS- Non-significant

population of 20.06 and 24.13 per 3 leaves after 3DAS and 7DAS respectively with mean population of 22.10 aphids per 3 leaves. Similar trend was noticed during 2020-21 also (Table 2 and Fig.1) as acetamiprid was the best among the different treatments with 98 per cent reduction of aphid population over control. The present findings are in accordance with Udikeri *et al.* (2009) who observed effective control of aphids in cotton ecosystem system.

Whitefly population

The whitefly population was distributed uniformly among the different treatments with no significant difference among the treatments and the pest load ranged from 20.00 to 20.27 (Table 1). Observations made on whitefly population 3DAS and 7DAS showed significant difference among the different treatments. Acetamiprid 20% SP recorded lowest whitefly population (10.40/3 leaves and 8.46/3 leaves at 3DAS and 7DAS, respectively) with the mean whitefly of 9.43 per 3 leaves (Table 1) and 62 per cent reduction over control (Fig.1), followed by, imidacloprid 17.8 SL recorded 12.47 and 10.53 whiteflies per three leaves on 3DAS and 7DAS respectively, with mean population of 11.15 whiteflies per three leaves, during 2019-20 (Table 1). thiamethoxam 25% WG, dimethoate 30 EC and profenosfos 50 EC recorded higher mean whitefly population (13.66, 15.73 and 18.76, respectively). Among all the other treatments, Untreated control registered significantly highest whitefly population (an average of 24.34/3 leaves). Present findings are in line with the results of Aina *et al.* (2017) who found acetamiprid 20 SP as a very good option in controlling whiteflies. Similar trend was observed during 2020-12 (Table 2). Acetamiprid resulted in highest reduction of whiteflies over control (62%).

Natural enemy population

The observations on natural enemies *viz*., Green lace wings and coccinellids during 2019-20 and 2020-21 revealed that acetamiprid 20% SP is safe and had lesser deleterious effect on the predatory population compared to other treatments (Table 3). Present findings are in line with Sonali and Yadu (2018) who reported that acetamiprid 20 SP as one among the safer chemicals in chilli ecosystem with lesser deleterious effect on natural enemies. Acetamiprid 20% SP has recorded highest fruit yield (13.45 t/ ha) which was followed by, imidacloprid 17.8 SL (12.20 t/ha). Untreated control recorded a minimum fruit yield of 4.30 t/ha (Table 3) during 2019-20. Similar trend was noticed during 2020-21 (Table 3).

CONCLUSION

Acetamiprid 20% SP was found to be best among different treatments in controlling sucking insect pest complex *viz*., thrips, leafhoppers, aphids and whiteflies with higher yield and was found to be relatively safe to predators.

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Isolation and characterization of beewax degrading enzymes from the digestive guts of greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae)

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ABSTRACT: The greater wax moth, *Galleria mellonella* (L.) is one of the most destructive honeycomb pests in the world. The digestive gut enzymes, esterase and lipase are a group of enzymes that hydrolytically catalyzes the esters and long-chain fatty acid linkage of wax components of beeswax. Final instar stage larvae were selected and homogenized for enzymes activity assay and subsequent analysis. The enzymes were purified by combination of ammonium sulfate precipitation and gel filtration using Sephadex G-100. The amount of protein and specific activity of lipase were 0.01 ± 0.002 mg/mL and 1.63 ± 0.88 mol/min/mg protein, respectively, with a 163-fold purity and 33.3 percent recovery, while for esterase14.71 ± 1.46 and 11.23 ± 1.41 mol/min/mg protein, respectively, with a 1.97-fold purity and 34.383 percent recovery. Furthermore, biochemical characterization esterase and lipase were carried out through testing its activities against factors, such as different temperatures and pH ranges with the use of *p*-nitrophenyl butyrate (PNPB) *p*-nitrophenyl palmitate substrates. The highest activities of enzymes were determined at the temperature ranges of 35–40 °C and pH ranges of 7–9.Insect pest damage to agricultural products can be reduced by using inhibitors against digestive enzymes. The findings of this research establish the role of esterase and lipase in the physiological function of *G. mellonella* in the degradation of beeswax and developing novel inhibitors against these enzymes can be implemented to control *G. melonella*. Isolation, purification, and characterization of esterases and lipases from the guts of *G. mellonella* could be a crucial step toward a better understanding of their action and the establishment of a safe and effective control method for *G. mellonella* as well as other insect pests.

Keywords: *Galleria mellonella*, Isolation, Characterization, Beeswax, Esterase and Lipases.

INTRODUCTION

Beeswax honeycomb is a hexagonal prismatic wax cell structure constructed by honey bees and made up of long fatty acids, esters and hydrocarbons (Blomquist *et al.,* 1980). It is a complex substance made up of wax esters, fatty acids and hydrocarbons (Piek, 1964; Tulloch, 1970). The composition of beeswax varies depending on place of production (Europe, Asia, or Africa), species of honeybee, and age of wax (Tinto *et al.,* 2017). Generally, unhydrolyzed & unrefined beeswax contains hydrocarbons (15%), esters (71%), free acids (8%), and other compounds (6%) (Tulloch, 1970; Parish *et al.,* 2002). The beeswax comprises ~70% esters in its composition mainly monoesters (35%), diesters (14%), triesters (3%), hydroxymonoesters (4%), hydroxypolyesters (8%), acid esters (1%) and acid polyesters (2%). In addition, beeswax also contains hydrocarbons (14%), free fatty acids (12%), free alcohols (1%) and miscellaneous groups (6%). This wide range of biochemical composition offers beeswax many distinctive features (Goodman, 2003) and also prevents us from fully comprehending the production and secretion process.

The greater wax moth (GWM)*, Galleria mellonella* (L.) (Lepidoptera: Pyralidae) is one of the most damaging pests of honey bee colonies around the world (Oh *et al.,* 1995). Beekeepers suffer significant financial losses as a result of the damage to wax combs. The devastation of the comb by *G. mellonella* often considered to be the cause of honey leaking/ contamination, bee larvae death and the spread of honey bee diseases (Caron, 1992). Wax moth larvae do not harm bees directly (i.e., they do not feed on any life stages of the), but they do attack beeswax combs, which are an important component of the honey bee colony (Abou El-Ela, 2014; Berry and Delaplane, 2001; Hamby, 2006; Swamy, 2008a). Greater wax moth infestations can drive weak colonies to abscond, or quit the nest entirely, and can also overrun seemingly robust colonies, but this is rare (Swamy, 2008b). Greater wax moth infestation is also more common in stored combs and weak colonies, resulting in huge financial losses for

Table 1. Purification process of lipase from the guts of *G. mellonella* larvae

Purification step	Protein[a] (mg/ml)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude extract	2.33 ± 0.19	0.03 ± 0.004	0.01 ± 0.0008	1	100.00
40 % SAS*	1.21 ± 0.17	0.02 ± 0.009	0.02 ± 0.009	2	66.66
80 % SAS*	0.21 ± 0.01	0.01 ± 0.011	0.11 ± 0.04	11	33.33
Sephadex G-100	0.01 ± 0.002	0.01 ± 0.007	1.63 ± 0.88	163	33.33

[a] Protein content was measured by the Bradford method (1976); *SAS= Saturated Ammonium Sulfate; [b]one unit of activity corresponds to 1µmol of pNP released per min using 50mM *p*-NPP as the substrate at 37 °C; Data are showed as (mean ± SE), n = 3 replicates.

beekeeping industry (Swamy *et al.,* 2010). However, no comprehensive control methods for this pest have been developed to date. Currently practiced physical and chemical techniques are highly ineffective (Burges, 1978). As a result, numerous studies have been conducted in order to find alternate ways to regulate this pest.

In recent past, the digestive enzymes of several insect pests have been studied as target molecules for pest management. Particularly studies on the use of enzyme inhibitors are indeed a priority to develop optimal and effective control measures involving advanced biotechnological methods (Oh *et al.,* 1995; Pereira *et al.*, 2005). As the beeswax that serves as major food source for the larvae of GWM comprises a high concentration of esters, long-chain fatty acids and lipids as mentioned earlier, it is crucial to understand the gut enzymes of the greater wax mothlarvae that can depolymerize the complex substrate (= beeswax). In general, the specific enzymes which act on these substrates are usually esterases and lipases which hydrolyzes short-chain and long chain esters respectively at different reaction rates (Chahinian and Sarada, 2009). Esterases are hydrolytic enzymes that break down the ester bonds in a wide range of biomolecules, are widely present across the animals, plants, and microorganisms (Oakeshott *et al.,* 2005; Upadhya *et al.,* 1985). Esterases present in the larval gut hydrolyzes the esters to an alcohol moiety and fatty acids which are further broken down (Niemierko and Wlodawer, 1950). Insect lipases, on other hand are classified into phospholipases, triacylglycerol lipases (TAG-lipases), alkaline and acid phosphatases (Terra and Ferreira, 2012) that are divided into two categories: lysosomal (intracellular) and digestive lipases (Miled *et al.,* 2000). Usually, triacylglycerols (TAG) are hydrolyzed by intracellular lipases and are preserved as lipid droplets, while digestive lipases hydrolyze TAGs in the diet. Both esterases and lipases can attack the same substrate depending on the physical state of the substrate.

The extraction of digestive esterase from the guts of *G. mellonella* larvae was attempted earlier (Juraimi, 1986). Esterases of the GWM larvae are categorized as carboxylesterases that converts the beeswax esters into various acids and alcohols (Jacobson et al., 1968; Niermerko, 1959). However, only about 50% of the ingested wax is utilized and metabolized by theGWM larvae and the excess acid resulting from the high lipid diet is excreted (Vonk *et al.,* 1984).While, the lipases of GWM larvae are mostly triacylglycerol lipase, that perhaps hydrolyses bees wax TAGs into various fatty acids and glycerol (Mahdy *et al.*, 2020).Similarly, β-glucosidase, a type of digestive enzyme that hydrolytically catalyzes the beta-glycosidic linkage of glycosides was also extracted earlier from the guts of *G. mellonella* (Kara *et al.,*2014). They found that β-glucosidase degrades the β-glycosides components of beeswax and comprehended that establishing its role could be a prospective control procedure for *G. mellonella.* As a result, very limited information is available on the nature of the enzymes that are responsible for the hydrolysis of carboxylic acid esters and fatty acids of beeswax in *G. mellonella.* Therefore, the present study was initiated as an attempt to isolate, identify, characterize and measure the activity of esterase and lipase enzymes from the guts of *G. mellonella* larvae.

MATERIALS AND METHODS

Insects rearing

Galleria mellonella larvae (7–8thinstar) were obtained from the Department of Entomology, University of Agricultural Sciences, GKVK, Bengaluru, India and maintained at the Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bengaluru continuously as per the procedures described earlier (Li *et al.*, 2019; Cassone *et al.*, 2020). Larvae of *G. mellonella* were reared on honeycombs of Indian honey bee, *Apis cerana indica* Fab. in a dark plastic container (20 × 20 ×

Table 2. Purification process of esterase from the guts of *G. mellonella* larvae

Purification step	Protein[a] (mg/ml)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude extract	81.24 ± 2.62	464.20 ± 11.26	5.71 ± 0.04	1	100
50 % SAS	45.17 ± 2.87	349.30 ± 8.32	7.81 ± 0.68	1.37	75.24
80 % SAS	31.53 ± 2.58	268.43 ± 8.30	8.60 ± 0.59	1.50	57.82
Sephadex G-100	14.71 ± 1.46	161.71 ± 1.39	11.23 ± 1.41	1.97	34.83

[a] Protein content was measured by the Bradford method (1976); *SAS= Saturated Ammonium sulfate; [b]one unit of activity corresponds to 1µmol of pNP released per min using 50mM *p*-NPB as the substrate at 37 °C. Data are showed as (mean ± SE), n = 3 replicates

30.5 cm; length × width × height), at ambient conditions (27 ± 1°C, 75 ± 2% RH and 14L: 10D h photoperiod). The larvae during their final instar stage (7^{th}-8^{th}) were collected for further experiments.

Chemicals

The substrates for enzyme activity studies [*p*-nitrophenyl acetate (CAS No. 830-03-5), *p*-nitrophenyl palmitate (CAS No. 1492-30-4)] and protein assay reagents were purchased from Sigma-Aldrich, India.

Enzyme Preparation

A total of 20 larvae of *G. mellonella* (58 g weight) were rinsed in cold distilled water and blotted with filter paper, homogenized in a pre-cooled pestle and mortar in 25 mL of 50 mMTris-HCl buffer at pH 7.0. The homogenate (~25 mL) was centrifuged at 4°C for 15 min at 10,000 g and the supernatant collected (~20 mL) was used directly as enzyme source and the pellet discarded. The activity of the enzymes was tested in triplicates. Crude enzymes were stored at -80 °C until further use.

Estimation of protein concentration

Protein concentration of crude enzymes was determined as described by Bradford (1976) with bovine serum albumin (BSA) as standard protein.

Determination of lipase activity

Lipase activity was measured based on procedures described by Tsujita *et al.* (1989) and Zuo *et al.* (2010) with slight modifications. The reaction mixture that consisted 250 µL of 100 mM sodium phosphate buffer (pH 7.0), 30 µL of 5 mM *p*-nitrophenyl palmitate as a substrate (PNPP)and 30 µL of crude enzyme were thoroughly mixed and incubated at 37°C for 30 minutes. Later, 100 µl of distilled water were added to each tube (control and treatment). The reaction was stopped after incubation with 50 µL of 3.5% SDS solution and the color that developed due to liberation of the *p*-nitrophenols were immediately measured using a $T80^{+}$ UV/VIS spectrometer (PG Instruments, UK), at 410 nm approximately for 5 min. For negative control tubes, samples were placed in a boiling water bath for 15 min to destroy the enzyme activity then cooled. One unit of esterase activity was defined as the rate at which 1 µmol of *p*-nitrophenol is liberated per min under assay conditions (Rúa *et al.,* 1997).

Determination of esterase activity

Esterase activity was measured based on the procedures described by Zuo *et al.* (2010) and Wheeler *et al.* (2010) with slight modifications. The reaction mixture contained 250µL of 100 mM PBS buffer (pH 7.0), 30 µL of 5 mM of *p*-nitrophenyl butyrate (PNPA) to which 30 µL of crude enzyme was added. The mixture was thoroughly mixed and incubated at 37°C for 30 minutes and a total of 100 µl of distilled water were added to each tube (control and treatment). For negative control tubes, samples were placed in a boiling water bath for 15 min to destroy the enzyme activity then cooled. Spectrophotometer reading at 410 nm was measured immediately after incubation of the reaction mixture. One unit of esterase activityas defined as the amount of enzyme that produced one micromole of product, *p*-nitrophenol per minute in a substrate (5mM of PNPB) solution at 37°C and pH 7.5. Specific activity was expressedas the ratio of units of enzyme activity per milligramof protein in the solution (Juraimi,1986; Rúa *et al.,* 1997).

Purification of lipase and esterase

The purification of gut lipase and esterase was done based on the procedure described by Zibaee *et al.,* (2012) and Orscelk *et al.,* (2007) with minor modifications. All the purification steps were performed at 4 °C as mentioned below and in each step the activity of enzyme and content of protein were determined.

Ammonium sulfate precipitation

Samples were first exposed to ammonium sulfate

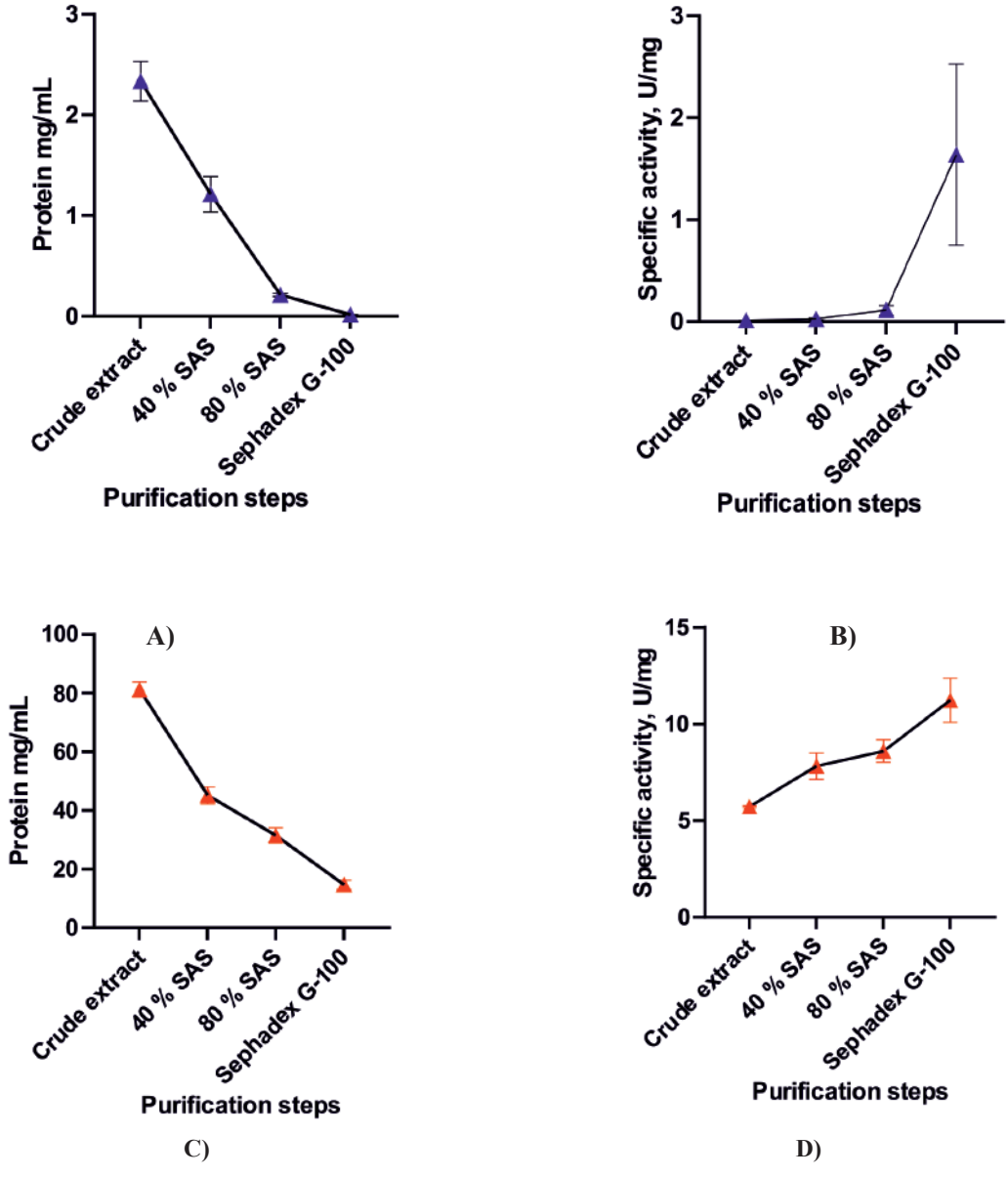

Fig 1. The purification of the lipase (A and B) and esterase (C and D) of *G. mellonella* extracted from the different fractions. *SAS= Saturated Ammonium sulfate.

precipitation by making use of 40% and 80% of saturated ammonium sulfate solution (SAS) fraction was then gathered and centrifuged at 12,000 rpm for 10 min and further subjected for gel filtration as mentioned below.

Sephadex G-100 gel filtration chromatography

The final fraction of ammonium sulphate was gel filteredon a dried Sephadex G-100 column. The resultant dried gel was incubated at 70°C for 5 hours in distilled water. After cooling and removing air, the gel was put into the column [12 × 2 cm, equilibrated with 50mM universal buffer (pH 10) and 50mM $(NH_4)_2SO_4$ at 30°C. With the same buffer, five milliliters of enzyme fractions were collected at a flow rate of 20 mL/h. Protein content and enzyme activity were determined for each fraction.

Determination of biochemical characteristics of the purified esterase and lipases

Determination of Optimal pH

The pH effect on the activity of purified lipase and esterase was measured using purified enzyme diluted in 25µL universal buffer with the pH range from 2 to 13 (Mahdy *et al.,* 2020; Stauffer, 1989). Lipase & esterase activity was assayed as described above, after incubation for 1 h at each pH value.

Determination of Temperature

Esterase and lipase were diluted in 25 µL of buffer (50 mM Tris–HCl pH 7–7.5) and incubated for 1 hour at temperatures ranging from 20 to 70°C. The activity of lipase and esterase was evaluated immediately after incubation using PNPP and PNPA as substrates, as described before.

Statistical analyses

Data were analysed using the Graph Pad Prism software, V.9.0 and expressed as mean ± standard error (SE) of three replicates for each determination. Before conducting the analysis, all the data were subjected for normality distribution test to confirm the normality. As the data confirms normal distribution, the difference between means was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, when $P \leq 0.05$.

RESULTS

Purification of esterase and lipase from the *G. melonella* larvae

Partial purification of the esterase and lipase activity of the *G. melonella* larvae was attempted through ammonium sulfate fractionation. In case of lipase, the amount of protein decreased from 2.33 ± 0.19 mg in crude extract to 1.21 ± 0.17 in 40% ammonium sulfate fraction with the specific activity of 0.02 ± 0.009 µmol/min/mg protein, 66.66% recovery and 2-fold purification. While with 80% ammonium sulfate fraction, the quantity of lipase decreased to 0.21 ± 0.01 mg, with 0.11 ± 0.04 µmol/min/mg protein, 33.33% recovery and 11-fold purification. The protein amount and the specific activity of digestive lipase from the fractions of Sephadex G-100 column were 0.01 ± 0.002 mg/mL and 1.63 ± 0.88 µmol/min/mg protein, respectively with 33.33% recovery and 163-fold purification. Statistically, One-way ANOVA with Tukey's multiple comparison test revealed that, there was a significant difference in protein concentration between crude sample and sample after ammonium sulfate precipitation steps[40% and 80%] and Sephadex G-100 (One-way ANOVA; $P<0.0001$, $df=3$, $F_{(3,8)} = 64.63$,Fig. 1A). The specific activity of lipase in Sephadex G-100 was found to be significant over40% SAS, 80% SAS fractions and crude extract (One-way ANOVA; $P = 0.008$, $df = 3$, $F_{(3,8)} = 8.19$, Fig. 1B). This clearly exhibits that Sephadex G-100 step was more effective in the purification process and the specific activity of lipase was more with the substrate, *p*-PNPP (Table 1).

Similarly, in case of esterase, the amount of protein decreased from 81.24 ± 2.62 mg in crude extract to 45.17 ± 2.87 in 40% ammonium sulfate fraction with the specific activity of 7.81 ± 0.68 µmol/min/mg protein, 75.24% recovery and 1.37-fold purification, while with 80% ammonium sulfate fraction, the quantity of esterase decreased to 31.53 ± 2.58 mg, with 8.60 ± 0.59 µmol/min/mg protein, 57.82% recovery and 1.50-fold purification. The protein amount and the specific activity of digestive esterase of fractions from Sephadex G-100 column were14.71 ± 1.46 mg/ml and 11.23 ± 1.41 µmol/min/mg protein, respectively with 34.83% recovery and 1.97-fold purification. Significant difference was observed in protein concentration between crude sample, sample after ammonium sulfate [40% and 80%] precipitation steps and Sephadex G-100 (One-way ANOVA; $P<0.0001$, $df = 3$, $F_{(3,8)} = 133.5$, Fig. 1C). Whereas, the specific activity of esterase in Sephadex G-100 was found significantly higher than40% SAS and crude extract (One-way ANOVA; $P = 0.004$, $df = 3$, $F_{(3,8)} = 9.79$, Fig. 1D). However, no significant difference was observed between the Sephadex G-100 and 40% SAS ($P=0.12$) indicating the Sephadex G-100 step was more effective in the purification process of protein and the specific activity of esterase was more with the substrate, *p*-PNPB (Table 2).

Biochemical characterization of purified lipase and esterase

The effect of pH on lipase and esterase activity

The effect of pH on lipase and esterase activity is depicted in (Fig. 2A and B). The activity of lipase gradually increased from 5 to 8 and then reduced until pH 14. Activity was high when assayed at pH 7–10, with the maximal activity at pH 8. Lipase activity was decreased at pH 13. While in case of esterase, the activity gradually increased from 6 to 8 and then reduced until pH 13. The specific activity of esterase was found high at pH 8-8.5.

The effect of temperature on lipase and esterase activity

The effect of temperature on the activity of pure lipase and esterase was investigated throughout a temperature range of 20 to 70 °C (Fig. 2 C and D). Purified lipase activity increased gradually as incubation temperature was raised from 20 to 40 °C, then declined till 70 °C. The Maximum activity under these conditions was found at 37 °C. Similarly, esterase activity was also increased gradually with raise in incubation temperature from 20 to 40 °C, then declined till 70 °C. The Maximum activity under these conditions was at both 35 °C and 40 °C.

DISCUSSION

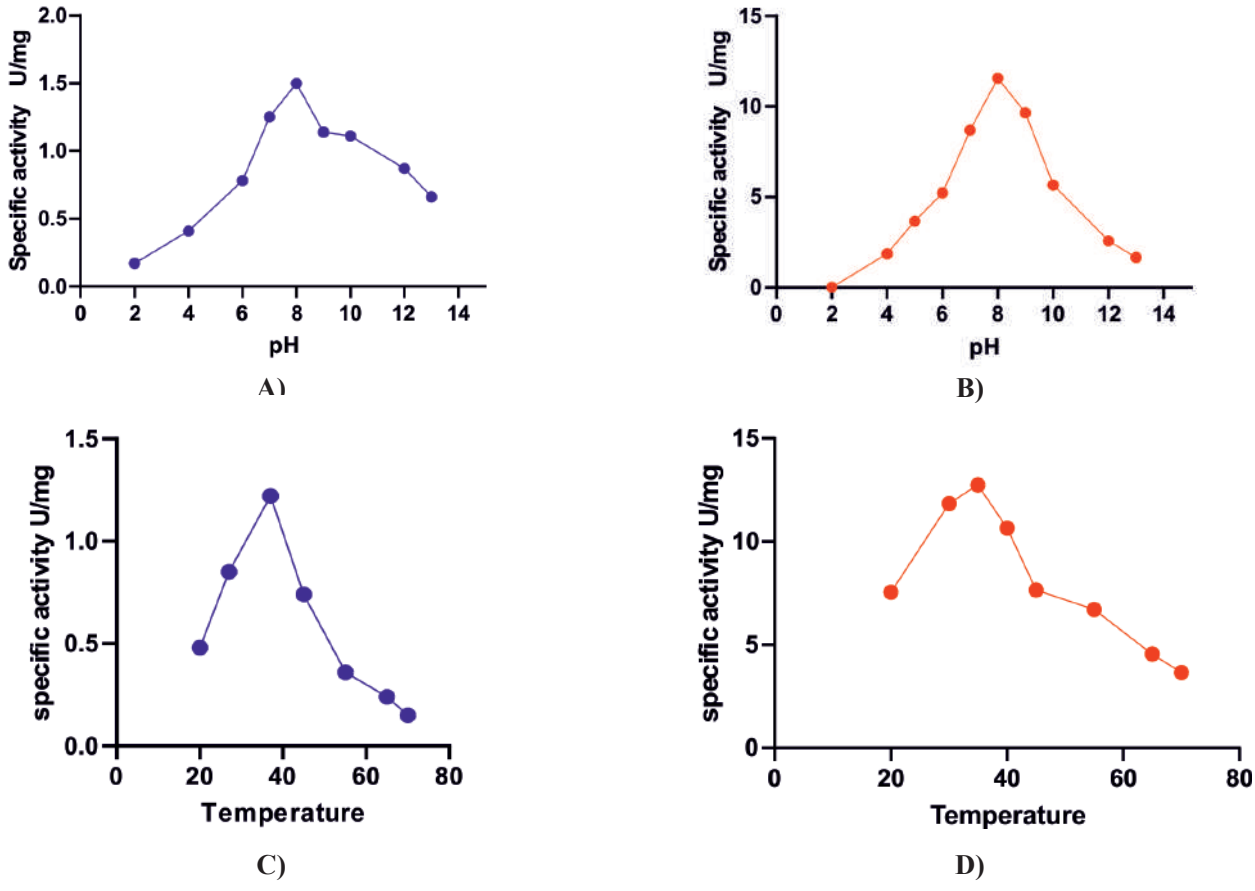

Fig 2. Effect of pH and temperature on the lipase and esterase from larvae of *G. mellonella*. Effect of pH on activity of lipase (A) and esterase (B) using different pH ranges. Effect of temperature (° C) on activity of lipase (C) and esterase (D) using different temperature ranges.

The greater wax moth is considered as a global challenge to the bee health and the beekeeping industry, however received very less attention in terms of research to control its damage. Several management approaches are developed to control *G. melonella* damage such as temperature, insecticides, entomopathogens (*Bacillus thuringiensis*), natural enemies (employing larval (*Bracon hebetor* Sayand *Apanteles galleriae* Wilkinson) and egg (*Trichogramma pretiosum* Riley, *Trichogramma evanescens* Westwood and *Trichogramma minutum* Riley) parasitioids, Sterile Insect Technique (SIT) and semiochemicals (Kwadha *et al.,* 2017), which are found to be ineffective and unsatisfactory. Greater wax moths are voracious wax feeders, and their guts may contain enzymes that break down wax containing long-chain fatty acids and carboxyl esters. Degrading these complicated long chain esters and fatty acids is usually a difficult and time-consuming task, and it's still unclear why and how these tiny larvae of GWM use beeswax as their regular dietary substance.

The present study describes the activity of gut digestive enzymes of *G. mellonella* namely esterase and lipases. The extracted lipase and esterase from *G. mellonella* larval guts were purified by using two steps involving SAS precipitation and gel filtration. These two procedures are generally employed to isolate and purify various enzymes including lipase and esterase (Mahdy *et al.,* 2020).In the present study, salting-out using SAS helped to remove the non-protein components from proteins, as addingSAS reduced the number of solvent molecules that interact with proteins resulting in coagulation and precipitation of proteins (Robinson, 2015). Earlier studies mentioned that this process can also be employed to separate a particular enzyme from other proteins (Mahdy *et al.,* 2020).

In the present study, we were able to isolate and purify specific gut enzymes namely esterase and lipase from the larval guts of *G. mellonella*. The enzyme activity of crude extracted lipase and esterase was assayed with the substrates *p*-nitrophenyl butyrate and *p*-nitrophenyl acetate. For both substrates, the total enzyme activity for lipase and esterase was found to be significantly high, with a 100% yield of protein. The enzyme activity was gradually decreased after precipitation with ammonium

sulphate saturation and purification with gel filtration. However, the specific activity of lipase and esterase was gradually enhanced with the purifying forms of the enzymes. The highest specific activity was observed with the gel filtered purified enzyme for both lipase and esterase. Similar results were observed by Zibaee *et al.* 2012 on rice green caterpillar, *Naranga aenescen* Moore, where they found the highest specific activity of lipase in the 3rd instar stage larva on substrate *p*- nitrophenyl butyrate. In 1986, Juraimi demonstrated the extraction of digestive esterase from the guts of *G. mellonella* larvae and reported its activity on substrates namely acetylsalicylic acid, and acetyl-p-hydroxybenzoic acid and tentatively classified esterase of *G. mellonella* as carboxylesterase. He reported highest specific activity of the purified esterase on these substratesat 70 % SAS. Mahdy *et al.* (2020)for the first time extracted, purified and characterized the fat body lipase from the guts of *G. mellonella* larvae and reported highest specific activity of purified lipase with the substrates, trioleoylglycerol, triton-X100 and *p*-nitrophenol butyrate. Similar procedures were employed to extract and purify the gut lipases from *Ectomyelois ceratoniae* Zeller (Ranjbar *et al.,* 2015) and *Antheraea mylitta* Drury (Marepally and Benarjee, 2016) larvae where they reported the highest specific activity of lipase on selective substrate, *p*-nitrophenol butyrate. Wheeler *et al.*(2010) identified and purified four esterase genes and quantified esterase activity from the guts of termites, *Reticulitermes flavipes* Kollar to understand its metabolism.

The pH is one of the most important variables in biochemical reactions. In our study, the activity of digestive lipase of *G. mellonella* was found to increase with pH from 6.0 to 8.5 with the highest activity at pH 8.0. While the most activityof lipase was lost at pH 12.0-13.0. This shows that lipases from *G. mellonella* have a slightly alkaline optimum pH similar to studies reported in the kissing bug *Rhodnius prolixus* Stal which have high lipase activity at pH 7.0 - 7.5 (Grillo *et al.,* 2007) and *Manduca sexta* (L.) at pH 7.9 (Arrese and Wells, 1994).In case of esterase, the highest activity was recorded at pH from 8.0 to 9.5, while most activity decreased gradually from thereon (pH 10.0-13.0). This indicates that the esterase of *G. mellonella* is active in the mild alkaline optimal pH range (8.5 to 10.0; 7.5 to 8.0) which is observed similarly in rice green caterpillar *Naranga aenescens* Moore and *G. mellonella* (Zibaee *et al.*, 2012; Nemec and Zenka, 1996).

