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COVER PHOTOS : Infestation of a new scale, *Aulacaspis madiunensis* on *Cycas circinalis*
Photo Courtesy: Dr. Sunil Joshi (ICAR-NBAIR, Bengaluru)

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CONTENTS 29 (2)

RESEARCH ARTICLES

Pest Management in Horticultural Ecosystems
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Aulacaspis madiunensis (Zehntner) (Hemiptera: Diaspididae) – an additional danger to the endangered *Cycas circinalis* L.

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ABSTRACT: Armored scale insects (Hemiptera: Diaspididae) are major economic pests and are among the world's most invasive species. Most armoured scale insect pests are invasive and with increased international trade, these concealed and cryptic pests pose a serious threat to the world agricultural economy causing economic losses in the US to be as high as $1 billion annually. *Aulacaspis* Cockerell is one of the largest genera of armoured scale insects with 151 species known worldwide, and 23 species from India. Five species of *Aulacaspis* are associated with *Cycas* spp. throughout the world. Through this correspondence, we report the occurrence and severe infestation of *A. maduinensis* (Zehntner) on *C. circinalis* from India, though it has been reported to infest other species of *Cycas* from elsewhere. We provide information about nature and extent of damage, field and taxonomic diagnostic characters which will be helpful in quick diagnosis of the pest, monitoring its spread to different geographical areas, and generation of awareness about the pest species to avoid further economic loss.

Keywords: Armoured scale insects, cycads, taxonomy, diagnostic characters, outbreak

INTRODUCTION

Armored scale insects (Hemiptera: Diaspididae) are major economic pests and are among the world's most invasive species (Nomark *et al*., 2019). Out of a total 8373 scale insects (Superfamily Coccoidea) recorded throughout the world, 640 species (7.6 percent) are considered as pest species. Of these, 7.6 percent, armoured scale insects top the list with 222 species (34 percent) being considered pests in agriculture, horticulture, and forestry (Kondo and Watson, 2022). Most armoured scale insect pests are invasive and with increased international trade, these concealed and cryptic pests pose a serious threat to the world agricultural economy causing economic losses in the US to be as high as $1 billion annually (Miller and Davidson 2005).

In India, 236 species under 66 genera of armoured scale insects have been reported so far. Recently, *Aulacaspis crawii* (Cockerell) was reported to have invaded and caused heavy damage to *Melia dubia* Cav. (Meliaceae) plantation of Kerala (Joshi *et al*., 2022), similarly, a heavy incidence of *Rutherfordia major* (Cockerell) on lovi-lovi *Flacourtia inermis* Roxb. (Salicaceae) was reported in Kerala (Prathapan, 2022). Earlier to this, *Lopholeucaspis japonica* (Cockerell) was reported as an emerging pest of pomegranate in Gujarat (Harsur *et al*. 2018). In south India, a serious infestation of *Myrtaspis ramakrishnai* (Rao) covering the whole trunk and branches of *Syzygium cumini* and *S. calophyllifolium* is a regular sight in homesteads (Sankaran, 1984). Of late, a newly described species *Aulacaspis elettaria* Joshi & Nafeesa was reported causing substantial damage to economically important *Elettaria cardamomum* (L.) Maton (Zingiberaceae) in Idukki high ranges of Kerala (Joshi *et al*., 2023).

The genus *Cycas* (Cycadales: Cycadaceae), with approximately 98 known species, belongs to one of the oldest plant groups. Due to its structure, *Cycas* species are utilized as ornamental plants in urban gardens shaping the landscape of different cities (Valverde, 2015). It is a Red Listed Endangered species (IUCN Red List: Varghese *et al*., 2009) and apart from other reasons, incidence of insect pests is cited as an important factor for its declining population. In south India, young leaves are used as food whereas, mature leaves are used for thatching and during religious and cultural events for decorations. Seeds, pith and male cones are also used as insect repellent (Verghese and Ticktin, 2006).

It is considered as Indian endemic gymnosperm species restricted to the Western Ghats and hills of the southern peninsular, as far north-east as Chennai, in the states of Kerala, Karnataka, Tamil Nadu and Maharashtra (Hill 1995; Hill *et al*., 2003), but it has been claimed that the plant is widely cultivated in Hawaii for its appearance in landscapes and interiors, and for cut foliage (Ruth and Rauch, 1988). However, Lindstrom and Lindstrom stated *C. circinalis* to be native to southern India and Sri Lanka, they further specified that the species name was formerly widely used for similar cycads in Southeast Asia, which leads to confusion about its distribution in the world. The specimens described as *C. circinallis* in Indonesia and New Guinea are now recognized as *C. rumphii*, while the taxon formerly described as the subspecies *C. circinalis* ssp. *riuminiana* from the Philippines is now regarded as a separate species, *C. riuminiana* (Lindstrom *et al*., 2008; Lindstrom *et al*., 2009). The table 1 enlists scale insect pests reported on *C. circinalis* throughout the world (García Morales *et al*., 2016), which also indirectly indicates presence of *C. circinalis* in 16 countries, however, the authenticity of identification of the host plants in these studies has to be confirmed.

Aulacaspis Cockerell is one of the largest genera of armoured scale insects with 151 species known worldwide, and 23 species from India, 13 of which are known from south India. Five species of *Aulacaspis* are associated with *Cycas* spp. throughout the world (García Morales *et al*., 2016). Through this paper, we report another species *Aulacaspis maduinensis* (Zehntner) severely infesting *C. circinalis* in Kerala, though it has been reported to infest other species of *Cycas* from elsewhere (Suh, 2016).

MATERIALS AND METHODS

Collection of insects

Scale insect-infested plant parts were collected and brought to the laboratory. The females of insects were carefully collected by removing the armour and preserved in 70 % ethanol. Parts of the infested plants were kept as such in the containers, each covered with a lid ventilated with wire mesh, for emergence of parasitoids and predators.

Mounting and identification

The field-collected preserved specimens were slide mounted in Canada balsam following Williams and Watson (1988). Morphological terminology also follows the same authors. The species was identified based on the description of *Aulacaspis* spp. attacking mangroves and cycads (Takagi and Faveri, 2009) and grasses and herbs (Takagi, 2015). Observations on the morphology of the slide-mounted female were based on 52 specimens mounted on 4 slides.

Photographic illustration

Photographs of the live coccids were taken using a camera (Leica DFC 420) mounted on a stereozoom microscope (Leica M205A). Slide-mounted adult females were observed through a microscope (Nikon Eclipse 80i) and photomicrographs were captured with a camera (Nikon DSVi1) mounted on the microscope. Figure 1 h, i and j were taken using a 60 mm Micronikon lens mounted on a Nikon D3000 camera body. All the plates were generated using Adobe Photoshop CS2.

Material examination

Kerala: Kozhikode, Vatakara, Chekkiad, 11.731716°N, 75.627268°E, 3 ♀♀ on *Cycas circinalis* (Cycadaceae), 18.viii.2023, Aparna Gokul Coll.; Kerala: Kozhikode, Vatakara, Thuneri, 11.706951°N, 75.629963°E, 4 ♀♀ on *Cycas circinalis* (Cycadaceae), 25.viii.2023, Aparna Gokul Coll.; Kerala: Kozhikode, Vatakara, Mudavantheri, 11.724145°N, 75.630161°E, 6 ♀♀ on *Cycas circinalis* (Cycadaceae), 29.viii.2023, Aparna Gokul Coll.; Kerala: Kannur, Panoor, Chokli, 11.708365°N, 75.572491°E, 39 ♀♀ on *Cycas circinalis* (Cycadaceae), 18.xi.2023, Sachin, K. Coll.

Voucher specimen deposition

Voucher specimens of *A. maduinensis* (accession nos NBAIR/HEM/Aula/180823-1-3; NBAIR/HEM/Aula/250823-4-7; NBAIR/HEM/Aula/290823-8-13; NBAIR/HEM/Aula/181123-14-52) are deposited in ICAR – National Bureau of Agricultural Insect Resources, Bengaluru.

RESULTS AND DISCUSSION

Incidence of *A. madiunensis* was first observed in March 2023 and several farmers from North Kerala brought the news of the incidence and spread of the new insect pest on *Cycas* to our notice. Our field surveys revealed that the infestation of the scale insect began from the base of the rachis and then spread on both the upper and lower surfaces of the leaflets, completely covering it with a crowded population of female and male scale insects (Fig. 1a). Later the infestation spread on megasporophyll and the surface of the nuts (Fig. 1b). In severe cases, the scale insects were seen to gather on the stem covering the whole length of the tree (Fig. 1c). As a result of continuous sap sucking from the parenchyma tissues of different plant parts, the leaflets, nuts, and complete crown started giving a dried appearance (Fig. 1d, e & f). At some locations, the whole crown decayed

which resulted in the death of the trees (Fig. 1g). Five to ten numbers of females and males were found to settle in a 1 cm^2 area of both leaf and bark. The panchayats viz., Chekkiad, Valayam, Tuneri, Nadapuram, Purameri, Kunnumal and Eramala of Kozhikode district and Tripangoottur, Panoor and Kunnothuparambu of Kannur district were severely affected. More than 260 trees were found to be heavily infested in the above locations and 90 percent of the heavily infested trees were more than 50 years old.

Field characters: Adult female cover flat, circular or oval, greyish white (Fig. 1h); shed skin marginal or submarginal, yellowish-brown. Male cover elongate, parallel-sided, bright white (Fig. 1h – marked with arrows), felted, with two longitudinal ridges on submarginal areas; shed skin marginal, yellow or brown. Body of adult female elongate to oval, with reddish brown prosoma and pygidium and pale yellow anterior abdominal segments (Fig. 1i). Marginal gland spine distinctly visible, pale whitish yellow. Eggs reddish yellow (Fig. 1j). Heavy infestation on leaf sheath, petiole, rachis, upper and lower surfaces of leaflets. Females initially crowded and overlapping around midrib of the leaflets and later disperse on the surfaces.

Slide-mounted specimens: Completely grown adult female with swollen prosoma (Fig. 2a), distinctly broader than metathorax, roughly trapezoid, with lateral margins oblique and straight; prosomatic tubercles slightly developed; abdominal segment II strongly produced laterally, as broad as metathorax, abdominal segment I recessed between them. Pygidium triangular, with four pairs of lobes (Fig. 1b). Median lobe sunken into apex of pygidium; mesal margin close together, nearly parallel or a little separated basally, then weakly divergent; each lobe minutely serrated on mesal and lateral margins, rounded apically; basal zygosis in an arch. Lobules

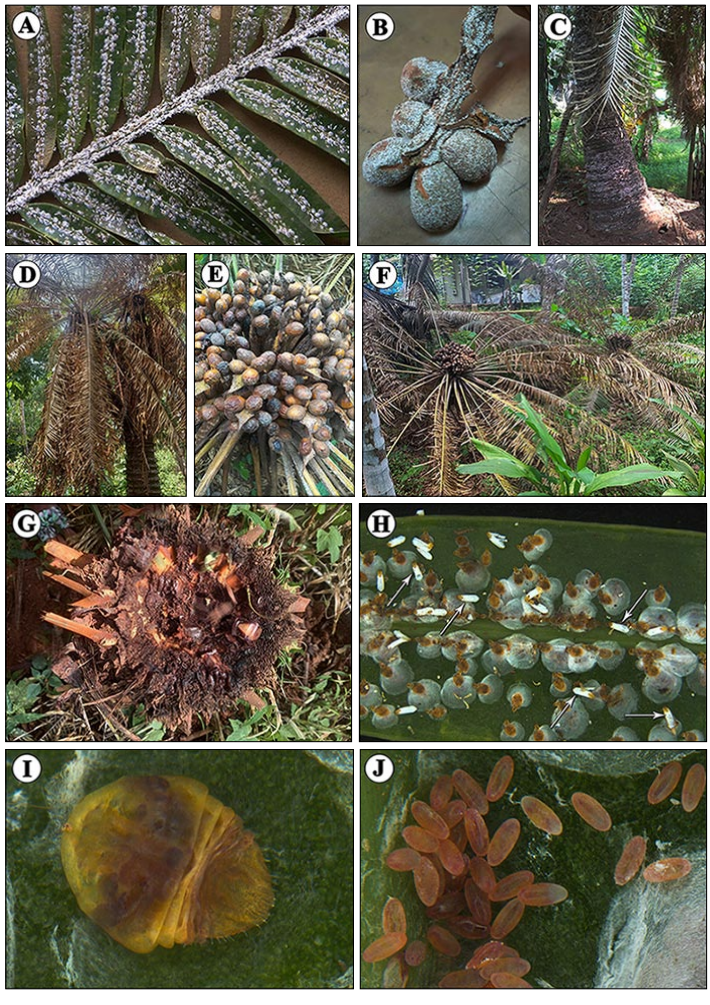

Fig 1. Damage caused by *Aulacaspis madiunensis* (Zehntner): A, Heavily infested leaf; B, infestation on megasporophyll and nuts; C, pest-ridden tree trunk; D, dried foliage; E, dried and shrunken nuts; F, field showing withered trees due to the pest; G, dead crown; H, close-up of females and males; I, mature female with scale cover overturned; J, eggs of the scale insect

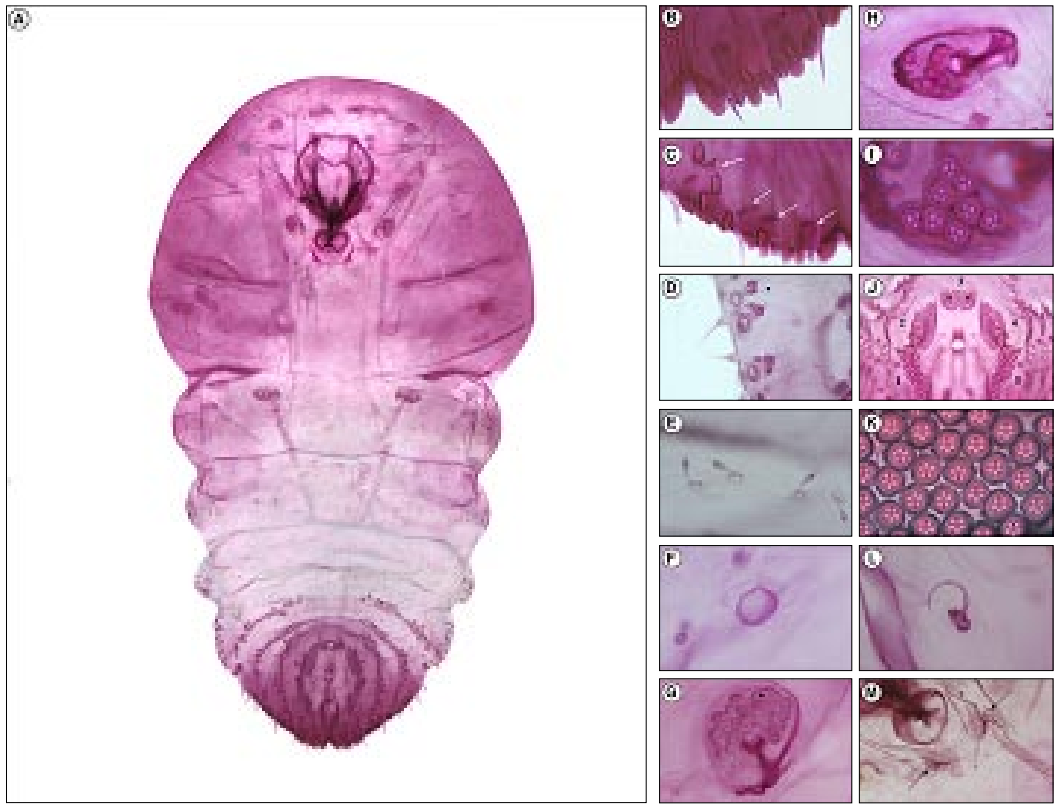

Fig 2. Diagnostic features of *Aulacaspis madiunensis* (Zehntner): A, Female derm; B, dorsal macroducts associated with pygidial margin; C, apex of the pygidium showing lobes and gland spines; D, microducts; E, antenna; F, anterior spiracle; G, close-up of trilocular pores associated with spiracles; H, posterior spiracle; I, perivulvar pores in five groups; J. close-up of perivulvar pores; K. lateral macroducts; L, dorsal boss; M. peribuccal sclerosis

of the second lobe well developed, the lateral a little smaller than the mesal; third lobe with broader lobules Gland spines singly between median and second lobes, between second and third lobes, singly on segment 5 and 6 and numerous around edges of segments 2 to 4. Gland spine formula 1–1–1 (Fig. 1b). Pore prominence (Fig. 2c) on abdominal segment VI produced nearly the same as mesal lobules of third lobe; pore prominence on VII hardly produced to level of apex of mesal lobule of second lobe. Marginal processes occurring laterad of pore prominence on abdominal segment IV and those on V broad, flattish, and serrate. Prepygidial gland spines slender. Dorsal ducts in submedian and submarginal rows (Fig. 1c – marked with arrows) on segments 3 to 5, and in a submarginal group of 3-4 on segment 6 (Fig. 2d). A few smaller ducts on margins of segments 2 and 3. Minute ducts present anteriorly on margins to head (Fig. 2e). Lateral bosses present in some specimens on segments 1 and only in two specimens on segment 6 (Fig. 2f). Anterior spiracle with compact group of 18 – 32 disc pores (Fig. 2g); posterior spiracle with relatively smaller group consisting of 7 – 17 pores (Fig. 2h). Spiracular pores trilocular (Fig. 2i). Perivulvar pores in five groups (Fig. 2j): 21 – 37 in anterolateral group, 20 – 32 in posterolateral group and 14 – 24 in the medial group. Perispiracular pores quinquelocular (Fig. 2k). Microducts in rows on pygidium and on margins of free abdominal segments. Minute ducts present near margins of thorax. Antenna each with a single seta (Fig. 2l). Prebuccal sclerosis well developed (Fig. 2m). Ventral side of abdominal segments II–V with flower-shaped segmental scars for muscle attachment.