Temperature is another variable that affects biological responses. In the current study, the temperature effect on lipase and esterase activity was assessed over a range from 20 to 70 °C. The maximum activity of lipase was at 37 °C while the minimum activity was at 70 °C, whereas esterase activity increased gradually with increase in the temperature from40 - 60°C and then dropped down after 70 °C to till 100 °C. This revealed that the enzyme activity for lipase of *G. mellonella peaked* at the most optimal temperature range (37 °C), then reduced as the temperature was raised until the enzyme denatured and the activity dropped significantly. Similar results were reported in other insect gut lipases, as in gypsy moth *Lymantria dispar* (L.) (Mrdaković *et al.,* 2008) and *Rhynchophorus palmarum* (L.) (Santana *et al.,* 2017). Our results indicated that the enzyme activity for esterase was eventually high with the raise in the temperature and dropped at the denaturation point of enzyme at higher temperature. Parallel results were observed from the guts of *Triatoma infestans* Klug (De Malkenson *et al.* (1984) and eastern subterranean termites, *Reticulitermes flavipes* Kollar (Davis *et al.,* 1995). In contrast, Zhu *et al.* (1990) observed a decrease in the esterase activity from the guts of *Lygus hesperus* Knight after 40 °C of temperature. This suggests that the esterase activity perhaps is temperature independent and varies between the species, particularly in *G. mellonella*, due to its nature of feeding beeswax.

The use of enzyme inhibitors was indeed a priority segment in integrated pest management programs due to the widespread use of synthetic pesticides and the emergence of environmental concerns such as pest recurrence, pesticide resistance, and harmful effects on non-target organisms. Detailed investigations on the identification, characterization, and inhibition of specific digestive enzymes across a wide range of economically significant insect species will aid our understanding of insect nutrition, growth/development, and mortality. Therefore, employing the inhibitors that target the specific enzymes is likely to be more effective provided the holistic knowledge about the insect digestive enzymes is available. Our finding is the first step in this direction, and it will aid future research into understanding the physiological function of the identified esterase and lipase of *G. mellonella* in the degradation of beeswax and envisages identifying novel enzyme inhibitors to control the pest damage.

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Biosafety assessment of nanemulsion of hexanal on *Mallada boninensis* Okamoto (Chrysopdae: Neuroptera)

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ABSTRACT: Experiments were carried out under laboratory conditions to assess the effect of nano emulsion of hexanal on egg and grub stage of green lacewing, *Mallada boninensis* Okamoto (Neuroptera: Chrysopidae) through laboratory bioassay. The results showed that, nanoemulsion of hexanal was found to cause egg hatchability up to 90.04 percent in higher concentrations of 0.06 percent. Egg hatchability at field recommended concentration of 0.02% was found maximum of 94.18 percent respectively. In addition nanoemulsion of hexanal at field recommended concentration 0.02% tested against grubs of *M. boninensis* results revealed that 6.67 percent grub mortality in dry film method at 48h after exposure it was least toxic to grub of *M. boninensis*. The maximum percent pupation and adult emergence was recorded in nanoemulsion of hexanal @ 0.02% showed 95.33 and 95.0 percent, respectively. The results clearly indicated that all the concentration of nano formulation of hexanal was harmless to *M. boninensis* which recorded a mortality of < 30 percent as per the threshold prescribed by IOBC.

Keywords: Mango, post-harvest losses, hexanal, nanoemulsion, biosafety

INTRODUCTION

India ranks first in the world production of mango with the production of 19.27 million tonnes from 2.5 million hectares which is the largest in the world and it accounts for 21 percent of the total fruit production in the country (FAOSTAT. 2011). Post harvest losses are one of major constraints in the production and marketing of mango. Many biologically active volatile compounds like hexanal are found to reduce the post harvest losses by checking the over ripening. The key enzyme involved in the ripening of fruits is Phosholipase D (PLD). The use of hexanal by inhibits PLD production in fruit skin which turn delays post harvest deterioration. (Paliyath, G. and Subramanian, J. 2008). Formulation of hexanal as nanoemulsion would be more effective for fruit preservation, which shall be uniform droplet sizes (<100 nm), high kinetic stability and optical transparency. Biosafety of nano product is one of most important prerequisite to be assessed against beneficial and non-target organisms. (Karthika*et al.*, 2015; Mohan *et al.*, 2017) There are several natural enemies in mango ecosystem among them the *Mallada boninensis*is a most important generalist predator of many soft bodied insect pests of several crops (Geetha and Swamiappan, 1998) and it can be easily mass cultured in laboratory (Ridgway *et al.,* 1970). The grubs are voracious and efficient predators for various phytophagous arthropods which include whiteflies, aphids, scales, mealy bugs, leafhoppers, psyllids, thrips, mites, small caterpillars, eggs of moths and other soft bodied insects (McWen *et al.,* 2001). The biosafety of nanoemulsion of hexanal on the natural enemies associated with mango cropping system need to be studied and Hence, the present studies was undertaken on safety of nanoformulation of hexanal on *M. boninensis* under laboratory condition.

MATERIALS AND METHODS

Laboratory experiments were carried out at the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2015-2017, to study the toxicity of nanoemulsion of hexanal to *M. boninensis.* Safety of nanoemulsion of hexanal was compared with pure form of hexanal which is a patentable product of Canada. The susceptibility of *M. boninensis* to nanoemulsion of hexanal at different concentrations was evaluated along with control in a Completely Randomized Design (CRD) replicated three times.

Preparation of nanoemulsion of hexanal

Preparation of hexanal nanoemulsion involves mixing of hexanal:Tween 20 and ethanol in the ratio of 1:10:10 v/v basis and were sonicated using sonicator at 20 kHz for 15 min for good emulsion as per the standard method. (Jafariand Bhandari, 2006).

Statistical analysis

The data on percentage values were transformed in to arcsine values and the population recorded as numbers were transformed into $\sqrt{x+0.5}$ before statistical analysis. The data obtained from laboratory experiments were analyzed in completely randomized design. The

mean values were separated using Duncan's Multiple Range Test (DMRT). The corrected percent mortality was worked out using the formula (Abott, 1925)

$$\text{Corrected per cent mortality} = \frac{P_0 - P_c}{100 - P_c} \times 100$$

P_0 - Observed mortality in treatment

P_c - Observed mortality in untreated check

Effect of hexanal formulation on the eggs of *M. boninensis*

Laboratory studies were conducted to assess the effect of nano emulsion of hexanal formulation on the eggs of *M. boninensis*as per the method described by Krishnamoorthy (1985). The eggs along with stalks on brown paper strips were sprayed with hand atomizer at different concentrations separately as mentioned in table 1. Each treatment was replicated three times with 100 eggs per treatment. Untreated check was maintained by spraying distilled water. The number of grubs hatched from each treatment was recorded and per cent hatchability worked out.

Effect of hexanal formulation on grubs of *M. boninensis*– Poison food techniques

The UV treated *Corcyra* eggs was sprayed different concentrations of hexanal formulation separately using hand atomizer as mentioned in table 1. The treated eggs were shade dried for 30 minutes and then transferred to rectangular multi cavity tray @ 2 cc per tray. The untreated check was maintained by spraying distilled water. Second instar *M. boninensis* grubs were transferred to these cavity trays @ one per cavity. After the grubs completely fed the hexanal treated eggs, the grubs were provided with untreated *Corcyra* eggs till pupation. Observation on mortality of grub at 12, 24 and 48 h after treatment were recorded. In addition, the percent pupation and percent adult emergence of live grub were also noted.

RESULTS AND DISCUSSION

Effect of hexanal on eggs of *M. boninensis*

The effect of nanoemulsion of hexanal on eggs of *M. boninensis* was studied under laboratory condition. The data in table 2 represents the hatching of *Mallada* eggs

Table 1. Ovicidal effect of nanoemusion of hexanal on the eggs of *Mallada boninensis*

Treatment	Egg hatchability (%)	Percent reduction over control
T_1- Nanoemusion of hexanal @ 0.02%	94.18 (76.86)^c	4.82
T_2- Nanoemusion of hexanal @ 0.04%	94.26 (76.49)^c	5.74
T_3- Nanoemusion of hexanal @ 0.06%	90.04 (71.65)^d	9.95
T_4- Pure hexanal @ 0.02%	88.74 (70.52)^{de}	11.26
T_5- Pure hexanal @ 0.04%	88.47 (70.18)^{de}	11.53
T_6- Pure hexanal @ 0.06%	87.32 (69.19)^e	12.68
T_7- Tween 20 @ 0.2%	99.32 (85.27)^b	0.68
T_8- Ethanol @ 0.2%	94.44 (76.37)^c	5.56
T_9- Control	100 (90.00)^a	
Mean	**92.97**	**7.90**
S.ED	**7.83**	
CD (0.05%)	**16.46**	

Mean of three observations ; In a column means followed by a common letter are not significantly different at p = 0.05 by DMRT;

Figures in parentheses are arcsine √ P transformed value

Table 2. Effect of nanoemusion of hexanal on the development of II instar grub of *Malladaboninensis*

	Grub mortality (%)						Pupation (%)	Adult emergence (%)
	12 HAT	Corrected mortality	24HAT	Corrected mortality	48HAT	Corrected mortality		
T_1- Nanoemusion of hexanal @ 0.02%	3.33 (10.51)[a]	0.00	6.67 (14.97)[a]	0.00	6.67 (14.96)[a]	0.00	95.33 (77.52)[ab]	95.00 (77.17)[b]
T_2- Nanoemusion of hexanal @ 0.04%	3.33 (10.51)[a]	0.00	6.67 (14.97)[a]	0.00	6.67 (14.96)[a]	0.00	95.33 (77.52)[ab]	95.00 (77.17)[b]
T_3- Nanoemusion of hexanal @ 0.06%	3.33 (10.51)[a]	0.00	6.67 (14.97)[a]	0.00	13.33 (14.96)[a]	7.14	94.44 (76.37)[bc]	92.13 (76.61)[bc]
T_4- Pure hexanal @ 0.02%	6.67 (14.97)[b]	3.34	10.0 (18.43)[a]	3.57	16.66 (24.09)[a]	10.70	92.00 (73.57)[c]	91.11 (76.37)[bc]
T_5- Pure hexanal @ 0.04%	10.0 (18.43)[c]	6.67	13.33 (21.41)[c]	7.14	16.67 (24.10)[c]	10.71	90.70 (72.25)[cd]	90.00 (71.57)[c]
T_6- Pure hexanal @ 0.06%	13.33 (21.41)[d]	10.0	20.0 (26.57)[d]	14.28	26.67 (31.09)[d]	20.00	90.00 (71.57)[d]	88.89 (70.53)[d]
T_7- Tween 20 @ 0.2%	6.67 (14.97)[b]	3.34	10.0 (18.43)[b]	3.57	6.67 (14.97)[b]	0.00	95.65 (78.17)[a]	100.00 (90.00)[a]
T_8- Ethanol @ 0.2%	3.33 (10.51)[a]	3.33	6.67 (14.97)[a]	0.00	6.67 (14.97)[c]	0.00	95.33 (77.52)[ab]	100.00 (90.00)[a]
T_9- Control	3.33 (10.51)[a]	-	6.67 (14.97)[a]	-	6.67 (14.96)[a]	-	96.29 (78.94)[a]	100.00 (90.00)[a]
Mean	**5.98**	**3.35**	**9.26**	**3.74**	**11.25**	**5.71**	**93.71**	**94.68**
S.ED	**0.57**	**0.37**	**0.86**	**0.46**	**1.145**	**0.70**	**7.90**	**7.97**
CD (0.05%)	**1.21**	**0.78**	**1.81**	**0.98**	**2.40**	**1.47**	**16.61**	**16.76**

*Values in the parentheses are arcsine transformed values; In a column means followed by a common letter(s) are not significantly different by DMRT (P=0.05);

*Mean of three replications; HAT- Hours after treatment

in different concentrations of nano emulsion of hexanal. The results indicated that Cent per cent egg hatchability was observed in untreated check 48 hours after treatment (HAT) followed by the surfactant Tween 20 and solvent absolute ethanol @0.2% which recorded 99.32 and 94.44 percent egg hatchability respectively. The nanoemulsion of hexanal @ 0.02, 0.04 and 0.06 percent showed 94.18, 94.26 and90.40 percent egg hatchability at the same period of observation. The standard check purehexanal @ 0.02, 0.04 and 0.06 % percent showed 88.74, 88.47 and 87.32 percent egg hatchability, respectively (Table 1).

Effect of nanoemulsion of hexanal formulation on II instar grub of *M. boninensis*– Poison food techniques

The data presented in table 3 represents the mortality of grubs, percent pupation and adult emergence. The results of the study indicated that nano emulsion of hexanal at 0.02 percent was on par with 0.2 percent of Tween 20 and ethanol, 0.02 percent pure hexanal and untreated check with the grub mortality of 6.67 percent. The percent grub pupated was maximum (96.29) in untreated control which was on par with nano emulsion of hexanal at 0.02 and 0.04 percent (95.33%) and standard checks Tween 20 (95.65) and ethanol at 0.2 percent (95.33). The adult emergence recorded in all the concentrations (0.02, 0.04 and 0.06%) of nano emulsion of hexanal ranged 92.2- 95 percent were next best to untreated control, Tween 20 and ethanol at 0.2 percent which recorded cent percent adult emergence.

Based on the toxicity levels of the newer molecules tested against beneficial organisms, they are classified as harmless (mortality <30%), slightly harmful (> 30% and <79%), moderately harmful (> 80% and <99%), and harmful (>99%). The mortality of *M.boninensis*

recorded in nano emulsion of hexanal 0.02% at field recommended dose being significantly lesser than the standard check and control with mortality < 30 percent very close to the threshold prescribed by IOBC for the test product shall be claimed as harmless (Hassan *et al.,* 1992). The results indicated maximum hatchability of predator eggs at all concentrations of nanoemulsion of hexanal. The findings of Karthika et al. (2015) who showed the safety of nanoemulsion of hexanal to *C. zastrowi* eggs can also be related to the present finding. But several authors who have tested chemical pesticides reported that *M.boninensis* showed higher sensitivity to newer molecules at adult stage than the egg, larval and pupal stages which possess eggshell to protect the immature stage of bio control agents.

Kulkarni and Patil (2012) reported that egg hatchability and adult emergence of C. zastrowi were higher, when treated with castor leaf extract (5%), garlic bulb extract (2%), neem (1%) and flufenoyuron (0.5%). Vasanthakumar *et al.* (2012) observed that the neem formulation azter (Azadiractin 0.15%) and neem kernel aqueous extract (NKAE) were safer to predator M. boninensis with no adverse effect on the predatory efficiency. The above findings reported on the biosafet of plant products to Chrysopids were similar to the present finding.Uthamasamy *et al.* (2003) reported that acetamiprid concentrations tested on *C. carnea* showed less than 10 percent mortality in contact toxicity method against 22.5 percent mortality in feeding toxicity method. This has clearly indicated the poison on ingestion will have more adverse effect than physical contact. The above factor can be attributed to the safety of nanoemulsion of hexanal which was evaluated through contact toxicity showing least adverse effect. Likewise, the studies on the attraction of *C. zastrowisillemi* and *M. boninensis* using the synthetic herbivore induced plant volatile (methyl salicylate) indicating no adverse effect can be related to the plant derived hexanal showing no adverse effect on *M. boninensis*.

In the present study it was concluded that the different concentrations of nanoemulsion of hexanal tested on *M. boninensis* was least toxic to eggs and grubs. This information can be used in the development and improvement of IPM programmes to reduce harm to beneficial insects from hexanal applications.

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Seasonal incidence of ginger shoot borer, *Conogethes punctiferalis* (Guenee) and its correlation with abiotic factors

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ABSTRACT: A field experiment was carried out at the Department of Entomology, Nagaland University, Medziphema campus during 2018 and 2019 to study the seasonal incidence of ginger shoot borer, *Conogethes punctiferalis* (Guenee) and its correlation with abiotic factors. The results revealed that the incidence of *C. punctiferalis* was observed from 120 DAP (Days after planting) in D_1 (15th February planting) which falls in the second week of June and for D_2 (17th March planting) the incidence of *C. punctiferalis* was observed from the second week of July and for D_3 (16th April planting) the incidence of *C. punctiferalis* was observed from the second week of August respectively for both the years. In both the experimental year, the highest incidence of *C. punctiferalis* was observed at 210 DAP in D_3 which falls in the second week of November whereas the lowest incidence was recorded at 120 DAP in D_1 which falls in the second week of June respectively. Among the weather parameters, maximum temperature and rainfall showed negative correlation whereas maximum relative humidity (RH) showed positive correlation on the incidence of *C. punctiferalis* for both the years respectively.

Keywords: *Conogethes punctiferalis,* seasonal incidence, abiotic factors

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) belonging to the family *Zingiberaceae* is a herbaceous perennial and an important cash crop grown for its rhizome which is used as a spice and for its medicinal value. Ginger can be grown both under rain fed and irrigated conditions. However, being an exhausting crop it is not desirable to grow ginger in the same land year after year. The crop grows well at a temperature of 19°C- 28°C and a humidity ranging from 70-90% (Jayashree *et al.*, 2015). The productivity of most of the spice and condiment crops is considerably low in India due to many reasons among which infestation by pests and pathogens is a major factor which causes significant yield losses (Devasahayam *et al.*, 2012). Bacterial and fungal diseases, insect pests and parasitic nematodes causes economic losses in ginger cultivation(Nada *et al.*, 1996). Among the several insect pests reported on ginger, the shoot borer, *Conogethes punctiferalis* Guenee, is the most severe. Crop yield can be significantly affected when more than 45% of shoots in a clump are damaged (Devasahayam *et al.*, 2010). Information on incidence of ginger shoot borer at each stages of the crop and their relation with weather parameters will help to take up appropriate control measures. The meteorological parameter like temperature play a pivotal role in the biology of any insect pest and is the most crucial abiotic factor influencing the rate of growth and development of insect pests and is important for timing of effective control measures. The study on the relationship between weather parameters and incidence of insect pests helps to find out under what weather conditions, pest would appear, which will help to forewarn the farmers to resort to preventive measures against such pest in time. Such information is therefore essential in developing integrated pest management systems with ecological and economical balance. Therefore, an attempt has been made to study the effect of the changes in abiotic factors in relation to shoot and fruit borer on ginger crop under Nagaland Agro-climatic conditions.

MATERIALS AND METHODS

To study Seasonal incidence of ginger shoot borer and its correlation with abiotic factors a field experiment was carried out in the Experimental farm, Department of Entomology, School of Agricultural Sciences and Rural Development (SASRD), Nagaland University, Medziphema campus during 2018 and 2019.The ginger variety Nadia was used in the experiment. The ginger crop was raised according to recommended agronomic practices. Plots measuring 14.5 m x 2.8 m size each replicated 3 times was maintained in order to study the seasonal incidence of ginger shoot borer under natural condition. Ginger were planted based on three different assigned dates of planting (15th February, 17th March and 16th April). For this purpose, five numbers of plants was selected randomly from each plot and tagged to assess the incidence of insect pests during the course of crop growth.

The extent of incidence of the pest was recorded by counting the number of infested shoots to the total number of shoots on the five randomly selected plants at fortnightly intervals and expressed as percentage.

The status of the pest was considered based on number of insects occurring on the plant. To study the effect of major abiotic factors *viz.,* maximum and minimum temperature, morning and afternoon relative humidity, rainfall and sun shine hours on pest infestation, a correlation coefficient and multiple linear regression was worked out taking fortnightly larval population as dependent variable with the standard week mean meteorological data as independent variables. The important weather parameters were collected from ICAR Research Complex, NEH Region, Jharnapani, Nagaland to correlate with infestation of ginger shoot borer.

RESULTS AND DISCUSSION

Seasonal incidence of ginger shoot borer (*Conogethes punctiferalis*)

The data from the two year experimental trial (Table 2) reveals that the incidence of *Conogethes punctiferalis* in the year 2018 was observed from 120 DAP (Days after planting) with 10.70 larvae per plant in D_1 (15th February planting) which falls in the second week of June followed by D_2 (17th March planting) with 11.65 larvae per plant which falls in the second week of July and D_3 (16th April planting) with 12.72 larvae per plant which falls in the second week of August.

The incidence of the *C. punctiferalis* in the year 2019 was also observed from 120 DAP (Days after planting) with 11.40 larvae in D_1 (15th February planting) which falls in the second week of June followed by D_2 (17th March planting) with 12.42 larvae per plant which falls in the second week of July and D_3 (16th April planting) with 13.50 larvae per plant which falls in the second week of August respectively.

In both the years of experimental trials, the incidence of *C. punctiferalis* showed an increasing trend till 210 DAP and then decreases thereafter. In both the experimental year, the highest incidence of 18.90 and 19.28 of *C. punctiferalis* larvae per plant was observed at 210 DAP in D_3 which falls in the second week of November whereas the lowest incidence of 10.70 and 11.40 larvae per plant was recorded at 120 DAP in D_1 which falls in the second week of June for both the years respectively.

Pooled data (Table 1) reveals that the highest total mean population of 19.09 larvae per plant was observed in third date of planting (D_3) and the least mean population

Table 1. Incidence of ginger shoot borer, *Conogethes punctiferalis* on ginger variety Nadia at different dates of sowing during 2018 and 2019

Date of sowing	Shoot borer incidence (%)														
	120 DAP			150 DAP			180 DAP			210 DAP			240 DAP		
	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled	2018	2019	Pooled
15th February: (D_1)	10.70 (19.09)	11.40 (19.73)	11.05 (19.41)	12.22 (20.46)	12.87 (21.02)	12.55 (20.74)	14.15 (22.10)	14.72 (22.56)	14.44 (22.33)	16.52 (23.98)	17.05 (24.39)	16.79 (24.18)	15.47 (23.16)	15.90 (23.49)	15.69 (23.33)
17th March: (D_2)	11.65 (19.95)	12.42 (20.62)	12.04 (20.29)	13.50 (21.55)	14.10 (22.05)	13.80 (21.80)	15.24 (22.97)	15.80 (23.41)	15.52 (23.19)	17.75 (24.92)	18.10 (25.17)	17.93 (25.04)	16.50 (23.96)	16.97 (24.32)	16.74 (24.14)
16th April: (D_3)	12.72 (20.89)	13.50 (21.55)	13.11 (21.22)	14.75 (22.58)	15.35 (23.06)	15.05 (22.82)	16.40 (23.88)	17.26 (24.55)	16.83 (24.21)	18.90 (25.77)	19.28 (26.05)	19.09 (25.91)	17.62 (24.82)	18.16 (25.22)	17.89 (25.02)
SEm±	0.27	0.30	0.20	0.28	0.33	0.22	0.32	0.35	0.24	0.29	0.31	0.21	0.27	0.31	0.20
CD (P= 0.05)	1.07	2.78	0.66	1.09	2.78	0.71	1.24	2.78	0.77	1.15	2.78	0.69	1.04	2.78	0.66

Note: Figures in the table are mean values and those in parenthesis are arc sine transformed values.

Table 2a. Correlation coefficient (r) of ginger shoot borer, *Conogethes punctiferalis* incidence with abiotic factors during June to December 2018

Pearson correlation coefficient	Ginger shoot borer incidence		
	15th February: (D_1)	17th March: (D_2)	16th April: (D_3)
Maximum temperature (ºC)	-0.327	-0.730	-0.815
Minimum temperature (ºC)	0.447	-0.690	-0.885*
Maximum relative humidity (%)	0.485	0.899*	0.938*
Minimum relative humidity (%)	-0.944*	-0.613	-0.921*
Rainfall (mm)	-0.828	-0.839	-0.934*

Table 2b. Correlation coefficient (r) of ginger shoot borer, *Conogethes punctiferalis* incidence with abiotic factors during June to December 2019

Pearson correlation coefficient	Ginger shoot borer incidence		
	15th February: (D_1)	17th March: (D_2)	16th April: (D_3)
Maximum temperature (ºC)	-0.454	-0.763	-0.818
Minimum temperature (ºC)	-0.428	-0.658	-0.819
Maximum relative humidity (%)	0.864	0.762	0.988**
Minimum relative humidity (%)	0.723	-0.308	-0.686
Rainfall (mm)	-0.173	-0.510	-0.821

of 11.05 larvae per plant of *C. punctiferalis* was observed in first date of planting (D_1).

The findings of the present study are in line with that of Koya (1984), who stated that the percentage of shoots bored by *C. punctiferalis* in ginger was at the minimum of 5% in July and it steadily increased reaching a peak of 14.8% in November. It is also in partial agreement with the work of Jacob (1981), who reported that incidence of *C. punctiferalis* in ginger was highest during September to October. Patel *et al.* (2015) also stated that higher activity of the pest was observed from first week of November to second week of January, with a peak level (20.04) on third week of November.

Influence of weather parameters on ginger shoot borer (*Conogethes punctiferalis*)

The correlation (Table 2a) of *C. punctiferalis* with the abiotic factors for the year 2018 had revealed a non significant negative effect with maximum temperature on all the three planting dates. Correlation of minimum temperature with the incidence of *C. punctiferalis* showed a significant positive effect on D_1, negative non-significant on D_2 whereas significant negative effect on D_3. Correlation of maximum relative humidity (RH) with the incidence of *C. punctiferalis* showed a non-significant, positive effect on D_1 whereas, significant positive effect on D_2 and D_3. Correlation of minimum relative humidity (RH) with the incidence of *C. punctiferalis* showed a significant negative effect on D_1 and D_3 respectively whereas non significant negative effect on D_2. Correlation of rainfall with the incidence of *C. punctiferalis* showed a non-significant negative effect on D_1 and D_2 whereas significant negative effect on D_3 respectively.

The correlation (Table 2b) of *C. punctiferalis* with the abiotic factors for the year 2019 also revealed a non significant negative effect with maximum temperature on all the planting dates i.e D_1, D_2 and D_3 respectively. Correlation of minimum temperature with the incidence of *C. punctiferalis* showed a non-significant negative effect on D_1, D_2 and. Correlation of maximum relative humidity (RH) with the incidence of *C. punctiferalis* showed a non-significant positive effect on D_1, and D_2 whereas significant positive effect on D_3. Correlation of minimum relative humidity (RH) with the incidence of *C. punctiferalis* showed a non-significant positive effect on D_1 whereas non-significant negative effect on D_2 and D_3 respectively. Correlation of rainfall with the incidence of *C. punctiferalis* showed a non-significant negative effect on all the three planting dates respectively.

The present finding is in line with the work of Goel and Kumar (1990) who have stated that maximum and minimum temperature showed significant positive effect on per cent infestation of capsule by shoot and capsule borer, *C. punctiferalis*. It is also in line with the work of Kasareddy *et al.* (2018) who have mentioned that population of *C. punctiferalis* was significant and positively correlated with relative humidity and rainfall. Rashmi (2014) stated that the incidence of borer showed significant positive correlation with maximum temperature, while the relative humidity and rainfall showed a negative correlation with the incidence of shoot borer. Stanley *et al.* (2009) reported that increasing relative humidity increases damage caused by *C. punctiferalis*. Madhuri (2005) reported that incidence of *C. punctiferalis* showed significant and positive correlation with maximum temperature, whereas relative humidity showed significant and are negatively correlation while rainfall showed non significant and negative correlation which agrees to the present findings.

CONCLUSION

The knowledge on seasonal incidence of pest and their correlation with abiotic factors will help in developing integrated pest management systems and indicating at what stage of the crop the management practices should be taken up to reduce shoot borer infestation which cause heavy losses to cultivators.

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Effect of *Bacillus pumilus* IIHR Bp-2 1% A.S. and *Pseudomonas putida* IIHR Pp-2 1% A.S. on *Meloidogyne incognita* infecting okra (*Abelmoschus esculentus* (L.) Moench)

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ABSTRACT: Field studies were conducted for three seasons from 2015-16 to 2017-18 on bio-management of root knot nematode, *Meloidogyne incognita* in okra. The study revealed that liquid formulation of BCAs, *Bacillus pumilus* IIHR Bp-2 and *Pseudomonas putida* IIHR Bp-2 were at par with each other in reducing the nematode population and increasing okra yield. It was found that seed treatment with BCAs at 10 ml kg $^{-1}$ followed by soil application of Farm Yard Manure (20 tons ha^{-1}) after enrichment with liquid formulations @ 5 lit recorded 66.51 to 67.57 per cent decrease in nematode population and 29.44 to 30.83 per cent increase in okra yield with cost benefit ratio of 1: 1.91 to 1: 1.93. It was followed by chemical treatment with carbofuran @ 1 kg a.i. ha^{-1} which revealed 50.76 to 62.42 per cent reduction in soil nematode population and 16.19 to 16.38 per cent increase in okra yield. Hence, this study proves that liquid formulations of *Bacillus pumilus* IIHR Bp-2 and *Pseudomonas putida* IIHR Bp-2 can be used as promising components in integrated nematode management packages for successful management of *M. incognita* in okra.

Keywords: *Bacillus pumilus*, *Pseudomonas putida, Meloidogyne incognita,* nematode management, okra

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench), is an important vegetable crop of the tropical and sub-tropical countries including India. It is mainly grown for green tender fruits which are rich in vitamins, proteins, calcium and other minerals (Santos *et al.*, 2013). India is the biggest producer of okra and ranks first in the world contributing 62 per cent of total global production. It occupies nearly 509 thousand hectares area with production of 6095 thousand metric tonnes and productivity of 12 metric tonnes/ ha in India (Anonymous, 2018). However, productivity of okra is hindered due to yield losses caused by several insect pests, diseases and nematodes. In India, Kumar *et al.* (2020) estimated annual crop losses due to plant-parasitic nematodes at 21.3% amounting to Rs. 102,039.79 million (1.58 billion USD), annually. In okra, root-knot nematodes (*Meloidogyne* spp.) are the most serious pests (Hussain *et al.*, 2011; Mukhtar *et al*., 2017) causing yield losses up to 20.4 per cent (Ravichandra, 2012) and monetary yield losses are estimated at Rs. 2480.86 million annually (Kumar *et al.*, 2020). They cause severe growth reductions and damage the roots by formation of characteristic galls. The damage severity is aggravated due to association of nematodes with soil-borne fungal and bacterial pathogens resulting in disease complex situations (Kayani *et al.*, 2018).

Inadvertent use of chemical nematicides is popular among vegetable growers for their ease in application and instantaneous action to mitigate nematode menace. However, owing to their negative impact on environmental and human health, many pesticides are being withdrawn from the market. Hence, biological control agents (BCAs) are steadily gaining importance as an alternative to chemical pesticides due to their capability to antagonize nematodes by different modes of action (Rao *et al.,* 2015a).Many plant growth promoting rhizobacteria (PGPR) including fluorescent pseudomonads and *Bacillus* spp. are reported not only to enhance plant growth by colonizing the plant root system, but also exhibited excellent nematicidal activity against a multitude of phytoparasitic nematodes (Abd-Elgawad, 2016; Zhao *et al.*, 2018).

Pseudomonas spp. serve as ideal candidates for commercial exploitation as they possess many desirable qualities and reportedly increased plant growth and reduced nematode damage in several crops (Tian*et al.*, 2007; Umamaheswari *et al.,* 2020). BCAs belonging to of *Bacillus* group are considered as 'microbial factories' due to their ability to produce a vast spectrum of biologically active molecules and anti-microbial compounds that are antagonistic to a wide range of plant pathogenic microbes and nematodes (Ongena and Jacques, 2008; Stein, 2005). Keeping in view their antagonistic potential, field trials were conducted under All India Coordinated Research Project (Vegetable Crops) to evaluate the efficacy of liquid formulations of *Bacillus pumilus* IIHR Bp-2 (1% A.S.) and *Pseudomonas putida* IIHR Pp-2 (1% A.S.) in the management of root knot nematode, *Meloidogyne incognita* infecting okra.

MATERIALS AND METHODS

Field trials were conducted in nematode sick experimental plots at ICAR-Indian Institute of Horticulture Research, Bengaluru, Karnataka for three consecutive years during 2015-16, 2016-17 and 2017-18. Okra cv. Arka Anamika seeds were sown in plots as per the treatment schedule mentioned in Table 1. Liquid formulations of *Bacillus pumilus* (IIHR Bp-2) 1% A.S. and *Pseudomonas putida* (IIHR Pp-2) 1% A.S prepared in Nematology Laboratory, Division of Crop Protection were evaluated after seed treatment @ 10 ml kg $^{-1}$okra seed, alone and in combination with soil application of 20 tons Farm Yard Manure (FYM) after enrichment with bacterial formulations @ 5 litha^{-1}. For proper enrichment, the liquid formulations were mixed in FYM and maintained under shade for 15 to 21 days at optimum moisture of 25-30%. The entire heap was mixed intermittently to ensure uniform enrichment of BCAs and later applied to soil according to treatments. As standard check, chemical treatment with carbofuran 3 G @ 1 kg a.i. ha^{-1}was evaluated alone and in combination with FYM. The experiment was laid out in a randomized block design with eight treatments and four replications. Regular crop management practices were followed throughout the season as per the package of practices of bhindi (Anonymous, 2020).

Initial root knot nematode population (second stage juveniles –J2) per 100 c.c soil was recorded before sowing and final nematode population per 100 c.c soil was recorded after termination of the experiment in all the three trials. Nematodes were extracted from soil by Cobb's wet sieving and decanting technique and modified Baermann's funnel technique (Southey, 1986). At termination, plants were uprooted and the roots were carefully observed for root knots or galls and recorded for the gall index on 1-5 scale (Heald *et al.*, 1989). After staining the roots with acid fuchsin, number of females in 10 g roots was estimated (Bridge *et al.,* 1982). Cumulative marketable yield per plot was recorded and expressed in tons ha^{-1}. The data of the three seasons were pooled and cost benefit ratio was calculated.

All the data were statistically analyzedusing ANOVA and means separated with the Duncan Multiple Range Test as per Panse and Sukhatme (1989).