In general, characters observed in the material collected from India agree well with the description of the species by Williams and Watson (1988), however, they could observe lateral dorsal bosses on segments 1, 4 and 6, while in the specimens examined by us, dorsal boss was observed mainly on abdominal segment 1 and only in two specimens on abdominal segment 6. In several specimens, bosses were present only on one side of the specimens, indicating this character to be of highly irregular occurrence.

This species can be separated from highly invasive cycad scale *A. yasumatsui* by (characters of *A. yasumatsui* given in the parentheses) (i) two or three macroducts on abdominal segment 6 (1 or 2 with mean 1.1); (ii) well developed peribuccal sclerosis (absent); (iii) anterior

and posterior spiracle with 18-32 and 7-17 perispiracular pores (corresponding number – 10-14 and 6-15); (iv) median, anterolateral and posterolateral groups of perispiracular pores with 14-24, 21-37 and 20-32 pores (corresponding numbers – 12-17, 14-26 and 14-25).

Cycas circinalis L. is attacked by 17 scale insect species throughout the world, of which 12 belong to Diaspididae family, and from India, the soft scale *Saissetia coffeae* (Walker) (Coccidae) and an armoured scale *Poliaspis media* Maskell have been reported earlier (table 1). Table 1 also indicates that, though *C. circinalis* is considered an endemic species to India, the records of natural enemies of scale insects infesting *C. circinalis* have been recorded throughout the world. Either there is erroneous identification of the host plant in those studies or *C. circinalis* should not be considered as an endemic species to India.

No natural enemies were reported in the present study, however, Rao and Sankaran (1969) have reported *Rhyzobius pulchellus* (Montrouzier) (Coccinellidae) as an important predator of this pest from India on sugarcane. Apart from this, seven parasitoids belonging to Aphelinidae and Encyrtidae and a predator belonging to Coccinellidae have been recorded from different parts of the world (García Morales *et al*., 2016). No information on the life cycle of this scale insect is available in the

Table 1. Scale insect species recorded from *Cycas circinalis* from different countries

Scale insect species	Family	Country	Reference
Aulacaspis yasumatsui Takagi	Diaspididae	Britain	Malumphy & Marquart, 2012
Ceroplastes floridensis Comstock	Coccidae	Cuba	Ballou, 1926
Ceroplastes rubens Maskell	Coccidae	Sri Lanka	Vithana, *et al*., 2018
Chrysomphalus aonidum (Linnaeus)	Diaspididae	República Dominicana	Gómez-Menor Ortega, 1941
Chrysomphalus dictyospermi (Morgan)	Diaspididae	República Dominicana	Gómez-Menor Ortega, 1941
		Spain	Martín, 1983
Crypticerya multicicatrices Kondo & Unruh	Monophlebidae	Ecuador	Arias de López *et al*., 2022
Furchadaspis zamiae (Morgan)	Diaspididae	Switzerland	Kozár & Hippe, 1996
Hemiberlesia palmae (Cockerell)	Diaspididae	Tropical South Pacific Region	Williams and Watson, 1988
Lepidosaphes cocculi (Green)	Diaspididae	Micronesia	Takahashi, 1939
Lepidosaphes laterochitinosa Green	Diaspididae	Micronesia	Beardsley, 1966
		Japan	Takagi, 1970
Lindingaspis misrae (Laing)	Diaspididae	California	McKenzie, 1943
Parlatoria proteus (Curtis)	Diaspididae	Hawaii	Nakahara, 1981
Poliaspis media Maskell	Diaspididae	India	Rao and Kumar, 1952
Prococcus acutissimus (Green)	Coccidae	Hawaii	Nakahara, 1981
Pseudaulacaspis cockerelli (Cooley)	Diaspididae	Florida	Dekle, 1965
Saissetia coffeae (Walker)	Coccidae	India	Ali, 1968; Ali, 1971
		Cuba	Ballou, 1926
		Netherlands	Jansen, 1995
		Denmark	Kozarshevskaya & Reitzel, 1975
Selenaspidus articulatus (Morgan)	Diaspidiae	California	McKenzie, 1956

literature. This scale insect has been reported to infest 16 host plants belonging to five families and 12 genera with plants belonging to Poaceae as major host plants (García Morales *et al*., 2016). *Cycas circinalis*, is a new host, however, we could not observe any other host plants with its infestation, in the areas surveyed.

CONCLUSION

Operating a conservation action plan for an endemic *Cycas* population that has become threatened by this new pest attack requires an understanding of a combination of phenomena. Also, it is necessary to know the exact distribution of *C. circinalis* in India and elsewhere in the world. Way back in 1996, the causes of the rapid decline in the population of cycads were enlisted and their conservation strategies were proposed (Pant, 1996), which are valid even today. Apart from *A. madiunensis*, highly invasive *A. yasmatsui* is associated with cycads in several parts of the world, and invasion of this species to different parts of the world is the greatest concern amongst quarantine and plant protection officers, hence rapid identification of armoured scales in every newly infested location should be conducted by an expert taxonomist to rule out additional species involved in the damage caused to various species of cycads. Several pesticides are being recommended for the containment of this pest, however, farmers are hesitant due to the expenditure involved. Because of the immense seriousness of this pest in cycad-growing areas of India, it would be desirable to conduct surveys, record natural enemies, and study the life cycle for future use in the fields to develop an appropriate management action plan. Publicizing new outbreaks is essential to enable the most effective response from the plant protection agencies.

Characters of the scale insect in the field and the mounted condition described, and key to the Indian species of *Aulacaspis* discussed herein will be helpful in quick diagnosis of the pest, monitoring its spread to different geographical areas, and generation of awareness about the pest species to avoid further economic loss.

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Key to adult females of the Indian species of *Aulacaspis*

Key to the species of *Aulacaspis* from India has recently been published by Joshi *et al.* (2023). As this paper is not easily accessible to Indian researchers, we are reproducing the key with the permission of the copyright holder ©Magnolia Press, Auckland, New Zealand.

Note: Although recorded from India, *A. malayala* Varshney has not been included in this key because, in his checklist of South Asian scale insects, Varshney (2002) named this 'sp. nov.' based on a brief comment added by Takagi & Williams (1998) to their notes on *A. vitis* and related forms. He designated no specimen as the holotype, nor any specimens as a type series, and since there was no indication of where his material was deposited, the species is considered a *nomen nudum* (García Morales *et al*., 2016). The key below is derived from keys to the species of *Aulacaspis* in Wei *et al.* (2016) and Tian & Xing (2022). In the present key, *A. yasmatsui* has been classified under the species with swollen prosoma as per the original description by Takagi (1977), however, later Takagi and Faveri (2009) discussed variation in this species and illustrated *A. yasmatsui* without swollen prosoma.

1(0) Abdominal segment VI without dorsal macroducts 2
- Abdominal segment VI with dorsal macroducts 3

2(1) Abdominal segment II with dorsal macroducts and segment III with submedian macroducts ..... *A. australis* Brimblecombe
- Abdominal segment II without dorsal macroducts and segment III without submedian macroducts ..... *A. litzeae* (Green)

3(1) Prosoma not swollen 4
- Prosoma swollen 9

4(3) Body shape unique, narrowly oblong, with an incompletely formed prosoma in which mesothorax is clearly demarcated from fused head and prothorax ..... *A. loranthi* (Green)
- Body shape not as above 5

5(4) Abdominal segment III with only 1 submedial macroduct on each side ..... *A. hedyotidis* (Green)
- Abdominal segment III with 3 or more submedial macroducts on each side ……..... 6

6(5) Posterior spiracle associated with disc pores encircling anterior, lateral and posterior parts of peritreme. Abdominal segment II with dorsal macroducts ..... *A. elettaria* Joshi & Nafeesa

- Posterior spiracular pores present only anteriorly or laterally or anterolaterally to peritreme 7

7(6) Posterior spiracle with 1–4 associated disc-pores situated anterior to spiracle. Median lobes (L1) rounded, with basal zygosis represented by a pair of small sclerotizations ..... *A. anaimala* Takagi
- Posterior spiracle with associated pores situated lateral to spiracle. Shapes of median lobes and their basal zygoses various 8

8(7) Posterior spiracle with 6–20 associated disc-pores in a compact lateral group. Median lobes (L1) slender, narrowly separated, with basal zygosis horseshoe shaped and produced anteriorly beyond bases of L1 ..... *A. malabarica* Takagi
- Posterior spiracle with only 5–8 associated disc-pores in a lateral group. Median lobes widely separated; specimens from twigs with basal zygosis horseshoe shaped but specimens from leaves with basal zygosis represented by a pair of small sclerotized patches ..... *A. nilagirica* Takagi

9(3) Posterior spiracle without associated disc-pores .. *A. vitis* (Green)
- Posterior spiracle with associated disc-pores 10

10(9) Prosoma without lateral tubercles 11
- Prosoma with lateral tubercles 16

11(10) Pygidial margin with pore prominences poorly developed. Short dorsal microducts present in submarginal band around free abdominal segments and on prothorax ..... *A. tegalensis* (Zehntner)
- Pygidial margin with pore prominences well developed. Dorsal microducts not distributed as above 12

12(11) Abdominal segment II with submedial macroducts ..... *A. litseae* Tang
- Abdominal segment II without submedial macroducts 13

13(12) Submedial macroducts numbering fewer than 3 on abdominal segment III ..... *A. herbae* (Green)
- Submedial macroducts numbering more than 3 on abdominal segment III 14

14(13) Lateral macroducts on abdominal segment II numbering more than 4 ..... *A. rosae* (Bouché)
- Lateral macroducts on abdominal segment II numbering fewer than 4 15

15(14) Derm remaining membranous except for pygidium and small patches on cephalothorax, even in very mature specimens. Perivulvar pores numbering 37–57 on each side of body ..... *A. yasumatsui* Takagi
- Complete prosoma tending to be sclerotized with maturity, with many small patches. Perivulvar pores numbering 35–77 on each side ..... *A. litsearum* Takagi

16(10) Submedian macroducts absent from abdominal segment II 17
- Submedian macroducts present on abdominal segment II 19

17(16) Posterior spiracle associated with up to 25 spiracular pores ..... *A. gudalura* (Green)
- Posterior spiracle associated with no more than 12 spiracular pores 18

18 (17) Peribuccal scleroses not formed ..... *A. ferrisi* Scott
- Peribuccal scleroses well developed ..... *A. madiunensis* (Zehntner)

19(16) With 4 or more macroducts on abdominal segment VI 20
- With 1 or 2 macroducts on abdominal segment VI 21

20(19) Abdominal segment VI with about 8 macroducts. Total number of perivulvar disc-pores 136–245 . ..... *A. crawii* (Cockerell)
- Abdominal segment VI with about 4 macroducts. Total number of perivulvar disc-pores 95–125 ... . *A. rosarum* Borschsenius

21(20) Median lobes (L1) robust and produced but sunken into pygidium, strongly divergent from bases linked by a strong zygosis; lobes with mesad margins parallel sided ..... *A. tubercularis* Newstead
- Median lobes (L1) small, partly recessed, divergent and bluntly pointed [further details not available] ..... *A. orientalis* (Green)

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Management of thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) in mango using botanicals: a multilocation study

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ABSTRACT: Over the past decade, thrips *Scirtothrips dorsalis* Hood has emerged as a significant pest affecting mango cultivation across India. Multilocation field studies were carried out at six major mango belts in India, *viz.,* Bengaluru (Karnataka), Mohanpur (West Bengal), Paria (Gujarat), Rahuri (Maharashtra), Sangareddy (Telangana), and Vengurla (Maharashtra) to manage mango thrips using botanicals from 2019-20 to 2021-22. The findings indicated that in Karnataka, Gujarat, and Maharashtra, the application of neem soap spray at a concentration of 10g/l proved to be the most effective, while in West Bengal and Telangana, the treatment with azadirachtin at 10,000 ppm and a concentration of 3g/l was identified as the most effective.

Keywords: Botanicals, mango, *Scirtothrips dorsalis*, thrips.

INTRODUCTION

The mango crop is found to be infested by more than 100 insect and non-insect pests (Butani, 1962; Tondon and Verghese, 1985; Chavan *et al*., 2009; Narangalkar *et al*., 2018). Among these pests, thrips, a group of sucking insects, have emerged as a significant threat to fruit crops, causing substantial yield losses (Sithanantham *et al*., 2007; Kumar *et al*., 1994; Reddy *et al*., 2019; Munj *et al*., 2020). Although thrips were considered minor pests of mango until the last decade, their status has transitioned to major pests in recent years due to the excessive use of synthetic insecticides (Chavan *et al*., 2009; Munj *et al*., 2012; Patel *et al*., 2013; Bana *et al*., 2015; Munj *et al*., 2020). Notably, three new species of thrips, *Thrips florum* Schmutz (inflorescence and fruits), *Bathrips jasminae* Ananthakrishnan (leaves), and *Haplothrips ganglbaueri* Ananthakrishnan (inflorescence), have been reported infesting various parts of mango plants (Reddy *et al*., 2020). Utilizing their rasping and sucking mouthparts, thrips inflict damage on tender plant parts, such as leaves, flower buds, flowers, panicle raches, and fruits. This leads to blackening of leaf veins, a dusty appearance of affected leaves, weakness, leaf curl, and eventual leaf fall. Thrips also cause browning and shedding of flower buds and flowers, resulting in reduced fruit set (Pena *et al*., 1998; Grove *et al*., 2000; Munj *et al*., 2012; Salvi *et al*., 2018; Reddy *et al*., 2020; Munj *et al*., 2020; Patel *et al*., 2020). Mango fruit epidermis laceration by thrips leads to the development of grey-colored patches on fruits (Chavan *et al*., 2009; Munj *et al*., 2012; Gawade *et al*., 2014; Munj *et al*., 2020).

Various synthetic insecticides have been recommended for mango thrips management, along with bio-pesticides such as *Beauveria bassiana* and *Metarhizium anisopliae* (Kumar *et al*., 1994; Munj *et al*., 2012; Patel *et al*., 2013; Gawade *et al*., 2014; Bana *et al*., 2015; Reddy *et al*., 2019; Munj *et al*., 2020). Additionally, botanicals like neem seed kernel extract and neem oil have been suggested for effective thrips management (Aliakbarpour *et al*., 2011; Gundappa and Shukla, 2020). However, to assess their efficacy against mango thrips, it was imperative to evaluate different botanicals, leading to the initiation of the present multi-location study.

MATERIAL AND METHODS

The field experiments were conducted at six prominent research centers, namely the Indian Institute of Horticulture Research in Bengaluru (Karnataka), Bidhan Chandra Krishi Vishvavidyalaya in Mohanpur (West Bengal), Agriculture Experiment Station in Paria

(Gujarat), Mahatma Phule Krishi Vidyapeeth in Rahuri (Maharashtra), Fruit Research Station in Sangareddy (Telangana), and Regional Fruit Research Station in Vengurla (Maharashtra). These experiments were carried out as part of the All India Co-ordinated Research Project on Fruits from 2019-20 to 2021-22. The primary objective was to assess the effectiveness of various botanicals in managing thrips infestations on mango crops. The experiments were designed using a randomized block design, with three replications and seven treatments. Details of the treatments are provided in Table 1.

Spray schedule:

1st Spray: At panicle initiation stage

2nd spray:15 days after 1st spray

3rd spray:15 days after 2nd spray

4th spray:15 days after 3rd spray (need based)

5th spray:15 days after 4th spray (need based)

Observations

The pre-treatment pest population was counted a day before spray and the post treatment pest population was counted seven days after each spray. For counting thrips, the panicles were given single tap on a plain paper and the fallen thrips were counted with the help of hand lance. At the time of harvesting, the fruit yield per tree was recorded and the B:C ratio was worked out.

RESULTS AND DISCUSSION

The data collected on the management of mango thrips across the years 2019-20, 2020-21, and 2021-22 were pooled and subjected to analysis (Table 2). The results indicate the significant effectiveness of all treatments in thrips management. Pre-treatment observations made a day before insecticide application were statistically non-significant, suggesting a uniform pest population throughout the experimental area. Notably, the treatment with the standard check (T_6) consistently exhibited the lowest thrips population across all centers, with a population of 1.85 thrips/panicle in Bengaluru, 1.41 in Mohanpur, 2.32 in Paria, 0.33 in Rahuri, 1.50 in Sangareddy, and 0.03 in Vengurla. Among the various botanical treatments, the application of azadirachtin (T_1) emerged as the most effective in reducing thrips populations at Paria and Vengurla. In Paria, T_1 recorded 3.73 thrips/panicle seven days after the last spray, while in Vengurla, T_1 recorded 2.83 thrips/panicle. Treatment T_1 outperformed all botanical treatments significantly in Paria, while in Vengurla, it was comparable to T_3. At Mohanpur, Rahuri, and Sangareddy, T_5 (T_1 followed by T_3 followed by T_4 followed by T_2) demonstrated the highest efficacy in managing thrips, with population figures of 2.86, 1.13, and 2.25/panicle, respectively.

In Bengaluru, T_3 was found to be the most effective treatment for mango thrips management (2.45 thrips/panicle), standing on par with T_5, T_1, and T_4. Conversely, the untreated control exhibited a significantly high thrips population seven days after the last spray across all centers: Bengaluru (20.47), Mohanpur (6.19), Paria (11.90), Rahuri (11.43), Sangareddy (10.75), and Vengurla (10.73). Examining the mean percentage reduction in thrips population seven days after the last spray (Table 3), the standard check consistently recorded the highest reduction percentage at all centers. Among the botanical treatments, T_3 exhibited the highest percentage reduction in Bengaluru (88.04%). At Mohanpur, Paria, and Vengurla, T_1 demonstrated the highest reduction

Table 1.Treatment details

Treatment No.	Treatment details
T_1	Azadirachtin 10,000 ppm @3 ml/l of water
T_2	Botanical formulation "AAVYA" @ 4 g/l of water
T_3	Neem soap (IIHR product) @ 10g/l of water
T_4	Pongamia soap (IIHR product) @ 10g/l of water
T_5	T_1 followed by T_3 followed by T_4 followed by T_2
T_6	As per University/Institute recommendation for sucking pest (Standard check)
T_7	Control

Table 2. Efficacy of botanicals against mango thrips at different centres (2021-22)

Treatments	Number of thrips /tap/panicle											
	Bengaluru		Mohanpur		Paria		Rahuri		Sangareddy		Vengurla	
	Pre count	7 days after last spray	Pre count	7 days after last spray	Pre count	7 days after last spray	Pre count	7 days after last spray	Pre count	7 days after last spray	Pre count	7 days after last spray
T_1- Azadirachtin	13.28 (3.64)	2.98 (1.72)	7.14 (2.85)	2.89 (1.97)	7.09 (2.65)	3.73 (1.92)	8.63 (3.01)	3.13 (1.89)	8.25 (3.03)	2.75 (1.91)	9.13 (3.18)	2.83 (1.95)
T_2-"AAVYA"	15.48 (3.93)	5.25 (2.29)	6.68 (2.77)	5.20 (2.49)	6.55 (2.53)	7.38 (2.71)	9.05 (3.08)	2.40 (1.70)	8.37 (3.06)	2.50 (1.87)	8.90 (3.14)	4.17 (2.27)
T_3-Neem soap	14.26 (3.77)	2.45 (1.56)	6.59 (2.76)	2.97 (1.99)	5.36 (2.28)	6.04 (2.46)	8.80 (2.96)	1.23 (1.31)	8.25 (3.03)	3.12 (2.20)	9.93 (3.30)	3.00 (2.00)
T_4-Pongamia soap	15.67 (3.95)	3.02 (1.73)	6.87 (2.80)	3.43 (2.11)	6.90 (2.61)	6.35 (2.52)	7.90 (2.89)	2.05 (1.59)	8.62 (3.10)	3.00 (1.99)	10.67 (3.41)	4.47 (2.33)
T_5 -(T_1+T_3+T_4+T_2)	16.34 (4.04)	2.84 (1.68)	7.16 (2.86)	2.86 (1.97)	6.50 (2.54)	5.41 (2.32)	9.15 (3.10)	1.13 (1.27)	8.25 (3.03)	2.25 (1.79)	9.63 (3.26)	4.10 (2.26)
T_6-Standard check	14.27 (3.77)	1.85 (1.36)	6.93 (2.82)	1.41 (1.54)	7.26 (2.67)	2.32 (1.52)	8.40 (2.98)	0.33 (0.91)	8.12 (3.01)	1.50 (1.57)	8.83 (3.13)	0.03 (1.01)
T_7-Control	15.24 (3.90)	20.47 (4.52)	7.19 (2.86)	6.16 (2.68)	6.06 (2.43)	11.90 (3.45)	9.03 (3.08)	11.43 (3.45)	8.12 (3.01)	10.75 (3.42)	10.60 (3.40)	10.73 (3.44)
SEm±	-	0.11	-	0.07	-	0.05	-	0.05	-	**0.13**	-	0.07
CD at 5%	NS	0.33	NS	0.28	NS	0.15	NS	0.16	NS	**0.3**8	NS	0.20

*figures in parenthesis are square root transformed values

Table 3. Mean per cent reduction in thrips population (Pooled)

Treatments	Mean per cent reduction in thrips population					
	Bengaluru	Mohanpur	Paria	Rahuri	Sangareddy	Vengurla
T_1: Azadirachtin	85.44	69.43	68.68	70.86	71.63	84.40
T_2: AAVYA"	74.38	27.25	38.03	72.75	73.90	74.48
T_3: Neem soap	88.04	62.73	49.29	78.41	73.07	79.67
T_4: Pongamia soap	85.26	56.93	46.62	71.99	73.40	73.26
T_5: (T_1+T_3+T_4+T_2)	86.14	64.17	54.56	78.29	77.07	75.76
T_6 : Standard check	91.00	76.87	80.50	87.48	88.45	99.66
T_7: Control	-	-	-	-	-	-

Table 4. Yield recorded at different centers (Pooled)

Treatments	Yield (kg/tree)					
	Bengaluru	Mohanpur	Paria	Rahuri	Sangareddy	Vengurla
T_1: Azadirachtin	81.95	94.73	43.15	24.78	21.08	21.18
T_2: AAVYA"	67.84	71.53	24.17	22.79	22.39	17.21
T_3: Neem soap	80.54	79.96	34.01	31.53	18.11	19.43
T_4: Pongamia soap	78.32	73.54	28.28	26.16	17.29	16.45
T_5: (T_1+T_3+T_4+T_2)	78.54	88.00	27.84	32.78	23.85	17.05
T_6 : Standard check	84.21	97.63	51.53	42.28	25.23	26.96
T_7: Control	32.15	63.14	11.65	19.22	11.01	13.66
S.E	1.81	2.74	3.06	1.17	0.55	0.59
C.D	5.43	8.19	9.10	3.46	2.11	1.82

Table 5. Benefit- cost (B:C) ratio (Pooled)

Treatments	B.C. ratio					
	Bengaluru	Mohanpur	Paria	Rahuri	Sangareddy	Vengurla
T_1-Azadirachtin	2.55	2.68	1.71	1.57	2.40	1.38
T_2-"AAVYA"	1.14	1.88	1.58	1.52	2.15	1.35
T_3-Neem soap	3.46	2.19	1.80	2.04	1.19	1.42
T_4-Pongamia soap	2.87	2.04	1.61	1.69	1.27	1.23
T_5-(T_1+T_3+T_4+T_2)	2.50	2.52	1.62	1.90	1.99	1.29
T_6-Standard check	3.10	3.03	3.85	2.36	4.74	2.27
T_7-Control	-	-	-	-	-	-

percentages, recording 69.43%, 68.68%, and 84.40%, respectively. Rahuri and Sangareddy saw the maximum reduction with T_5 (78.29% and 77.07%, respectively).

The marketable fruit yield at harvest (Table 4) revealed that the standard check treatment (T6) consistently produced the highest fruit yield at all centers. Among the botanical treatments, T1 recorded the maximum fruit yield in Bengaluru (81.95 kg/tree), Mohanpur (94.73 kg/tree), Paria (43.15 kg/tree), and Vengurla (21.18 kg/tree). However, T_1 was comparable to other treatments in some centers, indicating its effectiveness. Economic analysis and the calculation of the Benefit-Cost (B: C) ratio (Table 5) showed that the standard check treatment (T_6) consistently yielded the highest B: C ratio at all centers except Bengaluru. Among the botanical treatments, T_3 exhibited a higher B:C ratio in Bengaluru (3.46), Paria (1.80), Rahuri (2.04), and Vengurla (1.42). In Mohanpur and Sangareddy, T_1 had a higher B: C ratio (2.68 and 2.40, respectively). These findings indicate that for effective mango thrips management with botanicals, the treatment involving neem soap (IIHR product) at 10g/l (five sprays at 15-day intervals, starting from panicle initiation) is most effective in Bengaluru, Paria, Rahuri, and Vengurla. Conversely, for Mohanpur and Sangareddy, the treatment of 10,000 ppm azadirachtin at 3ml/l proves most effective. Similar results have been reported by Aliakbarpour *et al*. (2011), Bana *et al*. (2015), and Gundappa and Shukla (2020).

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ICT-based surveillance of hoppers and thrips in mango orchards of Maharashtra, India

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ABSTRACT: The present global mango production faces various kinds of environmental and ecological fluctuations including biotic and abiotic stresses. Therefore in order to develop suitable management techniques, it is essential to have thorough understanding of the population dynamics and damage potential of the mango pests. Hence the present experiment was carried out to study the influence of particular seasonal months on the incidence of mango hoppers and thrips under field conditions. The highest degree of infestation by hoppers was detected in March (22.210 hoppers per shoot/panicle), followed by February (21.336) and January (18.863). The peak prevalence of the thrips were observed in the month of February (26 - 50% of fruit area damaged) followed by January and March (1 - 25% of fruit area damaged). The occurrence and seasonal prevalence data generated from the present study can be used to manage the population of hoppers and thrips on mango and this study have far-reaching implications in pest management strategy and the data so generated would help in the forecast of hoppers and thrips.

Keywords: Mango, ICT, seasonal prevalence, hoppers, thrips, forecast, pest management

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important tropical fruits in the world. Mango output in the world is anticipated to be more than 26 million tonnes per year, with India leading the way with 40% of total mango production (APEDA, 2020). During the vegetative and reproductive stages, more than 300 insect pest species plague mango crops globally (Pena *et al.,* 1998), with 188 of these documented from India (Tandon and Verghese, 1985). Mango hoppers are a significant, serious and ubiquitous insect problem in the Indian Mango ecosystem throughout the year. Hoppers species, especially *Idioscopus clypealis* (Lethierry), *Idioscopus nitidulus* (Walker) and *Amritodus atkinsoni* (Lethierry), remain active and cause damage at all stages of mango starting from fresh flush emergence to flowering, fruiting and harvesting results in losses of up to 100 per cent (Gundappa *et al.,* 2015; Babu *et al.,* 2002).). Both nymphs and adults of hoppers have been recorded sucking cell sap from young leaves, fragile shoots, inflorescences or panicles and the rachis of young fruits, resulting in the dropping of immature fruits. Hoppers also produce a lot of honeydew, which causes sooty mould to grow and as a result, hinders plant photosynthesis (Kumar *et al*., 2014).

Thrips are a growing hazard to mango production, causing significant economic loss in mango orchards. *Scirtothrips dorsalis* and *Thrips hawaiiensis* have been identified as serious pests of several vegetables, fruits and ornamental crops in Eastern Asia (Reynaud *et al.,* 2008). *Thrips palmi* was found on mango inflorescences in India (Verghese *et al.,* 1988) and three thrips species, *Megalurothrips distalis* (Karny), *Thrips hawaiiensis* and *Haplothrips tenuipennis* have been found on mango in Andhra Pradesh (Kannan and Rao, 2006). Kumar and Bhatt (1999) found two species of thrips, *Scirtothrips mangiferae* and *S. dorsalis* on mango in Gujarat. *Scirtothrips mangiferae*, *S. dorsalis* and *Rhipiphorothrips cruentatus* were found on mango by Kumar *et al* (2002) in Cuttack. *R. cruentatus* was discovered in Haryana by Dahiya and Lakra (2001). Patel *et al.* (1997) discovered three thrips species on mango rootstock: *Pantachaetothrips* sp., *Selenothrips rubrocinctus* and *Caliothrips impurus* in addition to *Scirtothrips dorsalis*. *S. rubrocinctus* was found on mango by Ananthakrishnan and Muraleedharan (Ananthakrishnan and Muraleedharan, 1974). Recently *Frankliniella schultzei* and *Thrips subnudula* were found on mango inflorescence from Tamil Nadu (Krishnamoorthy *et al.,* 2012).

To design an early warning weather-based system for any pest in a given agro-ecosystem, a basic understanding of pest population dynamics and seasonal occurrence in relation to the most prevalent weather parameters is required. This will make it simpler to decide when to act and how to apply the best pest management techniques. It is well known that weather variations have a significant impact on pest dynamics, making site-

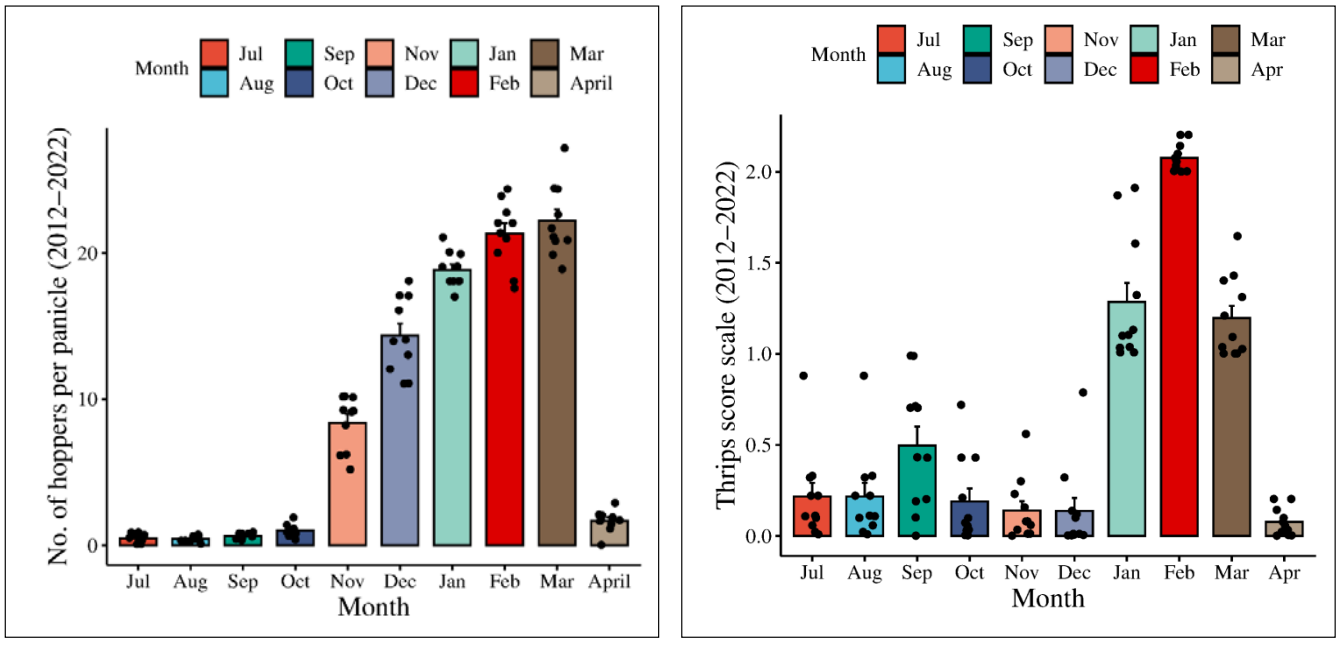

Fig. 1. Seasonal activity and damage of hoppers (top) and thrips (bottom) in ango (2012-2022)

specific research even more important. That is the reason why this current study was carried out to investigate the seasonal occurrence of mango hoppers and thrips. Based on work carried out between 2012 and 2022, we have compiled an inventory of hoppers and thrips prevalence on mango trees in Maharashtra. This is a step in studying the seasonal occurrence of mango hoppers and thrips in order to develop appropriate defense strategies against the pests, thereby contributing to increased Mango production.

MATERIALS AND METHODS

The investigation was carried out during the crop-growing seasons from 2012 to 2022. The pest surveillance programme was implemented in the four districts of Maharashtra, namely Aurangabad, Beed, Osmanabad, Raigad, Ratnagiri, Shindhudurga and Thane and it was made possible through the use of information technology, which aided in the development of an e- pest surveillance programme by recording pest occurrence data with the assistance of scouts and pest monitors employed by the Department of Horticulture, Govt. of Maharashtra.

A system based on three-tier architecture was developed, consisting of three functional components, namely a mobile app for data collection, a central database and a web-based pest reporting and consulting application. This system was designed with the challenges of pest monitoring and internet connectivity in remote areas of the state in mind. The pest observers were trained to capture pest observations in farmers' fields via a mobile app. The app has the built-in feature to automatically sync the collected data to the central database of the National Research Center for Integrated Pest Management in New Delhi once the device enters an internet-connected area.