RESULTS

Before the start of experiments, initial nematode (J2) population was estimated at 122 ± 3, 114.3 ± 2.1 and 121.3 ± 1.6J2 per 100 cc soil for the three consecutive seasons, respectively. The data from three year's trials showed similar trend and hence pooled analysis was done and treatments were compared. In all the three trials, the

Table 1: Treatment schedule

Treatment	Details
T1	Seed treatment with *Bacillus pumilus* (IIHR Bp-2) 1% A.S. @ 10 mlkg^{-1} seed
T2	Seed treatment with *Pseudomonas putida* (IIHR Pp-2) 1% A.S. @ 10 ml kg^{-1} seed
T3	T1+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Bacillus pumilus*(IIHR Bp-2) 1% A.S.
T4	T2+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Pseudomonas putida*(IIHR Pp-2) 1% A.S.
T5	Application of 20 tons ha^{-1} of FYM
T6	Chemical treatment (carbofuran at 1 kg a.i. ha^{-1})
T7	Chemical treatment (carbofuran at 1 kg a.i.ha^{-1}) +Recommended dose of FYM (20tonsha^{-1})
T8	Control without treatment

Table 2. Effect of *Bacillus pumilus* (IIHR Bp-2) 1% A.S. and *Pseudomonas putida* (IIHR Pp-2)1% A.S. on soil and root population of *M. incognita* infecting okra

Treatment	Final Nematode population (J2) per 100 cc soil				No. of *M. incognita* femalesin 10 g roots			
	2015-16	2016-17	2017-18	Pooled	2015-16	2016-17	2017-18	Pooled
T1	123.50^{b}	124.0^{c}	125.8^{c}	124.42^{c}	20.75^{b}	22.3^{b}	25.8^{c}	23.58^{c}
T2	129.00^{b}	129.3^{c}	131.0^{c}	129.75^{c}	21.75^{b}	21.5^{b}	26.0^{c}	24.33^{c}
T3	93.75^{a}	79.8^{a}	78.3^{a}	83.92^{a}	14.00^{a}	12.5^{a}	13.0^{a}	13.25^{a}
T4	99.00^{a}	81.5^{a}	79.5^{a}	86.67a	14.25^{a}	11.8^{a}	13.3^{a}	13.58^{a}
T5	176.50^{c}	212.8^{d}	216.3^{d}	176.00^{d}	30.25^{c}	37.8^{c}	42.0^{d}	31.92^{d}
T6	101.50^{a}	103.3^{b}	102.5^{b}	127.42^{c}	17.75^{ab}	21.3^{b}	23.5^{bc}	25.25^{c}
T7	96.50^{a}	95.3^{b}	95.0^{b}	97.25^{b}	20.25^{b}	19.0^{b}	21.5^{b}	19.75^{b}
T8	254.00^{d}	259.8^{e}	262.5^{e}	258.75^{e}	36.00^{d}	42.5^{d}	49.8^{e}	43.00^{e}
CD (5%)	8.36	11.68	8.28	6.94	5.07	4.08	3.06	2.87
SE	4.02	5.61	3.98	3.34	2.44	1.96	1.47	1.38

*Numericals followed by same alphabets are not significantly different at P=0.05

[T1-; Seed treatment with *Bacillus pumilus*1% A.S. @ 10 ml kg^{-1} seed; T2 - Seed treatment with *Pseudomonas putida* 1% A.S. @ 10 ml kg^{-1} seed; T3 - T1+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Bacillus pumilus;* T4 - T2+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Pseudomonas putida*; T5 - Application of 20 tons ha^{-1} of FYM; T6 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}); T7 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}) + Recommended dose of FYM (20tons ha^{-1}); T8 - Control without treatment]

Table 3. Effect of *Bacillus pumilus* (IIHR Bp-2) 1% A.S. and *Pseudomonas putida* (IIHR Pp-2)1% A.S. on nematode gall index and yield of okra

Treatment	Gall index at termination (1 to 5 scale)				Yield (t ha^{-1})				Benefit: Cost ratio
	2015-16	2016-17	2017-18	Pooled	2015-16	2016-17	2017-18	Pooled	
T1	2.45^{b}	2.43^{b}	2.55^{b}	2.44^{b}	30.55^{b}	29.70^{b}	14.9^{b}	25.05^{b}	1.25
T2	2.48^{b}	2.53^{b}	2.60^{b}	2.48^{bc}	30.70^{b}	30.38^{ab}	14.8^{b}	25.28^{b}	1.28
T3	1.78^{a}	1.75^{a}	1.78^{a}	1.75^{a}	34.40^{a}	34.13^{a}	16.1^{a}	28.22^{a}	1.93
T4	1.83^{a}	1.80^{a}	1.80^{a}	1.79^{a}	34.65^{a}	33.10^{a}	16.0^{a}	27.92^{a}	1.91
T5	3.73^{c}	3.93^{c}	3.98^{c}	3.28^{d}	29.10^{bc}	27.33^{c}	13.6^{c}	23.35^{c}	1.12
T6	1.95^{a}	2.15^{ab}	2.05^{a}	2.67^{c}	30.78^{b}	29.53^{b}	14.9^{b}	25.06^{b}	1.54
T7	1.95^{a}	1.88^{a}	1.95^{a}	1.94^{a}	29.78^{b}	30.25^{ab}	15.3^{b}	25.10^{b}	1.61
T8	4.93^{d}	4.90^{d}	4.95^{d}	4.93^{e}	26.78^{c}	25.60^{d}	12.3^{d}	21.57^{d}	
CD (5%)	0.30	0.40	0.30	0.21	2.75	0.71	0.56	0.98	
SE	0.14	0.19	0.14	0.10	1.32	0.34	0.27	0.47	

*Numericals followed by same alphabets are not significantly different at P=0.05

[T1-; Seed treatment with *Bacillus pumilus*1% A.S. @ 10 ml kg^{-1} seed; T2 - Seed treatment with *Pseudomonas putida* 1% A.S. @ 10 ml kg^{-1} seed; T3 - T1+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Bacillus pumilus;* T4 - T2+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Pseudomonas putida*; T5 - Application of 20 tons ha^{-1} of FYM; T6 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}); T7 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}) + Recommended dose of FYM (20 tons ha^{-1}); T8 - Control without treatment]

lowest nematode population in soil (83.92 per 100 c.c soil) and female nematode population in roots (13.25 per 10 g roots) was observed in T3 wherein okra seeds were treated with *Bacillus pumilus* (IIHR Bp-2) 1% A.S. @ 10 ml kg^{-1} followed by soil application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Bacillus pumilus* (IIHR Bp-2) 1% A.S. This was at par with T4. i.e. seed treatment with *Pseudomonas putida* (IIHR Pp-2) 1% A.S.@ 10 ml kg^{-1} followed by soil application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Pseudomonas putida* (IIHR Pp-2) 1% A.S. which recorded 86.67 J2 per 100 c.c soil and 13.58 females per 10 g roots of okra. Chemical treatment with carbofuran 3G recorded 127.42 J2 and 97.25 J2 in 100 CC soil and 25.25 and 19.75 females in 10 g okra roots, when incorporated alone and in combination with FYM, respectively. Seed treatment with *B. pumilus*1% A.S. and *P. putida*1% A.S. also recorded significantly lower nematode population as 124.42 and 129.75 J2 in soil and 23.58 and 24.33 females in okra roots, respectively, as compared to untreated control which recorded the highest nematode population in soil (258.75 nematodes per 100 cc soil) and roots (43 females per 10 g roots) (Table 1).

Pooled analysis of three year's trials revealed that a maximum of 67.57 and 66.51 per cent reduction in soil nematode population was recorded in T3 and T4, respectively (Fig.1). The lowest gall index (1.75) and the highest yield (28.22 tons ha^{-1}) coupled with maximum cost benefit ratio (CBR) of 1:1.93 was recorded in T3. This was at par with T4 which recorded significantly lower gall index (1.79) and 29.44 per cent increase in yield, with CBR as 1:1.91 (Fig.1 and Table 2).

This was followed by chemical treatment with carbofuran (T6) which recorded gall index of 2.67 and yield of 25.06 tons ha^{-1} in okra. When applied together with FYM (T7), there was significantly lesser gall index (1.94) and 16.38 per cent increase in okra yield with CBR of 1:1.61. Seed treatment with *B. pumilus*1% A.S. (T1) and *P. putida*1% A.S. (T2) were at par in recording significantly lower gall index as 2.44 and 2.48 and higher yield as 25.05 and 25.28, respectively, as compared to untreated control which recorded the highest gall index (4.93) and lowest yield (21.57 t ha^{-1}).T2 and T1 caused 49.86 to 51.92 per cent reduction in nematode population and 16.15 to 17.19 per cent increase in okra yield with a CBR of 1:1.25 to 1:1.28 (Table 2; Fig. 1).

Application of FYM alone @ 20 t/ha (T5) revealed 31.98 per cent reduction in nematode population and 8.27 per cent increase in yield, compared to control.It recorded an average of 176 J2 per 100 cc soil and 31.92 females per 10 g roots, in all three trials. Gall indices from 3.73 to 3.98 and yield from 13.6 to 29.1 tons ha^{-1} was recorded in this treatment (Table 1, 2; Fig. 1).

DISCUSSION

In the present study, *B. pumilus* IIHR Bp-2and *P. putida* IIHR Pp-2 applied as seed treatment and soil application were found promising in reducing the nematode population and gall index. This falls in line with the findings of Ali *et al.* (2002) and Elbanna *et al.* (2011) who also reported significant reduction in root knot nematode population and gall index due to *P. putida*. Rao *et al.* (2017a) reported a similar trend by seed treatment with *P. putida* at 20 ml kg^{-1} followed by soil application of 5 tons ha-1 of FYM enriched with 5 lit *P. putida* which caused 61.02 to 61.95 per cent reduction in *M. incognita* population and 77.9-78.5 per cent reduction in disease incidence of *Fusarium oxysporum* f. sp. *vasinfectum* in okra. Similarly Priti *et al.* (2018) revealed the antagonistic potential of *B. pumilus, P. monteilii*and *Trichoderma harzianum* against *M. incognita* and *F. oxysporum* f. sp. *cepae* in onion under field conditions which also increased onion yield by 26.1 to 28.8 per cent. Furthermore, Sowmya and Rao (2012) reported that gladiolus treatment with *P. putida*and *Paecilomyces lilacinus* reduced the disease incidence of *M. incognita* and *F. oxysporium* f. sp.*gladioli* by 66% and 57%, respectively and increased crop yield by 23per cent.

Ann (2013) examined *Bacillus* spp. and confirmed the production of protease enzyme which degraded the nematode cuticle and completely destroyed *M. incognita* juveniles within 12 h. *Bacillus* spp. were also reported to produce a wide range of nematicidal volatile compounds such as benzene acetaldehyde, 2-nonanone, decanal, 2-undecanone and dimethyl disulphide, which exhibited larvicidal and ovicidal action against J2 of *Meloidogyne* spp. (Huang *et al.*, 2010). Lipopeptides of *Bacillus* spp. *viz.* surfactins, iturins and fengycins are well documented biomolecules for their nematicidal and fungicidal action (Kavitha *et al.*, 2012; Sarangi and Ramakrishnan, 2016).

P. fluorescence is capable of altering specific root exudates which control nematode behaviour (Oostendorp and Sikora, 1989). Fluorescent pseudomonads are reported to exhibit nematicidal activity through production of metabolites that reduce egg hatching and cause J2 mortality; alteration of specific root exudates which control nematode behaviour and hinder host finding ability and enhancement of the defence mechanism in plants leading to the induction of systemic resistance (Sikora and Hoffmann-Hergarten, 1993; Hallmann *et al.*, 2001; Siddiqui *et al.*, 2001). Araujo *et al.* (2005) detected phytohormones, IAA and ABA in metabolites of *B. Subtilis* which are responsible for enhanced growth in soybean. These mechanisms exhibited by *Bacillus* spp. and *Pseudomonas* spp. might be responsible for suppression of nematode population and increase in okra yield in our study.

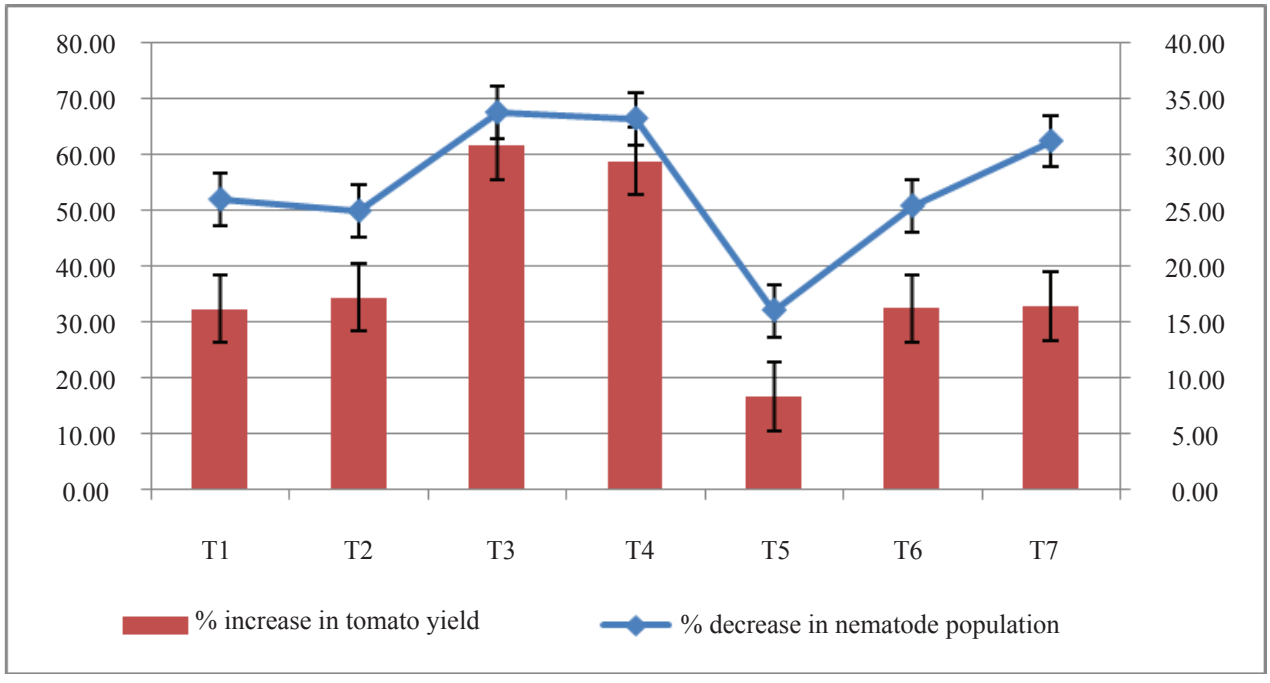

Fig 1. Effect of liquid formulations of *Bacillus* bioagents on nematode gall index and yield of okra (pooled data of three trials)

[T1-; Seed treatment with *Bacillus pumilus*1% A.S. @ 10 ml kg^{-1} seed; T2 - Seed treatment with *Pseudomonas putida* 1% A.S. @ 10 ml kg^{-1} seed; T3 - T1+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Bacillus pumilus;* T4 - T2+ application of 20 tons of FYM enriched with 5 lit ha^{-1} of *Pseudomonas putida*; T5 - Application of 20 tons ha^{-1} of FYM; T6 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}); T7 - Chemical treatment (carbofuran at 1 kg a.i. ha^{-1}) + Recommended dose of FYM (20 tons ha^{-1}); T8 - Control without treatment]

Also in the present study, soil application of BCAs after enrichment in FYM recorded significantly higher reduction in nematode population and increase in yield compared to application of FYM alone. Mass multiplication of BCAs in organic composts is gaining importance among the farming community as it improves soil health, reduces the cost of crop protection and provides sustainable solutions for disease management (Rao *et al.*, 2015b). Application of vermin compost enriched *B. subtilis* IIHR BS-2 liquid formulation increased carrot yield and reduced nematode and soft rot disease complex (Rao *et al.*, 2017b). Sharma (2002) reported that soil application of *P. fluorescens* enriched farm yard manure and vermicompost significantly reduced bacterial wilt in tomato. Similarly, application of *B. cereus* along with organic fertilizers showed enormous nematicidal activity in tomato and muskmelon (Xiao *et al.*, 2013). As reported by Oka (2010), use of manures supports growth of microorganisms, improves soil physiology and decreases nematode population by production of nematicidal compounds through decomposition process. Hence, in the current study, application of FYM as such or after enrichment with biopesticides recorded 31.98 to 67.57 reduction in nematode population.

CONCLUSION

The present study proves the antagonistic potential of liquid formulations of *Bacillus pumilus* IIHR Bp-2 (1% A.S.) and *Pseudomonas putida* IIHR Pp-2 (1% A.S.) against the root knot nematode, *M. incognita* and achieved yield enhancement in okra. As there is growing demand among the public for organic products, there is a large scope for exploiting these microbial biopesticides as safe alternatives for chemical nematicides in conventional and hi-tech horticultural crop production systems.

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Dissipation kinetics, decontamination and risk assessment of cyantraniliprole in/on cabbage using UHPLC

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ABSTRACT: A simple and steadfast analytical method was developed and validated for the determination of cyantraniliprole residues in cabbage by using Ultra High Performance Liquid Chromatography (UHLC) – photo diode array detector. The method developed had a valid specificity (RSD < 5%), good linearity (R^2 = 0.999), recovery (90.96 – 95.92 %) and satisfactory repeatability. The Limit of Detection and Limit of Quantification values for cyantraniliprole in UHPLC were 0.015 and 0.05 µg g^{-1}, respectively. Under the optimized specifications, the developed method was utilized to examine the dissipation kinetics of from the collected field samples. The mean initial deposit of cyantraniliprole after the second spray was 1.67 and 2.15µg g^{-1} and reached BDL (0.05 µg g^{-1}) on 10 and 15 days with the calculated half-lives of 1.47 to 1.84 days at 60 g a.i. ha^{-1} and 120 g a.i. ha^{-1} doses, respectively. Boiling the cabbage (100 ºC for 10 min) reduced 78.06 % of cyantraniliprole residues as a result of decontamination study. The dietary Risk quotient (RQ) was also negligible to humans.

Keywords: Cabbage, cyantraniliprole, decontamination, dissipation, half-life, risk assessment

INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata*) is one of the important crucifer vegetables grown and consumed worldwide. Consumption of cabbage either raw or processed in different ways is popular because of its antioxidant, anti- inflammatory and antibacterial properties (Rokayya *et al.,* 2013). Fruitful cultivation of cabbage is impeded by a number of pests like diamond back moth (DBM), *Plutella xylostella,* leaf webber, *Crocidolomia binotalis*, cabbage webworm, *Hellula undalis,* tobacco cutworm, *Spodoptera litura,* aphids, *Brevicoryne brassicae* etc.(Srinivasan and Veeresh, 1986). Diamondback moth is a major constraint in the successful production which is reported to cause more than 90 per cent yield loss in areas of outbreak (Verkerk and Wright, 1997). For the speedy recovery and acceptable marketing, farmers resort to spraying a number of insecticides at frequent intervals. The major drawback of increased pesticide usage is the development of resistance in pests and residues in foodstuff. Thus monitoring of pesticide residues on food and use of pesticides with short half-life and waiting period at mandatory intervals is essential as it is less likely to build up after repeated applications.

Exploration of cyano-substituted anthranilic diamides lead to the second entry in the anthranilic class with the product, cyantraniliprole ((3-bromo-N-[4-cyano-2-methyl-6-[(methylamino) - hydroxy] phenyl]-1-(3-chloro-pyridine-2-yl)-1-H-pyridine-5-formamide) (Figure 1) (https://pubchem.ncbi.nlm.nih.gov/compound/Cyantraniliprole). Cyantraniliprole was developed with improved plant mobility and translaminar activity (Mandal, 2012) that has excellent cross spectrum activity against a wide range of lepidopteran and sucking pests (Seiby *et al.,* 2013). This compound activates the insect ryanodine receptor that affects the calcium homeostasis by unregulated release of internal calcium ions leading to muscle paralysis and finally death (Cordova *et al.,* 2006). This paper reports a simple and reliable UHPLC method developed to detect the residues of cyantraniliprole in cabbage for which a field study was carried out.

MATERIALS AND METHODS

Chemicals and reagents

The reference standards of cyantraniliprole (99.6 % purity) were purchased from Sigma Aldrich, Bangalore, India. Acetonitrile of LCMS and HPLC grade, sodium chloride and anhydrous magnesium sulphate of analytical grade were obtained from Merck, Mumbai, India. Primary secondary amine (PSA) (Bondesil 40 µm) and graphitized carbon black (GCB) were acquired from Agilent technologies, USA. HPLC grade water (18.2 MΩ) was collected with a Milli-Q water purification system. The commercial formulation of cyantraniliprole 10.6 OD was purchased from local pesticide shop at Coimbatore, India.

Preparation of standard solution

A primary stock solution (1000 µg mL^{-1}) of cyantraniliprole was prepared by dissolving 25.10 mg of analytical standards in 25 mL LCMS grade acetonitrile in a volumetric flask. An intermediate stock (100 and 10 µg mL^{-1}) was prepped from primary stock solution

and further the working standards were prepared from intermediate stock. The standard solutions required for constructing calibration curve (0.01, 0.05, 0.1, 0.2, 0.4 and 0.8 µg mL^{-1}) were prepped from the intermediate stock by serial dilutions with LCMS grade acetonitrile. All working standard solutions were stored at -20°C before use.

Field experiment

Supervised field trial was executed in a farmer's field located at Naraseepuram village, Coimbatore, TamilNadu, India (11°N latitude and 76°E longitude). The field trial has been followed with good agricultural practices that had no previous application of cyantraniliprole and the treatment was made up of three replicated plots along with untreated control. Cabbage raised on the trail plots were sprayed with cyantraniliprole at 60 g a.i. ha^{-1}(recommended dose) and 120 g a.i. ha^{-1}(double the recommended dose), and the control plots were sprayed with water. Two spraying was given at 10 days interval during 50 percent head formation stage using hand operated knapsack sprayer using 500L/ha as spray fluid.

Sample collection and preparation

Cabbage head samples (2kg) were drawn at specified intervals starting from 0 (1 hr) to until 15 days after application. The head sample was collected randomly from each replicate of cyantraniliprole treated plots at 0 (1 h), 1, 3, 5, 7, 10, 15 and 21 days after the second application including the control sample. The collected samples were transported to the laboratory, chopped into small pieces, mixed thoroughly and homogenized with the help of high volume blade homogenizer.

Extraction and cleanup

The residues of cyantraniliprole were extracted from the cabbage by following the modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Anastassiades *et al.,* 2003). A representative homogenized sample of 10 g from each treatment were taken in a 50 mL poly-propylene centrifuge tube and 20 mL of acetonitrile was added to it and the mixture was hand shaken vigorously, followed by vortexing for 2 min. subsequently 1g of NaCl and 4 g of anhydrous $MgSO_4$

Table 1. Recovery of cyantraniliprole at different fortified levels in/on cabbage

Pesticide	Spiked concentration (µg/g)	Recovered concentration (µg/g)	Recovery (%) ± SD	RSD (%)	Horwitz ratio (HorRat)
Cyantraniliprole	0.05	0.045	90.96 ± 3.38	3.72	0.15
	0.25	0.229	91.52 ± 2.45	2.68	0.14
	0.50	0.480	95.92 ± 2.74	2.86	0.16

*Mean of three replicates; SD – Standard Deviation, RSD- Relative Standard Deviation

Table 2. Persistence and dissipation of cyantraniliprole 10.6 OD residues in/on cabbage heads

Days after application	Residue (µg g^{-1})			
	Recommended dose (120 g a.i. ha^{-1})		Double the recommended dose (60 g a.i. ha^{-1})	
	Mean*±SD	Dissipation (%)	Mean*±SD	Dissipation (%)
Control	ND		ND	-
0 (1 hr)	1.67±0.118	-	2.15±0.125	-
1	1.02±0.096	38.59	1.32±0.107	38.73
3	0.50±0.063	70.17	0.70±0.130	67.53
5	0.17±0.017	89.68	0.27±0.034	87.42
7	0.07±0.012	95.78	0.14±0.017	93.55
10	BDL	100.00	0.06±0.011	97.30
15	BDL	100.00	BDL	100.00

ND – Not detected; BDL – Below detectable level (0.05 µg g^{-1})

were added to the sample mixture, vortexed for 2 min followed by centrifugation at 6000 rpm for 10 min. The supernatant (9 mL) aliquot was transferred into a test tube containing 4 g of $NaSo_4$. From this 6 mL of aliquot was transferred to a 15 mL prefilled centrifuge tube with 10 mg GCB, 100 mg PSA sorbent and 600 mg anhydrous $MgSO_4$. The mixture was vortexed for one minute and then centrifuged at 3000 rpm for 10 min and 4 mL of supernatant aliquot was transferred into a turbovap tube concentrated to dryness under a gentle stream of nitrogen by using the Turbovap LV set at 40ºC. The residue was redissolved using acetonitrile (1 mL) and was filtered by 0.2 µm membrane syringe filter and transferred into a 2.0 mL UHPLC auto sampler glass vials for analysis.

Instrument parameters

The quantification of cyantraniliprole residues were performed by UHPLC (Shimadzu, i series 2020) equipped with diode array detector (SPD-M30A) and auto-sampler. Chromatographic separation was achieved with reverse phase - C18 (Agilent) column, 250 mm length x 4.6 mm id x 5 µ particle size in a column oven, at 40˚C. The low pressure gradient condition employed with a mobile phase of acetonitrile and water (70:30, v/v) with a flow rate of 0.6 ml min^{-1}. The injection volume of 20 µl with the absorbance of 225nm, for cyantraniliprole was fixed with total run time of 10 minutes. Residues of insecticides were quantified by the comparison of peak area of standards with that of unknown or spiked samples run under identical conditions of operation. The cyantraniliprole was eluted at the retention time of 6.9 minutes.

Method validation

Linearity studies were performed by developing linearity curves of cyantraniliprole standard solutions with the concentrations of 0.01, 0.05, 0.1, 0.2, 0.4 and 0.8 µg mL^{-1} with each three replications. The sensitivity of the method was evaluated by arriving the limit of detection (LOD) and limit of quantification (LOQ) by spiking the cyantraniliprole with selected matrices at the lowest concentration level meeting the analytical method requirements. The LOD and LOQ were determined by considering the signal to noise ratio of three and ten, respectively with regarding the background noise from the blank matrices. The method description for sample preparation was validated by recovery investigation. Recovery studies were carried out on blank matrix of cabbage (10 g) by spiking them with known quantities of standard cyantraniliprole solutions at three different concentrations (0.05, 0.25 and 0.5 µg g^{-1}) with six replications. The precision of the method was performed in terms of repeatability (Relative Standard Deviation) for each spiked levels of 0.05, 0.25 and 0.5 µg g^{-1} of the matrix. The Horwitz ratio (HorRat) related to intralaboratory precision, which specifies the acceptability of a method with respect to reproducibility was calculated for cyantraniliprole as follows

HorRat = RSD/PRSD

Where, PRSD (Predicted RSD) = 2 $C^{-0.15}$, where C is the concentration expressed as mass fraction (10ng/g=10×10^{-9}) (Paramasivam and Bhuvaneswari, 2020).

Statistical analysis

The cyantraniliprole residues were calculated using

$$\frac{As \times Cstd \times S1 \times Vs}{Astd \times Ws \times Asj}$$

Where, As - Peak area of the sample; Cstd – Concentration of the standard in (µg ml^{-1}); S1 – injected volume of standard (µl); Vs –volume of the sample (final extract in mL); Astd - Peak area of the standard; Ws - Weight of the sample in g; Asj- Aliquot of the sample injected in µl.

The insecticide degradation pattern was analyzed by applying seven transformation functions as suggested by Hoskin (1961) and Timme *et al.,* (21). The half-life was calculated using Pesticide Residue Half-life Calculator software developed by Department of Soil Science, Tamil Nadu Agricultural University, Coimbatore based on Regupathy and Dhamu (2001) and best fit degradation model was determined.

Decontamination of pesticides in/on cabbage

Cyantraniliprole residues in cabbage heads (collected 1 hour after application) were reduced by adopting six simple culinary practices as decontamination treatments. Treatments include T1 - control; T2 – washing in tap water for 2 min; T3 – washing with 2% tamarind water for 2 min; T4 - washing with 2 % baking soda for 2 min; T5 - washing with 2 % lemon juice for 2 min and T 6 - boiling for 10 min. The decontamination solutions of tamarind, baking soda and lemon juice was prepared separately in 500 mL beaker and the cabbage samples were dipped in the solution for about 2 minutes and gently rubbed with hand. The same way tap water washing and boiling in 500 mL of water was also followed. After discarding the solutions the samples were air-dried on a filter paper to remove the moisture. The samples were subjected to analysis of residues following the above described method.

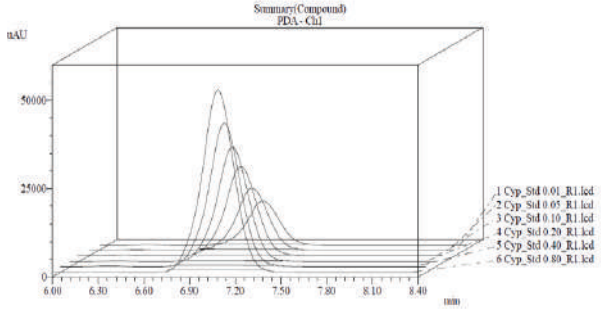

Fig. 1. Linearity chromatograms of cyantraniliprole in UHPLC-PDA

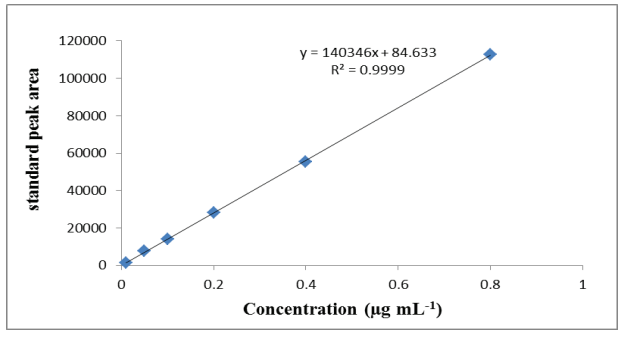

Fig. 2. Calibration curve of cyantraniliprole in UHPLC-PDA

Dietary risk assessment

The estimated daily intake (EDI) of cyantraniliprole residues were calculated by multiplying the highest residue concentration (mg/kg) with the food consumption rate (kg/day) divided by the mean body weight of an adult. Risk quotient (RQ) is derived for the long term risk assessment of intakes, by dividing the EDI by the relevant acceptable daily intake (ADI) (mg/kg body weight (bw)/day). ADI of cyantraniliprole is 0.03 mg/kg bw/day according to FAO. The average body weight of an Indian adult according to National Instituite of Nutrition is 60 kg and recommended vegetable consumption of an Indian adult is 300 g/day (NIN, 2011). The risk for long term human dietary intake of cyantraniliprole is acceptable when RQ is less than 1 and unacceptable if RQ is more than one.

RESULTS AND DISCUSSION

Method validation

Efficiency of analytical method was evaluated based on linearity and recovery studies. Standard calibration curve of cyantraniliprole was constructed by plotting concentration against peak area in the range of 0.01 to 0.8µg g^{-1} (Fig. 2). The concentrations injected observed linear signal with the r^2 value of 0.999 and the linear regression equations of cyantraniliprole was y = 140346x + 84.633 (Fig. 3). Limit of detection (LOD) and limit of quantification (LOQ) of cyantraniliprole were determined as 0.015 and 0.05 µg g^{-1}, respectively. The results of the recoveries and relative standard deviations (RSDs) of cyantraniliprole carried out at the levels of 0.05, 0.25 and 0.5 µg g^{-1} in cabbage is presented in Table 1. The mean recovery values of cyantraniliprole in cabbage heads ranged from 90.96 – 95.92%, with the standard deviation ranging from 2.68 - 3.72. Similar findings with the average percentage recoveries of cyantraniliprole from cabbage were 89.80 to 100.11% (Kumar *et al.,* 2021) and 88.9 to 96.5% of recoveries from tomato fruits (Malhat *et al.,* 2018) were also reported. Since the recovery percentage ranges within the acceptance limit of 80 and 120 % and relative standard deviation recorded less than 5.0 % which is in accordance with the SANTE guidelines (SANTE, 2020). The HorRat ratio for cyantraniliprole was below 0.5 for all the spiked concentrations (0.05, 0.25 and 0.5) which is acceptable according to AOAC guidelines (AOAC, 2016) that satisfy the intralaboratory precision and accuracy. Thus the method can be adopted for residue and dissipation study for cyantraniliprole in cabbage.