For pest monitoring, data formats were developed in consultation with plant experts to record pest observations in the fields. Field location details and pest information were key components of these data formats that were integrated into the mobile app. Each field was assigned a unique ID and its geographic coordinates were also recorded by the mobile app while pest information was collected from the field. Pest experts from state agencies reviewed the pest reports generated by the web-based reporting and advisory application for the collected data and submitted the appropriate pest management decisions to the system. SQL 2012, ASP.net, Android Studio and XML technologies were used to create the system (Ahuja and Chattopadhyay, 2015).

The orchards were selected one each on hill slope and on plane for fixed and random survey. From each selected orchard, randomly 4 trees were selected, and on each selected trees, 5 shoots/panicles were observed randomly for recording observation of hoppers. Two fixed orchards and two random orchards were selected by one scout who covers two villages per day. Observations on hoppers were recorded in structured sheet which was prepared for the pest scouts. Five shoots/panicles were selected per tree, one from each direction and centre of selected tree. Weekly observation on number of both nymphs and adults were recorded on selected shoots or panicles. Mean hoppers per shoot/panicle were calculated as Total

No. of values/20. Total number of fruits from pea nut stage onwards are recorded per shoot/ panicle selected.

From each selected orchard, 4 trees were randomly selected and from each selected tree, 5 shoots/panicles were randomly observed to record observation of hoppers. Two fixed orchards were selected by a scout covering two villages per day. Observations on hoppers were recorded in structured sheet which was prepared for the pest scouts. Five shoots/panicles were selected per tree, one from each direction and one from centre of the selected tree. Weekly observations of both nymph and adult numbers were recorded on selected shoots or panicles. Mean hoppers per shoot/panicle were calculated as Total No. of values/20. Total number of fruits from pea nut stage onwards are recorded per shoot/ panicle selected. Observations on the thrips population are recorded by tapping the panicles on white paper. At the beginning of fruit formation, damage caused by thrips on the fruit is assessed. Based on the surface area of the fruit damaged by thrips, the fruit observed is ranked on a scale from 0 to 4, as described below. Mean thrips per shoot/ panicle per plant (total number of scores / 20). The thrips scoring scale used was; 0 - healthy fruit, 1 - 1 - 25% of fruit area damaged, 2 - 26 - 50% of fruit area damaged, 3 - 51 - 75% of fruit area damaged, 4 - 76% and more of fruit area damaged (Ahuja and Chattopadhyay, 2015).

The statistical analysis was based on the seasonal incidence data of hoppers and thrips collected during the study period from 2012 to 2022. The data generated were subjected to an analysis of variance (ANOVA) and the statistical procedures were performed using the R program. Since it turned out that the seasonal mean incidence values of one or more months in the study years 2012 to 2022 were not similar, we performed the Shapiro-Wilk normality test. The change in pest infestation scenario was visualized with bar charts and a circular heat map using the R program. Total infestation percentage and average seasonal frequency figures were plotted using Google Colab by examining the Matplotlib library of Python program.

RESULTS AND DISCUSSION

Trends in Seasonal incidence of mango hoppers: The results showed a significant difference between the hoppers that infested mango orchards in the seasonal months from 2012 to 2022. The data revealed that the highest infestation of mango hoppers was recorded in the month of March with an average of 22.210 hoppers per shoot/panicle followed by February (21.336 hoppers per shoot/panicle). A significant infestation was observed in the month of January (18.863) and December (14.364) (Fig. 1A, Fig. 2A and Table. 1). In terms of per cent incidence, mango hoppers showed their peak incidence in March (24.5%) followed by February (23.5%). The total percentage of hoppers infestation in the months of July, August, September, October, November, December, January, April ranged from 0.9 to 20.8% for the years under study (Fig. 3A). Several previous studies also reported that the incidence of mango hopper reached its peak (12.41 hoppers/panicle) during 4th week of March (Gundappa *et al.,* 2016) and among the three species of mango hoppers, *Idioscopus clypealis* and *I. nitidulus* were the dominating species during full bloom period (January to March) where *Amrasca splendens* was also active during marble and stone sized fruit stage of the

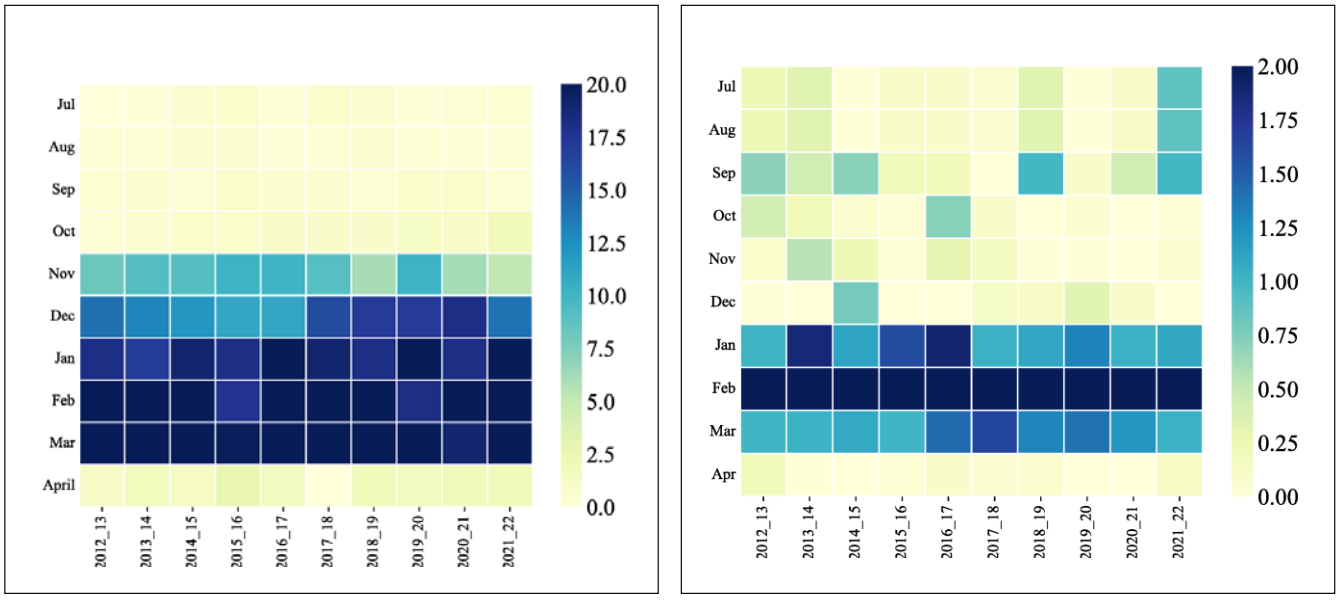

Fig. 2. Heat map depicting the seasonal abundance of hoppers and thrips in Mango (2012-2022)

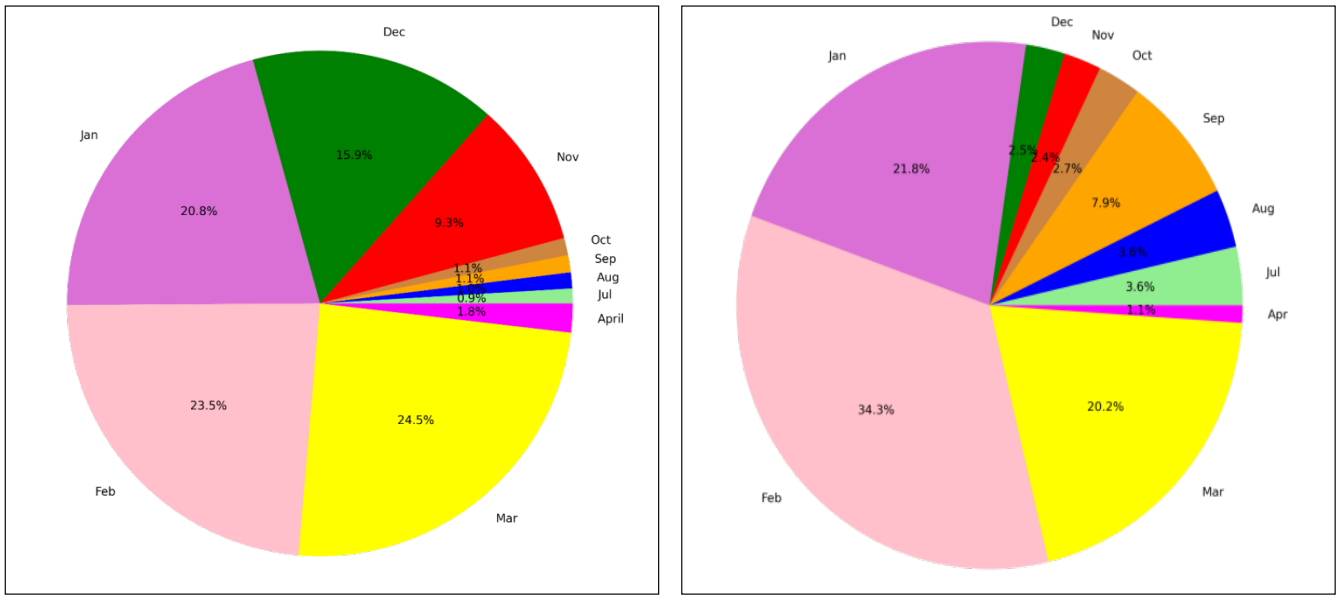

Fig. 3. Trend in percentage incidence of mango hoppers and thrips in Mango (2012-2022)

crop (March–April) (Bana *et al.,* 2018). Mango hoppers appeared during last week of October with 1.70 hoppers per twig/panicle. The hoppers population continued to build up and attained peak incidence (12.38/twig/panicle) during third week of February at flowering stage. Declining trend of hoppers population was recorded from last week of February, when tree enters in fruiting stage. The lowest hopper population (0.13/twig/panicle) was recorded during first week of April at fruit maturity stage (Anant *et al.,* 2019).

Results of our current investigation are in line with Kadavkar *et al.,* (2021) in which they revealed that incidence of the mango hopper was noticed from month of September corresponding to 36 M.W, (1.19 hoppers/panicle) and the peak incidence of mango hopper was found to be in the 49th M.W i.e. (12.31 hoppers/panicle) whereas the first minimum appearance of hoppers population were 13.75 and 13.40 on the branches/tree trunk of mango trees recorded in September. Peak hoppers population were 146.65 and 137.00 on the inflorescence of mango tree in second fortnight of March (Kaushik and Nirmalkar, 2021) and in the similar study, incidence of hopper population was noted from 4th week of January which reaches its peak (80 hoppers/20 panicles) during fourth week of February corresponding with 8th standard week. The hopper population declined from second week of March which disappeared during second week of May (Chaudhari *et al.,* 2017).

Trends in Seasonal incidence of thrips: The variation in the incidence of thrips on the mango crop over seasonal months from 2012 to 2022 showed significant differences and the data revealed that the highest infestation of thrips were recorded in the month of February (26 - 50% of fruit area damaged) followed by January (1 - 25% of fruit area damaged). A significant infestation of thrips were observed with 1 - 25% of fruit area damaged in the month of March whereas no significant differences were recorded during the other months viz., August, September, October, November, December and April (Fig. 1B, Fig. 2B and Table. 1). Furthermore the incidence in terms of overall percentage by thrips observed to be highest in the month of February (34.3%) and January (21.8%) followed by March (20.2%) (Fig. 3C). The overall percentage infestation of moths in the month of July, August, September, October, November, December and April ranged from 1.1-7.9% for the years under study. Similar studies on the seasonal incidence of mango thrips revealed that appearance of thrips started from the third week of January. Thereafter, the thrips population increased continuously till the last week of March and reached to peak at 13th standard week. The maximum population was recorded during 11th to 14th standard week, after which the population declined (Patel and Shukla, 2021). One another similar investigation with eight years study from 2012-19 on mango cv. Kesar, Alphonso and Amrapali revealed two peaks of incidence of thrips, first during flowering-cum-fruit setting stages and the second during new flush stage (Bana *et al.,* 2021). The maximum population of thrips observed on flower (43.33/panicle) and foliage (29.46/twig) on 15th and 42nd SW coinciding with stone sized fruit and emergence of new flush stages respectively (Patel *et al.,* 2018).

In conclusion, the information on the seasonal incidence of any insect pest in a specific ecological niche

Table 1. Trends in seasonal incidence of mango hoppers and thrips in a particular month of mango growing seasons of the years from 2012 to 2022 in the state of Maharashtra, India

Month	Mango hoppers	Thrips
July	0.477 [e]	0.215 [cd]
August	0.413 [e]	0.215 [cd]
September	0.627 [e]	0.475 [c]
October	1.000 [e]	0.165 [cd]
November	8.390 [d]	0.144 [cd]
December	14.364 [c]	0.149 [cd]
January	18.863 [b]	1.312 [b]
February	21.336 [a]	2.064 [a]
March	22.210 [a]	1.216 [b]
April	1.673 [e]	0.064 [d]

Note: Means in the same row followed by the same letters are not significantly different (P>0.05) using the Shapiro-Wilk normality test.

should be taken into consideration for the development of an eco-friendly pest management programme. Although similar studies on population dynamics have been conducted elsewhere but this is the first of its kind in the state of Maharashtra, exploring continuous pest monitoring over the seasons from 2012 to 2022. The current study compared the occurrence and severity of hoppers and thrips on Mango. The data so generated would help in the forecast of the pests and prove helpful in devising an effective strategy for their management.

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AUTHOR CONTRIBUTIONS

Niranjan Singh and Devaramane Raghavendra contributed to experimentation, data collection and original draft writing; Ramesh K. B analyzed the data, wrote the original draft and edited the manuscript; Subhash Chander edited the manuscript. All authors have read and agreed to the published version of the manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Diversity and damage of weevil species on fruit crops in Punjab, India

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ABSTRACT: Surveys and surveillances were carried out during 2004 to 2022 to record the biodiversity of weevil species on the fruit crops in the Punjab state, India. A total of eight species of weevils *viz., Arrhenodes* sp., *Deporaus marginatus* (Pascoe), *Hypolixus truncatulus* (Fabricius), *Mecopus hopei* Rosenschoeld*, Myllocerus undecimpustulatus* Faust, *Peltotrachelus cognatus* Marshall, *Rhynchaenus mangiferae* Marshall and *Xanthochelus major* (Herbst) were observed to occur on seventeen fruit crops *viz.,* kinnow mandarin, guava, mango, pear, litchi, ber, peach, plum, grapes, *jamun*, pomegranate, loquat, fig, bael or wood apple, *phalsa*, papaya and apple in different districts of Punjab. Highest number of species were observed in mango (5), followed by guava, ber, jamun and fig (2 each) while only one species was observed in kinnow mandarin, pear, litchi, peach, plum, grapes, pomegranate, loquat, bael, phalsa, papaya and apple. These weevils were observed to damage leaves, stem, bark, flowers and fruits. The damage caused by these weevils on different hosts ranged between 2-3 per cent for most species except, *D. marginatus* (10%) and *R. mangiferae* (8%). Further studies on their biology, life cycle, population dynamics and management are needed.

Keywords: Diversity, Survey, Fruit crops, Weevils, Punjab, Damage

INTRODUCTION

Fruit crops exhibit an area of 86673 ha and production of 1850259 million tonnes (MT) in Punjab, India. The major fruit crops of Punjab are Kinnow mandarin, guava, mango, pear, Sweet Orange, litchi, peach and ber while the minor fruit crops are limes/lemons, amla, grapes, plum, banana, pomegranate, phalsa, sapota and papaya (Anonymous, 2019). These fruit crops are attacked by number of insect pests such as borers, sucking insects, mealybugs, leaf rollers, leaf miners, fruit flies etc.

Among these pests, weevils are important insect-pests that belong to family Curculionidae, the largest family of the animals (Zarzaga and Lyal, 1999). Weevils are successful dwellers in the world, inhabiting almost all ecosystems, from Arctic to the Sub-ant-arctic and deserts. These weevils include several serious pest species of agriculture, fruits, stored grains and forest trees (Oberprieler *et al*., 2007). These insects have long snout bearing mandibulate type of mouthparts and antennae are geniculate type. They feed on internal tissues of plants with the help of snout. Snout is also used to prepare oviposition sites on plant parts. Nearly, all the species of weevils are phytophagous and feed on every part of plant from root to seed (Tara *et al*., 2010; Srivastava *et al*., 2020). Despite their importance, weevil diversity, distribution and biology have been little studied. There is lack of information about the weevil diversity of the fruit crops in Punjab. So, the present study was undertaken to study the biodiversity of weevil species occurring on different fruit crops in the Punjab, India.

MATERIALS AND METHODS

Rapid roving surveys were conducted since 2004 till date in the fruit growing areas of the Punjab, India (31.1471°N latitude and 75.3412°E longitude) to record the diversity of weevils along with their host plants. All the major fruit growing districts of Punjab were surveyed in all the agro-climatic regions such as submontane zone, central plain zone and arid-irrigated zone along with *Kandi* and *Bet* areas. The observation of weevil pests associated with fruits, flowers, leaves and trunk of fruit trees was done while collection. The observations were recorded at monthly intervals during each year**.** The weevils were collected by different methods as hand picking, hand nets and by stem beating. The collected weevils were then killed, pinned and labelled.

Population of weevils from different fruit plants was observed and per cent damage was also recorded on various plant parts *i.e.* flowers, leaves and fruits from twenty five trees/plants. Activity period of weevils on different hosts was observed based on their presence or absence on the tree. Various stages of these weevils were reared in the Fruit Entomology laboratory in the Department of Fruit Science, Punjab Agricultural University (PAU),

Ludhiana, Punjab (India). Adult specimens were sent for identification to Division of Germplasm Collection and Characterization, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka (India). The specimens were identified based on key of provided by Marshall (1916), Ramamurthy and Ghai (1988), Pajni (1990) and also compared with authentically identified specimens at National Insect Museum, ICAR-NBAIR, Bengaluru.

RESULTS AND DISCUSSION

Details of weevils recorded in the fruit crops grown in various districts of Punjab, India since 2004 till date have been discussed below and compiled in Tables 1 and 2.

Table 1. List of weevil species observed on fruit crops in Punjab since 2004

Common name of the weevil	Scientific name of the weevil	Host plants
Grey weevil	*Myllocerus undecimpustulatus* Faust	Kinnow mandarin, guava, mango, pear, litchi, peach, plum, grapes, jamun, pomegranate, loquat, fig, *bael*, *phalsa*, apple
Long brown weevil	*Xanthochelus major* (Herbst)	Guava, *ber*, papaya
Weevil	*Peltotrachelus cognatus* Marshall	Mango
Mango flea weevil or mango leaf weevil	*Rhynchaenus mangiferae* Marshall	Mango, *jamun*
Leaf cutting weevil	*Deporaus marginatus* (Pascoe)	Mango
Straight-snouted weevil	*Arrhenodes* sp.	Mango
Small brown weevil	*Hypolixus truncatulus* (Fabricius)	*Ber*
Weevil	*Mecopus hopei* Rosenschoeld	Fig

Table 2. List of fruit crops infested by various weevil species in Punjab since 2004

Common name of the host plant	Scientific name of the host plant	Common name of the weevil	Scientific name of the weevil	Plant part infested	Activity period	Location
Kinnow mandarin	*Citrus reticulata*	Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	April to July	Many districts in Punjab
Guava	*Psidium guajava*	Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	August to October	Many districts in Punjab
		Long brown weevil	*Xanthochelus major* (Herbst)	Leaves	November	Ludhiana
Mango	*Mangifera indica*	Weevil	*Peltotrachelus cognatus* Marshall	Leaves	June to December	Ludhiana and Hoshiarpur
		Mango flea weevil or mango leaf weevil	*Rhynchaenus mangiferae* Marshall	Leaves	June to December	Ludhiana, Hoshiarpur and Mohali
		Leaf cutting weevil	*Deporaus marginatus* (Pascoe)	Leaves	May to August	Ludhiana and Hoshiarpur
		Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	April to July	Many districts in Punjab
		Straight-snouted weevil	*Arrhenodes* sp.	Wood borer	June	Hoshiarpur

Pear		Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	April to July	Many districts in Punjab
Litchi	*Litchi chinensis*	Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	March to June	Many districts in Punjab
Ber	*Zizyphus jujube*	Long brown weevil	*Xanthochelus major* (Herbst)	Leaves and fruits	September and January	Ludhiana
		Small brown weevil	*Hypolixus truncatulus* (Fabricius)	Adult on leaves; larvae on stem	September	Ludhiana
Peach	*Prunus persica*	Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	April to July	Many districts in Punjab
Plum	*Prunus domestica*	Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	April to July	Many districts in Punjab
Grapes	*Vitis vinifera*	Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	November-December	Many districts in Punjab
Jamun	*Syzygium cumini*	Flea weevil or leaf weevil	*Rhynchaenus mangiferae* Marsahall	Leaves	June to September	Many districts in Punjab
		Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	May	Many districts in Punjab
Pomegranate	*Punica granatum*	Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	September and February-March	Ludhiana, Patiala
Loquat	*Eryobotrya japonica*	Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	March, September-November	Ludhiana, Pathankot
Fig	*Ficus carica*	Weevil	*Mecopus hopei* Rosenschoeld	Bark	October	Ludhiana
		Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	November-December	Ludhiana
Bael or wood apple	*Aegle marmelos*	Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	November	Ludhiana, Patiala
Phalsa	*Grewia robusta*	Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	September	Ludhiana
Papaya	*Carica papaya*	Long brown weevil	*Xanthochelus major* (Herbst)	Flowers	September	Ludhiana
Apple	*Malus domestica*	Grey weevil	*Myllocerus undecimpustulatus* Faust	Leaves	April	Ludhiana

***Myllocerus undecimpustulatus* Faust (Entiminae: Curculionidae)**

Synonym:

*Myllocerus*11-*pustulatus* Faust (1891)
*Myllocerus*11-*pustulatus* var. *pistor* Faust (1897)
Myllocerus maculosus Desbrochers des Loges (1899)
Myllocerus maculosus Stebbing (1914)
Myllocerus marmoratus Faust (1897)

Diagnostic: Body black in colour with pale grey scaling; Antennal second funicle segment very much longer than first; distance between the eyes greater than the distance between the scrobes; Prothorax basal margin more or less bisinuate and subparallel laterally; each elytron with black spots on the shoulder, one on interval fourth and ninth before the middle, one on eighth just behind the middle, one still further back on interval three and one at apex of fifth; sometime one or more these spots absent; hind femur tridentate (Ramamurthy and Ghai, 1988).

Nature of damage: This weevil feeds on leaves, starting from leaf margins to inwards and causing typical leaf notching type of symptoms. Adults were found to feed

on both new flush and mature leaves. Intense feeding by numerous weevils may cause plant decline or stunting (Neal, 2013). Larvae feed on the roots of plants and later pupate in soil (George *et al.*, 2019). This weevil is a poor flier (Srivastava *et al*., 2020).

Host plant: Rice, maize, pigeonpea, cotton, jute, sunflower, mango, pomegranate, *ber*, strawberry, apple, lucerne and Shisham (*Dalbergia sisoo*) (Ramamurthy and Ghai, 1988), litchi (Neal, 2013; Srivastava *et al*., 2020), avocado, peach, ornamental plants and palms (Neal, 2013).

Distribution: India, Indonesia, Pakistan (CABI, 2020).

India: Throughout India, especially tropical regions (Ramamurthy and Ghai, 1988), Delhi, Karnataka, Uttar Pradesh (CABI, 2020).

Seasonal activity: Damage was observed on the leaves of Kinnow (Fig. 1A), peach, plum and pear in many places in Punjab during April to July. On guava, severe damage of grey weevil was observed on leaves at Fruit Research Farm (FRF), PAU, Ludhiana during September-October. On mango, damage was observed during April to July at FRF, PAU, Ludhiana and during May-June in district Gurdaspur. Adult weevils were observed in large number on leaves of litchi plants and young fruits at FRS Gangian, Govt. Garden and Fruit Nursery, village Bhunga, and village Budhabar, district Hoshiarpur during March-June. Severe damage and high population of weevil was observed on new leaves of litchi at Ranjit Baag, district Gurdaspur in June. On grapes, damage was observed on leaves at FRF, PAU, Ludhiana during November-December. On *jamun*, damage and adults of weevil were observed on leaves at Govt. Garden and Fruit Nursery, Wazidpur, district Patiala during May. Minor attack of this weevil was observed on pomegranate leaves during September and February-March in the FRF, PAU, Ludhiana. On loquat, this weevil was observed during March and September to November. Damage was observed on *bael* leaves during November at FRF, PAU, Ludhiana and on fig, damage was observed in college orchard, PAU, Ludhiana during last week of August and again during November-December. On *phalsa*, damage was observed on the leaves in the FRF, PAU, Ludhiana during September and on leaves of apple during 3rd week of April at FRF, PAU, Ludhiana. The damage caused by this weevil ranged between 2-3 per cent on different fruit plants (Fig. 2).

Kumar (2017) reported *M. undecimpustulatus* as minor pest of *Dalbergia sissoo* during May to September. On litchi, this weevil was observed from August to April, with peak activity during September-October (Srivastava *et al*., 2015). It was reported that infestation of this weevil started with new flush growth and continued upto last week of December. Due to unavailability of food, weevil undergoes hibernation during winter months and again appeared in April. This weevil has also been recorded on cotton from April to November (Atwal, 1976). Mazumder *et al.,* (2014) recorded incidence of *Myllocerus discolor* during March to May on litchi in Assam. *M. discolor* was also observed on ber in Jammu and Kashmir region (Tara *et al*., 53). Peach and avocado are preferred host plants of *M. undecimpustulatus* over litchi and citrus (George *et al*., 2019).

***Xanthochelus major* (Herbst) (Lixinae: Curculionidae)**

Synonym:
Xanthochelus faunus (Olivier 1807)
Curculio major Herbst (1784)

Diagnostic: Adults stout, about 8 to 18 mm size; body ovate; rostrum convex dorsally; scrobes not reach the apex; prothorax broader than long, base much broader than apex; Elytra with strong shoulder, parallel side beyond the middle; protibial mucronate apically in both the sex. Female slightly larger than male and has dark brown snout. Legs similar in size and structure. Coxa large, globular, small trochanter, long femur, tibi short, thin, slender, four-segmented tarsi. Adults respond quickly to even a slight disturbance.

Larvae are whitish, apodous, C-shaped and pass through five instars. Teneral adults remain in the pupal chamber made within the gall for 5-7 days (Azam *et al*., 2009).

Nature of damage: Leaves get distorted, with various shaped holes in the middle of leaf lamina. The margins of leaves are eaten up. Adult weevils cause severe damage both to mature and immature leaves (Mazumder *et al*., 2015). They nibbled on leaves starting from the margin and ate small patches of leaves.

Host plant: *Ber* (*Ziziphus mauritiana* Lamk) (Tara *et al*., 2010), Kaliziri (*Saussurea heteromalla*) (Azam *et al*., 2009), Falconer's thistle (*Cirsium falconeri*) (Bhagat, 2016), Arjun (*Terminalia arjuna*), Indian Laurel (*T. tomentosa*).

Distribution: India: North East Himalaya (Lefroy, 1909), Uttar Pradesh (Gupta, 1980), Jammu and Kashmir (Azam, 2007; Azam *et al*., 2009), Rajouri (Iqbal, 2010), Samba, Sagal, Khandwal, Rajpura (Tara *et al*., 2010), Jharkhand (Singh *et al*., 2014b).

Seasonal activity: Adults of *X. major* were observed on leaves of guava during November and severe damage on

ber was observed during September at PAU, Ludhiana, farmers' orchard in village Hambran, district Ludhiana and at FRF, PAU, Ludhiana during first week of January. Adults and damage was observed on *ber* leaves (Fig. 1B) and fruits (Fig. 1C) during first week of March at FRF, PAU, Ludhiana. Attack of this weevil was also observed to occur on flowers of papaya in polyhouse at Village Badal, district Sri Muktsar Sahib during September. The damage caused by this weevil on different hosts varied between 2-3 per cent (Fig. 3). Azam *et al.* (2009) reported that the adults remain active from third week of March to August and population reaches its peak during May, coinciding with the host availability, and a mean incidence of 76.57±4.59% recorded. Total life-cycle takes about 63-70 days and at least two generations observed from March to August. Incidence of leaf eating weevil, *X. faunus* in ber (Shah *et al*., 1990; Tara *et al*., 2010; Mazumder *et al*., 2015), tropical pine (Gupta, 1980), medicinal plant (*Saussurea heteromalla*) (Mohammad *et al*., 2009), lac production system (Singh *et al*., 2014b) have also been reported. Mazumder *et al.* (2015) recorded its incidence on *ber* during June-September in Assam. Azam *et al.* (2009) observed its breeding in the flower buds of *S. heteromalla.*

***Rhynchaenus mangiferae* Marshall (1915) (Rhynchaeninae: Curculionidae)**

Diagnostic: Adults about 2.5 to 2.8 mm size; body ovate; antennae with short scape, six segmented funicle; with three segmented oval club; Prothorax broader than long, broadest at base surface closely punctate with white setae; Elytra closely punctate and covered with white setae; straie 7 and 8 not reach the base of the elytra; Hind femur enlarged.

Nature of damage: Grubs of this weevil bore into the leaves and as many as 20-30 grubs can be found in one leaf and such leaves dry up completely (Marshall, 1915). Adult weevils feed on leaves and result in skeletonization of leaves (Reddy *et al*., 2018). Tender leaves are most attacked by this weevil (Sathe *et al*., 2015). Grubs are also reported to bore into florets while adults nibbled florets and young fruits resulting in drying (Singh, 1988).

Host plant: Mango (Kannan and Rao, 2006), litchi, *jamun*

Distribution: India: Maharashtra, Tamil Nadu, Uttar Pradesh, Punjab, Karnataka (Peter and Balasubramanian, 1984), Andhra Pradesh (Kannan and Rao, 2006)

Seasonal activity: *Rhynchaenus mangiferae* was recorded on nursery plant of mango in college orchard and FRF, PAU, Ludhiana during June to September. Severe damage of this weevil was observed on leaves of mango (Fig. 1D) at Kairon Farm, Zirakpur, district Mohali and FRS Gangian, district Hoshiarpur during last week of April. On *jamun*, weevil was recorded from nursery plant in college orchard and new orchard, PAU, Ludhiana during June to September. This weevil caused up to 8 per cent damage on mango leaves and 6 per cent damage on *jamun* leaves (Fig. 4).

Young plants of mango (0-5 year) are preferred by *R. mangiferae* (Kannan and Rao, 2006). This weevil is considered as a minor pest of mango (Reddy *et al*., 2018). Sathe *et al*., (2015) reported this weevil damaging tender leaves on mango trees in Maharashtra.

***Mecopus hopei* Rosenschoeld (Baridinae: Curculionidae)**

Diagnostic: Adults are 7-13 mm in size. This weevil is spider like with grey and black colour dorsally and black, grey and white ventrally.

Nature of damage: The adults feed on barks of both living and dead trees. This weevil is active throughout the year.

Host plant: Fig (*Ficus* sp.), Jackfruit (*Artocarpus integrifolia*), Sal (*Shroea robusta*) (Sheikh, 1996).

Distribution: India

Seasonal activity: Adults were observed in fig plantation in college orchard, PAU, Ludhiana during October. This weevil caused up to 2 per cent damage to the fruits (Fig. 5). Grubs were observed inside the fig fruits.

***Arrhenodes* sp. (Brentidae: Curculionoidea)**

Diagnostic: Slender, very elongate rostrum and body, head abruptly excised almost direct behind the eyes and very small mandible and non-geniculate antennae. Larvae possess legs unlike most other weevils' larvae, and are very elongate.

Nature of damage: Larvae feed on wood (Fig. 1E) and result in small worm holes in the trunk of tree. Frass and sawdust is expelled from oviposition hole at the beginning of the gallery (EFSA, 2019). The diameter of galleries increases with the size of larvae. These holes almost reach the opposite side of the trunk of tree and then make a sharp U-turn toward the entrances. Weevils are attracted to wounds on the living trees (Thomas, 2007). Adults feed on the sap oozing from the trees and observed to congregate under loose bark at wounds (EFSA, 2019).

Host plant: They are found under the barks of various dead and decaying wood/ trees e.g. Maple (*Acer negundo*),

Honey locust (*Gleditisia triacanthos*) (Solomon, 1995), oak trees (*Quercus* sp.) (Thomas, 2007), Elm (*Ulmus* sp.), Beech (*Fagus* sp.) and Aspen (*Populus* sp.) (EFSA, 2019).

Distribution: Canada, USA (Thomas, 1996).

India: Usually found in tropical regions, Kerala (Tom and Kaippallil, 2016).

Seasonal activity: *Arrhenodes* sp. was observed on mango trees at Govt. Garden and Fruit Nursery, Bhunga, district Hoshiarpur, Punjab, India during June-July. About 3 per cent damage to trunks of mango trees was observed (Fig. 5). Solomon (1995) reported *Arrhenodes minutus* (Drury) as a vector of oak wilt fungus, *Ceratocyctis fagacearum* (Bretz) Hunt) in North America. In Florida, adult weevils were observed during February to November (Thomas, 1996) while EFSA (2019) reported its presence from early May to August. In Kerala, *Arrhenodes* sp. was observed during January to April (Tom and Kaippallil, 2016).

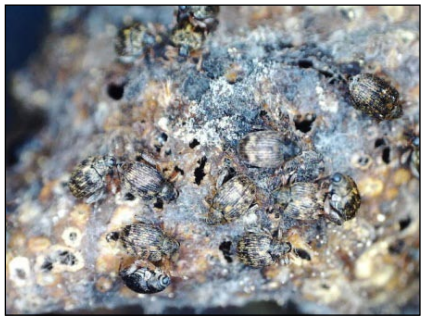
***Peltotrachelus cognatus* Marshall (Eremninae: Curculionidae)**

Diagnostic: Body black in colour with whitish scaling; whitish scale arranged in three transverse irregular bands on elytron; strial punctures of each elytra small and shallow; antennae with second funicle segment distinctly longer than first; prothorax broader than long, widest at base and deeply bisinuate (Pajni, 1990).