Dissipation of cyantraniliprole in cabbage

The results of persistence and dissipation of cyantraniliprole in/on cabbage sprayed at 60 and 120 g a.i ha^{-1} are presented in the Table 2. The mean initial deposits (1 hour after spraying) of cyantraniliprole on cabbage were found to be 1.67 and 2.15 µg g^{-1} at recommended and double the recommended doses, respectively. At recommended dose, residues persisted upto 7 days and reached Below Detectable Limit (BDL) of less than 0.05 µg g^{-1} on 10 days after treatment. At double the recommended dose, the mean residues persisted for 10 days and reached BDL (0.05 µg g^{-1}) on 15 days after treatment. The initial deposits of cyantraniliprole in tomato were 0.751 and 0.841 mg/kg in two different locations of Nile valley Delta region and it persisted upto 14 days after which the level has reduced below BDL (Malhat *et al.,* 2018). Dissipation pattern of cyantranilprole in cabbage was computed following seven transformations and the best fit observed was first order kinetics for both the doses (Table 3). The statistical parameters like intercept (a), slope of regression lines (b) and half-life were presented in Table 3. The half – life values of cyantraniliprole on cabbage were found to be 1.47 and 1.84 days at recommended and double the recommended dose respectively. This was in close proximity with the findings in watermelon with the half-life of 1.1 days for cyantraniliprole (Hu *et al.,* 2013). Close upon other reports with the half-life of cyantraniliprole were, 2.2 days in cucumber, 2.8 days

Table 3. Statistical parameters for dissipation pattern of cyantraniliprole 10.6 OD residues in/on cabbage heads

Function		Recommended dose @ 60 g a.i. ha^{-1}						Double the recommended dose @ 120 g a.i. ha^{-1}					
		a	b	T Half	r	r^2	Modified r^2	a	b	T Half	r	r^2	Modified r^2
First order		0.56	-0.47	1.47				0.70	-0.38	1.84			
	UCL	0.82	-0.41	1.67	-0.99**	0.99	0.99	0.87	-0.35	1.99	-0.99**	0.99	0.99
	LCL	0.30	-0.53	1.28				0.53	-0.41	1.69			
1.5th order		0.45	0.46	0.43				0.37	0.37	0.42			
	UCL	1.46	0.70	1.35	0.96**	0.92	-2.98	1.03	0.49	1.17	0.97**	0.95	-6.54
	LCL	-0.50	0.22	-0.49				-0.29	0.25	-0.34			
2nd order		-1.56	2.12	-0.74				-2.23	1.80	-1.24			
	UCL	6.11	3.99	4.42	0.90*	0.81	-2.42	4.17	2.96	4.87	0.91*	0.82	-5.05
	LCL	-9.24	0.25	2.94				-8.62	0.65	2.40			
RF First order		1.01	-1.27	0.30				1.30	-1.23	0.32			
	UCL	3.50	-0.52	0.65	-0.95*	0.91	0.63	3.24	-0.79	0.54	-0.97**	0.94	0.61
	LCL	-1.48	-2.02	-0.05				-0.64	-1.67	0.09			
RF 1.5th Order		0.14	1.17	0.02				-0.08	1.13	-0.01			
	UCL	2.29	2.37	0.65	0.87NS	0.76	- .96	1.63	1.95	-0.53	0.89*	0.78	-167714.4
	LCL	-2.02	-0.04	-0.61				-1.79	0.31	0.51			
RF 2nd order		-2.83	5.22	0.26				-3.91	5.24	0.56			
	UCL	10.67	12.76	3.22	0.78NS	0.62	-1.77	8.04	10.98	4.18	0.78NS	0.62	-0.95
	LCL	-16.32	-2.32	-2.63				-15.87	-0.50	-3.06			
Inverse PL		-0.37	0.40	5.75				-0.50	0.44	4.85			
	UCL	2.60	0.97	20.26	0.79NS	0.62	0.28	2.08	0.91	13.00	0.79NS	0.63	0.33
	LCL	-3.33	-0.17	-8.75				-3.08	-0.03	-3.30			

UCL- Upper Confidence Limit; LCL – Lower Confidence Limit; *Significant at 5 per cent level; ** significant at 1 per cent level

(Dong *et al.,* 2012), 2.6 days (Malhat *et al.,* 2018) in tomato and with a wide difference it was reported as 6.5 days in banana fruits (Qiang *et al.,* 2017).

Decontamination of insecticides in/on cabbage heads

The results of decontamination study revealed that, boiling for 10 min gives higher (78.06 %) reduction of residues of cyantraniliprole followed by washing with 2 % baking soda for 2 min (61.07 %), 2 min washing with 2 % lemon juice (51.16 %), 2 min washing with 2 % tamarind water (44.69 %) and 2 min tap water washing (34.01 %). In untreated sample the mean initial deposit of cyantraniliprole 10.6 OD @ 60g.ai / ha was 1.655µg g^{-1} (Table 4). In comparision with other diamides, chlorantraniliprole residues in cabbage and cauliflower heads were reduced upto 100% and 17 – 40 % by boiling and tap water washing, respectively (Kar *et al.,* 2012). Cowpea pods on treatment with slaked lime minimized the chlorantraniliprole residues to the level of 90% (Vijayasree *et al.,* 2013). Similarly, Vijayasree *et al.,* (2015) reported about 90 % of chlorantraniliprole residues were brought down on brinjal fruits by treating the samples with 2 % slaked lime and common salt solution.

Dietary risk assessment

For calculating the risk assessment of cyantraniliprole in cabbage, grown under open field conditions the residue dissipation data were used. Dietary risk quotient (RQ) was calculated based on the highest residue concentration obtained from the treated recommended dose. The RQ was less than one from 0 (1 hour after application) days after application of cyantraniliprole, (Table 5) which indicates the cabbage heads from field were safe for consumption. Likewise, the dietary RQ was also less than one for chlorantraniliprole in okra fruits (Paramasivam and Bhuvaneswari, 2020).

CONCLUSION

A simple and efficient residue analytical method using UHPLC for detection and monitoring the residues of cyantraniliprole in cabbage was developed, validated and evaluated. The LOQ of the method for cyantraniliprole was below MRL (2mg/kg) as given by CODEX ALIMENTARIUS (2014). More than 80 % of cyantraniliprole residues were dissipated on 5 and 7 DAS and recorded BDL after 7 and 10 DAS. Dissipation of the insecticide followed first order reaction kinetics and the calculated half-life was 1.47 to 1.84. The dietary risk of cyantraniliprole at recommended doses were negligible to humans, since the RQ value is less than one so that the vegetable can be safely consumed.

Table 4. Effect of decontamination methods on cyantraniliprole residues at 60 g a.i. ha^{-1} in/on cabbage heads

Treatment	Residue (µg g^{-1})	
	Mean ± SD	Per cent Reduction
T1 – Control (Samples without any treatment)	1.655 ± 0.045	-
T2 - Washing with tap water for 2 minutes	1.092 ± 0.034	34.01
T3 - Washing with 2 % tamarind solution for 2 min.	0.916 ± 0.028	44.69
T4 - Washing with 2 % baking soda solution for 2 min.	0.644 ± 0.042	61.07
T5 - Washing with 2 % lemon juice for 2 min.	0.808 ± 0.048	51.16
T6 - Boiling with water for 10 minutes	0.363 ± 0.079	78.06

Table 5. Dietary risk assessment of cyantraniliprole @ 60 g a.i. ha^{-1} and 120 g a.i.

Days after treatment	60 g a.i. ha^{-1}				120 g a.i. ha^{-1}			
	Maximum residue (mg/kg)	EDI (mg/kg bw/day)	ADI (mg/kg bw/ day)	Risk quotient (RQ)	Maximum residue (mg/kg)	EDI (mg/kg bw/day)	ADI (mg/kg bw/day)	Risk quotient (RQ)
0 (1 hr)	1.77	0.0089	0.03	0.2950	2.27	0.0114	0.03	0.3783
1	1.11	0.0056	0.03	0.1850	1.44	0.0072	0.03	0.2400
3	0.55	0.0028	0.03	0.0917	0.82	0.0041	0.03	0.1367
5	0.18	0.0009	0.03	0.0300	0.29	0.0015	0.03	0.0483
7	0.08	0.0004	0.03	0.0133	0.16	0.0008	0.03	0.0267
10	BDL	-	-	-	0.07	0.0004	0.03	0.0117
15	BDL	-	-	-	BDL	-	-	-

ADI – Acceptable daily intake, EDI – Estimated daily intake

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Dissipation and persistence pattern of chlorantraniliprole residues in tomato under open field conditions and its estimation through UHPLC-PDA

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ABSTRACT: Field experiment was carried out to study the dissipation and persistence of chlorantraniliprole in tomato (cv. Kashi Vishesh) sprayed @ 30 and 60 g a.i. ha^{-1} . The initial residues ranged from 0.54 to 0.74 and 0.92 to 1.20 mg kg^{-1}. Residues further declined below Limit of Quantification (LOQ) of 0.05 mg kg^{-1} after 5th day, respectively. The $T_{1/2}$ of chlorantraniliprole on tomato after the 2nd spray was calculated to be 1.02 and 1.32 days whereas after the 3rd spray it was observed to be 0.90 days at both the dosages. The safe waiting period for tomato was suggested to be 1 day after application, if followed the GAP.

Keywords: Chlorantraniliprole, residues, tomato, waiting period, dissipation, UHPLC

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is a solanaceous vegetable crop having a good source of nutrients. It is the world's largest consumed vegetable, widely grown throughout the world and ranked first among the canned vegetables (Choudhary, 1996). The fruits of tomato can be consumed both in raw form as well as in cooked form. It is grown under tropical and subtropical and temperate regions. Biotic and abiotic stresses are two major factors responsible for the reduction of quality and quantity production and productivity. In India, *Helicoverpa armigera* is the most serious pest and is responsible for huge economic losses by reducing the quantity, quality thereby market value (Singh *et al*., 2011; Reddy and Zeharm, 2004). In India around 5 to 55 per cent losses reported due to fruit borer in tomato growing regions. Under favourable conditions, damage caused by the pest may go up to 88 per cent (Selvanaryanan and Narayanasamy, 2006). Chemical control serves as an important tool of pest management that is employed largely against the management of above pests in tomato. The injudicious use of the chemicals leads to the problem of residue, resistance as well as the outbreak of secondary pests. This resulted in development of newer molecules such as chlorantraniliprole with a unique mode of action for the safety of human beings as well as other beneficial organisms on different plants, including tomatoes.

Chlorantraniliprole is a new insecticide with systemic action of anthranilic diamide group (Cordova *et al.,* 2006). The population of fruit borer larvae was minimum in plot with chlorantraniliprole 18.5 SC treated with mean larval population of 1.14 larvae/plant (Wasu *et al.,* 2020). The reduction per cent of fruit borer larval population was highest at 3rd day of after application with per cent reduction of 98.04. When rynaxypyr imposed @ 40 g a.i. ha^{-1} showed a high level of insecticidal activity against lepidopteran insect pests and less toxic to mammals attributed by its higher selectivity to insects over mammalian ryanodine receptors (Ghosal *et al.*, 2012). Besides its insecticidal activity against lepidopteran pest, it is also equally effective in controlling the population of whitefly, leaf miners, beetle, and termite species (Babu *et al.,* 2019). Chlorantraniliprole 18.5 per cent SC is used against fruit borers of chillies and tomato, bollworms in cotton, tobacco caterpillar, okra fruit borer and diamond back moth in cabbage (Anon., 2020).

The rational recommendation for an insecticide must achieve effective control of target pest without leaving residues on the produce. Pesticide use on any crop leads to its residues which further dissipate with time. The dissipation and persistence of pesticide residues depend upon the physico-chemical properties of the pesticide used, dose of pesticide used and nature of the crop on which sprayed and weather parameters of that locality. Most of the pesticides applied may affect non target organism may be due to inefficient application system which further increases the problems of pesticide residues in the environment. To safe guard the problems arising out of pesticide use, it is always advisable for judicious use of pesticide. The amount of residues present in different commodities for different pesticide, supervised field trails are to be conducted. The dissipation and persistence nature of pesticides on different crops can be obtained by conducting supervised field trials. Data from supervised trials give reliable estimates of the residue levels that are likely to persist in food commodities at the time of harvest. To ensure consumer safety, the

Table 1. Per cent recovery of chlorantraniliprole from spiked samples of tomato

Spiked Level (mg kg^{-1})	Replicates		Percent Mean Recovery ± SD	RSDr
	Amount recovered	Percent recovery		
0.05	0.042	84.00	88.00 ± 5.292	6.013
	0.043	86.00		
	0.047	94.00		
0.25	0.240	96.00	89.60 ± 5.769	6.438
	0.212	84.00		
	0.220	88.00		
0.5	0.459	91.80	90.60 ± 4.937	5.449
	0.473	94.60		
	0.425	85.00		

RSDr = (Relative Standard Deviation (Repeatability), SD = (Standard Deviation)

persistence pattern of pesticides on edible parts of crops must be known. The current field experiment was carried out with an objective to study the dissipation and persistence of chlorantraniliprole in tomato at different time intervals and also to suggest waiting periods for chlorantraniliprole on tomato.

MATERIALS AND METHODS

Insecticide: Analytical standard of chlorantraniliprole (purity 97.28 per cent) was obtained from Dr. Erhenstrofer, India, chlorantraniliprole 14.5 SC formulations were used for field application. Standard stock solutions of chlorantraniliprole (1mg mL^{-1}) were pre arranged with respect to HPLC grade acetonitrile, the standard solutions were further diluted to have different concentrations and injected into the instrument to see the linearity by plotting a calibration curve. The storage temperature for all these standard solutions were kept around -4^{0}C prior to use.

Raising of the crop*:* A field experiment with three treatments and three replications was carried out at Agricultural Research Farm, Tirhut College of Agriculture (TCA), Dholi, Muzaffarpur, Bihar, India. Tomato (cv. Kashi Vishesh) was raised in a randomized block design according following the recommended agronomic practices for this region. The seedlings were transplanted in the first week of November at proper spacing (30cm x 45cm) in 25 m^2 plots.

Application of insecticide: The chlorantraniliprole 18.5 SC was applied on tomato crops at doses of 30 and 60 g a.i. ha^{-1}, respectively by using High Pressure Lithium Battery Operate and Knapsack Sprayer of 15 L capacity. The 1st spraying was done at 50 per cent flowering/ fruit initiation stage & subsequently 2 sprays were done at 10 days interval. Amount of volume used while spraying was 500 litres ha^{-1}.

Sampling: About half kg samples of marketable size tomato fruits were randomly taken from each plot at "0 (2 hours), 1, 3, 5, 7 and 10 days after the second and 0 (2 hours), 1, 3, 5, 7, 10 and 15 days" after third application of the of insecticide. The collected samples were brought to the pesticide residue laboratory, Department of Entomology for further analysis. Samples were chopped and 15 g of macerated samples were kept in the refrigerator at 4 ^{0}C. The extraction and cleaned up was done on next day of sampling.

Residue analysis of tomato samples*:* "Quick, Easy, Effective, Rugged and Safe (QuEChERS)" technique with slight changes is used for processing of tomato samples for residue analysis (Anastassiades *et al.*, 2003). A grinded tomato sample (15g) was transferred to a 50 mL polypropylene centrifuge tube later kept overnight in refrigeration. Samples were taken from the refrigerator and 30 mL of ACN (HPLC grade) was added to each tube. To each centrifuge tube, for phase separation, (10 ± 0.1 g) NaCl was added and agitated for 10 minutes at 50 rpm on a rotospin (Tarson®). Sample was centrifuged for 3 min at 2,500 rpm. Moisture, if any, was removed from aliquot of acetonitrile by sodium sulphate anhydrous (10 ± 0.1 g) followed by clean-up through "dispersive solid phase extraction (DSPE)". For this, a polypropylene tube constituting "0.15 ± 0.01 g PSA sorbent and anhydrous $MgSO_4$ (0.90 ± 0.01 g) was prepared for an aliquot of 6 mL which was thoroughly mixed by vortex spinix (Tarson®). Once again centrifuged for 3 min at 2,500 rpm and finally a 3 mL aliquot was taken for estimation of residues of chlorantraniliprole for residue analysis.

Table 2. Residues of chlorantraniliprole in tomato

Days after spraying	2nd spray				3rd spray			
	Dose @ 30 g a.i. ha^{-1}		Dose @ 60 g a.i. ha^{-1}		Dose @ 30 g a.i. ha^{-1}		Dose @ 600 g a.i. ha^{-1}	
	Mean of Residue (mg kg^{-1}) ± SD	Per cent dissipation	Mean of Residue (mg kg^{-1}) ± SD	Per cent dissipation	Mean of Residue (mg kg^{-1}) ± SD	Per cent dissipation	Mean of Residue (mg kg^{-1}) ± SD	Per cent dissipation
Before application	<LOQ	-	< LOQ	-	< LOQ	-	< LOQ	-
0 (2hrs after spray)	0.60 ± 0.076	-	0.97 ± 0.047	-	0.70 ± 0.068	-	1.10 ± 0.083	-
1	0.18 ± 0.031	70.00	0.33 ± 0.053	65.97	0.21 ± 0.036	70.00	0.35 ± 0.055	68.18
3	0.07 ± 0.015	88.33	0.11 ±0.015	88.86	0.06 ± 0.015	91.43	0.10 ± 0.015	90.09
5	<LOQ	-	<LOQ	-	< LOQ	-	< LOQ	-
7	<LOQ	-	< LOQ	-	< LOQ	-	< LOQ	-
10	<LOQ	-	<LOQ	-	< LOQ	-	< LOQ	-
15	-	-	-	-	< LOQ	-	< LOQ	-

LOQ = Limit of Quantification (0.05 mg kg^{-1})

Table 3. Dissipation parameters of chlorantraniliprole residue in tomato

Dissipation parameters	Doses after 2nd spray		Doses after 3rd spray	
	30 g a.i. ha^{-1}	60 g a.i. ha^{-1}	30 g a.i. ha^{-1}	60 g a.i. ha^{-1}
K_1 (b)	-0.295	-0.227	-0.336	-0.336
K_2 (a)	1.687	1.889	1.770	1.976
$T_{1/2}$	1.02	1.32	0.90	0.90
T_{tol}	0.31	0.49	0.4	0.58
R^2	0.933	0.882	0.964	0.973
Y	-0.295x + 1.687	-0.227x + 1.889	-0.336x+1.7703	-0.336x+1.9767

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 of 0.6 mg kg^{-1} "
R^2 = "Coefficient of determination"

Estimation: The estimation of chlorantraniliprole was done through UHPLC (High Pressure Liquid Chromatography) developed with "Photodiode Array Detector (PDA)". The peak of chlorantraniliprole was detected at a λ_{max} of 260 nm when Mobile phase {ACN: HPLC water (70:30)} was given with a flow rate of 0.3 ml min^{-1}, injected at a volume of 20 µl using C_{18} column at a temperature of 40 °C.

Determination of residues*:* The residues of chlorantraniliprole in tomato were matched with the retention time of respective standards, whereas, estimated by peak area. Retention time for chlorantraniliprole was observed to be 4.327 min., correspondingly when injected under above mentioned conditions.

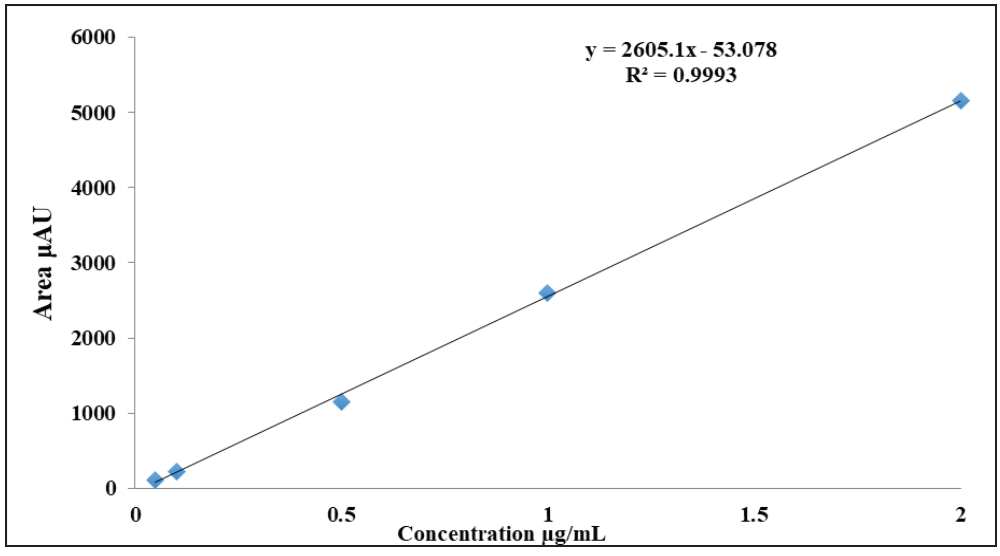

Fig. 1 Linearity curve of chlorantraniliprole standards

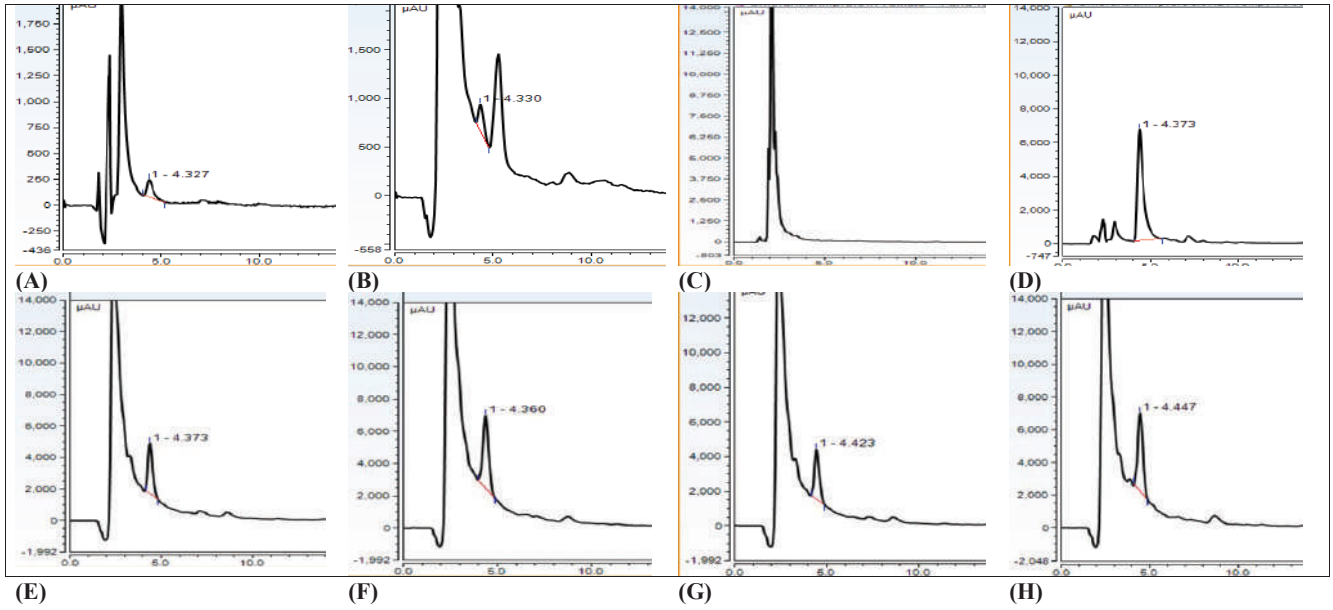

Fig 2. HPLC chromatograms for tomato samples of (A)Chlorantraniliprole standard @ 0.05 µg mL^{-1}; (B)Tomato spiked with chlorantraniliprole @ 0.05 µg mL^{-1}; (C)Tomato control; (D)Chlorantraniliprole standard 1µg mL^{-1}; (E)Chlorantraniliprole @ 30 g a.i.ha^{-1}after 2nd spray; (F)Chlorantraniliprole @ 60 g a.i.ha^{-1} after 2nd spray; (G)Chlorantraniliprole @ 30 g a.i.ha^{-1} after 3rd spray; (H)Chlorantraniliprole @ 60 g a.i.ha^{-1} after 3rd spray

RESULTS AND DISCUSSION

Limit of detectability of chlorantraniliprole residues in tomato*:* The full-scale deflection was obtained with 5 ng of the standard of chlorantraniliprole, respectively. Samples of tomato were processed and terminal volume was composed to 3 mL. The sample load 20 µL for chlorantraniliprole was injected to observe the maximum load of samples can be analysed without any interference peak in the area relating to the compound estimated. The LOQ (Limit of quantification) and LOD (Limit of detection) for chlorantraniliprole was found to be 0.05 mg kg^{-1} and 0.017 mg kg^{-1}, respectively.

Recovery of chlorantraniliprole in tomato: Mean recoveries of chlorantraniliprole in tomato samples spiked with 0.05, 0.25 and 0.5 mg kg^{-1} levels range about 84.00 to 96.00 per cent and found to be were greater than 80 per cent (Table 1 and Fig. 2). The quantitative determination of chlorantraniliprole tomato was validated as stated by

bio analytical method recommendations described in the SANCO guidelines (Anon., 2021).

The calibration curves in relation to chlorantraniliprole generate a linear relationship with different conc. of 0.05, 0.1, 0.5, 1 and 2 µg ml^{-1} (Fig. 1). The linearity curve was prepared taking different concentrations of standards starting with the concentration equal to LOQ. The curve shows good linearity with R^2 value of more than 0.99 implies that the detector used and standard prepared were fit for further analysis of chlorantraniliprole residues.

Determination of Repeatability (RSD_r) by spiking chlorantraniliprole through developed analysis method at different concentrations to different substrates. The repeatability (RSD_r) for chlorantraniliprole in tomato at 0.05, 0.25 and 0.5 mg kg^{-1} level correspond to 6.01, 6.43 and 5.44 per cent, respectively (Table 1).

Estimation of chlorantraniliprole residues in tomato*:* The quantitative estimates of chlorantraniliprole (30 and 60 g a.i. ha^{-1}) residues in tomato, at different time interval, after 2nd and 3rd spray were presented in Table 3. Similarly, the representative chromatograms for 0 day samples with reference to second and third sprays for recommended and double doses was presented in Fig. 2.

At recommended dose, the mean initial deposit of chlorantraniliprole after 2nd spraying was found to be 0.60 mg kg^{-1}. The residue of chlorantraniliprole in tomato fruits was found to be dissipated to a mean level of 0.18 mg kg^{-1} after one day of 2nd spray which represented a dissipation of about 70. 00 per cent residues. The mean level of residue after three days of spraying was found to be 0.07 mg kg^{-1} and represented a dissipation of about 83.00 per cent residues. After 5th days of spraying, the residues in tomato fruits were found below the limit of quantification (0.05 mg kg^{-1}) (LOQ) (Table 2, Fig. 2).

At double dose, the mean initial deposit of chlorantraniliprole after 2nd spraying was found to be 0.97 mg kg^{-1}. The residue of chlorantraniliprole in tomato fruits was found to be dissipated to a mean level of 0.33 mg kg^{-1} after one day of 2nd spray which represented a dissipation of about 65.97 per cent residues. The mean level of residue after three days of spraying was found to be 0.11 mg kg^{-1} and represented a dissipation of about 88.86 per cent residues. After 5th days of spraying, the residues in tomato fruits were found below the limit of quantification (0.05 mg kg^{-1}) (LOQ) (Table 2 and Fig. 2).

After 3rd spraying, the mean initial deposits of chlorantraniliprole @ 30 g and 60 g a.i/ha was found to be 0.70 mg kg^{-1} and 1.10 mg kg^{-1}, respectively. At recommended dose, dissipation of about 70. 00 and 91.62 per cent was observed after one and three days of spraying, respectively. Similarly, in double doses, dissipation of about 68.18 and 91.00 per cent was observed after one and three days of spraying. The residues reached below the limit of quantification after five days of spraying in both the recommended as well as in double doses (Table 2 and Fig. 2).

According to the findings of investigation, higher the doses of application led to higher amount of initial deposits. The overall results of the experiment revealed that, the mean initial deposits of chlorantraniliprole @ 30 g a.i. ha^{-1} and 60 g a.i. ha^{-1} varied from 0.54 to 0.74 mg kg^{-1} and 0.92 to 1.20 mg kg^{-1}, respectively. The residue of chlorantraniliprole both at a recommended and double dose dissipated to below LOQ after five days of spraying.

Similar results also reported by Paramasivam (2020) who studied the dissipation of chlorantraniliprole on tomato at Tamilnadu. Kabadad and Gali (2018) studied the dissipation pattern of four sprays of chlorantraniliprole at 0.20 ml L^{-1} (single dose) and 0.40 ml/L (double dose) on cabbage. Chlorantraniliprole residues reached BDL by 3rd day, showing 100 per cent dissipation of residue at respective dose. Reddy *et. al*. (2017) who observed that the mean deposits of 0.56 mg kg^{-1} chlorantraniliprole in chilli following application @ 30 g a.i. ha^{-1}. Vijayshree *et al.* (2012) also found that mean deposits of 0.55 mg kg^{-1} following application of chlorantraniliprole 18.5 SC on cowpea. But Singhla *et. al*. (2020) observed the initial deposits of 0.21 mg kg^{-1} and 0.46 mg kg^{-1} in okra fruits when fruits were sprayed with chlorantraniliprole @ 25 and 50 g a.i. ha-^{1}, respectively, which are less than the residues reported in this study may be because of lower dose applied. The amount of initial deposit of any pesticide residues mainly depends upon the amount of dose applied on that crop. Similarly, Kar *et al.* (2013) reported deposits of 0.18 and 0.29 mg kg^{-1} of residues of chlorantraniliprole on cauliflower following application @ 9.25 and 18.50 g a.i. ha^{-1}. Not only the dose but also the type of crop also influences the initial deposits. The results of present study particularly the deposit of chlorantraniliprole on tomato are different from the findings by Sonia (2019) who reported higher initial deposits of 1.17 mg kg^{-1} and 2.36 mg kg^{-1} at 30 and 60 g a.i. ha^{-1} in tomato, respectively.

Waiting period for chlorantraniliprole on tomato*:* The half-life values are described simply and broadly as the time required to dissipate initial residues to half (Gunther and Blinn 1955). Time taken for residue to reach below MRL (Ttol) and $T_{1/2}$ in days were computed by using the formula given by Hoskins (1961).

The Maximum Residue Limit (MRL) of chlorantraniliprole on tomato was approved at 0.6 mg kg $^{-1}$ (FSSAI). For chlorantraniliprole insecticide, a linear relationship was determined by plotted log concentration of residue against the time. It confirms, that the declination of the chlorantraniliprole residues showed first order kinetic reaction. The $T_{1/2}$ of chlorantraniliprole on tomato were calculated to be 1.02 days and 1.32 days when applied at the rate of 30 and 60 g a.i. ha^{-1} after 2nd spray, whereas after 3rd spray it was observed to be 0.90 days at both the dosages (Table 3). The mean initial deposits of chlorantraniliprole on tomato was observed to be below the MRL after 1 day of spraying at both the dosages after 2nd and 3rd spraying. Thus, the present study suggested that, a waiting period of one day for chlorantraniliprole at single and double dose is required, provided that followed good agricultural practices were followed.

These findings were in accordance with the (Sonia, 2019) who found that half live of chlorantraniliprole was 1.57 days and 1.98 days at recommended and double dosages on tomato, respectively. Similar results were also recorded by (Paramasivam, 2020) who analysed the tomato sample as well as soil sample treated with chlorantraniliprole and found that the half-life of chlorantraniliprole was 1.26 and 1.77 days in fruits and soil respectively. Similar result was also reported by (Kar *et. al.,* 2013), in cauliflower, who concluded a half-life value ($T_{1/2}$) at recommended dosages (9.25 g a.i. ha^{-1}) of chlorantraniliprole was found to be 1.36 days. Vijayasree *et. al.* (2013) reported a waiting period of 0.62 days when we go for the spraying of chlorantraniliprole 18.5 % SC and the half-life was found to be 1.31 days on cowpeas. Shams EL Din *et al.* (2015) found that the residues of chlorantraniliprole were higher in winter and summer seasons of 2012, the lowest residues were in 2013 for winter seasons and in 2014 for the summer seasons. The least half–life and PHI were 2.441 and 6 days in the summer season of 2014, whereas it was the longest in the winter season of 2012 with 2.988 and 7.400 days, respectively.

CONCLUSION

The residues of chlorantraniliprole in tomato dissipated and reached below LOQ after 5 days at recommended and double dose. After 2nd spray, the half-life values of chlorantraniliprole on tomato were calculated to be 1.02 and 1.32 day at 30 and 60 g a.i. ha^{-1} whereas after 3rd spray it was observed to be 0.90 days @ 30 and 60 g a.i. ha^{-1}. The present study suggested a waiting period of 1 day for chlorantraniliprole following application at recommended dose on tomato if followed good agricultural practices.

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Estimation of yield loss due to chilli leaf curl disease in chilli (*Capsicum annuum* L.) at different stages of the crop

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ABSTRACT: The yield loss assessment was done in chilli hybrids (Arka Meghana and Arka Khyati) against chilli leaf curl disease (ChiLCD) at Bengaluru, India. The ChiLCD infected plants were tagged at different stages of the crop from 20 to 60 days after transplanting on the onset of first appearance of the disease. The observations were recorded on different parameters such as plant height, number of branches, number of sub-branches, number of fruits per plant, number of unmarketable fruits, average ten fruit weight per plant, average ten fruit width per plant, average ten fruit length per plant, yield per plant in infected and healthy chilli plant on both the varieties. The analysis revealed that the fruit yield of chilli significantly was affected due to ChiLCD at different growth stages of the crop in both Arka Meghana and Arka Khyati. The highest (> 98%) per cent yield loss was recorded in the plants infested at 20 DAT while the lowest (< 43%) per cent yield loss was recorded in 60 DAT compared to healthy plants. The yield loss had gradually decreased when stage of infection progressed from 20 to 60 days after transplanting.