A. *Myllocerus undecimpustulatus* on Kinnow mandarin

B. *Xanthochelus major* on *ber* twig

C. *Xanthochelus major* on *ber* fruit



D. *Rhynchaenus mangiferae* on mango

E. *Arrhenodes* sp. on mango

F. *Peltotrachelus cognatus* on mango

G. *Deporaus marginatus* on mango

Fig 1. Different weevils observed feeding on fruit crops in Punjab. A. *Myllocerus undecimpustulatus,* B. *Xanthochelus major* on *ber* twig, C. *Xanthochelus major* on *ber* fruit, D. *Rhynchaenus mangiferae*, E. *Arrhenodes* sp., F. *Peltotrachelus cognatus*, and G. *Deporaus marginatus*.

Nature of damage: Adult weevils are known as defoliators of fruit trees (Siddappaji and Lingappa, 1977). The weevils start feeding from leaf margins to inwards leaving thick veins and midrib in an irregular fashion. Adults have the habit to congregate on the lower leaf surface during sunny hours and scatter during cool hours. Weevils prefer brittle leaves to tender ones (Siddappaji and Lingappa, 1977). Sometimes, weevils make round holes or C shaped cut on the leaves (Singh *et al*., 2014b).

Host plant: Cashew, sapota, mango, guava, pomegranate, peach, pear, plum, apple, cherry, mulberry (Singh *et al*., 2014a), teak, The Bombay ebony (*Diospyros montana)*, The Indian elm (*Holoptelea integrifolia)*, Takoli (*Dalbergia lanceolaria)* and the Indian rosewood (*D. latifolia*) (Siddappaji and Lingappa, 1977).

Distribution: India: Puducherry; Karnataka, Tamil Nadu, Kerala (Pajni, 1990).

Seasonal activity: This weevil was observed damaging mango leaves (Fig. 1F) at FRS, Gangian, district Hoshiarpur during June, and at PAU, Ludhiana and FRS Gangian during December. Only, up to 3 per cent damage was recorded on leaves (Fig. 5). Siddappaji and Lingappa (1977) observed the activity of *P. cognatus* from May to November. Severe infestation on mango grafts was observed during September-October. This weevil has been reported as pest on mulberry (Singh *et al*., 2014a) and on Kusum tree (Singh *et al*., 2014b). On Kusum trees, adult weevils appear during March and remain active till October-November.

***Hypolixus truncatulus* (Fabricius)**

Synonym: *Lixus truncatulus* Fabricius (1798)

Diagnostic: Medium sized weevil, adults with black in colour elytra, prothorax and head reddish brown. Eyes very prominent, snout straight, gradually widened at apex. Antennae geniculate type, funicle and club segments gradually increase in size. Body covered with whitish hairy setae. Legs slender, femora basal part brownish and remaining segments black (Tara *et al*., 2009).

Nature of damage: Larvae make tunnels in the stem of the tree in a zig-zag manner (Tara *et al*., 2009). Feeding by larvae result in the gall formation in the stem and adult weevils feed on leaves and epidermis of tender stem (Tara *et al*., 2010). Stem become weak and may breakdown in heavy winds (Kalia and Lal, 1999). At the place where larva forms its pupal chamber, galls are formed due to thickening of stem walls. Adults emerge from these galls by biting holes. The eaten leaves with deeply incised, irregular margins indicate the presence of adults (Tara *et al*., 2009).

Host plant: Polyphagous e.g. *Dalbergia sissoo* (Kalia and Lal, 19), *Amaranthus* spp. (Rajeshkanna *et al*., 2017), Gum Arabic tree, *Acacia nilotica* (Misra *et al*., 1994) and *Ziziphus mauritiana* (Singh *et al*., 2014b).

Distribution: India, Mexico, Pakistan, Thailand (Jackson, 2019).

India: Tamil Nadu, Kerala, Karnataka, Orissa, Arunachal Pradesh**,** Jharkhand, Bihar, Himachal Pradesh, Madhya Pradesh (Kalia *et al*., 1994), Uttar Pradesh (Agarwal, 1985), New Delhi (Phogat *et al*., 1994; Butani and Jotwani, 1983), Uttarakhand, Jammu (Tara *et al*., 2009).

Seasonal activity: Adults were observed on leaves of *ber* plants during September in PAU, Ludhiana and in farmers orchard in village Hambran, district Ludhiana, Punjab and damage upto 3 per cent was noticed (Fig. 5). This weevil has been considered as major pest of *Amaranthus* sp. in many countries (Aragon *et al*., 2011; Kagali *et al*., 2013). Maximum population of this weevil on *Amaranthus* sp. was observed during June-July by Tara *et al.* (2009). The weevil remains active from April to October and overwinters in the cracks and crevices

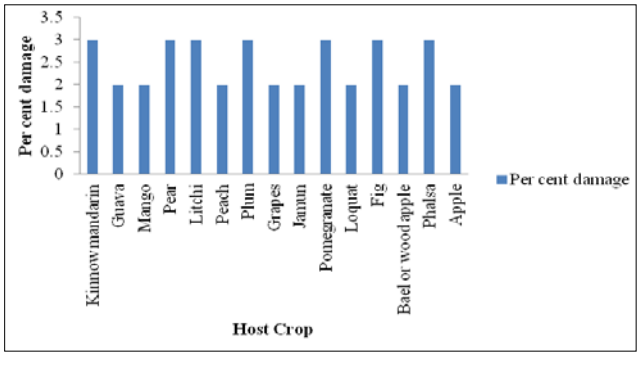


Fig 2. Per cent damage of *Myllocerus undecimpustulatus* on fruit crops

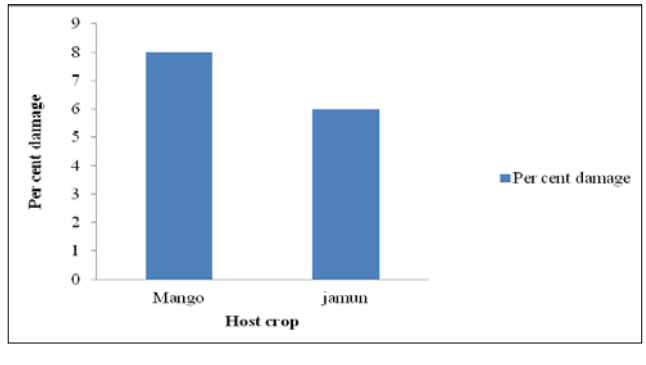


Fig 4. Per cent damage of *Rhynchaenus mangiferae* on fruit crops

of the walls or in dead, harvested stems or in stumps in the soil. Maximum oviposition was observed during June to September (Tara *et al*., 2009). This weevil was recorded from Jammu and Kashmir region by Tara *et al*. (2010) and by Singh *et al*. (2014b) as a pest of *ber* from Jharkhand.

***Deporaus marginatus* (Pascoe) (Attelabidae: Curculionoidea)**

Synonym: *Eugnamptus marginatus* Pascoe

Diagnostic: Very small weevil, adults with black in colour elytra, prothorax and head reddish brown (Fig. 1G). Eyes very prominent, snout straight, gradually widened at apex. Antennae non-geniculate type, funicle and club segments gradually increase in size. Body covered with whitish hairy setae. Legs slender, femora basal part brownish and remaining segments black.

Nature of damage: Adult weevils feed on the epidermis of young leaves and the affected leaves turn brown, curly and crumpled (Singh, 2014). Feeding by this weevil produces conspicuous 'windowpane' symptoms on the leaves and the infested shoots become almost leafless. The gravid female excavates small cavities on either side of midrib for egg laying and then cut the leaf near the base from one edge through the midrib to the other. Larvae mines into the tissues of fallen leaves. The most obvious symptom of attack by this pest is the presence of young leaf bits below the tree (Rashid *et al*., 2017).

Host plant: Mango (Kannan and Rao, 2006; Rashid *et al*., 2017) and cashew (NBAIR, 2019).

Distribution: India (Butani, 1979; Rafiquzzaman *et al*., 1999; Singh, 2014), Bangladesh (Uddin *et al*., 2003; Uddin *et al*., 2014), Sri Lanka (Hutson and Alwis, 1934) and Malaysia (Soh and Khoo, 1983; Tigvattnanont, 1988).

India: Andhra Pradesh (Kannan and Rao, 2006), Tamil Nadu, Kerala, Karnataka, Orissa, Arunachal Pradesh**,** Jharkhand (Singh, 2014), Bihar.

Seasonal activity: Adult weevils as well as damage of leaf cutting weevil, *D. marginatus* was observed on mango at Fruit Research Station (FRS), Gangian, district Hoshiarpur, Punjab during May-June and during last week of August at college orchard, PAU, Ludhiana. This weevil resulted in about 10 per cent damage to the leaves of mango (Fig. 5). It is reported to be a pest of young trees (0-5 year) of mango (Kannan and Rao, 2006). This pest is more active in the rainy season and its attack delays the growth of rootstock which hinders the development of new grafts. In Jharkhand state, the activity of this weevil started from June on ***ber*** (Singh, 2014). The activity was recorded in the form of egg laying in the cavities of leaves resulting in defoliation. Development of weevil synchronized with initiation of new leaves of mango in grafted young plants and more prevalent from June to October (Mukherjee *et al*., 2016). Tom and Kaippallil (2016) also recorded *D. marginatus* as pest of mango, observed during January to April in Kerala.

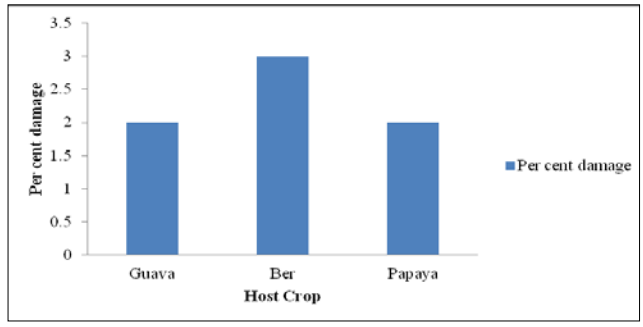


Fig 3. Per cent damage of *Xanthochelus major* on fruit crops

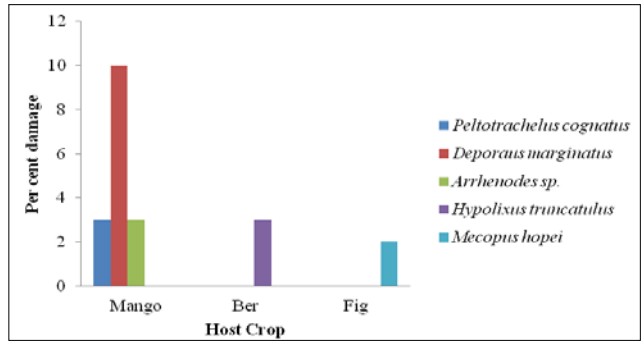


Fig 5. Per cent damage of different weevil species on fruit crops

CONCLUSION

This study reports the occurrence of eight species of weevils actively feeding on seventeen fruit crops of Punjab, India observed since 2004. Highest number of species were observed in mango (5), followed by guava, ***ber***, ***jamun*** and fig (2 each) while only one species was observed in Kinnow mandarin, pear, litchi, peach, plum, grapes, pomegranate, loquat, *bael*, *phalsa*, papaya and apple. These weevils were observed to damage leaves, stem, bark and flowers. The average damage caused by these weevils ranged from 2-3 per cent on different hosts except *D. marginatus* (10% damage on mango) and *R. mangiferae* (8% damage on mango). Further investigations are needed to study their biology, population dynamics, incidence level and management practices.

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Assessment of losses caused by major insect-pests and diseases of mango (*Mangifera indica* L) under humid tropics

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ABSTRACT: A study spanning five consecutive years (2016-20) was conducted to evaluate the damages inflicted on mango crops in humid tropical regions by primary insect pests and diseases. The findings revealed that hoppers, thrips, and fruit flies were the primary pests, while powdery mildew, anthracnose (pre and post-harvest), and stem end rot (SER) were the dominant diseases affecting mango trees. Notably, the population of hoppers and thrips remained significantly lower in orchards with protective measures (1.84 hoppers/panicle and 1.73 thrips/panicle/tap, respectively) compared to those without protection (11.58 and 8.84, respectively). Similarly, the incidence of pre-harvest diseases such as powdery mildew (2.28%) and anthracnose (5.24%), as well as post-harvest diseases like anthracnose (3.00%) and SER (3.75%), was notably reduced in protected orchards compared to unprotected ones (7.28, 14.30, 7.25, and 8.50, respectively). On average, % yield loss of 47.33 was documented in unprotected orchards compared to protected ones. This assessment holds the potential for enhancing the management of major insect pests and mango diseases, offering valuable insights for agricultural advisory services to aid farmers effectively.

Keywords: Anthracnose, mango hopper, stem end rot, thrips, unprotected orchard, yield loss

INTRODUCTION

Mango, *Mangifera indica* L. (Family: Anacardiaceae) is known for its delicious taste, attractive colour, savoring flavor, and high nutritional value of vitamins A and C, mineral and fiber content. The area under mango cultivation in India is 2.20 million hectares, with a total production of 18.64 million tonnes, sharing 40 percent of the total mango production of the world (NHB, 2015). Among Indian states, Gujarat covers 150 thousand hectares area with a total of 1.24 million tonnes of output with 8.10 tonnes/ha productivity (NHB, 2015). Various biotic (insect, disease, and weeds) and abiotic (temperature, humidity, wind, etc.) factors limit the potential productivity of mango in India. Crop loss assessment is the quantification of the impact of pests and diseases on crop yield. Among insects, more than 492 species of insect species are reported as pests on mangoes, of which 188 are reported from India (Tandon and Verghese, 1985). Hoppers, thrips, and fruit flies are recorded as a serious pest of mango in south Gujarat at flowering to fruiting harvesting stages and cause significant yield losses (Rahman and Kuldeep, 2007; Kumar *et al.,* 2014; Gundappa *et al.,* 2014; Mouly *et al.,* 2017; Bana *et al.,* 2017, Bana *et al.,* 2018; Bana *et al.,* 2021). Fruit fly incidence reduces the yield and quality and restricts the export of fruits to many countries (Patel *et al.,* 2013). In the highly humid and heavy rainfall zone of south Gujarat, direct damage is caused by fruit flies to fruits of mango and sapota to 16-40 and 2-4 percent, respectively (Patel and Patel, 2005).

Mango also suffers from several diseases, among them anthracnose, powdery mildew, and stem end rot (SER), which are recorded major from new flush to harvesting and storage conditions (Bana *et al.,* 2020; Nelson, 2008a, Karunanayake and Adikaram, 2020). Anthracnose is the major pre and post-harvest mango disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. About 25 to 30% of fruit loss in total mango production has been reported under humid conditions due to the incidence of anthracnose and SER (Uddin *et al.,* 2018). Powdery mildew, *Oidium mangiferae* Berthet, is a significant, serious, and widespread disease in India and causes up to 90 percent flower panicle damage when observed in the epidemic (Misra *et al.,* 2016; Nelson, 2008b; Prakash and Srivastava, 1987). Symptoms of this disease can be noticed on leaves, inflorescences, and young fruits (Nelson, 2008b and Bana *et al.,* 2020). The southern part of Gujarat is considered a major production hub of the delicious Kesar variety of mango in western India.

Estimating yield losses in mango orchards is a major predicament in the economical production of high-quality quantities of mango over the entire region. Database of loss estimates related to crops and pests are essential prerequisites for economic management of

pests and evaluating the efficacy of recommended crop protection practices. Pest and disease activity changes over time with respect to changing climate scenarios. In recent years, we have witnessed major climate changes, ultimately leading to changes in the status of pests. Therefore, quantification of yield losses with respect to changes in insect-pests status is very important for economic pest management. Strategies for using limited resources for management may be developed to optimize productivity and sustainability of production. Therefore, keeping in mind the above facts, the present experiment was carried out to estimate the yield losses of mangoes concerning major insect pests and diseases for developing cost-effective and ecologically benign pest management strategies under humid climatic conditions of south Gujarat.

MATERIALS AND METHODS

Experimentation and data observations

The study was conducted at the Agriculture Experimental Station, Navsari Agricultural University, Paria, India, on 20-year-old mango trees of *cv*. Kesar during five consecutive years (2016-20). Plant-to-plant and row-to-row distances were 8×8 and 10×10 m. The experiments were conducted by paired 't' tests with two treatments (fully protected and unprotected plots). For insect pests, the first spray was applied of acephate 75 SP (0.04%) at the panicle emergence stage, followed by a second spray (need basis) with spinosad 45 SC (0.0112%) and a third need-based spray of thiamethoxam 25 G (0.008%) and first spray of carbendazim 50 WP (1gm/l) at appearance of disease on newly emerge flushes followed by hexaconazole 5 EC (1ml/l) at panicle stage for diseases. Weekly observations of major insect pests and diseases were recorded on ten panicles or inflorescence per tree from twenty randomly selected trees (10 sprayed and unsprayed trees) per the NICRA manual (NICRA, 2011). Fruit fly incidence was observed in 5 fruits per tree at 60 days after fruit set and harvest stage in plucked fruits from protected and unprotected orchards. Fruit fly infestation (dropped fruits) was observed 60, 75, and 85 days after the fruit set. Protection against insect pests and diseases in protected treatments was given according to the recommended package and practices of the plant protection sub-committee of Navsari Agricultural University, Navsari, Gujarat (Anonymous, 2014; 2016).