Keywords: Chilli, ChiLCD, leaf curl, symptoms, yield loss estimation

INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important vegetable and spice crop grown across the tropical and subtropical regions of the world (Makari *et al,* 2009). In India, green chilli is grown in an area of 3.6 lakh hectares with a production of 34.06 lakh tones (Anon, 2017). Major chilli producing states of India are Karnataka, Bihar, Chhattisgarh, Madhya Pradesh and Maharashtra, Andhra Pradesh, Telangana, West Bengal and Orissa. India is the largest producer and exporter of chilli in the world (Anonymous, 2017). In Karnataka, chilli is grown in all districts with an area of 0.44 lakh hectares and production of 6.35 lakh tones. Viral diseases are major threat for chilli production resulting in low fruit quality and quantity. More than 65 viral diseases have been reported to infect chilli worldwide (Devi *et al.,* 2020). Of these, eleven viruses are reported from India which include, *Cucumber mosaic virus* (CMV), *Chilli veinal mottle virus* (ChiVMV), *Tobacco ring spot virus* (TRSV), *Potato virus* X (PVX), *Chilli leaf curl virus* (ChiLCV), *Tobacco leaf curl virus* (TbLCuV), *Potato virus* Y (PVY), *Pepper veinal mottle viru*s (PeVMV) and *Pepper vein bending virus* (PeVBV), *Capsicum chlorosis virus* (CaCV), *Chilli leaf curl virus* (ChiLCV), *Chilli leaf curl India virus* (ChiLCINV), *Chilli leaf curl Vellanad virus* (ChiLCVV), *Tomato leaf curl Joydebpur virus* (ToLCJV) and *Tomato leaf curl New Delhi virus* (ToLCNDV) (Rajamanickam *et al.,* 2020; Vinoth Kumar *et al.,* 2015, Wahyuni *et al.*, 1992; Zehra *et al.*, 2017). Among these, CMV, ChiVMV and ChiLCV were reported to be more serious pathogens causing up to 100% yield loss in marketable fruits and sometimes resulting in abandoning the fields prior to harvest.

Chilli leaf curl disease was first recorded during 1960s in India (Mishra *et al.,* 1963; Dhanraj and Seth, 1968) and has been threatening the commercial cultivation of chilli in the country. The disease occurs at all the growth stages of the crop and characterized by the symptoms *viz*., vein clearing, curling, puckering, distortion vein thickening, enation, boat shaped leaves with upward bending of leaf tips, blistering of inter-veinal areas and shortening of internodes (Arun Kumar, 2006). The amount of damage caused by disease depends on the stage of the crop at the time of infection. The plant infected at very early stage failed to produce flowers and plants infected at later stages compromised with the quality and quantity of the fruits produced (Senanayake *et al*., 2007; Senanayake *et al.*, 2012). Hence, the present study was carried to

estimate the losses due to Chilli leaf curl virus disease at different stages of crop growth.

MATERIALS AND METHODS

Field experiments were carried out at ICAR-Indian Institute of Horticultural Research (IIHR) farm at Hessaraghatta, Bengaluru, India to assess the yield losses in chilli due to chilli leaf curl disease infection during *kharif*, 2018. The crop was sown with a spacing of 25×40 cm in Randomized Block Design having plot size of 7×7 m using the cultivars Arka khyathi and Arka Meghana, which are susceptible to ChiLCuD. For the entire crop period, care was taken to follow the all cultural practices as per package of practices developed by the ICAR-IIHR. Ten virus infected plants were tagged when initiation of first symptoms (Yellowing of emerging leaves) at 20, 30, 40, 50 and 60 DAT. Similarly, 10 healthy plants were also tagged as a control treatment. The observations were recorded on different parameters such as plant height, number of branches, number of sub-branches, number of marketable fruits, number of unmarketable fruits, fruit weight, fruit width, fruit length, yield per plant in both infected and healthy chilli plants. Yield loss was calculated on the basis of total weight of the fruits obtained from healthy plants and virus infected plants and expressed in terms of percentage according to the formula (Sastry and Singh, 1973) given below.

$$\text{Per cent yield loss} = \frac{\text{Yield of healthy plants} - \text{Yield of diseased plants}}{\text{Yield of healthy plants}} \times 100$$

RESULTS AND DISCUSSION

The data on estimation of yield losses in chilli at different stages of crop growth revealed that the plant height was affected at all the stages of ChLCuD infection. However, there was significant difference among the period at which the first symptoms were appeared starting from 20, 30, 40, 50 and 60 DAT. The maximum plant height (98.6 and 99cm) was recorded in the healthy plants of Arka Meghana and Arka Khyati and it was significantly superior to all the plants with symptoms at different days after infection (Tables 1and 2) which was followed by 60 and 50 days after transplantation (DAT) with recorded height of 88.6-91 and 84.2-86.6cm in both hybrids Arka Meghana and Arka Khyati and lowest plant height was recorded in the plants infected at 20 DAT in both the hybrids.

The fruit yield of five plants was varying significantly at different days of first appearance of symptoms. The lesser fruit yields of 9.84, 151.0, 319.2, 399.2, 533.2 and 921.6 were recorded at 20, 30, 40, 50 and 60 days of first appearance symptoms when compared to highest seed yield of 372.2 g in healthy plants. Based on the fruit yield data, the yield loss was calculated and results varied from 98.93 to 42.14 per cent during the early days of first appearance symptoms. The highest loss was 98.93 per cent recorded when the crop was shown first appearance symptoms at 20 DAS and there after the loss reduced gradually from 83.61 to 42.14 per cent from 30 to 60 days after sowing (Table 1 and Fig. 2).

Similarly, the number of branches formed per plant was recorded at 20, 30, 40, 50 and 60 days of first appearance symptoms, It was observed that more number of main branches (7.2-7.8) was recorded in the plants where disease appeared at 50 and 60 DAT and healthy plants of Arka Meghana and Arka Khyati cultivars. Whereas the lowest number of branches (4.2) was recorded in the plants infected at 20 DAT in both the hybrids. The number of main branches decreased with early appearance of the disease in both Arka Meghana and Arka Khyati. With respect to the average number of fruits per plant was recorded at 20, 30, 40, 50 and 60 days of first appearance symptoms. More number of average fruits (262 - 261.8/plant) was recorded in healthy plants of both the hybrids (Table 1and 2). The average number of 148-152.2 fruits per plant was recorded at 60 DAT (days after transplanting) in the diseased plants of Arka Meghana and Arka Khyati respectively, whereas 107.6 fruit per plant recorded at 50 DAT in both hybrids. The average less number of fruits per plant was recorded (54.8-85.8) in disease appeared at 30 and 40 DAT and the least average number of fruits per plant was recorded in plants where disease was recorded as early as 20 DAT in both hybrids.The average number of fruits per plant was less when the plants affected by leaf curl disease at early stage. The delay in the appearance of the disease will minimize the loss with respect to the number of fruits produced per plants.

The maximum number of unmarketable fruits (23.2-24.2) were observed in the plants where symptoms started as early as 30 DAT in Arka Meghana and Arka Khyati respectively (Tables1 and 2). When there was delay in the appearance of disease from 30 to 60 DAT, there was reduction in the number of unmarketable fruits from 23.2 to 11.2 and 24.2 to 11.6 in Arka Meghana and Arka Khyati, respectively. At 60 DAT the unmarketable fruits were 11.2 in Arka Meghana and at 50 and 60 DAT 11.6-12.2 fruits in Arka Kyathi. While less number (8-9.80) of unmarketable fruits were recorded in healthy plants. The number of unmarketable fruits was decreased when stage of infection increased from first appearance of symptoms at 20 to 60 DAT plants in both

Table 1. Yield parameters of chilli cv. Arka Meghana in relation to the stage of first Chi LCD appearance at different days after transplanting

Days after transplanting	Plant height (cm)	Number of main branches	Number of sub branches	Number of fruits per plant	Number of unmarketable fruits	Fruit weight (10 fruits) (g)	Fruit width (10 fruits) (mm)	Fruit length (10 fruits) (cm)	Yield (average of 5 plants) (g)	Yield loss (%)
20	40.4^{d}	4.2^{c}	9.2^{c}	4.75^{f}	4.4^{d}	1.72^{f}	0.82^{d}	3.38^{d}	9.82^{f}	98.90
30	61.8^{c}	5.2^{bc}	9.8^{c}	54.8^{e}	23.2^{a}	3.12^{e}	0.98^{c}	4.3^{cd}	166^{e}	81.55
40	67.4^{c}	5.8^{b}	9.8^{c}	83.0^{d}	16.6^{b}	3.76^{d}	1.02^{c}	5.06^{bc}	300^{d}	66.66
50	84.2^{b}	7.2^{a}	12.6^{b}	107.6^{c}	16.4^{b}	4.2^{c}	1.3^{b}	5.58^{ab}	402^{c}	55.33
60	88.6^{b}	7.8^{a}	13.8^{b}	152.2^{b}	11.2^{c}	5.3^{b}	1.32^{b}	6.1^{ab}	532^{b}	40.88
Healthy	98.6^{a}	7.8^{a}	17.6^{a}	262^{a}	8.0^{c}	5.6^{a}	1.36^{a}	6.52^{a}	900^{a}	-
SEM±	14.6	0.35	0.76	1.98	1.41	0.6	0.3	0.38	17.57	-
CD (P=0.05)	4.99	1.0	2.2	5.8	4.1	0.19	0.09	1.1	51.5	-
CV (%)	11.76	9.64	10.89	3.1	18.38	8.44	13.52	12.93	7.92	-

Table 2. Yield parameters of chilli cv. Arka Khyathi in relation to the stage of first Chi LCD appearance at different days after transplanting

Days after transplanting	Plant height (cm)	Number of main branches	Number of sub branches	Numberof fruits per plant	Number of unmarketable fruits	Fruit weight (10 fruits) (g)	Fruit width (10 furits) (mm)	Fruit length (10 furits) (cm)	Yield (average of 5 plants) (g)	Yield loss (%)
20	38.8^{d}	4.2^{c}	8.4^{d}	3.0^{f}	3.4^{e}	1.5^{e}	0.82^{c}	3.28^{e}	9.84^{f}	98.93
30	63.6^{c}	5.2^{bc}	10.8^{c}	54.2^{e}	24.2^{a}	3.24^{d}	1.00^{bc}	4.18^{d}	151.0^{e}	83.61
40	65.4^{c}	5.6^{b}	11.0^{c}	85.8^{d}	19.4^{b}	3.76^{cd}	1.06^{abc}	4.86^{cd}	319.2^{d}	65.36
50	86.6^{b}	7.6^{a}	11.8^{c}	107.6^{c}	12.2^{c}	4.46^{bc}	1.32^{a}	5.44^{bc}	399.2^{c}	56.68
60	91.0^{ab}	7.6^{a}	12.6^{b}	148.0^{b}	11.6^{cd}	5.12^{ab}	1.34^{ab}	5.92^{ab}	533.2^{b}	42.14
Healthy	99.0^{a}	7.6^{a}	17.6^{a}	261.8^{a}	9.8^{d}	5.68^{a}	1.38^{a}	6.58^{a}	921.6^{a}	-
SEM±	2.73	0.34	0.65	3.4	0.71	0.23	0.9	0.24	12.17	-
CD (P=0.05)	8.0	1.0	1.9	10.0	2.1	0.7	0.3	0.7	35.7	-
CV (%)	6.43	9.24	9.17	5.36	9.09	10.24	14.25	8.36	5.34	-

the varieties. In 20 DAT, plants recorded the highest percentage of unmarketable fruits followed by 30 DAT plants in Arka Meghana and Arka Khyati respectively. Similarly healthy chilli plots recorded more fruit weight (5.6-5.68g) in both the varieties and it was on par with 60 DAT in Arka Khyati. At 30, 40 and 50 DAT recorded moderate fruit weight *i.e*., 3.12-4.46 in both the cultivars. Where as in the plots infected after 20 DAT recorded less than 2g of fruit weight in both the cultivars. Whereas the lowest fruit weight per plant was recorded at 20 DAT in both the varieties (Table 1and 2). The fruit weight was increased when stage of infection increased from first appearance of symptoms at 20 to 60 DAT plants in both the varieties. Similar trend was observed with respect to fruit width and fruit length *i.e*., highest fruit length and width was recorded in healthy plants, while lowest was recorded in the plants infected after 20 DAT (Table 1and 2). The fruit width was increased when stage of infection increased from first appearance of symptoms at 20 to 60 DAT plants.

The highest yield per plant of 900-921.6 g was recorded in the healthy plants of Arka Meghana and Arka Khyati cultivars respectively. It was significantly superior to rest of the parameters at different infection stages, followed by 60 DAT (532-533.2g). At 50 DAT the fruit yield was 402 and 399.2g in Arka Meghana and Arka Khyati respectively. The fruit yield at 40 DAT in both the varieties was 300-319.2g. The lowest (9.82-9.84g) yield was recorded in the plants infected at 20 DAT (Tables 1and 2).The yield had gradually increased when stage of infection advanced from 20 to 60 days after transplanting.

The literature survey showed that ToLCV infected plants produced very few fruits when infected within 20 days after planting and resulting up to 92.3 per cent yield loss in tomato and plants infected at 35-50 days after transplanting resulted in 23-74 per cent yield loss (Sastry and Singh, 1973). Similar in case of sunflower affected by sunflower leaf curl virus disease, significantly affected head diameter, 100gm seed weight, oil content depending on the growth stages at which first symptoms appears. In the plants, first appearance of symptoms at 30 DAS, the diameter of the head, 100gm seed weight, oil content and weight of seeds/ 10 heads were reduced to maximum extent as compared to healthy plants (Sinha and Chakrabarthi, 1978; Deepa *et al.,* 2015). Further they also showed that the plant height was reduced at all the stages of sunflower due to leaf curl disease infection. However, significant difference was observed when the first appearance of symptoms at 30 DAS or earlier (72.60 cm plant height) as compared to the first appearance of symptoms at 45, 60, 75 and 90 DAS with 89.80 cm, 115.30 cm, 129.6 cm and 142.2 cm plant height respectively. Significant reduction in size of the head, 100 seed weight, oil content and weight of seeds/10 heads as compared to the healthy control plant (Deepa *et al.,* 2015). Simialry, Gopal and Upadhyaya (1991) reported that, early infection of peanut bud necrosis disease caused heavy reduction in dry pod yield, 100 seed weight, and shelling percentage compared to late infection in groundnut.

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Induction of systemic acquired resistance in *Capsicum annuum* L. against *Chilli Veinal Mottle Virus* by foliar spray of salicylic acid

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ABSTRACT: Management of viral diseases requires an integration of several methods aimed at preventing or delaying infection of crops, as the viruses overcome the resistance due to their genome plasticity. Plants have evolved a variety of active and passive mechanisms to defend themselves against viral pathogens. One such mechanism is systemic acquired resistance; a safekeeping system a plant has preserved to combat various pathogen attacks. The possibility of inducing resistance in plants against viruses with chemicals or beneficial microorganisms deserves more interest. This study was conducted to evaluate the efficacy of salicylic acid (SA) foliar spray against *Chilli Veinal Mottle Virus* (ChiVMV) infecting *Capsicum annuum* L. which induces systemic acquired resistance. Salicylic acid was used as a foliar spray in different concentrations against ChiVMV with reference to disease incidence, percentage inhibition of virus, virus quantification, plant growth and yield parameters, and internal SA accumulation, under pot conditions. Minimum disease incidence and maximum percentage inhibition were recorded when plants were sprayed with 100 ppm salicylic acid 24 h before challenge inoculation. SA 100 ppm foliar spray not only delayed the virus symptoms but also reduced the virus concentration with enhanced growth and yield. Exogenous SA increased the internal SA accumulation, a key factor for SAR.

Keywords: Pepper, systemic acquired resistance, induced resistance, *Capsicum annuum, Chilli Veinal Mottle Virus*, salicylic acid

INTRODUCTION

Both hot and sweet peppers (*Capsicum annuum* L.) are of commercial importance worldwide. Known for their traditional medicinal properties, they are used as fresh vegetables, dried spices, food colorants, and flavorants (Kenyon *et al.* 2014). After tomatoes, they are the world's second most wholesome consumed vegetable (Nkansah *et al.* 2017). Despite the economic and rich nutritional value of *Capsicum* spp., its production is severely hampered by various pathogens, which not only reduces yield and fruit quality but also increases the production of clean planting materials (Arogundade *et al.* 2020). Amongst these, Potyvirus is a large genus (Family: *Potyviridae*) comprising several important species that are the most prevalent viral pathogens causing economically devastating diseases in diverse tropical and subtropical fruits and vegetables including peppers (Kenyon *et al.* 2014). At least, eleven species of potyvirus are predominant viruses that affect peppers globally, among which *Chilli Veinal Mottle Virus* (ChiVMV) is the most prevalent virus infecting peppers in Asia (Tsai *et al.* 2008). First reported by Brunt *et al.* (1996), ChiVMV is a linear, positive-sense ssRNA of around 10 kb with a VPg structure at its 5' end and a poly (A) tract at its 3' end. RNA is encapsulated by a single type of coat protein and the open reading frame encodes a large polyprotein, co-translated into ten functional proteins (Banerjee *et al.* 2016). The virus particles are flexuous filaments of around 680 - 900 nm long which are transmitted mechanically and by several aphid species in a non-persistent manner. ChiVMV characteristic symptoms include dark green vein banding, mettling leaf, small and distorted leaves, and stunted growth (Anindya *et al.* 2004) causing more than 50% yield loss in quality and quantity (Riaz *et al.* 2021).

Current management prospects for ChiVMV infection in peppers are by the integration of several methods like protected nurseries, removal of solanaceous weeds, application of insecticides to control the aphids, early identification and timely disposal of infected plants, use of resistant varieties (Kalimuthu *et al.* 2022) But the conventional phytosanitary practices are inefficient as ChiVMV are rapidly spread by several aphids and have a broad host range. Enhancing plant resistance using elicitors, is an alternative, cheaper, and more fruitful approach to combat plant diseases (Yu *et al.* 2022). Plants have evolved several complex mechanisms to fight pathogen attacks by inducing various defense

responses. One of the conserved mechanisms is systemic acquired resistance (SAR) involving various defense-related genes. Succeeding the primary infection, the plant becomes resistant to subsequent pathogen attacks. This phenomenon, called SAR, has attracted scientific attention for more than 60 years (Zhu *et al.* 2014). First depicted by Ray and Beauverie independently (1901), SAR is a "whole-plant resistance". It is also called "Broad-spectrum resistance", as it confers a long-lasting guard against a wide range of pathogens (Conrath 2006).

Elicitors like DL-β-aminobutyric acid (BABA), 2, 6 dichloroisonicotinic acid (INA), N-cyanomethyl-2-chloroisonicotinamide (NCI), azelaic acid, benzothiadiazole (BTH), jasmonic acid, salicylic acid mimic microbial attack and results in physiological changes in plants inducing SAR. (Faoro and Gozzo 2015). The objective of this study is to evaluate plant defense response using salicylic acid (SA) as a foliar spray against ChiVMV infection in sweet pepper.

MATERIALS AND METHODS

All the experiments were carried out at the Division of Crop Protection, ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, India during 2019-21. All the chemicals used for the experiments were purchased from Sigma-Aldrich, USA molecular biology grade.

Source of the virus, sap inoculation, and culture maintenance

ChiVMV symptomatic leaf samples were collected from the infected *Capsicum* plants around IIHR and were used as a source of inoculum for mechanical sap inoculations. *Datura stramonium* was used as a primary and propagation host. Simultaneously, a month-old *C. annuum* (Susceptible Cultivar: Arka Mohini) seedlings were mechanically sap inoculated. For inoculum preparation, 1g of leaf sample was pulverized in 0.1 M phosphate buffer (pH 7.0) with 0.01 M mercaptoethanol in a pre-chilled mortar and pestle (W/V). Carborundum powder, abrasive was dusted on the leaf surface pre-inoculation and sap inoculation was made by gently rubbing the prepared inoculum on the leaf surface followed by a water wash. Plants were maintained in an insect-proof greenhouse at a temperature range of 28-30 ^{o}C and relative humidity of 60-80%.

Confirmation of Virus

On the expression of symptoms, leaf samples were collected and were tested for the presence of the virus by DAC-ELISA using ChiVMV polyclonal antibodies (DSMZ, Germany). ELISA positive samples were further leaf dip prepared on the grid and examined under HT7700 transmission electron microscope (TEM) for confirmation of virus particles. RNA was extracted using TRI Reagent according to the manufacturer's instructions. Extracted RNA was reverse transcribed and the cDNA copies were amplified by RT-PCR followed by PCR using ChiVMV-specific primers (forward primer: ChVMF1133 5'-CACGCTGGAATGAACACCATG -3'; reverse primer: ChVMR2480 5'-CAGATGGGCGATAAACTGATCTC) (PCR conditions: Initial denaturation at 94 °C for 4 min followed by 35 cycles of 45 Sec denaturation at 94 °C, 1 min annealing at 56 °C and extension for 90 sec at 72 °C and a final extension of 20 min at 72 °C). Total RNA extracted from a healthy plant was used as a negative control. Amplified DNA fragments were subjected to gel electrophoresis on 1% agarose gel and visualized under a UV transilluminator. Eluted products were sent for sequencing at the sequencing facility of Medauxin, Bengaluru. Database search for ChiVMV sequences was carried out by the NCBI-BLAST program for confirmation of virus isolate.

Distribution of the treatments and experimental Design

Pepper seedlings were raised in portrays initially and were transplanted to pots after a month under controlled conditions. Salicylic acid (SA-mol.wt.: 132) in different concentrations (50, 100, 150 ppm) was used for foliar treatment, and water was used as a control (Table 1). After 24 hours of spray, the plants were challenge inoculated with ChiVMV (50 plants per treatment). Leaf samples were collected at 1, 3, 5, and 15 days post-inoculation (DPI) for further analysis.

Table 1. **Details of experimental treatments**

	Treatments
C	Healthy negative control
I	Infected positive control
SA100	100 ppm SA sprayed/uninoculated
SAC50	50 ppm SA sprayed/Inoculated
SAC100	100 ppm SA sprayed/Inoculated
SAC150	150 ppm SA sprayed/Inoculated

Disease incidence (DI)

DI, the percentage of plants that developed symptoms in each treatment compared to control, was scored visually at 15, 30, and 45 DPI.

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total plant population}} \times 100$$

Percentage disease inhibition (PDI)

The effect of SA on the plants was quantified based on the symptom expression after 30 days post-inoculation. The percent disease inhibition over control was calculated by using the formula given by Vincent (1947).

$$PDI\ (\%) = (C - T) \times 100 / C$$

Where, C = Percent disease in untreated control, T = Percent disease in treatment

Quantification of virus infection

Leaf samples were collected at 1, 3, 5, and 15 DPI. Virus infection in the treated and control plants was quantified by DAC-ELISA (Hobbs *et al.* 1987) using ChiVMV specific antibodies (DSMZ, Germany) at 405 nm using VERSA max microplate reader (Molecular Devices). The amount of virus infection was calculated from the calibration curve using the mean absorbance values of the respective sample (Khedhair 2016).

Plant growth and yield parameters

Plant growth and yield parameters like plant height (cm), number of fruits per plant, individual fruit weight (g), and fresh fruit yield (g/plant) were recorded after 90 days from 10 plants per treatment.

Quantification of salicylic acid (SA) by LCMS

SA was extracted according to the procedure described by Pan *et al.* (2008). 1g leaf sample was homogenized in hormone buffer containing 1-propanol/ H_2O/concentrated HCl (2:1:0.002, v/v/v), sonicated for 30 min, and incubated overnight at 4 °C. An equal volume of dichloromethane was added to the homogenate, sonicated for 30 min, and then centrifuged at 12,000 rpm for 10 minutes. Water traces from the bottom layer was removed with the help of sodium sulphate and evaporated using a flash evaporator. After completely dried, the sample was dissolved in 1m L of methanol-0.05% formic acid (1:1, v/v). The solution was filtered using a nylon filter paper and injected into LCMS (Waters Acquity UPLC H class coupled with TQD MS/MS) for further analysis.

Statistical Analysis

Values presented are of at least three independent replicates. The significance of differences was determined using analysis of variance (ANOVA), one way ANOVA (Holm-Sidak's multiple comparisons test) for PDI, and two-way ANOVA (Tukey's multiple comparisons test) for DI, ELISA, and growth and yield parameters, using GraphPad V8.0.1 for Windows 10.

RESULTS

Culture maintenance and virus confirmation

Virus culture was successfully sap inoculated and maintained on *D. stramonium* and *C. annuum* L. (Fig. 1). Inoculated plants showed characteristic ChiVMV symptoms of dark green vein banding, mosaic, mottling, and distorted leaves. Infected samples exhibited a strong positive reaction to ChiVMV-specific antisera in the DAC-ELISA test. O.D. values of infected samples were 3 times higher than that of healthy and buffer control samples. Leaf dip preparation of virus-infected leaf extract observed under TEM showed the presence of flexuous filaments of around 650 nm in size (Fig. 2). RT-PCR/ PCR resulted in amplification of 1.2 kb DNA fragment from infected plants, but not healthy control plants (Fig. 3). Cloned and sequenced PCR products were subjected to a Blast search which showed nucleotide identity with ChiVMV – Bangalore isolate.

Fig. 1. Mechanical sap inoculation
Pepper plant showing characteristic ChiVMV symptoms of dark green vein banding, mosaic, mottling and distorted leaves.

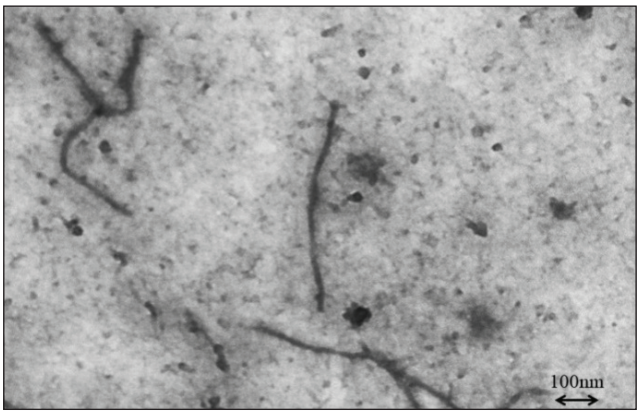

Fig. 2. Virus confirmation on TEM
TEM image depicting flexuous filaments of around 650 nm in size.

Disease incidence (DI)

Pre-treatment of salicylic acid foliar spray on *C. annuum* L. delayed and reduced the symptom severity of ChiVMV. The progress of the disease in the untreated/ challenge inoculated control plants were rapid and the symptoms appeared around 15 DPI with 95.6% mean disease incidence. But 50 ppm (SAC50) foliar spray delayed the symptoms by 3-5 days (18- 20 DPI) whereas 100 ppm (SAC100) and 150 ppm (SAC150) delayed it by around 8 to 10 days (23-25 DPI) **(Fig. 4)**. In 50 ppm SA sprayed plants the mean disease incidence was around 38.3%. 100 ppm and 150 ppm SA further decreased the disease incidence to around 25.3% and 26% **(Fig.5).**

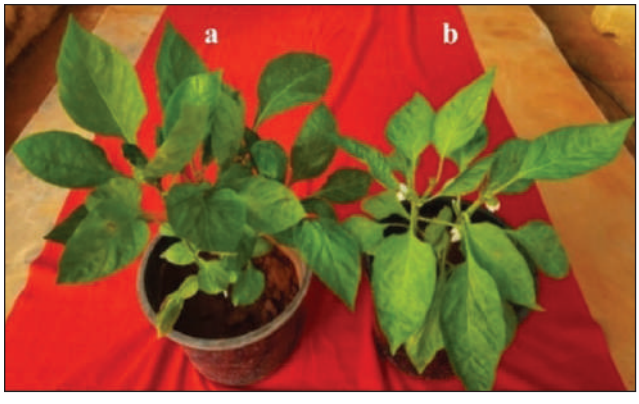

Fig. 4. Delayed and reduced ChiVMV symptoms on pepper plants (a) 100ppm SA sprayed ChiVMV challenge inoculated pepper plant with delayed/ reduced symptoms and enhanced growth when compared to (b) ChiVMV challenge inoculated pepper plant showing symptoms.

Percentage disease inhibition (PDI)

In 50 ppm salicylic acid-treated and challenge inoculated plants the disease was inhibited by 58.50 % after 30 DPI. Whereas, 100 ppm and 150 ppm SA sprayed/ challenge inoculated plants showed 72.79% and 72.11 % disease inhibition respectively **(Fig. 6)**. Also, the symptoms were restricted and did not spread to other leaves.

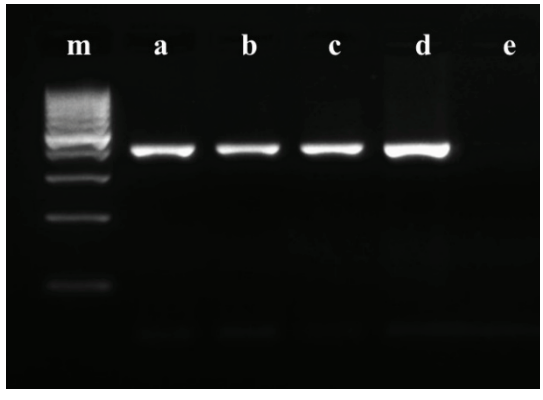

Fig 3. PCR confirmation of the virus (m) Lambda DNA/ECOR1+Hind III Marker; (a-d) 1.2 kb amplified fragment from symptomatic pepper samples specific to ChiVMV specific primer, (e) Healthy pepper leaves (Negative control);

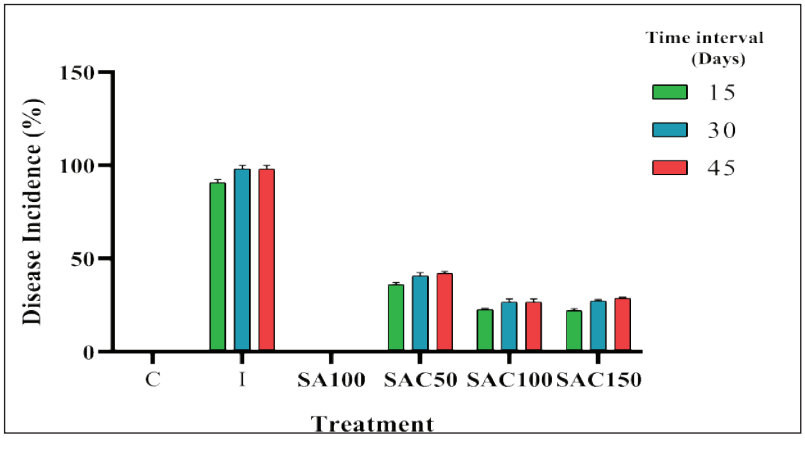

Fig. 5. Disease incidence at 15, 30, 45 DPI. The x-axis indicates different treatments. Vertical bars refer to mean ± SEm (n = 3), p-value <0.0001.

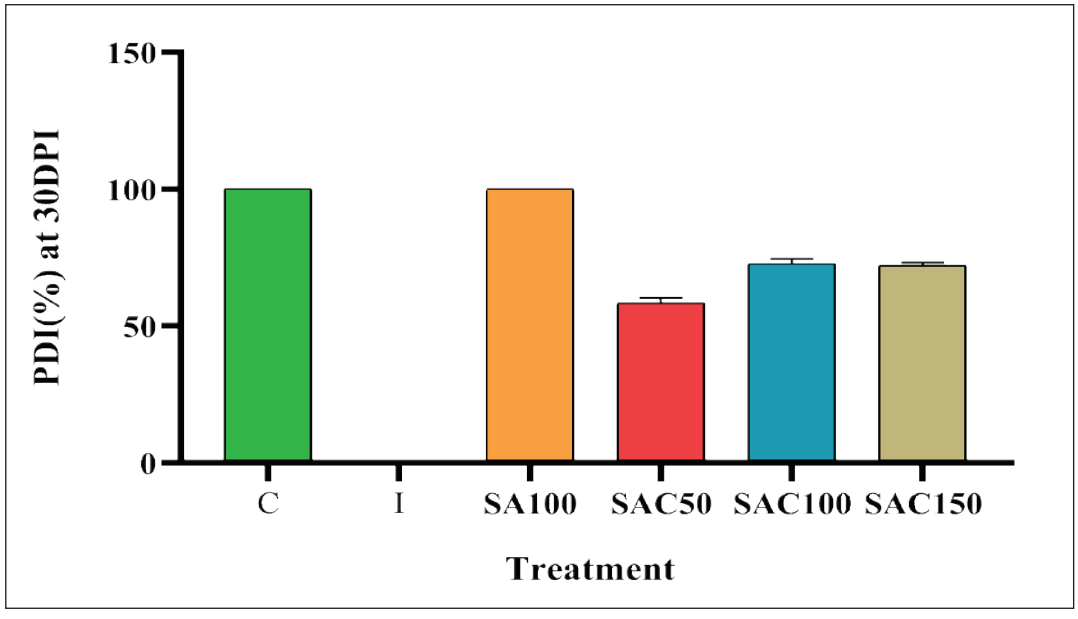

Fig 6. Percentage disease inhibition at 30 DPI. The x-axis indicates different treatments. Vertical bars refer to mean ± SEm (n = 3), p-value <0.0001.

Quantification of virus infection

The DAC-ELISA values of SA treated pepper seedlings showed a significant reduction in the viral concentration when compared with positive control plants. SAC100 significantly reduced the viral concentration over the period when compared to SAC50. There was no significant difference between SAC100 and SAC150. Healthy and SA100 seedlings contained no detectable ChiVMV **(Fig. 7)**.

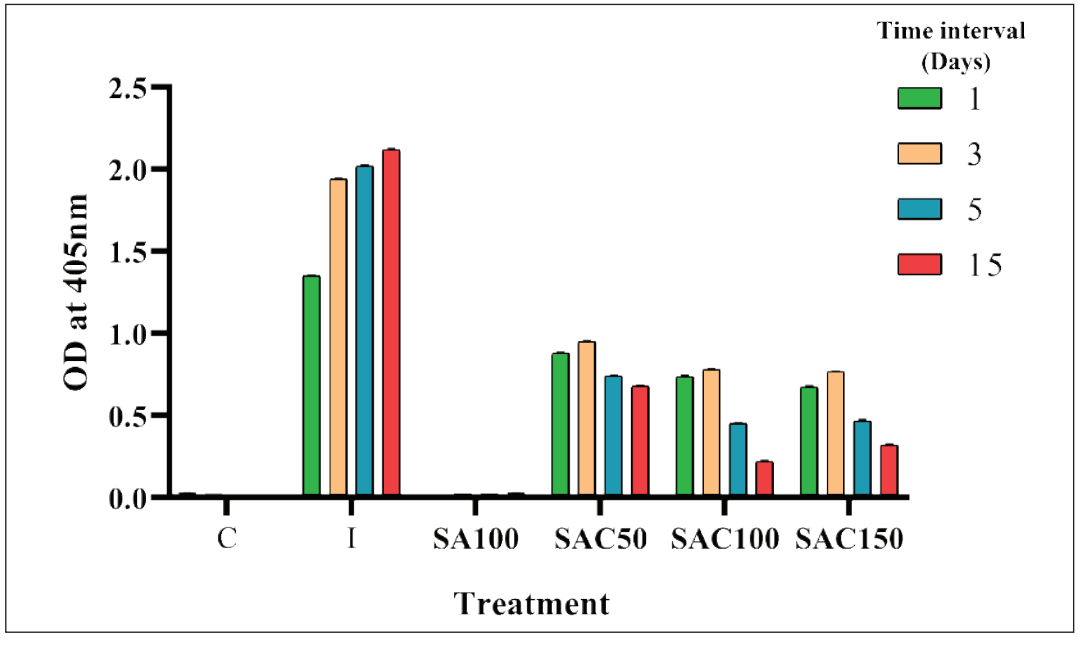

Fig 7. Quantification of virus infection. SA treated pepper plants showing significant reduction in the viral concentration when compared with positive control plants (I). The x-axis indicates different treatments. Vertical bars refer to mean ± SEm (n = 3), p-value <0.0001.

Plant growth, and yield parameters

Salicylic acid 100 ppm foliar spray on healthy plants increased the plant height when compared with untreated **(Fig. 8)**. The infected plants had characteristic stunted growth. There was a significant increase in the plant height treated with salicylic acid/ challenge inoculated when compared to healthy control. But there was no significant difference between the different concentrations. The number of fruits per plant increased with SAC100 and SA100 when compared with healthy control. There was a significant difference between SAC50 and SAC100/ SAC150, but not between SAC100 and SAC150. The same was with fruit length and width. Fruit weight in SAC100 and SAC150 increased three times when compared to infected plants (I). SA100 enhanced the fruit weight even compared to healthy control. There was a significant yield enhancement in SAC100 when compared not only with infected, but with healthy control too (Table 2).

Fig. 8. Plant growth (a) 100ppm SA sprayed ChiVMV challenge inoculated pepper plant with increased plant height when compared to (b) ChiVMV challenge inoculated pepper plant.

Table 2. Effect of foliar spray of SA on growth and yield parameters of pepper plants at 90 days after transplanting, p-value <0.05

Treatment	Plant height (cm)	No. of fruits/ plant	Fruit length (cm)	Fruit width (cm)	Avg. fruit weight (g)	Yield/plant (kg)
C	71.1	4.7	6.8	4.9	106	0.59
I	32.2	2.1	1.8	1.6	42	0.23
SA100	76.3	5.6	7.8	5.5	118	0.73
SAC50	77.1	3.1	4.8	3.4	72	0.32
SAC100	76.7	4.9	7.6	5.2	116	0.62
SAC150	76.6	4.8	7.7	5.2	115	0.61
SEm±	7.28	0.54	0.97	0.62	12.69	0.08
CD at 5%	20.71	1.53	2.76	1.76	36.06	0.23

Quantification of total salicylic acid (SA)

Salicylic acid accumulation increased internally in the plants treated with exogenous SA when compared to the infected plants. SAC100 proved to be more significant than SAC50. Though there was no significant difference between SAC100 and SAC150, SAC100 treated plants showed a slightly higher concentration of internal SA accumulation. SA accumulation increased from day 1 to day 3 and gradually decreased. In all the treatments SA accumulation reached the maximum on day 3 and gradually decreased. Control plants showed a negligible amount of internal SA accumulation **(Fig 9).**

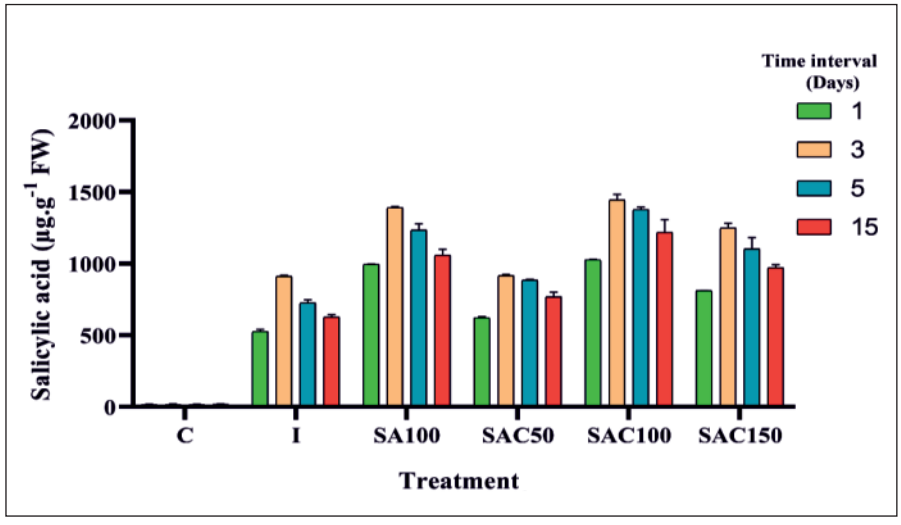

Fig 9. Quantification of total salicylic acid (SA) Effects of exogenous SA application on internal SA accumulation in pepper plants. The x-axis indicates the different treatments. Vertical bars refer to mean ± SEm (n = 3), p-value <0.001.

DISCUSSION

Boundless economic losses occur due to virus diseases worldwide. Plants fight against them in assorted tactics. Treatment of plants with biotic or abiotic agents can stimulate the immune system and develops resistance which may further restrict the pathogen growth and decrease the disease severity. Inducing systemic acquired resistance in the plants against the viruses is a superlative approach to managing the viruses (Zhang *et al.* 2011). Foliar spray increases growth and yield both quantitatively and qualitatively. It also increases soil fertility, nutrient uptake, and nitrogen fixation. Several compounds are used for foliar fertilization amongst which salicylic acid is advantageous for plants in multiple ways.

Salicylic acid, a natural growth regulator is said to influence several physiological and metabolic processes. Exogenous application of SA is known to enhance plant growth and yield (Ibrahim *et al.* 2019). 150 ppm SA spray enhanced mungbean growth with the highest seed yield/ha (Ali and Mahmoud 2013). 75 mg/ L SA spray enhanced the plant growth and yield in a few chili cultivars grown in arid regions (Nafees *et al.* 2019). 1.5 g /L SA increased the fruit weight and yield by 15.9 to 27.7% in various sweet pepper cultivars (Ibrahim *et al.*, 2019).

The salicylic acid foliar spray may increase the yield by reducing stress-induced growth control. Foliar spray of SA is effective against various pests and fungi with increased plant growth and yield (Thakur *et al.* 2014; Dixit *et al.* 2018; Yousif 2018; Bakr *et al.* 2020). SA induced resistance in resistant and susceptible tomato cultivars against the *Tomato yellow leaf curl virus* (Li *et al.* 2019). In resistant and susceptible *Vigna mungo*, SA induced resistance against *Mungbean Yellow Mosaic Virus* with reduced disease incidence and increased seed yield (Sahni and Prasad 2021).

Our data showed that foliar application of salicylic acid on *C. annuum,* 24 hours before inoculation reduced the disease severity and enhanced the growth and yield. The highest percentage inhibition of 72.9% was with 100 ppm SA. SA spray also delayed the onset of symptoms on the pepper plants. SA foliar spray delayed the onset of CMV infection on squash plants due to the inhibition of cell-to-cell movement of the virus (Mayers *et al.* 2005). 100 ppm SA reduced the virus concentration as well. There was a significant difference between 50 ppm and 100 ppm, but no significant difference was observed in the tests between 100 ppm and 150 ppm. Though there was not much difference between the different concentrations concerning plant height, considering other growth and yield parameters 100 ppm was considered the desired concentration.

Salicylic acid, a defense-related hormone, accumulates internally and induces SAR, which is correlated with the expression of SAR genes, including pathogenesis-related (PR) proteins and defense-related genes. These PR proteins and internal SA accumulation play an important role in resistance responses (Zhang *et al.* 2011). Our data showed a significant increase in internal SA accumulation when treated with exogenous SA 100 ppm, thereby enhancing the plant host resistance to ChiVMV in pepper plants.

To conclude, 100 ppm salicylic acid foliar spray was effective against *Chilli Veinal Mottle Virus* management in *C. annuum* by inducing systemic acquired resistance. Exogenous application of SA delayed and also reduced the symptom severity. Also, SA 100 ppm enhanced the plant growth and yield under controlled conditions. There was a significant increase in the internal SA accumulation, a key activator of the SAR pathway which will provide long-term resistance against the pathogens.

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Molecular characterization of *Peanut Bud Necrosis Virus* infecting tomato in Tamil Nadu

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ABSTRACT: Tomato bud blight caused by *Peanut bud necrosis virus* (PBNV) is an economically important disease affecting tomato causing great yield loss to the farming community. In the present study, the PBNV virus isolate, (GBNV CBE T02) was purified from the infected plant through mechanical transmission on cowpea cv. CO7, which produced chlorotic and necrotic local lesions on 4th days post inoculation. The pathogenicity of the virus on tomato cv. PKM1 was also proven via sap transmission. Transmission electron microscopy of infected tomato samples revealed spherical virus particle with diameter of 58 to 82 nm at 80000 X magnification. Amplification of the various genomic components of PBNV in the infected leaf samples through RT-PCR using gene specific primers produced an amplicon of 800 bp, 903 bp, 613 bp, 831 bp and 790 bp for RdRp, NSm, Gn/Gc, NP and NSs respectively. Sequence analysis of movement protein (NSm) (Gen Bank Acc. No. ON 529555) and Non-structural small protein (NSs) (Gen Bank Acc. No. ON529556) revealed that the isolate shared 98.98 % and 98.04 % identity respectively with other isolates in the GenBank database.

Keywords: Tomato, PBNV, TEM, RT-PCR, Sequence, Phylogeny

INTRODUCTION

Tomato (*Solanum lycopersicum* L., Family-*Solanaceae*), is one of the most important and popular vegetable crops grown throughout the world. It is rich in vitamins A & C, minerals, organic acids and is the richest source of the dietary antioxidant lycopene. Edible portion (100 g) of tomato contains 3.9 % carbohydrates, 0.9 % proteins, 2.6 % sugar, 1.2 % fibre, 0.2 % fat, 18.9 mg vitamin C, and 12.58 mg lycopene (Hanif *et al.,* 2006). In India, tomato is being cultivated in an area of 851.69 thousand ha with a production of 21002.81 thousand tonnes and productivity of 24.66 MT/ha. In Tamil Nadu, tomato is cultivated in an area of 82.98 thousand ha with the production of 2370.58 thousand tonnes and productivity of 28.57 MT/ ha (Indiastat, 2021). Biotic and abiotic stresses are the major limiting factors in tomato cultivation throughout the world (Abu Qamar *et al.,* 2013). Tomato is affected by various diseases, among which *Tospoviruses* are a major constraint creating huge economic loss in commercial cultivation (Scholthof *et al.,* 2011). More than 20 *Tospoviruses* have been reported globally (Zhu *et al.,* 2019), among which *Peanut bud necrosis virus* (syn. *Groundnut bud necrosis orthotospovirus,* GBNV) infecting tomato cause severe destruction to the crop (Mandal *et al.*, 2012).

Peanut bud necrosis virus belongs to the genus *Orthotospovirus* (Maes *et al.,* 2018), family *Tospoviridae* and order *Bunyavirales*, has tripartite ss RNA in their genome (Pappu *et al.,* 2009). They are transmitted by thrips in a circulative propagative manner (Gopal *et al.,* 2011). PBNV is characterized with enveloped isometric virus particle of diameter 80-120 nm (Radhakrishnan *et al.,* 2016). The genome includes negative sense large RNA (L-8.9 kb) encoding for virus replicase protein (RNA dependent RNA polymerase- RdRp) of 337 kDa (Gowda *et al.,* 1998); ambisense medium RNA (MP-34kDA kb) encoding movement protein (NSm or MP) of 127 kDa in sense orientation and glycoproteins (Gn/Gc) of 127 kDa in antisense orientation (Saritha and Jain, 2007) ; ambisense small RNA (S-3.05 kb) encoding non-structural small protein (NSs) of 49.5 kDa in sense orientation and nucleocapsid protein (N or CP) of 30.6 kDa in antisense orientation (Satyanarayana *et al.,* 1996).

PBNV infected tomato, show chlorotic and necrotic spots on young leaf, stem, petioles followed by drying of young bud (Umamaheshwaran *et al.,* 2003). Early infection in plants results in yellowing of leaves, stunted growth, and death. The infected plants set fruits with chlorotic concentric rings and reduced size. More than 80 percent yield loss has been reported due to PBNV in India (Dasgupta *et al.,* 2003). Now-a-days PBNV infection has become a serious disease in Tamil Nadu, Karnataka and Andhra Pradesh (Mandal *et al.,* 2017). The disease eventually causes death of the plant which

Table 1. Primers sequence used for detection of PBNV

RNA Segment	Gene	Sequence	Amplicon size
L RNA	RdRp	F: 5' CCTTTAACAGTDGAAACAT 3'	800 bp
		R: 5' CATDGCRCAAGARTGRTARACAGA 3'	
M RNA	NSm	F: 5' ATGTCTCGCTTDTCTAAHGTB 3'	903 bp
		R: 5' TTATATTTCAAGAAGATTATC 3'	
M RNA	Gn/Gc	F: 5' TGGATTAAAGTCAGTTTGCGCC 3'	613 bp
		R: 5' CTTTCTGTAAGGTTGTCTCGTG 3'	
S RNA	NSs	F: 5'CTAGCTAGCCATATGTCAACTGCAAAGAATGC 3'	790 bp
		R:5' CCCTCGAGGGTTATTCTGCTTTCACAATGAAGTG3'	
S RNA	N	F: 5' ATGTCTAACGT(C/T)AAGCA(A/G)CTC 3'	831 bp
		R: 5' TTACAATTCCAGCGAAGGACC 3'	

later affects the market value of tomato (Kunkalikar *et al.,* 2011). Accurate identification of this virus disease in the early stage of the crop is highly essential which will help to overcome losses in the later stage. Virus recombination occurs frequently under field conditions and hence sequencing of the various genomic components is highly essential. Based on this, in the present study, PBNV infecting tomato at Coimbatore district of Tamil Nadu was characterized through molecular methods.

MATERIALS AND METHODS

Survey and sample collection

A field survey was conducted in different locations of Coimbatore district, Tamil Nadu during 2022 to document the spotted wilt symptoms in tomato. At each stage, totally 60 plants were observed in each field and the percent disease incidence was calculated using

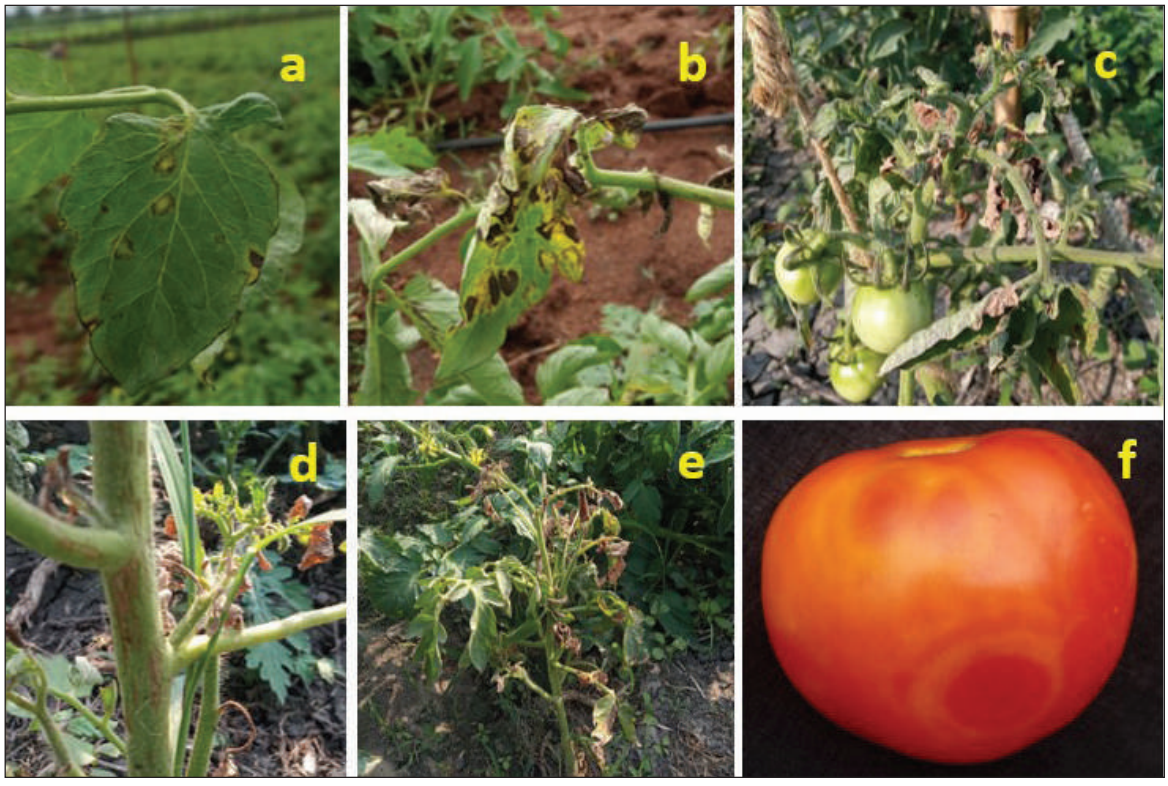

Fig. 1. Symptoms of PBNV in tomato observed in the field: a. Chlorotic ring spots on tomato leaves, b. Necrotic ring spots on tomato leaves which eventually coalesce leading to drying of leaves, c. Bud necrosis symptom, d. Stem necrosis, e. Stunting and wilting of young plant, f. Chlorotic ring spots on tomato fruit

Table 2. Tomato Spotted wilt incidence in Coimbatore district, Tamil Nadu

Location	Latitude	Longitude	Bud blight incidence (%)
Devarayapuram	10.7798° N,	77.0068° E	18.33
Madampetti	10.9698° N	76.8598° E	26.67
Narasipuram	10.9880° N	76.7740° E	8.33
Thenkarai	10.9366° N	76.8433° E	33.33
Thondamuthur	10.9899° N	76.8409° E	30.00
TNAU Orchard	11.0069° N	76.9309° E	11.67
Viraliyur	10.9978° N	76.7843° E	36.67

the *formula (Reddy *et al.,* 2008). The tomato plants exhibiting symptoms of PBNV *viz*., bronzing of leaves, chlorotic and necrotic lesions in the stem were collected from the field. About four to five symptomatic leaves were collected per plant. The samples were stored at -80°C and used for further studies.

$$\text{Percent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total no. of plants observed}} \times 100$$

Sap inoculation and pathogenicity test

The infected samples collected from the field were maintained in cowpea plants (*Vigna ungiculata* cv. CO7) through mechanical inoculation since cowpea leaves produce characteristic local lesion symptoms within 3-4 days after inoculation (Manjunatha *et al.,* 2010). The seeds of cowpea cv. CO7 were raised in PL480 glasshouse, Department of Plant Pathology, TNAU, Coimbatore under insect-proof condition. The sap transmission of GBNV was carried out by extraction of crude sap from infected tomato leaves using 0.01 M Sodium phosphate buffer (pH- 7.2) with 0.1% ß- merca ptoethanol in pre-chilled pestle and mortar. Seven days old cowpea plants were pre-dusted with carborundum powder (600 mesh) and the sap was gently rubbed on the leaves. The inoculated leaves were washed with distilled water and kept for observation. Similarly, the virus was inoculated in 25 days old tomato plants (*Solanum lycopercicum* cv. PKM1) for proving the pathogenicity of the virus.

Characterization of PBNV through Transmission Electron Microscopy (TEM)

TEM of the viral particle was obtained with the partially purified GBNV samples ground with phosphate buffer amended with 2 % polyvinylpyrroli done and 0.2 % sodium sulfite (Zechmann and Zellnig, 2009). Carbon coated copper grid was subjected to positive staining with a few drops of 0.05 M of potassium phosphate buffer for 10 min followed by dipping with partially purified samples for 15 min, allowing binding of the virus. Then the sample coated grid was subjected to negative staining with 2 % uranyl acetate for 5 min. After negative staining, the grid was washed three times with sterile distilled water and the copper grid was dried in desiccator to remove excess moisture. The sample coated copper grid was visualized under transmission electron microscope (Tecnai Sprit G2, FEI, Netherlands) at the Department of Nano Science and Technology, TNAU, Coimbatore.

RNA isolation

The total RNA was extracted from cowpea plants exhibiting local lesions of GBNV using the Trizol method (Chomczynski and Sacchi, 1987). Leaf samples (100 mg) showing symptoms of PBNV along with healthy samples was homogenized using liquid nitrogen. One

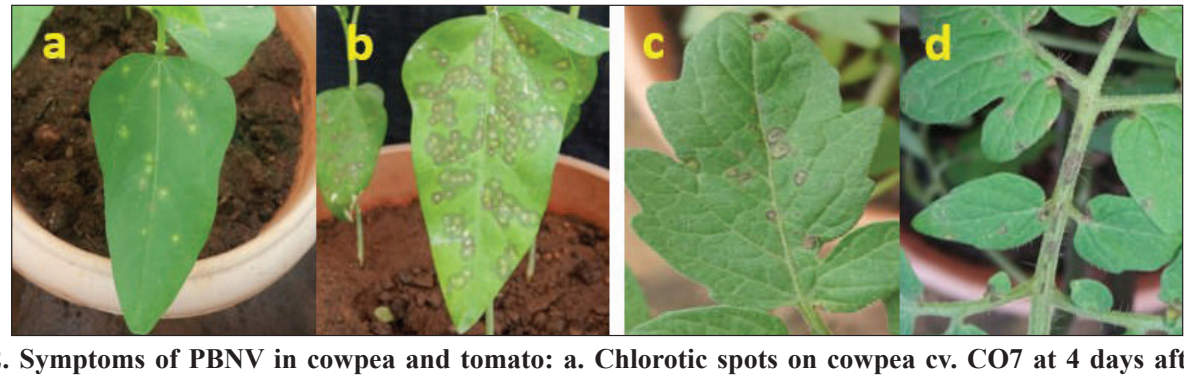

Fig. 2. Symptoms of PBNV in cowpea and tomato: a. Chlorotic spots on cowpea cv. CO7 at 4 days after sap inoculation with PBNV, b. Necrotic spots on cowpea at 8 days after virus inoculation, c. Chlorotic spots on tomato var. PKM 1 at 6 days after sap inoculation with PBNV, d. Necrotic streaks on stem of tomato plants 21 days after virus inoculation.

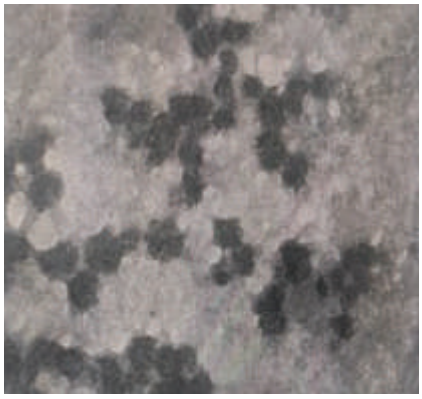

Fig. 3.Transmission electron microscopy (TEM) of virus: Spherical virus particle with diameter 58 to 82 nm observed in infected tomato samples at 80000X nm magnification

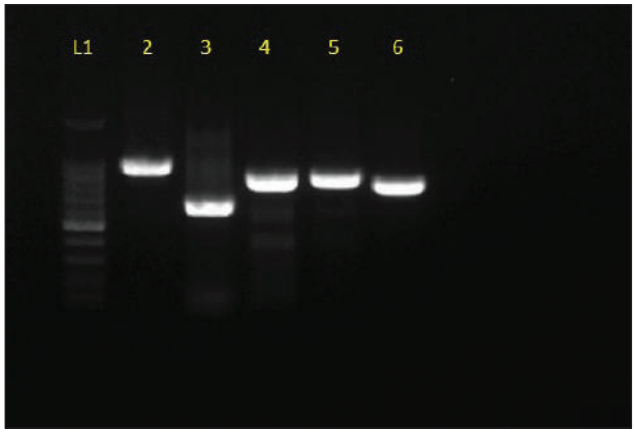

Fig. 4. Agarose gel electrophoresis of RT-PCR analysis with gene specificprimers. L1- 100bp ladder, 2- Nsm (903bp), 3- Gn/Gc (613bp), 4- RdRp (800bp), 5- N (831bp), 6- NSs (790bp)

ml of Trizol reagent was added, transferred to a 1.5ml centrifuge tube and incubated under room temperature for 2 min and then centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was transferred to a new 1.5ml centrifuge tube, and 250 µl of chloroform was added, mixed well and centrifuged at 12000 rpm for 15 min. The aqueous layer was transferred to another new tube and 250 µl of ice cold isopropanol, 250 µl 5M NaCl were added. Then it was incubated over the ice for 15 min and then centrifuged at 12000 rpm for 15 min. The supernatant was discarded and the pellets retained in the tube were washed with 70 % ethanol. Excess ethanol was removed and the pellet was dissolved in 30 µl RNA ase free water.

Reverse transcription

RNA was quantified using Nanodrop and the quality of RNA was assessed by A260/A280 ratio. First strand cDNA synthesis was carried out using first strand cDNA synthesis kit (Thermo scientific RevertAid first strand cDNA synthesis kit, USA). The reaction mixture contains Reaction buffer - 4 µl, dNTPs - 2 µl, random primer – 1 µl, reverse transcriptase - 1 µl, RNase inhibitor - 1 µl and total RNA- 3 µl (1800 ng) which was made up to 20 µl with DEPC treated water. The contents were incubated at 45 ºC for 60 min followed by 70 ºC for 5 min. The resultant cDNA was used to amplify the various genomic components of PBNV.

Amplification of genomic components of PBNV through RT-PCR

Amplification of the genomic components of PBNV was carried out through Reverse transcription- polymerase chain reaction (RT-PCR) using gene specific primers (Balol and Patil, 2016; Saritha and Jain, 2007. Table 1). The PCR reaction contains Master mix- 20 µl, 4 µl each of forward and reverse primers (5 µM), nuclease free water- 8 µl and cDNA- 4 µl. The PCR was carried out in a thermal cycler (C1000 Biorad Ltd.) using the following PCR conditions : initial denaturation of 94°C for 5 min, 35 cycles of denaturation for 94°C for 1 min, annealing for 50°C for 1 min, extension for 72°C for 1 min and a final extension of 72°C for 10 min. The RT-PCR product was analysed on 1.2% agarose gel, stained with ethidium bromide and viewed under gel documentation unit.

Sequence analysis

Partial sequencing of the amplified products of PBNV was done in Biokart Pvt. Ltd., Bangalore, India, analysed and submitted in the NCBI Genbank database and accession numbers were obtained. Nucleotide sequences were aligned using CLUSTAL X 1.81 and phylogenetic analysis of the sequence was carried out by comparing the reference sequence retrieved from the Genbank database with 1,000 bootstrap replicates using MEGA 11 software (Tamura *et al.*, 2021).

RESULTS AND DISCUSSIONS

Survey and sample collection

A survey was carried out in tomato fields in the Coimbatore district, Tamil Nadu during 2021and the results are presented in Table 2. The disease incidence ranged from 8.33 % (Narasipuram village) to a maximum of 36.67 % (Viraliyur village) (Table 2). The disease occurred in all the stages of the crop from young stage to flowering stage. The survey implies the natural distribution and symptomatology of PBNV in tomato. PBNV initially produced chlorotic ring spots (Figure 1a) which later turned to necrotic ring spots (Figure 1b) on the leaves. Severe infection on young shoots lead to bud

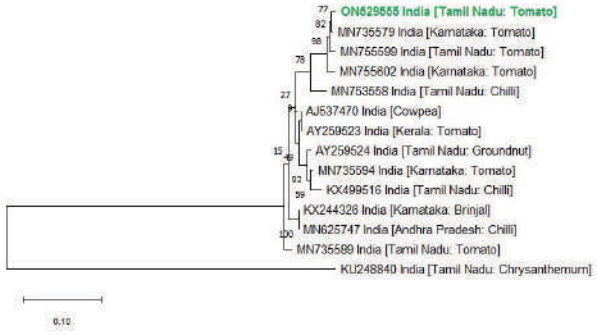

Fig. 5. Phylogenetic relationship of GBNV isolate (ON529555) from Tamil Nadu with other GBNV isolates reported based on movement protein gene. Phylogenetic tree generated using MEGA 11 software using maximum likelihood tree with 1000 replicates and TSWV (KU248840) isolate used as out group.

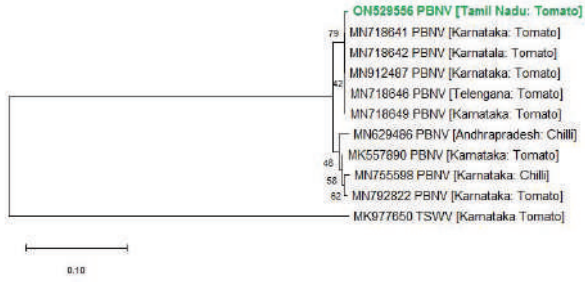

Fig. 6. Phylogenetic relationship of GBNV isolate (ON529556) from Tamil Nadu with other GBNV isolates reported based on Non structural small protein gene. Phylogenetic tree generated using MEGA 11 software using maximum likelihood tree method with 1000 replicates

blight necrosis (Figure 1c). On stem and petioles, PBNV caused necrotic streaks (Figure 1d). PBNV infection on early crop stage caused wilting and stunting of the whole plant (Figure 1e). On the fruit, it caused chlorotic ring symptom (Figure 1f). In Tamil Nadu, the prevalence of PBNV on Tomato was reported with the symptoms of chlorotic and necrotic ringspots on leaves followed by necrotic streaks on stems, petioles and chlorotic rings on infected fruits (Suganyadevi *et al.,* 2018).

Sap inoculation and Pathogenicity test

The virus sample collected from the field was maintained through sap inoculation on cowpea plants. The same was done in tomato plants for proving pathogenicity of PBNV. On cowpea, the virus produced chlorotic spots at 4 days after inoculation (Figure 2a) and necrotic ring spots (Figure 2b) at 8 days after inoculation. On tomato, the virus produced chlorotic spots on leaves (Figure 2c) on 6 days after sap inoculation and necrotic streaks on the stem (Figure 2d) proving the Koch postulates. Vanthana *et al.* (2019) has reported that the PBNV sap inoculated cowpea plants (CO7) exhibited chlorotic ring spots which later turn to necrotic spots within 4-5 dpi and tomato plants inoculated with PBNV has shown necrotic rings on leaves and necrotic streaks on stem similar to field symptoms.

Characterization of virus by TEM

Characterisation of the virus particles through TEM analysis revealed that the virions possess spherical virus particle with diameter 58 to 82 nm in infected tomato samples at 8000 X magnification (Figure 3). Pant *et al.* (2019) alsoreported the presence of tospovirus-like particles measuring 80-110 nm in diameter in PBNV infected samples through TEM analysis.

Molecular detection of PBNV in infected plants

RNA was extracted from the infected plants and RT-PCR was carried out using PBNV gene specific primers. The isolate, GBNV CBET 02 could amplify all the genomic components of PBNV. RT-PCR amplified different genes with a product size of 800 bp, 903 bp, 613 bp, 831 bp, and 790 bp for RdRp, NSm, Gn/Gc, N and NSs gene respectively (Figure 4). Suganyadevi *et al.* (2018) also reported that the replicase gene of PBNV produced an amplicon of 800 bpin all the infected samples. The results was in accordance with Sangeetha *et al.,* (2020) and Rajamanickam *et al.,* (2020), where the coat protein gene of PBNV was amplified with a size of 830 bp. Similarly, Amplified products of NSm and NSs genes were partially sequenced and analyzed. The sequences are available in NCBI database under accession numbers ON529555 and ON529556 respectively. The BLASTn search revealed 98.98 % identity for movement protein and 98.06 % for Non-structural small protein with the available PBNV sequences in the Genbank database.