Percent Disease Index (PDI) was recorded by scoring all the individual ten panicles on each plant on a 0-5 rating scale (0= inflorescence healthy, or no disease, 1=<20% inflorescence covered by disease in panicles, 2=21-40% of the inflorescence covered by disease in panicles, 3=41-60% of the inflorescence covered by diseases in panicles, 4=61-80% of the inflorescence covered by disease in panicles and 5=81-100% of the inflorescence covered by disease). Anthracnose PDI was recorded by scoring all the individual ten leaves or twigs on each plant on a 0-5 rating scale (0=no visible symptoms, 1=1 % leaf area affected, 2=1.1 to 10 % leaf area affected, 3=10.1 to 25 % leaf area affected, 4=25.1 to 50 % leaf area affected, 5=More than 50 % leaf area affected by the disease). Further, the PDI was calculated using the McKinney formula (1923).

$$\text{PDI (\%)} = \frac{\text{Sum of all the disease ratings } (\Sigma N)}{\text{No. of panicles observed x Maximum disease rating}} \times 100$$

Post-harvest diseases were observed on 100 plucked fruits in both treatments (protected and unprotected). Freshly harvested fruits at physiological maturity were stored at room temperature for 15 days to observe post-harvest diseases. The disease incidence (%) was calculated as the number of fruit infected (n) with post-harvest diseases out of the total number of fruits (N) observed.

$$\text{Disease incidence (\%)} = \frac{n}{N} \times 100$$

The avoidable crop loss and economics loss were calculated for each treatment by using the following formula,

$$\text{Avoidable loss (\%)} = \frac{\text{Yield in treated plot (kg ha}^{-1}\text{) - Yield in control plot (kg ha}^{-1}\text{)}}{\text{Yield in treated plot (kg ha}^{-1}\text{)}} \times 100$$

$$\text{Avoidable economic loss (\%)} = \frac{\text{Income in treated plot (kg ha}^{-}\text{)- Income in control plot (kg ha}^{-}\text{)}}{\text{Income in treated plot (kg ha}^{-}\text{)}} \times 100$$

Statistical analyses and data interpretation

Major pests (hopper, thrips, and fruit flies) and diseases (powdery mildew, anthracnose, and stem end rot) were recorded for five consecutive years (2016-20) in protected and unprotected orchards. Significant differences among the data were calculated using the paired 't' test. Avoidable losses were calculated in protected and unprotected orchards.

RESULTS AND DISCUSSION

Incidence of major insect-pests and diseases were observed during the study periods. Significant difference was observed among the imposed treatments in protected and unprotected orchards.

Incidence of major insect-pests in protected and unprotected orchards

The results presented in Table 1 showed that the

Table 1. Incidence of mango hopper in protected and unprotected plot

Hopper population/ panicle													
Year (s)		**2015-16**		**2016-17**		**2017-18**		**2018-19**		**2019-20**		**Pooled**	
Treatment details		UP	P	UP	P	UP	P	UP	P	UP	P	UP	P
BS	BS***	6.84	7.72	6.03	6.46	6.42	5.76	7.32	8.26	6.47	6.56	6.62	6.95
	SD****	1.76	2.43	1.08	1.66	1.84	1.62	2.29	1.96	2.25	2.01	0.70	0.81
	T-Statistic	0.936^{NS}		0.75^{NS}		0.93^{NS}		1.12^{NS}		0.09^{NS}		1.38^{NS}	
Ist spray	7 DAS	8.46	1.16	7.12	2.07	7.39	2.23	8.19	1.95	7.92	1.78	7.82	1.84
	SD	2.17	0.70	1.41	1.17	1.42	0.79	2.27	0.70	1.52	0.54	0.57	0.33
	T-Statistic	12.80**		11.87**		12.29**		11.05**		10.15**		33.51**	
	14 DAS	10.13	3.19	8.52	2.78	9.17	2.12	9.47	3.40	8.95	2.98	9.25	2.89
	SD	2.19	1.56	1.52	0.69	1.57	1.00	1.62	0.79	1.74	1.12	0.93	0.49
	T-Statistic	8.49**		14.00**		13.16**		11.12**		11.46**		22.35**	
IInd spray	7 DAS	12.07	0.67	9.84	1.10	10.08	1.34	10.96	0.86	11.02	0.55	10.79	0.90
	SD	2.95	0.49	1.68	0.59	1.34	0.83	1.74	0.41	2.04	0.48	0.75	0.33
	T-Statistic	12.71**		13.31**		15.96**		20.71**		15.91**		40.26**	
	14 DAS	13.18	1.73	11.23	1.14	10.67	0.91	13.41	1.46	12.95	0.82	12.29	1.21
	SD	2.35	0.77	1.88	0.67	1.99	0.53	2.15	0.80	2.13	0.36	1.01	0.30
	T-Statistic	14.17**		14.13**		13.75**		14.89**		17.18**		29.32**	
IIIrd spray	7 DAS	14.13	0.94	11.42	0.73	-	-	13.89	1.23	12.85	1.06	13.07	0.99
	SD	3.12	0.63	1.94	0.48	-	-	2.48	1.04	2.56	0.67	0.90	0.38
	T-Statistic	14.06**		15.91**				13.26**		12.90**		39.90**	
	14 DAS	13.92	1.21	12.18	0.92	-	-	14.87	1.60	15.20	1.89	14.04	1.41
	SD	2.03	0.68	2.05	0.65	-	-	2.27	1.18	2.31	0.95	0.86	0.37
	T-Statistic	18.52**		14.15**				14.64**		15.26**		35.84**	
Pooled	7 DAS	11.55	0.92	9.46	1.30	8.74	1.79	11.01	1.35	10.60	1.13	10.27	1.30
	SD	1.79	0.30	0.91	0.56	1.25	0.55	1.26	1.35	1.77	0.38	0.47	0.21
	T-Statistic	20.21**		23.18**		16.82**		23.22**		15.05**		57.92**	
	14 DAS	12.41	2.04	10.64	1.61	9.92	1.52	12.58	2.15	12.37	1.90	11.58	1.84
	SD	0.98	0.58	1.23	0.57	1.47	0.45	1.24	0.48	1.76	0.70	0.72	0.29
	T-Statistic	27.79**		17.29**		17.95**		20.95**		16.62**		35.37**	

Significant at 0.01 level; NS, Non-significant; UP, Unprotected; P, Protected; BS*, Before spray; SD****, Standard deviation

population of mango hoppers remained non-significant in both orchards (protected and unprotected) before the imposition of sprays. Based on the four-year consecutive study (irrespective of different post-spray intervals), the hopper population was found to be significantly lower in protected (1.30 & 1.84 hoppers /panicle) as compared to unprotected orchards (10.27 & 11.58 hoppers/panicle) after 7 and 14 days of sprays, respectively (P<0.01).

During the study periods, the thrips population was recorded in 2016- 17, and results revealed that populations were found to be non-significant before the imposition of sprays. The lowest thrips population was

recorded in protected trees (1.23 and 1.73 thrips /panicle/ tap) over to unprotected trees after 7 and 14 DAS (8.19 and 8.84 thrips/panicle/tap), respectively (Table 2). Both treatments were found to be non-significant in fruit fly infestation in plucked fruits during study periods, while a significant difference was observed in dropped fruits (Table 3). In dropped fruits, the significantly lowest fruit fly incidence was observed in the protected plots (5.27%) over to the unprotected plots (15.22%). Similarly, more or less trends were recorded during the study periods. Based on the pooled results, both treatments were significant in fruit fly infestation (P<0.01).

The Integrated Pest Management module comprised the first spray of acephate 75 SP (0.04%) at the panicle emergence stage, followed by spinosad 45 SC (0.004%) after 21 days. A third need-based spray was the most effective treatment in reducing the mango hopper and thrips population reported by Bana *et al.* (2015a) and Bana *et al.* (2015b). The maximum number of fruits at the marble stage (425.63 fruits/100panicles) was recorded with the treatment over to control (153.50 fruits/100panicles). Verghese *et al.* (2006) reported that pre-harvest IPM combination of male annihilation technique (using methyl eugenol as a lure) + sanitation brought down fruit flies infestation to 5.00% from an infestation ranging from 17–66% in control. The untreated fruits, which were also exposed to gravid females, showed 30% and 5.5% infestations in 2004 and 2005 at IIHR, Bengaluru.

Incidence of pre-and post-harvest disease in protected and unprotected orchards

After 15 days of spray, powdery mildew and anthracnose intensity were significantly lower in the protected plot (3.80 and 8.80%) than in the unprotected plot (12.20 and 20.40%). Similar trends were observed during succeeding years. Based on pooled results, minimum severity was recorded in protected plots (2.28 and 5.24 %) over in unprotected plots (7.28 and 14.30 %), respectively. Sayiprathap *et al.* (2018) surveyed ten districts of Karnataka. They reported that the highest percent disease index (PDI) was observed in Srinivas

Table 2. Incidence of mango thrips in protected and unprotected plot during 2016-17#

Treatment details	Thrips population/panicle/tap																	
	I st Spray						II nd Spray				III rd Spray				Pooled			
	BS		7 DAS		14 DAS		7 DAS		14 DAS		7 DAS		14 DAS		7 DAS		14 DAS	
	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P
Mean	5.11	4.32	5.47	2.26	6.32	3.10	8.86	0.59	9.51	0.92	10.24	0.84	10.69	1.17	8.19	1.23	8.84	1.73
SD	1.11	0.69	1.40	1.05	1.82	0.80	2.20	0.35	1.76	0.48	1.55	0.56	1.20	0.81	0.96	0.45	1.02	0.41
T-Statistic	1.73^{NS}		4.92**		5.18**		11.24**		13.97**		19.62**		21.18**		24.17**		22.19**	

** Significant at 0.01 level; NS, Non-significant

#Thrips population was recorded in 2016-17, rest years incidence low or negligible in plot.

Table 3. Fruit fly incidence in plucked and dropped fruits on protected and unprotected plot

Treatment details	Plucked fruits (% Fruit fly infestation)*											
	2015-16		2016-17		2017-18		2018-19		2019-20		Pooled	
	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P
Mean	13.00	6.00	8.00	3.00	9.00	5.00	10.00	2.00	9.00	4.00	9.80	4.00
SD	11.59	8.43	6.32	4.83	7.38	5.27	11.54	4.21	7.38	6.99	3.71	2.49
T-Statistic	1.12^{NS}		1.86^{NS}		1.80^{NS}		2.22^{NS}		1.34^{NS}		4.11**	
	Dropped fruits (%Fruit fly infestation)*#											
Mean	15.22	5.27	13.14	6.84	17.58	4.02	16.65	4.32	21.58	6.89	10.42	2.70
SD	6.20	1.98	4.71	5.77	5.86	2.50	5.24	3.47	4.46	2.70	2.83	1.07
T-Statistic	5.15**		2.92**		6.47**		6.67**		9.97**		7.48**	

** Significant at 0.01 level; NS, Non-significant

Hopper population on twigs, sooty mold symtoms on panicles and thrips infested pea sized fruits

Thrips damage on leves, and infested and dropped fruits by fruit fly

purtaluka (33.60 %) of Kolar district with a mean PDI of 32.40, followed by Chikkaballapur district with a mean PDI of 31.62. Dembele *et al.* (2019) reported that anthracnose disease incidence and severity varied from locality to locality in dry and rainy seasons. Disease intensity was higher in the rainy season than in the dry season.

Anthracnose and SER rot were reported as major devastating post-harvest diseases during the study periods. The results (Table 4 and Figure 3) showed that the minimum incidence of anthracnose and SER was reported in collected fruits from protected plots (3 and 2%) over to unprotected plots (11 and 6%), respectively. Subsequently, anthracnose and stem end rot post-harvest disease losses reached up to 4 and 6% in protected fruits, compared to un-protected plot collected fruit (9 and 13%). In pooled results, the incidence of anthracnose and SER were observed at 3& 3.75% in protected and 7.25 and 8.50% in unprotected fruits, respectively. Prusky *et al.* (2009) reported that postharvest diseases of mango reduce fruit quality and cause severe losses, resulting in entirely unmarketable fruits and increases during the ripening stage due to physiological changes. The disease was also more severe on fruits than on leaves (Dembele *et al*., 2019). Terao *et al.* (2018) reported that SER is a severe threat to the mango industry in Brazil and caused significant losses during transportation and storage. Pre- and post-harvest anthracnose correlated significantly, indicating that infection was initiated in the field and remained latent until fruit repining. In Israel, SER causes a 30–40% loss of harvested mango fruit (Diskin *et al.,* 2017). In Sri Lanka, postharvest losses of mangoes exceed 30 - 40% due to extensive rotting of harvested fruits.

Yield and economic losses

Results presented in Tables 5 & 6 showed that the significantly highest mango yield (73.60 kg /tree) was recorded in the protected plots compared to the unprotected plots (46.30 kg/tree) during 2015-16. Subsequently, the maximum yield was obtained in the protected plot (69.90 kg/tree) over to the unprotected plot (35.60 kg/tree). A similar trend was observed during consecutive years; the highest yield was recorded in the protected plots (62.43, 60.43, and 65.25 kg/tree) as compared to unprotected plots (29.65, 25.18, and 18.30 kg /tree) during 2017-18 to 2019-20, respectively. In pooled results, the maximum yield was recorded in the protected plot (66.32 kg /tree) compared to the unprotected plot (31.01kg/tree). The maximum net return was recorded in the protected plot compared to the unprotected plot (Rs. 1205943 over to 104880), and the net profit over control is Rs. 97463. Overall, 47.33 percent yield losses were recorded in the unprotected plots compared with the protected plots in the Kesar variety of mango in humid tropics (Table 6).

CONCLUSION

This study showed that mango hopper, thrips, and

Table 4. Intensity of powdery mildew and anthracnose in protected and unprotected plot

Treatment details	Powdery mildew PDI (%)											
	2015-16		2016-17		2017-18		2018-19		2019-20		Pooled	
	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P
Mean	12.20	3.80	7.80	3.00	6.20	1.80	5.40	1.20	4.80	1.60	7.28	2.28
SD	6.42	2.39	5.12	2.16	3.59	1.75	2.98	1.39	3.68	1.83	1.42	1.00
T-Statistic	4.78**		2.51*		3.50**		3.71**		2.67**		12.90**	
Anthracnose PDI (%)												
Mean	20.40	8.80	14.80	6.20	9.40	3.20	12.60	4.20	8.60	3.80	14.30	5.24
SD	5.95	4.73	3.01	3.05	3.77	1.93	4.11	1.98	5.25	2.40	1.98	0.87
T-Statistic	5.81**		6.61**		4.39**		5.44**		2.30*		17.47**	
Post-Harvest Diseases (%)*												
Anthracnose	11	3	4	3	9	4	5	2	8	3	7.25	3.00
Stem end rot	6	2	7	4	13	6	8	3	12	5	8.50	3.75

*, Significant at 0.05 level; **, Significant at 0.01 level

Table 5. Yield in protected and unprotected plot

Treatment details	Yield (Kg/tree)											
	2015-16		2016-17		2017-18		2018-19		2019-20		Pooled	
	UP	P	UP	P	UP	P	UP	P	UP	P	UP	P
Mean	46.30	73.60	35.60	69.90	29.65	62.43	25.18	60.43	18.30	65.25	31.01	66.32
SD	8.52	12.15	6.67	9.95	7.41	13.21	4.97	10.49	4.95	11.28	2.16	5.51
T-Statistic	5.99**		12.43**		8.06**		11.39**		14.65**		20.03**	

** Significant at 0.01 level

Powdery mildew symptoms on inflorescences **External symptoms of SER on ripe mango fruits**

Fig. 2: Symptoms of different major pre and post-harvest disease of mango under humid tropic

fruit fly are recorded as significant insect pests, and anthracnose, powdery mildew, and SER are primary pre and post-harvest fungal diseases that threaten the production and marketing of mango fruits in humid conditions. Hopper and thrips populations remained significantly lower in protected orchards (1.84 hopper/panicle and 1.73 thrips/panicle/tap, respectively) than in unprotected orchards (11.58 and 8.84). Similarly,

Table 6. Economics of yield losses in protected and unprotected plot

Treatment (s)	UP	P
Yield (kg/ha)	4017.78	8239.20
Yield loss (kg/ha)	4221.42	0.0
Gross return (kg/ha)	108480.06	222458.40
Pesticides cost and spraying charges	0.00	16514.86
Net return	108480.06	205943.54
Net profit over control	0.00	97463.48
Avoidable losses (%)	47.33	0.0

Cost of pesticides: Acephate 693/kg, Spinosad 1380/75ml, Thiamethoxam 887/kg, Carbendazim 506/kg and Hexaconazole 310/lit., price of mango Rs. 27 per kg.

the incidence of pre-harvest diseases, *viz*., powdery mildew (2.28%), anthracnose (5.24%), and post-harvest diseases, *viz*., anthracnose (3.00%) and SER (3.75%) was also found significantly lower in protected orchards as compared to un-protected orchard (7.28, 14.30, 7.25 and 8.50, respectively). The average yield losses of 47.33 percent were recorded in unprotected trees compared to protected trees in the Kesar variety of mango under humid tropics.