Phylogenetic analysis

The nucleotide sequence of PBNV NSm gene available under accession number ON529555 was compared with other isolates of PBNV infecting wide range of hosts which revealed three groups (Figure 5). The study isolate was closely related to PBNV isolated from tomato at Karnataka (Accession no: MN35579), tomato at Tamil Nadu (Accession no: MN55599) and tomato at Karnataka (Accession no: MN755602) whereas *tomato spotted wilt virus* tree of partial segment of NSs gene (Accession number: ON529556) is closely related to the isolates MN718641 at Karnataka and MN718642 at Karnataka (Figure 6). The comparison of PBNV nucleotides revealed that PBNV isolates are not divergent among different host throughout India.

CONCLUSION

In this study, PBNV infecting tomato in Western region of Tamil Nadu was characterized. The pathogenicity of the virus isolate was proved through sap inoculation. The virus was purified and the infected virions were characterized through TEM analysis. Besides, amplification of the five genomic components of PBNV was carried out through RT-PCR. Sequencing of the NSm gene and NSs gene showed its close identity with the PBNV isolate from Tamil Nadu and Karnataka thus confirming the presence of the pathogen.

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Cross infectivity of *Colletotrichum* spp. on tropical fruit crops and *Ageratum* spp. (weed host) in Southern Karnataka and Andhra Pradesh

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ABSTRACT: Species of *Colletotrichum* are associated with many host plants of diverse ecosystems including tropical, sub- tropical and temperature climatic conditions. Isolates of *Colletotrichum* were obtained from orchards of cultivated tropical fruit crops in Andhra Pradesh and Southern parts of Karnataka, India.Cross infectivity studies proved the wide host range of different isolates in seven fruit crops and one weed host (*Ageratum* spp.) tested. Most of the isolates displayed high virulence on seven alternative fruit crops and *Ageratum* spp. also. IIHR_COL_C6 isolated from mango recorded high virulence on guava fruits than other isolates. IIHR_COL_C8 displayed unique nature with cross infection *i.e.,* except on the original host isolated from, it showed least infection on all other alternative hosts tested showing its host specificity. Isolates IIHR_COL_C2, IIHR_COL_C3, IIHR_COL_C6, IIHR_COL_C7 and IIHR_COL_C9 recorded high virulence on their original hosts of isolation *viz*., banana, custard apple, mango, papaya and pomegranate respectively indicating the adaptation to main host and less preference of alternative hosts. A weed host *Ageratum* spp.was tested for its susceptibility to *Colletotrichum* isolates and found to be susceptible to all nine *Colletotrichum* isolates in the artificial inoculation. Most virulent isolates were IIHR_COL_C7 and IIHR_COL_C1 isolated from papaya and banana (4.76 cm and 4.68 cm), indicating the ability of *Colletotrichum* spp. in colonizing weeds. Isolates of *Colletotrichum* spp. from tropical fruit crops in this study can cause disease and might survive in off season alternatively on other fruit crops and also on weeds.

Keywords: *Ageratum*, *Colletotrichum*, cross infectivity, fruits, weed

INTRODUCTION

Colletotrichum species cause anthracnose disease in many crops including tropical fruit crops.They have world-wide distribution reported in all climatic conditions. *Colletotrichum* spp. were nominated as the eighth most important plant pathogen in the world on account of its scientific and economic importance (Dean *et al.*, 2012). This disease is considered as the most important fungal disease of *Annonaceous* species due to the losses ranging up to 70-100% during the periods of extended rainfall at the time of flowering, production of fruits (Junqueira and Junqueira, 2014; Kamei *et al.*, 2014).

A single species is potent to infect many host plants, similarly multiple species are potent to infect a single host plant genus (Fuentes-Aragon *et al.*, 2020). Cross infection studies unravel the *Colletotrichum* spp. life cycle like alternative hosts and survival of pathogen required for epidemic development with different species. The implication of identification and their potential to cross infect other fruit crops has direct impact on control measures as demonstrated by the host specificity and aggressiveness by *C. kahawae* to infect even green berries of coffee (Batista *et al.*, 2017) and differential sensitivity of *C. gloeosporioides* and *C. acutatum* to benomyl (Freeman *et al.*, 2000). *C. gloeosporioides* species complex was reported in association with 283 plant species of 212 genera in which the majority share of eudicots (80.6%) (Talhinhas and Baroncelli, 2021). Cross infection potential of isolates of *Colletotrichum* isolated from different tropical fruit crops on other hosts have been reported (Alahakoon *et al.*, 1994; Sanders and Korsten, 2003; Freeman *et al.*, 2000, 2001; Lakshmi *et al.*, 2011; Rampersad, 2011; Cruz *et al.*, 2020, Wu *et al.*, 2020).

In the present study we report the cross-infection potential of *Colletotrichum* isolates isolated from banana, custard apple, grapes, guava, mango, papaya, pomegranate on other fruit crops other than the host from which they had been isolated. Besides, the ability to infect a common weed host Ageratum that helps them to survive in the tropical fruit crop ecosystem also is also reported.

MATERIALS AND METHODS

Collection of anthracnose samples and isolation of *Colletotrichum* species

Anthracnose samples from the infected fruits, leaves, shoots from different fruit crops were collected

systematically based on the visible anthracnose symptoms from Andhra Pradesh and Southern Karnataka by personal visit. The samples were collected in plastic bags and brought to ICAR-IIHR, Bengaluru for isolation of *Colletotrichum* spp. and washed thoroughly with tap water and later rinsed with distilled water and dried on the blotting paper folds. All the specimens were subjected to tissue isolation.

Isolation and identification of the pathogens at genus level

Colletotrichum spp. isolations were made from the leaves, blossom or fruit samples of banana (*Musa* spp.), custard apple (*Annona squamosa* L.), grapes (*Vitis vinifera* L.), guava (*Psidium guajava* L.), mango (*Mangifera indica* L.), papaya (*Carica papaya* L.), pomegranate (*Punica granatum* L.) and showing typical symptoms of anthracnose. Isolations were made as per the standard procedures on potato dextrose agar plates. Pure cultures were maintained on PDA. To obtain the pure colonies, isolates were sub-cultured onto fresh PDA plates *i.e.,* discs of 5 mm diameter from growing tips or periphery of the colony were transferred to fresh PDA plates and incubated at 25 ± 1°C with photoperiod of 24 h for seven days.

The pathogenicity of the purified isolates was confirmed by proving the Koch's postulates. After the development of characteristic anthracnose symptoms on host parts, re-isolation was made from the infected portion of the host part and cultured on PDA. Isolates that were fulfilled the pathogenicity test were used for further studies. The identity of the pathogen was tentatively confirmed based on the morphological and cultural characteristics as described by Sanders and Korsten (2003), Damm *et al.* (2012) and Weir *et al.* (2012).

Cross inoculation on alternative fruit crops and weed host

Cross inoculation study was undertaken in order to determine the host range of the isolates within the host genera studied. For all isolates, cross infectivity was carried out as same as pathogenicity tests. Healthy susceptible, freshly harvested, untreated, mature but un-ripened fruits, leaves or flowers were washed under running tap water for 1 min followed by surface sterilization with 70% ethanol for 3 min, 1% sodium hypochlorite solution for 3 min and then rinsing three times in sterile distilled water and air dried on sterile tissue paper. The surface sterilized leaves, flowers and fruits were placed in a plastic box that contained moist blotting paper to maintain 95% relative humidity. They were inoculated by making wound method with pin-pricking on the leaves, flowers or fruits with a sterile needle and then placing mycelial disc of 5 mm diameter onto the wound and incubated at room temperature for seven days as described by Mo *et al.* (2018). Fruits of banana, papaya and guava, flowers of pomegranate and leaves of custard apple, mango and grapes were used for the inoculation. They were observed for symptom development up to 10-13 days. Isolates were considered pathogenic if lesion size expanded beyond initial wound site. Virulence was assessed by measuring lesion length at 7-11 days post inoculation (DPI). Significant differences in virulence caused were assessed by an analysis of variance, and when significant treatment effects were found, means were compared in CRD. The host range studies were carried out with a weed host *Ageratum* spp. also which was highly prevalent in the fruit orchards ecosystem at Hessaraghatta, Bengaluru.

RESULTS AND DISCUSSION

All the nine representative isolates of *Colletotrichum* spp. in this study were tested for their host range on seven fruit crops *viz*., banana, custard apple, grapes, guava, mango, papaya, pomegranate and one weed host *Ageratum* species. They produced typical anthracnose symptoms upon artificial inoculation (Fig. 1) within 7-11 days, though there was variation in virulence or aggressiveness in producing the symptoms. Symptoms like brown to black necrosis of host tissue due to infection from artificially inoculated *Colletotrichum* isolates were measured seven days post inoculation (DPI) on fruits of banana, guava and flowers of pomegranate, eight DPI on papaya fruits, nine DPI on mango leaves and *Ageratum* spp., ten DPI on grapes leaves, eleven DPI on custard apple leaves. The mean lesion diameters recorded with nine isolates of *Colletotrichum* spp. on seven fruit crops and one weed host *Ageratum* spp. is presented in Table 1. No infection was recorded in control fruits, flowers or leaves at the site of pin pricking. The response of different hosts to the nine isolates tested is described below.

Response of different host plants to *Colletotrichum* isolates from other hosts

Banana: Most severe infection on banana was recorded with IIHR_COL_C2 isolated from same host *i.e*., banana with a mean lesion diameter of 3.14 cm followed by infection with IIHR_COL_C1 and IIHR_COL_C6 isolated from banana and mango without significant difference between them (2.37 cm and 2.37 cm respectively); IIHR_COL_C4 and IIHR_COL_C7 isolated from grapes and papaya without significant difference between them (2.06 cm and 2.06 cm respectively). IIHR_COL_C3 and IIHR_COL_C5 isolated from custard apple and guava were on par in

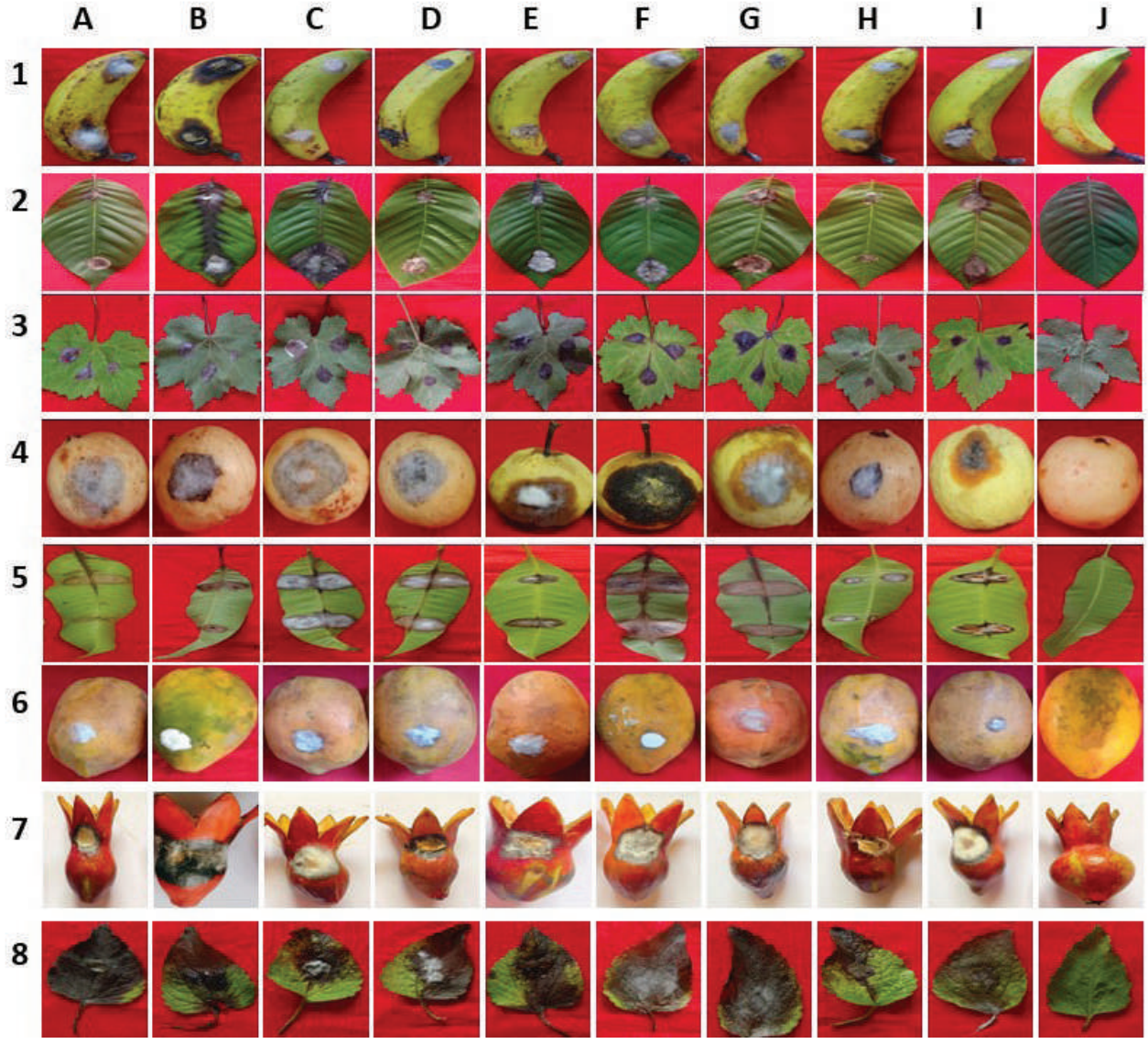


Fig. 1.Cross infection by isolates of *Colletotrichum* spp. (A to I, J-control) from different tropical fruit crops and one weed host *Ageratum*

1- Banana, 2-Custard apple, 3-Grapes, 4-Guava, 5-Mango, 6-Papaya, 7-Pomegranate, 8- *Ageratum*; A. IIHR_COL_C1, B- IIHR_COL_C2, C- IIHR_COL_C3, D- IIHR_COL_C4, E- IIHR_COL_C5, F- IIHR_COL_C6, G- IIHR_COL_C7, H- IIHR_COL_C8, I- IIHR_COL_C9, J-control

lesion diameter (1.72 cm and 1.77 cm respectively). IIHR_COL_C8 isolated from papaya caused poor infection on banana (1.59 cm). Varying symptoms observed were brownish black lesion coupled with pure white mycelium growth (C1), small salmon pink acervuli on the necrotic lesions (C2), black necrotic lesion without any mycelium growth (C4) and necrotic lesion coupled with dull white mycelium growth with other isolates.

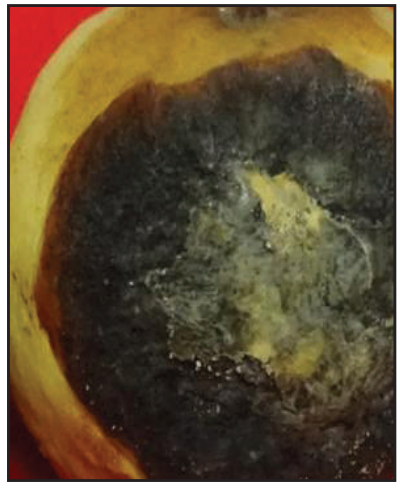
Custard apple: Most severe infection on custard apple was recorded with IIHR_COL_C3 isolated from same host *i.e.*, custard apple with a mean lesion diameter of 5.35 cm, followed by infection with IIHR_COL_C7 isolated from papaya (4.1 cm). Least cross infection on custard apple was recorded with IIHR_COL_C8 (1.8 cm) isolated from papaya. Other isolates had lesion size in between 2.29-3.6 cm. Highly virulent isolate C3 produced black necrotic, nearly spherical lesion, large in size with comparison to other isolates with small salmon pink acervuli while other isolates produced necrotic lesions without visible mycelium growth.

a. Banana with IIHR_COL_C1

b. Banana with IIHR_COL_C2

c. Custard apple with IIHR_COL_C3

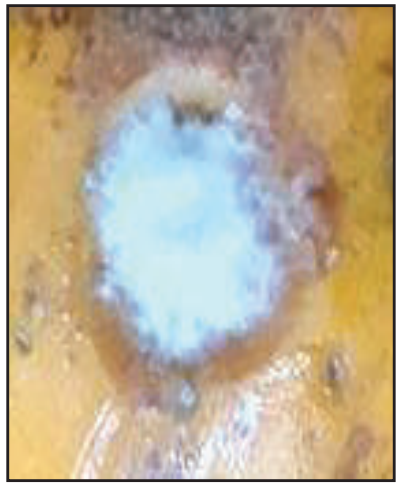
d. Grapes with IIHR_COL_C7



e. Guava with IIHR_COL_C6

f. Mango with IIHR_COL_C6

g. Papaya with IIHR_COL_C7



h. Papaya with IIHR_COL_C8

i. Pomegranate with IIHR_COL_C9

Fig. 2. Close view of typical anthracnose symptoms (some with acervuli) on host tissues of fruit crops with highly virulent isolates in this study.

Table 1. Host range of *Colletotrichum* isolates on fruit crops and *Ageratum* spp. on artificial inoculation

Isolate	Host	Mean lesion diameter (cm) on inoculated leaves, flowers and fruits							
		Banana (Fruit)	Custard apple (Leaves)	Grapes (Leaves)	Guava (Fruit)	Mango (Leaves)	Papaya (Fruit)	Pomegranate (Flower)	*Ageratum* spp. (Leaves)
IIHR_COL_C1	Banana	2.37	2.34	2.40	3.53	2.08	2.56	1.72	4.68
IIHR_COL_C2	Banana	3.14	2.56	1.85	2.40	2.33	3.27	1.98	3.41
IIHR_COL_C3	Custard apple	1.72	5.35	3.09	3.70	2.82	3.82	2.05	3.46
IIHR_COL_C4	Grapes	2.06	2.29	2.15	2.92	2.25	3.17	1.95	4.45
IIHR_COL_C5	Guava	1.77	3.00	2.35	3.39	1.76	3.41	2.02	4.08
IIHR_COL_C6	Mango	2.37	3.64	3.23	4.15	3.13	2.88	1.95	4.30
IIHR_COL_C7	Papaya	2.06	4.10	3.75	2.81	2.65	4.13	2.22	4.76
IIHR_COL_C8	Papaya	1.59	1.80	1.29	1.87	1.70	3.45	1.33	1.96
IIHR_COL_C9	Pomegranate	2.19	3.30	2.46	2.92	2.35	2.42	2.28	3.98
SE(m)±		0.02	0.02	0.03	0.02	0.04	0.03	0.02	0.09
C.D (0.05)		0.08	0.08	0.10	0.08	0.12	0.09	0.06	0.28
C.V %		2.35	1.59	2.43	1.55	3.20	1.61	1.79	4.25

Grapes: Maximum cross infectivity on grapes was recorded with IIHR_COL_C7 isolated from papaya with a mean lesion diameter of 3.75 cm followed with IIHR_COL_C6 (3.23 cm) isolated from mango. Least cross infection on grapes was recorded with IIHR_COL_C8 isolated from papaya (1.29 cm). Other isolates produced lesions of intermediate range. Purplish black necrotic, nearly circular lesions with sparse mycelium over the lesions with salmon pink colour sporulation (acervuli) were recorded with all the nine isolates upon artificial inoculation on grapes leaves. Shot hole like breaking of the infected lesions was observed due to drying of the necrotic region of lesions with IIHR_COL_C1.

Guava: Maximum cross infection on guava was recorded with IIHR_COL_C6 isolated from mango with a mean lesion diameter of 4.15 cm followed with IIHR_COL_C3 from custard apple (3.70 cm). Minimum cross infection on guava was documented with IIHR_COL_C8 isolated from papaya (1.87 cm). Other isolates resulted in lesions of intermittent range of 2.4 – 3.5 cm. The symptoms observed were dull or pure white mycelium growth with all isolates and acervuli with mango and guava isolates.

Mango: Maximum infection on mango was recorded with IIHR_COL_C6 (3.13 cm) isolated from same host *i.e*., mango. It was followed with IIHR_COL_C2, IIHR_COL_C4 and IIHR_COL_C9 isolated from custard apple and papaya respectively (2.33, 2.25 and 2.35 cm) which did not differ significantly in cross infectivity on mango. Least cross infection on mango was recorded with IIHR_COL_C5 (1.76 cm) and IIHR_COL_C8 (1.70 cm) isolated from papaya and guava. The symptoms included brown colour necrotic lesions surrounded with dark chocolate brown colour margins with no visible mycelium growth over the lesions. All lesions were nearly spherical in shape. Many small salmon pink sporulation's (acervuli) were observed in the necrotic regions of lesions for all isolates except with IIHR_COL_C8.

Papaya: Maximum infection on papaya was recorded with IIHR_COL_C7 isolated from same host *i.e*., papaya with a mean lesion diameter of 4.13 cm followed with IIHR_COL_C3 isolated from custard apple (3.82 cm). Least infectivity on papaya was recorded with IIHR_COL_C9 isolated from pomegranate (2.42 cm). The lesion diameter of other isolates ranged between 2.42 – 3.17cm. Ample dull white mycelium growth covered over the sunken brown colour lesions were recorded in all isolates while with C8 black colour sporulation was observed.

Pomegranate: Maximum infection on pomegranate with a mean lesion diameter of 2.28 cm was caused with IIHR_COL_C9 isolated from same host i.e., pomegranate followed with IIHR_COL_C7 (2.22 cm) isolated from papaya. Least infection of 1.33 cm on pomegranate was recorded with IIHR_COL_C8 isolated from papaya. Other isolates resulted in lesion size of 1.33 – 2.05cm. Black colour necrotic sunken lesions, with sparse mycelium growth over the lesions were recorded. Many small salmon pink sporulation's (acervuli) were observed in the necrotic regions of lesions for all isolates except with IIHR_COL_C8 with sparse mycelium growth with little infection.

***Ageratum* species- The weed host**

A weed host *Ageratum* spp. observed to be widely prevalent in the ecosystem of the fruit orchards in the areas of collection was tested for its susceptibility to all nine isolates of *Colletotrichum* spp. and was found susceptible to all nine *Colletotrichum* isolates in the artificial inoculation. Among the isolates tested, most virulent were IIHR_COL_C7 and IIHR_COL_C1 isolated from papaya and banana with mean lesion diameters of 4.76 cm and 4.68 cm respectively. Least infection on *Ageratum* spp.was recorded with IIHR_COL_C8 that was isolated from papaya (1.96 cm). Other isolates resulted in lesion size of intermediate range. Lesions were irregular shape. They were with brown or black necrotic in nature. to nearly spherical lesion shape in cross infectivity studies on fruit crops. No visible sporulation was observed within the necrotic lesions with all isolates on *Ageratum* spp. suggesting their endophytic life style (Freeman *et al.*, 2000).

As a whole, most of the isolates displayed high variation in aggression when inoculated on alternative fruit crops and one weed host tested. A close view of typical anthracnose symptoms with acervuli on host tissues of fruit crops with maximum infecting isolates in this study are presented in Fig. 2. In banana, custard apple, mango and pomegranate, the isolates from original hosts caused maximum lesion size. In case of grapes, isolate C7 from papaya caused severe infection with purplish-black lesion with presence of numerous small salmon pink acervuli formed in concentric rings. Similarly isolate from mango caused severe infection on guava. This clearly indicates that the pathogen can survive on other hosts and can move to other hosts depending on the availability of the host during the crop season. The results suggest that these isolates have good adaptation or specificity to certain hosts and less preference of other alternative hosts.

Another significant observation was that IIHR_COL_C8 isolated from papaya had falcate shaped conidia and greyish- black mycelium. Apart from the distinctiveness of morphological and cultural characters with other isolates, it also showed unique nature in cross infection. Except on the original host isolated from, it showed least infection on all other alternative hosts tested. IIHR_COL_C8 isolate's poor infection on alternative hosts also gives implications of host- specificity, possibility of pathogen elicitors ability to receipt only certain hosts before infection. The isolate from papaya resembled *C. truncatum* while all others resembled C. *gloeosporioides* complex that was confirmed with molecular data (Teja, unpublished data).

Cross infection between two or three hosts viz., mango and custard apple (Lakshmi *et al.*, 2011), mango and sweet pepper reported the cross infection between anthracnose pathogens of mango, papaya and custard apple (Rampersad, 2011); and avocado, durian and mango (Alahakoon *et al.*, 1994). Host range studies reported earlier also suggest the wide host range of *Colletotrichum* especially in fruit crops viz. avocados, mango, strawberry, guava, citrus and papaya (Sanders and Korsten, 2003; De Souza *et al.*, 2013, Cruz *et al.*, 2020, Wu *et al.*, 2020).

High variation in virulence observed in the present study is indicating the isolate variations from tropical fruit crops. Therefore, the isolates of *Colletotrichum* spp. from tropical fruit crops in this study can cause disease and might survive alternatively on other fruit crops and also on weeds. It has implications for researchers to identify the interaction responsible for poor cross infectivity, host specific nature and impart resistance to the cultivars.

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Biocontrol potential of endophytic *Pseudomonas stutzeri* isolated from watermelon (*Citrullus lanatus* Thunb) against *Colletotrichum orbiculare* causing anthracnose disease in watermelon

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ABSTRACT: The biocontrol potential of bacterial endophytes isolated from watermelon leaf was evaluated against the pathogen causing anthracnose disease in watermelon. Totally ten *Colletotrichum* isolates were isolated using tissue segment method and confirmed as *Colletotrichum* sp. based on the cultural and morphological characteristics. The virulence of the pathogenic isolates was confirmed through pathogenicity test. Among the 10 isolates, WEC 2 was identified as a highly virulent isolate. Eighteen bacterial endophytes were isolated from healthy watermelon plants and identified as *Bacillus* spp. (10 isolates) and *Pseudomonas* spp. (8 isolates) based on the cultural characters. The effect of 18 bacterial endophytes was tested against the highly virulent *Colletotrichum* isolate WEC 2 using dual culture technique. Among the 18 bacterial endophytes, isolate WE 8 was found to be highly potential in inhibiting the pathogenic isolate WEC 2. The next best isolate was WEC 17, which exhibited 57.78 % inhibition of pathogen over control. The least mycelial reduction was depicted by the isolate WE 16. The pathogenic isolate (WEC 2) was further confirmed at molecular level using the universal primers ITS 1 and ITS 4 as *Colletotrichum orbiculare* (WEC 2) and the potential bacterial endophytic isolate WE 8 as *Pseudomonas stutzeri* (WE 8) using ITS 27 F and ITS 1492 R primers.

Keywords: Endophyte, watermelon, anthracnose, *Colletotrichum orbiculare, Pseudomonas stutzeri*, molecular characterization

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) is an important cucurbitaceous fruit crop and is widely consumed around the world (Naveen kumar *et al.*, 2017). The crop is grown commercially in areas with long frost-free warm periods in the tropics and subtropics (Prohens and Nuez, 2008). India is the second largest producer of watermelon among the Asian countries (Tiamiyu *et al.*, 2015). Moreover, it is mainly cultivated in Karnataka, Madhya Pradesh, Maharastra, Punjab, Rajasthan, Tamil nadu and Uttar Pradesh. In Tamil nadu, the crop is cultivated in Ariyalur, Erode, Thiruvallur, Pudukkottai, Namakal and Villupuram (Lilly, 2013). Watermelons are susceptible to several diseases that attack the roots, foliage and fruits. The main concern is related to leaf blight (*Alternaria cucumerina*), gummy stem blight (*Didymella bryoniae*), anthracnose (*Colletotrichum orbiculare*) and fusarium wilt (*Fusarium oxysporum* f.sp. *niveum*) (Bulajic *et al.,* 2008). Among the various fungal diseases, anthracnose caused by *C. orbiculare* is considered as one of the important diseases. Economic losses caused by the disease are mainly attributed to lowering of fruit quality and marketability (Zivkovic*et al.*, 2017). Anthracnose caused by the fungi *Colletotrichum* species complex is one of the most significant diseases of cucumber (*Cucumis sativus* L.), melons (*Cucumis melo* L.), pumpkin (*Cucurbita pepo* L.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai). The disease is widespread under both greenhouse and field cultivations and cause severe infection on seedlings, leaves, petioles, stems and fruits of *Cucurbitaceae* and other herbaceous hosts belonging to the families such as *Asteraceae, Fabaceae* and *Malvaceae* (Farr and Rossman, 2013).

The structure and ecosystem of plant and soil have been seriously damaged by the overuse of chemical fertilizers and fungicides in agriculture (Abbamondi *et al.*, 2016). Therefore, a great attention has been taken. to reduce the use of these chemicals and to develop microorganisms as biological and environmentally friendly control agents in agricultural disease management. Many microorganisms have been reported to be beneficial to agriculture (Tao *et al.*, 2019). Among them, endophytic bacteria play a significant role in plant growth because of their abilities in solubilizing mineral phosphate and

producing a diverse range of secondary metabolites, such as indole 3- acetic acid (IAA) and siderophore. In addition, endophytic bacteria have the inhibitory effect against plant pathogens and effectively control the plant diseases caused by pathogens, and further promote plant growth (Yang *et al.,* 2021). Hence, the present study was under taken to investigate the biocontrol potential of bacterial endophytes isolated from watermelon against the anthracnose pathogen.

MATERIALS AND METHODS

Isolation and identification of the pathogen

The fungal pathogen was isolated from the infected tissues by employing standard isolation method with slight modification (Zivkovic *et al.*, 2017). The infected tissues were cut into small pieces and surface sterilized with 1% sodium hypochlorite for 30 seconds. Then the leaf bits were serially washed thrice with sterile distilled water (SDW) and dried between two layers of blotting papers. After that the bits were plated onto PDA medium containing 100 ppm streptomycin in sterile Petri dishes and incubated at room temperature (28±2°C). The fungal growth appeared around the bits were sub-cultured on PDA medium and maintained on slants for further studies.

Pathogenicity test

Preparation of spore suspension and inoculation

The pathogenic isolates were tested for their pathogenicity under glass house conditions. One month old watermelon plants were artificially inoculated with conidial suspension ($2x10^6$ cfu/ml) of *Colletotrichum* isolates. On cool evening hours, the plants were squirted with conidial suspension and moistened with damp cotton. Such inoculated plants were covered with polythene bags for seven days. Control plants were retained only by spraying with sterile distilled water. For each isolates three replications were maintained. After seven days of inoculation, these plants were observed for symptom development and the per cent disease index (PDI) was assessed as per the standard grade chart described by Pandey *et al.* (2003). Based on the PDI, virulence of the isolates were assessed and the isolate with maximum PDI was considered as the virulent one. Re-isolation was done from the inoculated plant and compared with the original culture for confirmation of the pathogen. The per cent disease index (PDI) was worked out using the formula Described by Mc Kinney (1923).

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves graded}} \times \frac{100}{\text{Maximum grade}}$$

Table 1. Survey on the incidence of anthracnose disease in different watermelon growing areas of Tamil Nadu

Location	District	Latitude	Longitude	Isolate code	Disease Incidence (PDI)	PDI* under pot culture
Perode	Erode	11.38756	77.629739	WEC 1	29.11	39.25^{d} (38.79)
Chittode	Erode	11.389647	77.63415	WEC 2	28.4	60.15^{a} (50.85)
Kadamathur	Thiruvallur	13.140167	77.505988	WTC 3	34.6	28.49^{f} (32.25)
Chithamur	Pudukkottai	10.37^{0}N	78.89^{0}E	WPC 4	15.32	31.57^{e} (34.18)
Alambadi	Vilupuram	12.006865	79.299048	WVC 5	21.36	57.61^{b} (49.37)
Perumandampalayam	Nammakal	11.114390	78.108255	WNC 6	21.74	25.69^{g} (30.45)
Nochipatti	Nammakal	11.125560	78.117814	WNC 7	19.66	31.57^{e} (34.18)
Tindivanam	Villupuram	12.202319	79.838118	WVC 8	18.34	19.78^{h} (26.40)
Nagamangalam	Ariyalur	11.079468	79.189324	WAC 9	14.83	33.16^{e} (35.15)
K. Kaikatti	Ariyalur	11.123505	79.136905	WAC 10	17.24	42.21^{c} (40.51)

PDI: Per cent Disease Index. *Values are mean of three replications. Values in parentheses are arc sine transformed values. In a column, means followed by a common letter are not significantly different at 5 % levels by LSD.

Table 2. Bacterial endophytic antagonists isolated from watermelon and their antifungal activity against *Colletotrichum* sp.

Location	District	Latitude	Longitude	Isolate code	Mycelial growth (cm)*	Per cent reduction over control
Perode	Erode	11.387549	77.629784	WE 1	5.1^{gh}	43.33 (41.15)
Perode	Erode	11.38756	77.629739	WE 2	4.4^{ef}	51.11 (45.61)
Perode	Erode	11.390023	77.631818	WE 3	4.0^{bc}	55.56 (48.17)
Perode	Erode	11.386433	77.628815	WE 4	5.9^{k}	34.44 (35.91)
Chittode	Erode	11.389655	77.634126	WE 5	5.6^{ij}	37.78 (37.90)
Chittode	Erode	11.388652	77.63447	WE 6	4.1^{cd}	54.44 (47.52)
Chittode	Erode	11.389647	77.63415	WE 7	4.6^{f}	48.89 (44.34)
Kadamathur	Thiruvallur	13.140167	77.505988	WE 8	3.7^{a}	58.89 (50.10)
Pallapattu	Thiruvallur	13.084008	80.017779	WE 9	5.4^{ij}	40.00 (39.21)
Ponneri	Thiruvallur	13.083959	80.017742	WE 10	5.6^{j}	37.78 (37.91)
Ponneri	Thiruvallur	13.0834566	80.01755	WE 11	6.2^{l}	31.11 (33.88)
Nochipatti	Namakkal	11.125560	78.117814	WE 12	4.3^{de}	52.22 (46.25)
Nagamangalam	Ariyalur	11.079468	79.189324	WE 13	5.3^{hi}	41.11 (39.86)
Alangudi	Pudukkottai	10.371200	78.895400	WE 14	4.9^{g}	45.56 (42.43)
Chithamur	Pudukkottai	10.574055	79.009224	WE 15	4.2^{cde}	53.33 (46.88)
Alambadi	Vilupuram	11.993934	79.28052	WE 16	6.9^{m}	23.33 (28.87)
Alambadi	Vilupuram	12.006865	79.299048	WE 17	3.8^{ab}	57.78 (49.45)
Alambadi	Vilupuram	12.01155	79.301629	WE 18	5.1^{g}	43.33 (41.14)
CD (p = 0.05)					0.26	1.22

Values are mean of three replications. Values in parentheses are arc sine transformed values.
In a column, means followed by a common letter are not significantly different at 5 % levels by LSD.

Isolation of antagonistic endophytic bacteria from watermelon

Healthy leaf samples were collected in sterile paper bags from different watermelon growing areas of Tamil Nadu. Leaves were cut into pieces and sterilized with 5% sodium hypochlorite for 3 min, followed by washing in distilled water for 3 times and dried by using sterile filter paper. The leaf bits were then crushed in a sterile mortar and the sap was serially diluted and streaked on to nutrient agar (NA) plates. The sterile water obtained from the last washing step was streaked on NA plates as control. This was followed by the incubation of the plates at 27 °C for 3 days. The selection of bacterial colonies was done based on distinct morphological features (colour, size, shape) and pure cultures were obtained by streak-plate technique (Al-Hussini*et al.*, 2019).

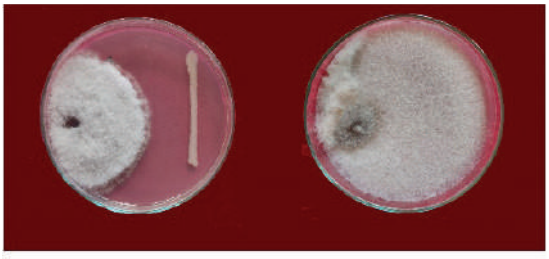


Fig 1. Antifungal activity of endophytic bacterial isolates (WE 8) against the *Colletotrichum* sp.

***In vitro* screening of antagonistic bacteria against *Colletotrichum* sp.**

Eighteen endophytic bacterial isolates were investigated *in vitro* for their antagonistic vitality against the virulent isolate (WEC 2) of anthracnose pathogen (*Colletotrichum* sp.) by dual culture technique. A bacterial colony was streaked at one end of a 9-cm Petri dish containing PDA medium. A 5-mm mycelial disc of a 7-day-old culture of the test fungal pathogens was placed on the other end and incubated at 27 °C until mycelial growth of the test fungus covers the entire Petri plate in the control. At the end, the inhibition zone was measured by measuring the distance between the 2 sides of the tested Petri dish (Al-Nadabi *et al*., 2021)**.**

Molecular characterization

Anthracnose pathogen

Total genomic DNA was isolated from mycelium of the virulent isolate (WEC 2) as described by White el at (1990).The isolated DNA was re-suspended in 50 µl of milliQ water or 1X TE buffer and stored at -20°C for further use. To check the quality of isolated DNA, 2.5µl of total DNA was resolved in the 1% agarose gel.

Amplification and sequencing of ITS region of rDNA

Polymerase chain reaction (PCR) was performed in a total volume of 50 µl using the Emerald Amp® GT PCR master mix using genomic DNA isolated from *Colletotrichum* sp. as a template. The intermediate regions of the 5.8S ribosomal gene were amplified using primer pairs ITS1 (5'TCCGTAGGTGAACCTGCGG 3') and ITS4 (5'TCCTCCGCTTATTGATATGC 3'). PCR cycle includes 4 minutes at 94 °C for Initial denaturation, followed by 35 cycles of 1 minute at 94 °C, 1 minute at 55 °C for annealing and 1 minute at 72° C with a final 7 minute extension at 72° C. The PCR products were resolved by electrophoresis in 1% agarose gel and it was sequenced at Bioserve Biotechnologies India Pvt Ltd, Bangalore.

Molecular characterization of potential bacterial antagonist

The genomic DNA from potential antagonistic bacterial isolates was isolated using the standard protocol of Cetyl trimethyl ammonium bromide (CTAB) method (Wilson, 2001).

Two milli liter of actively grown broth culture was taken in 2 ml centrifuge tube and centrifuged at 6,000 rpm for 5 min at 4°C. The supernatant was removed and the pellet was suspended in 1 ml TE buffer. To that, 0.5 ml of 1-butanol was added and vortexed well to mix the cells thoroughly (to remove extra cellular materials) and centrifuged at 6000 rpm for 5 min at 4°C. The supernatant (both supernatant layer and aqueous layer) was discarded and the pellet was re-suspended in 2 ml of TE buffer and centrifuged at 6,000 rpm at 4°C for 5 min to remove all traces of butanol. Again the pellet was re-suspended in 1ml TE buffer. To that, 100 µl of lysozyme (10 mg ml^{-1} freshly prepared) was added and incubated at room temperature for 5 min. After incubation, 100 µl of 10 % SDS and 25 µl of 100 µg ml^{-1} proteinase K were added, mixed well and incubated at 37°C for one hour. To the above mixture, 200 µl of 5M NaCl was added and mixed well. To this mixture, 150 µl of CTAB solution was added, mixed well and incubated at 65°C for 10 min. The mixture was extracted with 1ml of phenol: chloroform mixture in the ratio of 25:24, mixed well and centrifuged at 14000 rpm for 15 min at 4°C. The aqueous layer was transferred carefully to a 2 ml micro-centrifuge tube and DNA was precipitated by adding equal volume of ice cold iso-propanol by incubating overnight at - 20°C. The DNA was pelleted by centrifugation at 14,000 rpm for 15 min at 4°C. The pellet was washed with 70 per cent ethanol for 2 times and dried under vacuum or kept on water bath at 50- 55°C for 10 mins and re-suspended in 50 µl of TE buffer. The DNA was stored at -20°C for further use. The bacterial genome DNA was verified on agarose gel electrophoresis method as described earlier.

16S rDNA sequencing of endophytic bacteria

Polymerase Chain reaction was performed in a total volume of 50 µl using Emerald Amp® GT PCR master mix using genomic DNA of endophytic bacteria as a template. The small sub unit 16S region was amplified with universal primers. The primer pair used were 27F + 1492r. PCR conditions followed were, initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 sec, annealing temperature at 50°C for 30 sec and extension at 72°C for 2 min, and a final extension at 72°C for 10 min. The reaction was carried out in Eppendorf MasterCycler gradient PCR machine. The PCR products were then resolved by electrophoresis in 1% agarose gel. Later the PCR products were purified using FavorPrep

GEL/ PCR purification kit and sequenced at Bioserve Biotechnologies India Pvt. Ltd. Bangalore. (Isik*et al.*, 2014).

The Primers sequence used for amplification of 16S rRNA region, 27f - 5′ AGAGTTTGATCTGGCTCAG 3′ (forward primer) 1492r - 5′ TACGGYTACCTTGTTACGACT 3′ (reverse primer)

Statistical analysis

All the experiments were done in triplicates. The treatment mean differences were analysed using ANOVA and Duncan`s Multiple Range Test with a 5% significance level (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Isolation, cultural and morphological characterization of anthracnose pathogen

Anthracnose pathogen (*Colletotrichum* sp.) was isolated from the infected leaves of watermelon collected during the survey (Table 1). Numerous fungal colonies were obtained from infected leaves, totally of 10 *Colletotrichum* isolates were confirmed through microscopic examination based on their conidial structure. All isolates resembled as dark gray to white mycelium on PDA medium after incubation at 26^{o}C for seven days in the dark.

Pathogenicity test

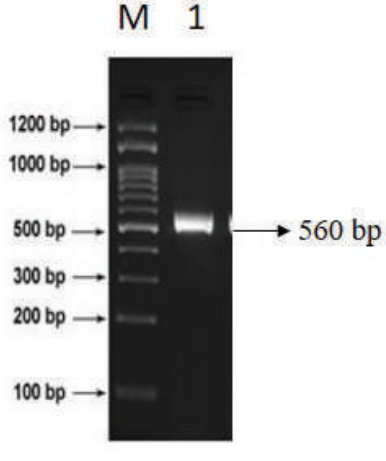
Pot culture experiment was conducted to study the virulence of different *Colletotrichum* isolates. The results showed that all the isolates induced symptoms on the inoculated watermelon leaves as like the natural symptoms. Among the 10 isolates, WEC 2 isolate collected from Erode district was found to be highly virulent with the PDI of 60.15 per cent. The isolate WVC 5 from Vilupuram stood next with the PDI of 57.61 per cent, whereas infection in other isolates ranged from 19.78 to 42.21 per cent (Table 1). The virulent isolate (WEC 2) exhibited initial development of dried up infected area and became dark brown, while necrotic areas broke and shattered at the centre of the lesion. The isolate (WVC 8) was identified as least virulent isolate, which exhibited a PDI of 19.78 per cent.

Isolation of bacterial endophytes from watermelon

Endophytic bacteria were isolated from healthy leaves and stems of watermelon crop. Totally 18 isolates were isolated by using serial dilution method on nutrient agar medium. Out of 18 endophytic bacteria, two different bacterial colonies were identified based on their morphology and biochemical characterization. The bacterial colonies were cultured on separate Petri plates containing NA media for getting pure culture and maintained at 4^{o}C for further studies (Table 2).

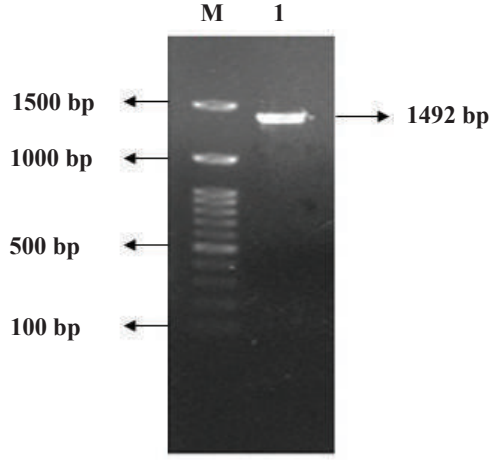
***In vitro* antifungal activity of endophytic bacterial isolates against *Colletotrichum* sp.**

In vitro antagonistic effects of bacterial endophytes were tested against *C. orbiculare* using the dual culture assay. Among the eighteen endophtyic bacteria, WE 8 isolate showed the maximum growth reduction of *C. orbiculare* by 58.89 per cent over the control and it was followed by WE 17 isolate which recorded PDI of 57.78 per cent. The minimum mycelial growth reduction



M- Marker (1000bp); 1- *Colletotrichum orbiculare* (WEC 2)

Fig 2. Molecular identification of virulent isolate of pathogen (*Colletotrichum* sp.)



M- Marker (1000bp); 1- *Pseudomonas stutzeri* (WE 8)

Fig 3. Molecular identificationof effective endophytic bacteria (WE 8)

of 23.33 per cent was observed in the WE 16 isolate (Table 2 & Fig.1). Rakotoniriana *et al.* (2012) isolated thirty-one endophytic bacteria from healthy leaves of *Centella asiatica* and screened against *Colletotrichum higginsianum*. The result showed that endophytic bacteria *Pseudomonas fluorescens* BCA08 inhibited the mycelial growth of *Colletotrichum higginsianum* by 82 per cent. According to Silva *et al.* (2016),the endophytic bacteria isolated from the guarana seed had growth inhibition percentage of 52.41% against *C. gloeosporioides.*

Molecular characterization of *Colletotrichum* sp.

The virulent isolate of *Colletotrichum* sp. (WEC 2) was observed macroscopically and microscopically and confirmed as *Colletotrichum* sp. by morphological and cultural characters at genus level. Further, its identity was confirmed through molecular technique using ITS sequence analysis.

The Internal transcribed spacer (ITS) region of *Colletotrichum* sp. isolate WEC 2 was amplified with primers ITS 1 and ITS 4 and the products obtained was detected as a single band in agarose gel stained with ethidium bromide. The size of the PCR fragments was approximately 600 bp (Fig 4). The DNA fragment obtained was sequenced at Bioserve Biotechnologies India Pvt, Ltd. The full length 16S rRNA sequences obtained was BLAST searched in the database of National Centre for Biotechnology Information (NCBI) and identified as *C. orbiculare*. Zivkovic *et al.* (2017) reported that *Colletotrichum* Serbian isolates LC1, LC2 and LC3 produced approximately 600-800 bp fragment, which had 100 % similarity with *C. orbiculare* isolate retrieved from the GeneBank database. Kumar *et al.*, (2010) identified and confirmed 21 isolates of *Colletotrichum* sp. using universal primers (ITS 1 and ITS 4) which produced a fragment of 600 bp and it had 95 % to 100 % similarity with the sequences of isolates retrieved from the GeneBank database.

Molecular characterization of effective endophytic antagonistic bacteria

The 16S rDNA sequence analysis is one of the commonly used molecular methods for the identification of bacteria at species level. The 16S rRNA from four virulent isolates WE 8 isolated using CTAB method. Single band of intact genomic DNA was visualized on the agarose gel. The 16S rRNA region of these bacterial isolates was amplified with primer pairs 27f and 1492r using a thermo cycler and the products produced were visualized as a single band in agarose gel strained with ethidium bromide. The size of the PCR fragments was approximately 1492 bp length for primer pair 27f and 1492r. The PCR products were sequenced at Bioserve Biotechnologies India Pvt, Ltd. The full length 16S rRNA sequence obtained for potential bacterial species was BLAST searched in the database of National Centre for Biotechnology Information (NCBI). The potential isolates were molecularly identified at the species level as *Pseudomonas stutzeri* (Fig. 3). Similarly, Islam *et al*. (2016) isolated ten bacterial isolates from the endophere region of cucumber and assigned isolate code *viz.,* PP B1,PPB2,PPB3,PPB4,PPB5,PPB8,PPB9,PPB9,PPB1 0,PPB11 and PPB12 and they were identified through morphological and biochemical methods. Further they were molecularly characterized with partial sequencing of 16s rRNA and identified as *Pseudomonas stutzeri, Bacillus subtilis, Stentotrophomonas maltphilia* and *Bacillus amyloliquefaciens*. Isolate PPB1 had 99% homology with *Pseudomonas stutzeri* and was submitted to Gen Bank under accession number KJ959616. Yuwantiningsih *et al.* (2015) isolated the endophytic bacteria from the forest crop and molecularly identified through universal primers (27F and 1492R) which produced fragments of 1500 bp and it had 99 % similarity.

CONCLUSION

The present study illustrated the effectiveness of 18 endophytic bacteria against anthracnose pathogen,C*olletotrichum orbiculare*. Among the 18 endophytic bacterial isolates, one isolate WE 8 showed the maximum antagonistic activity against the pathogen and was confirmed as *Pseudomonas stutzeri* through molecular approach.

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RESEARCH NOTE

Screening of cumin germplasm against *Fusarium* wilt under wilt-sick plot conditions

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ABSTRACT: Wilt caused by *Fusarium oxysporum* f. sp. *cumini* is the most serious disease of cumin (*Cuminum cyminum* L.) in India. Cumin germplasm was screened against *Fusarium* wilt at S. D. Agricultural University, Gujarat, India. Out of 105 genotypes evaluated, none of the genotype was found to be immune to wilt. The genotypes *viz.,* JC-18-11 and GC-5-1 were highly resistant with a minimum wilt incidence of 5.00 per cent while the genotypes *viz*., JC-18-01 (17.25%), JC-18-07 (18.25%), GP-5 (20.05%) and GP-7 (20.00%) were resistant. The genotypes *viz.,* JC-18-03 (25.15%), JC-18-05 (25.00%), JC-18-06 (25.00%), GP-3 (25.00%), GC-3(c) (25.06%), JC-2010-5 (30.25%), Sanand 5 (30.50%), Sanand 6(30.25%) and GP-4 (30.14%) were found moderately resistant. The genotypes *viz.,* JC-18-04 (35%), GP-2 (35%), GC-5-2 (35%), GC-5 (c) (35%) JC-18-02 (40%), JC-2000-28-1 (40%), JC-16-07 (40%), JC-18-08 (45%), GC-4 (c) (45%) and J-Cum-2-2017 (50%) were found susceptible to the pathogen. The remaining germplasm accessions with the wilt incidence of more than 50% were susceptible to highly susceptible.

Keywords: Cumin, germplasms, *Fusarium* wilt, screening, resistant, susceptible

Cumin (*Cuminum cyminum* L.) is a small herbaceous plant in the family Apiaceae (Umbelliferae), commonly called as "zeera". In India, cumin is exclusively cultivated in Gujarat and Rajasthan. The share of Gujarat in total area and production was 36.7 and 46.3 per cent, respectively (Vinod Kumar, 2017). Gujarat produced 319.9 thousand MT from 3.5 lakh hectare area during the year 2018-19(Anonymous, 2020). The main constraint to achieve high productivity is susceptibility of cumin to devastating diseases *viz*., *Fusarium* wilt, *Alternaria* blight and powdery mildew (Pandey *et al.*, 2019). Cumin is seriously affected by wilt caused by *Fusarium oxysporum* f. sp. *cumini* which causes up to 80 per cent yield loss (Divakara Sastry and Anandaraj, 2013). Wilt remained a serious, destructive and widespread disease of the crop and has threatened the cumin cultivation in Gujarat and Rajasthan. As *Fusarium* wilt is a soil borne disease, it is difficult to eradicate as the asexual fungal spores such as chamydospores survive up to six years in soil even in the absence of suitable host. As the wilt pathogen is soil borne, it is difficult to manage with fungicides or with any single management tactics. Hence there is a need to explore resistant sources in the existing genotypes. So, present investigation was carried out to find out resistant genotypes for the management of cumin wilt.

A total of one hundred and five (105) cumin genotypes were evaluated in *Fusarium* wilt sick plot at Seed Spices Research Station, Sardarkrushinagar Dantiwada Agricultural University, Jagudan, Gujarat, India. The cumin seeds were sown in wilt affected field having a wilt pathogen population of 1.3×10^4 cfu/g of soil. The infected soil was used because it permits the assessment of field resistance by allowing the infection process to take place under natural conditions, with realistic doses of naturally produced inoculums. Sowing of cumin was done in *rabi* season during the year 2019-2020. The size of each plot was 0.90 m × 4.0 m (2 rows of each genotype) with row spacing of 30cm. Each of the test entries was alternated by susceptible check. The recommended agronomic practices were followed. The statistical design used was augmented method. Based on the proportion of plants exhibiting *Fusarium* wilt symptoms in susceptible germplasm, the data was recorded for healthy and wilted plants from different genotypes and per cent disease incidence was calculated. Germplasm accessions were categorised as highly resistant (0-10% disease), resistant (11-20%), moderately resistant (21-30%), susceptible (31-50%) and highly susceptible (> 50%) as per the scale given by Iqbal *et al.* (2005).

The results presented in Table 1 revealed that out of one hundred and five cumin genotypes tested, two were highly resistant (HR), four were resistant (R), nine were moderately resistant (MR), ten were susceptible (S) and eighty were found highly susceptible (HS). None of the genotypes was immune to wilt. The germplasm accessions *viz.,* JC-18-11 and GC-5-1 were highly resistant with a

Table 1. Per cent wilt incidence in different germplasm accessions of cumin

Entry	Wilt incidence (%)	Reaction	Entry	Wilt incidence (%)	Reaction
JC-18-01	17.25	R	Sanand 5	30.50	MR
JC-18-02	40.00	S	Sanand 6	30.25	MR
JC-18-03	25.15	MR	GP-1	65.12	HS
JC-18-04	35.12	S	GP-2	35.44	S
JC-18-05	25.00	MR	GP-3	25.00	MR
JC-18-06	25.00	MR	GP-4	30.14	MR
JC-18-07	18.25	R	GP-5	20.05	R
JC-18-08	45.50	S	GP-6	90.62	HS
JC-18-09	90.75	HS	GP-7	20.00	R
JC-18-10	60.14	HS	GP-8	95.00	HS
JC-18-11	5.00	HR	GP-9	100.00	HS
JC-17-08	60.32	HS	GP-10	100.00	HS
CUM-40	70.50	HS	GP-12	100.00	HS
CUM-41	90.68	HS	GP-13	100.00	HS
CUM-42	70.25	HS	GP-14	100.00	HS
CUM-43	65.41	HS	GP-15	100.00	HS
JC-16-03	75.20	HS	GP-16	100.00	HS
JC-16-10	80.72	HS	GP-17	100.00	HS
JC-16-07	40.11	S	GP-18	100.00	HS
JC-2010-5	30.25	MR	GP-19	100.00	HS
GP-20	100.00	HS	JC-2000-28-2	70.33	HS
GP-21	100.00	HS	JC-2000-57	80.27	HS
GP-22	100.00	HS	JC-2002-09	95.00	HS
GP-23	100.00	HS	JC-14-2	90.25	HS
GP-24	100.00	HS	J-Cum-1-2017	95.04	HS
GP-25	100.00	HS	J-Cum-2-2017	50.41	S
GP-26	100.00	HS	JC-2010-05	90.62	HS
GP-27	100.00	HS	GC-5-1	5.00	HR
GP-28	100.00	HS	GC-5-2	35.13	S
GP-29	100.00	HS	Mutation-2	95.24	HS
GP-30	100.00	HS	Mutation-3	100.00	HS
GP-31	100.00	HS	Mutation-4	100.00	HS
GP-32	100.00	HS	Mutation-5	95.25	HS
GP-33	100.00	HS	Mutation-6	95.36	HS
GP-34	100.00	HS	Mutation-7	95.12	HS
GP-35	100.00	HS	Mutation-8	95.48	HS
GP-36	100.00	HS	Mutation-9	95.26	HS
GP-37	100.00	HS	Mutation-10	95.26	HS
GP-38	100.00	HS	Mutation-11	100.00	HS
GP-39	100.00	HS	Mutation-12	100.00	HS

GP-40	100.00	HS	Mutation-13	100.00	HS
GP-41	85.25	HS	Mutation-14	100.00	HS
GP-42	90.12	HS	Mutation-15	100.00	HS
GP-43	80.24	HS	Mutation-17	100.00	HS
GP-44	100.00	HS	Mutation-18	100.00	HS
GP-45	100.00	HS	Mutation-19	100.00	HS
GP-46	100.00	HS	Mutation-20	100.00	HS
GP-47	100.00	HS	GC-1 (c)	70.15	HS
GP-48	100.00	HS	GC-2 (c)	73.28	HS
GP-49	100.00	HS	GC-3 (c)	25.06	MR
GP-50	100.00	HS	GC-4 (c)	45.14	S
JC-2000-5	95.17	HS	GC-5 (c)	35.04	S
JC-2000-28-1	40.21	S			

HR- Highly resistant, R- Resistance, MR- Moderately resistance, S- Susceptible
HS- Highly susceptible

minimum wilt incidence of 5.00 per cent. The genotypes *viz*., JC-18-01 (17.25%), JC-18-07 (18.25%), GP-5 (20.05%) and GP-7 (20.00%) were found resistant. The genotypes *viz.,* JC-18-03 (25.15%), JC-18-05 (25.00%), JC-18-06 (25.00%), GP-3 (25.00%), GC-3(c) (25.06%), JC-2010-5 (30.25%), Sanand 5 (30.50%), Sanand 6 (30.25%) and GP-4 (30.14%) were found moderately resistant. The germplasms *viz.,* JC-18-04 (35.12%), GP-2 (35.44%), GC-5-2 (35.13%), GC-5 (c) (35.04%) JC-18-02 (40.00%), JC-2000-28-1 (40.21%), JC-16-07 (40.11%), JC-18-08 (45.50%), GC-4 (c) (45.14%) and J-Cum-2-2017 (50.41%) were found susceptible to the pathogen.

The remaining genotypes *viz.,* JC-18-10 (60.14%), JC-17-08 (60.32%), CUM-43 (65.41%), GP-1 (65.12%), CUM-40 (70.50%), CUM-42 (70.25%), GC-1 (c) (70.15%), JC-2000-28-2 (70.33%), GC-2 (c) (73.28%), JC-16-03 (75.20%), JC-16-10 (80.72%), GP-43 (80.24%), JC-2000-57 (80.27%), GP-41 (85.25%), GP-6 (90.62%), JC-18-09 (90.75%), CUM-41 (90.68%), GP-42 (90.12%), JC-14-2 (90.25%), JC-2010-05 (90.62%), GP-8 (95.00%), JC-2000-5 (95.17%), JC-2002-09 (95.00%), J-Cum-1-2017 (95.04%), Mutation-2 (95.24%), Mutation-5 (95.25%), Mutation-6 (95.36%), Mutation-7 (95.12%), Mutation-8 (95.48%), Mutation-9 (95.07%) and Mutation-10 (95.26%) were found highly susceptible. The genotypes *viz.,* GP-9, GP-10, GP-11, GP-12, GP-13, GP-14, GP-15, GP-16, GP-17, GP-18, GP-19, GP-20, GP-21, GP-22, GP-23, GP-24, GP-25, GP-26, GP-27, GP-28, GP-29, GP-30, GP-31, GP-32, GP-33, GP-34, GP-35, GP-36, GP-37, GP-38, GP-39, GP-40, GP-44, GP-45, GP-46, GP-47, GP-48, GP-49, GP-50, Mutation-3, Mutation-4, Mutation-11, Mutation-12, Mutation-13, Mutation-14, Mutation-15, Mutation-17, Mutation-18, Mutation-19 and Mutation-20 recorded cent per cent wilt incidence and were categorized as highly susceptible.

Twelve lines of cumin were screened against wilt pathogen *F. oxysporum* f. sp. *cumini* and highest resistance was recorded in UC-220 and UC-231 Arora *et al.*, (2004). Deepak *et al.,* (2008) screened 25 cumin germplasms but, none them shown resistant to wilt and found that the maximum resistance to wilt was observed in UC- 220, EC-220, EC- 232684 and UC-63 lines. The lines JC-2000-21 and JC-2000-22 were found moderately susceptible. The findings of Deepak and his co-workers match with the result of present study. The lines JC-2000-21 and JC-2000-22 in both the investigations are reported as moderately susceptible to *F. oxysporum* f. sp. *cumini*.

The genotypes *viz.,* JC-18-11 and GC-5-1 were found highly resistant with a minimum wilt incidence of 5.00 per cent. The genotypes *viz*., JC-18-01 (17.25%), JC-18-07 (18.25%), GP-5 (20.05%) and GP-7 (20.00%) were found resistant. The genotypes *viz.,* JC-18-03 (25.15%), JC-18-05 (25.00%), JC-18-06 (25.00%), GP-3 (25.00%), GC-3(c) (25.06%), JC-2010-5 (30.25%), Sanand 5 (30.50%), Sanand 6(30.25%) and GP-4 (30.14%) were found moderately resistant. The varieties GC-2 and GC-3 which are recommended to cultivate in Gujarat state were found to be highly susceptible to *F. oxysporum* f. sp. *cumini* under field screen studies.

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RESEARCH NOTE

A report on incidence of leaf gall psyllid, *Pauropsylla tuberculata* on Scholar tree, *Alstonia scholaris* in Rajasthan, India

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ABSTRACT: This research paper reports the gall infestation on an ornamentsal tree *Alstonia scholaris,* commonly called Scholar tree, caused by *Pauropsylla tuberculata* (Order: Hemiptera). The infested leaves bearing galls were collected from *Alstonia* trees and observed under dissecting microscope. Under this study we assessed the morphology of gall infested leaves, insect and damage symptoms. Theses pouch gall enclosed different development stages of insect *P. tuberculata*. The nymphal stage of the gall insect is completed within the galls and later imago exits from the galls through a small opening in the gall. Galls initially are greenish but later on get brownish on maturity.

Keywords: *Alstonia scholaris,* leaf gall, psyllid, Rajasthan*, Pauropsylla tuberculata*

Alstonia scholaris (Scholar Tree) is an ornamental tree, popularly known as satpatia and belongs to family Apocynaceae. It is considered as an important medicinal tree in the traditional systems of medicine. The tree reaches a height of 50 to 80 feet, with a furrowed trunk and oblong leaves. The paste of bark is used topically in chronic skin ulcers, given to lactating mothers for increasing lactation, enhance digestive power and has antipyretic properties (https://www.dabur.com/ amp/ in/ en-us/ about/ science-of - ayurveda / herbal-medicinal plants /alstonia-scholaris-medicinal-uses retrieved on 8-11-21). As per Baliga (2010), *A. scholaris* possesses radiomodulatory, chemomodulatory, and chemopreventive effects and antioxidant, anti-inflammatory, antimutagenic, and immunomodulatory activities which prevents cancer. *A. scholaris* is an important source of pulai timber and used for pattern making, plywood and carving etc.

Some biotic factors which affect these tree include infestation by *Parotis marginata* (leaf skeletonizer), termites, pinhole and marine borers, lyctid borers (http://apps.worldagroforestry.org/treedb2/AFTPDFS/Alstonia_scholaris.PDF,retrieved on 8-11-21) but presently in Rajasthan infestation of leaf gall was noticed on *A scholaris,* caused by *Pauropsylla tuberculata*. As per Raman (2003), hemipteran induced galls are caused by insect belonging to four superfamilies; Aphidoidea, Psylloidea, Coccoidea and Aleyrodoidea. Within the family Psyllidae, there are about 350 widely distributed gall inducing insect species infesting leaves of dicotyledonous plants (Hodkinson, 1984). Psyllid galls may be found isolated or aggregated on leaf surface and having simple or complex structures (Hodkinson 1984, Dreger-Jauffret & Shorthouse 1992, Raman 2003). The present work aims to study infestation by a psyllid *Pauropsylla tuberculata*, causing leaf gall of *Alstonia scholaris*.

The infected plant leaves look craggy and disfigured. Pouch like globular galls were found on both upper and under side of the leaves. Gall formation initiates as the adult *P. tuberculata* oviposits on leaves. The initial noticeable change can be seen as slight discolorisation at the site of eggs deposition on the leaf surface. Progressively a small outgrowth in the form of gall appears on the leaf surface which later enlarges into a dome shaped structure to hold different development stages of insect. The immature galls are green in colour and bulbous in shape. The mature galls changes to brownish black in colour and become hard and woody and remain on the leaf long after the escape of the adult insect. When the leaves are heavily infested with galls, the lamina gets reduced to a single cluster of cells. With the severity of infestation more number of gall appear and leafs become crumbled and deformed. Morphological, anatomical and biochemical studies on the foliar galls of *Alstonia scholaris* induced by *Pauropsylla tuberculata* (Psyllidae) was also studied by Albert (2011).

P.tuberculata belongs to order Hemiptera and has egg, nymph and adult stage in life cycle. Adult female lays eggs close to the midrib or side veins on the upper surface of leaf. The nymphs are elliptical in shaped with clearly defined abdomen and thorax and hair like structures covering the body. At the time of emergence mature instars nymphs come out of the gall through an exit hole and moult into an adult. Maximum number of galls on the leaves is observed on the upper side near the midrib and side veins. The number of gall on leafs were

Fig. 1. ***Alstonia scholaris***

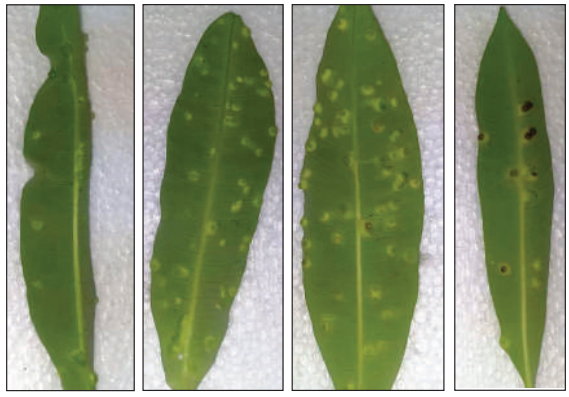

Fig. 2. Stages of leaf gall formation

Fig. 3. Nymph of ***P. tuberculata***

found to vary between 9- 47 per leaf. The infestation ranges from 35-45% per tree. Both young and mature leaves of *A. scholaris* are affected with leaf galls. The leaf galls occur strewed or in clusters resulting in the crumpling of the leaves. The higher infestation on leaf may lead to morphological deformities lowering the aesthetic value of Scholar tree *Alstonia scholaris* by forming galls on the foliage, which gives an unpleasant appearance to the tree thus destroying its beauty.

Albert *et al.* (2011) revealed that the nymph stage is the major cause of gall formation in various parts of the host plant which is in agreement of present findings. Krishnan *et al.* (2011) observed that *P.tuberculata* also causes galls on different parts of the plant *viz*., leaves, stems, fruits and inflorescence. Arya *et al.* (1975) reported that growth of gall tissues are related with the biochemical changes in the levels of carbohydrates, proteins, nucleic acids, phenols, IAA and enzymes.

This study on the leaf gall of *A. scholaris* describes the infestation and damage symptoms induced by *P. tuberculata*. The gall insect is host plant specific and completes its major part of life cycle within the galls on tree. Though minor infestation doesn't harm the tree, but in case of severe infestation the leaves gets crumbled and disfigured. Therefore eco-friendly measure to manage leaf gall of *A. scholaris* should be taken to lower down the number of infections before it reaches severity and effects tree health.

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