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Bio-efficacy of liquid bio-pesticides against major insect pests in Citrus nursery under Siang Valley of Arunachal Pradesh

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ABSTRACT: The field experiment on bioefficacy of biopesticides for the management of three major insect pests of citrus nursery *viz.,* citrus butterfly, leaf miner and whitefly was carried out on rough lemon seedlings at College of Horticulture and Forestry, Pasighat, Arunachal Pradesh during 2022-23. The highest efficacy against citrus butterfly caterpillar was observed in *Bacillus thuringiensis* after 4th spray at 7 and 14 days after spraying (DAS) in both parameters of population (0.80 and 0.66/plant) and defoliation percentage (3.00% and 3.90%), respectively. The entomopathogenic fungus *Lecanicillium lecanii* effectively reduced whitefly incidence to 3.10/ plant after the fourth spray at 14 days after sowing (DAS). Additionally, the botanical Azadirachtin at 1500 ppm concentration, applied at a rate of 2ml/ liter of water, significantly decreased leaf miner incidence by 11.40 percent at 7 DAS.

Keywords: Biopesticides, citrus nursery, insect pests, *Metarhizium anisopliae,* rough lemon.

INTRODUCTION

The citrus industry plays a vital role in global agriculture, providing substantial income and nutrition. Arunachal Pradesh stands out among the northeastern states of India for its significant production of Khasi Mandarin. The prosperity of citrus orchards hinges on the availability of high-quality seedlings from healthy nurseries. However, in recent years, citrus nursery pests have seriously threatened successful citrus tree propagation. The Siang Valley, a prominent Khasi Mandarin production area in Arunachal Pradesh, grapples with major incidences of citrus butterfly (*Papilio* spp.), whitefly (*Bemisia tabaci*), and leaf miner (*Phyllocnistis citrella*), resulting in significant crop losses. The *P. citrella* (4.00 to 49.27%) and *Papilio* spp. (3.32 to 27.89%) have been documented to cause substantial damage to Khasi Mandarin in Assam (Deka *et al*., 2016), while Krishna et al. (2021) reported yield losses of 30.70 to 53.20% in East Khasi Hills of Meghalaya due to *P. citrella* infestations.

Although synthetic chemical pesticides have been traditionally used to combat these pests, their widespread application has raised concerns about environmental pollution, pest resistance, and resurgence. Additionally, many farmers practicing natural or organic farming methods favor non-chemical solutions. Consequently, there is growing interest in adopting biopesticides for managing citrus nursery pests. Biopesticides, encompassing microorganisms, botanicals, and biorationals, offer environmentally friendly and sustainable alternatives to chemical pesticides (Rani *et al*., 2021). Derived from natural sources, they often exert minimal impact on non-target organisms and the surrounding ecosystem (Keerthi et al., 2022). With minimal residual effects and reduced harm, they are particularly suitable for pest management, especially in organic ecosystems of the northeastern region of India. Entomopathogenic fungi are considered crucial biological control agents for insect populations (Sharma and Malik, 2012). Moreover, these fungal agents possess biodegradable properties (Mishra et al., 2020). They could serve as viable alternatives to neem-based pesticides for managing pests in organic or natural citrus nursery production systems in Arunachal Pradesh.

Table 1. Efficacy of biopesticides against citrus butterfly and whiteflies

Treatments	Treatments	Dose	Number of Citrus butterfly caterpillar/seedling								Number of Whiteflies/seedling							
			1st spray		2nd spray		3rd spray		4th spray		1st spray		2nd spray		3rd spray		4th spray	
			7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS
TB_0	Control (Water spray)	-	3.60	4.10	4.40	3.98	3.85	3.68	3.15	2.90	9.85	11.90	13.60	15.10	16.80	18.10	15.80	15.50
TB_1	*Metarhizium anisopliae* NBAIR-Ma 35 (1×10^8 spores/ml)	5.0ml/L	1.95	2.02	1.73	1.80	1.41	1.48	1.14	1.08	7.50	7.95	6.20	6.44	5.66	4.96	4.80	4.60
TB_2	*Beauveria bassiana* NBAIR Bb-45 (1×10^8 spores/ml)	5.0ml/L	1.96	2.04	1.75	1.86	1.43	1.50	1.16	1.10	6.80	7.15	5.40	5.60	4.10	4.25	3.50	3.65
TB_3	*Lecanicillium lecanii* Vl- 8 (1×10^8 spores/ml)	5.0ml/L	1.80	2.30	2.05	2.50	1.85	2.02	1.80	1.92	7.51	7.60	6.50	6.80	4.80	4.95	3.40	3.10
TB_4	*Bacillus thuringiensis* NBAIR BTG4 (2×10^8 cfu/ml)	5.0ml/L	1.20	1.50	1.10	1.30	1.05	0.95	0.80	0.66	8.90	9.80	10.50	12.60	14.90	14.60	13.40	13.10
TB_5	Azadirachtin 1500 ppm	2ml/L	1.75	1.90	1.30	1.45	1.12	1.08	0.96	0.85	8.01	8.29	7.05	7.50	6.90	7.05	6.05	6.15
TB_6	Thiamethoxam 25WG	0.6ml/L	0.90	1.05	0.60	0.95	0.66	0.45	0.00	0.00	5.20	5.60	4.05	4.18	3.10	3.15	2.50	2.35
SE(m)±			0.02	0.01	0.04	0.02	0.04	0.05	0.02	0.01	0.07	0.06	0.09	0.09	0.09	0.10	0.11	0.09
C.D. (5%)			0.08	0.03	0.14	0.08	0.13	0.15	0.06	0.03	0.21	0.20	0.27	0.29	0.27	0.31	0.34	0.30
CV (%)			2.68	1.20	5.02	2.72	4.79	5.60	2.56	1.52	4.14	3.77	5.45	5.55	5.38	6.04	7.21	6.36

DAS: Days after spraying

MATERIALS AND METHODS

The present investigation was conducted at Fruit Nursery, Department of Fruit Science, College of Horticulture and Forestry, Pasighat located under Siang Valley of Arunachal Pradesh. The study employed a completely randomized design; with each of seven treatments being replicated thrice. The treatments were TB_0 (Control water spray), TB_1 (*Metarhizium anisopliae* NBAIR-Ma 35 (1×10^8 spores/ml) @ 5.0ml/L), TB_2 (*Beauveria bassiana* NBAIR Bb-45 (1×10^8 spores/ml) @ 5.0ml/L), TB_3 (*Lecanicillium lecanii* Vl- 8 (1×10^8 spores/ml) @ 5.0ml/L), TB_4 (*Bacillus thuringiensis* NBAIR BTG4 (2×10^8cfu/ml) @ 5.0ml/L), TB_5 (Azadirachtin 1500 ppm @ 2ml/L), TB_6 (Thiamethoxam 25 WG @ 0.6ml/L water). Rough lemon seedlings, with heights ranging from 8 to 10cm, were transplanted into pre-prepared polybags measuring 21×10 cm, with a capacity ranging from 1.20 to 1.30 kg. Various insect pest parameters such as the number of citrus butterfly caterpillars per plant, the number of whiteflies per plant, defoliation caused by citrus butterfly, incidence of leaf miner, number of natural enemies per plant, and plant vegetative parameters like plant height, internodal length, and number of leaves were meticulously recorded. Additionally, biochemical parameters including total chlorophyll (measured according to Arnon, 1949), carotenoids (also measured according to Arnon, 1949), total carbohydrates (following the methods outlined by Hedge and Hofreiter, 1962), and cellulose (analyzed using the procedures described by Updegraff, 1969) were assessed utilizing standard protocols.

Application of biopesticides

Treatments were given within the experimental framework at intervals of 15 days, and observations were carefully planned for the physical parameters for the 7th and 14th day after each treatment application. Observations were recorded 15 days after treatment for vegetative parameters and 25-day intervals for the biochemical parameters. The necessary solution amounts were determined by first spraying with water. A 500 ml solution was carefully made for each treatment plan and then given to a set of thirty plants. The quantity of biopesticide was precisely pipetted into a 500 ml laboratory-grade beaker, and the volume was then adjusted. Afterward, a sprayer was used to distribute the prepared solution evenly throughout the plant population. By ensuring uniform application, the methodological approach preserved the reproducibility and rigor of the experiments. To determine the significance of treatments, the percentage of pest infestation data were transformed into arcsine-transformed values, and the data on the number of pests were transformed into square root transformed values. The statistical analysis was done using OP stats software.

RESULTS AND DISCUSSION

The data displayed in the table 1 indicates that, the number of citrus butterfly caterpillar ranges from 0.00 to 4.40. The Thiamethoxam 25 WG @ 0.6ml/L was successful in reduction of citrus butterfly caterpillar to zero occurrence at the 7 and 14 DAS of 4th spray. The treatment *Bacillus thuringiensis* NBAIR BTG4 @ 5.0ml/L (0.80 and 0.66, respectively) recorded with significantly lower caterpillars among the biopesticides during 7 and 14 DAS of the 4thspray. The lower infestation may be attributed to the toxin produced by bacteria (*Bt*), a crystallized protein that creates perforations in the insect's gut lining upon ingestion. The insects eventually cease to feed and ultimately lead to death. Similar results were also documented in the studies conducted by Kalita *et al.* in 2015 and Swadener in 1994. The superiority of *Bt* over other treatments tested is also in agreement with Narayanamma and Savithri (2003).

Meanwhile at 14 DAS of the 4th spray, thiomethoxam 25WG @0.6 ml/L treatment imparted the lowest occurrence of whiteflies (2.35/seedling) (Table 1). Among the biopesticides, the EPF *Lecanicillium lecanii* Vl-8@ 5.0 ml/L was found to be the most successful treatment at 14 DAS with only 3.10 whiteflies per seedling. The *Beauveria bassiana* NBAIR Bb-45@ 5.0 ml/L was shown to be the second most effective treatment at 7 DAS of the fourth spray (3.50/seedling). The lowest population with thiomethoxam treated plants corroborates with the result of Kumar *et al.* (2017) in the study on efficacy of insecticides against whitefly in brinjal and revealed that application of thiomethoxam 25 WG @ 100g/ha gives zero population at the 3rd day of the 4th spray and Yadav *et al*. (2015) studied the efficacy of insecticides and bio-pesticides against sucking pest in black gram and found out thiomethoxam 25 WG @ 0.25g/L resulted best by reducing the whiteflies population from 2.33-3.60 to 0.11/3 leaves after 2nd spray. The result with biopesticides is consistent with research from by Kalita *et al.* (2015), Raghunandan *et al.* (2018) and Javed *et al.* (2019) documented that the enzymes and toxins released

Table 2. Efficacy of biopesticides on defoliation by citrus butterfly and incidence of leaf miner

Treatments	Treatments	Dose	Defoliation by citrus butterfly caterpillar (%)								Incidence of leaf miner (%)							
			1st spray		2nd spray		3rd spray		4th spray		1st spray		2nd spray		3rd spray		4th spray	
			7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS
TB_0	Control (Water spray)	-	23.70 (29.1)	25.00 (29.9)	27.50 (31.6)	29.80 (33.0)	33.10 (35.1)	32.05 (34.4)	29.50 (32.8)	26.10 (30.7)	35.10 (36.32)	36.90 (30.97)	28.50 (32.25)	31.40 (30.64(	29.20 (32.69)	30.00 (26.69)	24.70 (29.79)	26.20 (23.08)
TB_1	*Metarhizium anisopliae* NBAIR-Ma 35 (1×10^8 spores/ml)	5.0ml/L	13.30 (21.3)	15.80 (23.4)	10.80 (19.1)	12.10 (20.3)	11.50 (19.8)	13.70 (21.7)	12.05 (20.3)	11.75 (20.0)	22.70 (37.39)	23.90 (29.72)	21.8 (34.06)	23.50 (29.52)	15.30 (33.19)	16.40 (25.39)	13.80 (22.37)	12.50 (17.25)
TB_2	*Beauveria bassiana* NBAIR Bb-45 (1×10^8 spores/ml)	5.0ml/L	15.50 (23.1)	15.90 (23.4)	11.50 (19.8)	13.50 (21.5)	11.75 (20.0)	14.50 (22.3)	13.15 (21.2)	14.50 (22.3)	25.20 (28.43)	26.10 (30.51)	23.50 (27.81)	25.90 (30.38)	19.50 (23.01)	20.10 (25.90)	14.50 (30.78)	15.10 (19.99)
TB_3	*Lecanicillium lecanii* Vl- 8 (1×10^8 spores/ml)	5.0ml/L	15.70 (23.2)	18.00 (25.0)	16.50 (23.9)	20.75 (27.0)	18.60 (25.5)	22.50 (28.3)	18.10 (25.1)	20.10 (26.6)	20.70 (30.71)	22.60 26.69)	17.60 (30.58)	19.20 (24.64)	18.90 (26.62)	16.75 (21.95)	13.70 (20.35)	12.10 (19.71)
TB_4	*Bacillus thuringiensis* NBAIR BTG4 (2×10^8 cfu/ml)	5.0ml/L	11.20 (19.5)	11.70 (19.9)	7.10 (15.3)	8.70 (17.1)	4.20 (11.8)	5.30 (13.2)	3.00 (9.93)	3.90 (11.3)	25.60 (29.25)	26.50 (27.05)	23.40 (28.98)	26.00 (24.79)	19.60 (23.87)	20.20 (25.76)	14.70 (22.85)	15.40 (22.53)
TB_5	Azadirachtin 1500 ppm	2ml/L	11.50 (19.8)	11.90 (20.1)	8.40 (16.8)	9.20 (17.6)	5.30 (13.2)	6.00 (14.1)	3.30 (10.7)	4.10 (11.6)	24.60 (30.12)	25.80 (28.37)	24.30 (28.98)	25.60 (25.96)	18.40 (26.19)	19.10 (24.14)	11.40 (21.80)	11.70 (16.53)
TB_6	Thiamethoxam 25WG	0.6ml/L	10.20 (18.61)	10.40 (18.78)	6.80 (15.1)	7.20 (15.5)	3.80 (11.1)	4.20 (11.7)	1.50 (6.95)	2.00 (8.06)	20.20 (30.38)	22.70 (28.44)	17.40 (28.91)	16.50 (23.95)	14.00 (26.25)	10.50 (18.89)	8.80 (21.71)	6.50 (14.76)
SE(m)±			0.67	0.58	0.60	0.62	0.56	0.62	0.57	0.56	0.43	0.40	0.43	0.44	0.48	0.47	0.28	0.41
C.D(5%)			2.06	1.78	1.85	1.90	1.73	1.92	1.74	1.73	1.32	1.24	1.34	1.36	1.49	1.449	0.877	1.26
CV (%)			5.28	4.393	5.17	4.94	5.02	5.22	5.45	5.23	2.36	2.44	2.51	2.85	3.07	3.39	2.04	3.72

DAS: Days after spraying, Figures in the parentheses are arc sine transformed values

Table 3. Efficacy of biopesticides on natural enemies in citrus nursery

Trt. No.	Treatments	Dose	Number of natural enemies (spiders) per seedling							
			1st spray		2nd spray		3rd spray		4th spray	
			7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS
TB_0	Control (Water spray)	-	1.90	2.20	2.40	2.70	2.80	2.40	2.10	1.90
TB_1	*Metarhizium anisopliae* NBAIR-Ma 35 (1×10^8 spores/ml)	5.0ml/L	1.45	1.53	1.66	1.85 (1.5	1.90	1.74	1.53	1.35
TB_2	*Beauveria bassiana* NBAIR Bb-45 (1×10^8 spores/ml)	5.0ml/L	1.56	1.66	1.53	1.83	1.75	1.66	1.41	1.29
TB_3	*Lecanicillium lecanii* Vl-8 (1×10^8 spores/ml)	5.0ml/L	1.48	1.63	1.33	1.45	1.65	1.60	1.45	1.33
TB_4	*Bacillus thuringiensis* NBAIR BTG4 (2×10^8 cfu/ml)	5.0ml/L	1.43	1.51	1.33	1.75	1.85	1.75	1.66	1.51
TB_5	Azadirachtin 1500 ppm	2ml/L	1.35	1.50	1.43	1.66	1.53	1.38	1.33	1.21
TB_6	Thiamethoxam 25WG	0.6ml/L	0.70	0.80	0.90	0.70	0.40	0.30	0.10	0.00
SE(m)±			0.03	0.06	0.06	0.06	0.06	0.04	0.04	0.03
C.D (5%)			0.11	0.19	0.18	0.20	0.19	0.13	0.13	0.10
CV (%)			4.35	7.09	6.21	7.481	6.28	5.26	4.87	4.10

DAS: Days after spraying

by *L. lecanii* and *B. bassiana* spores penetrate the body of the insect through spiracles and cause internal organ and tissue damage. The population of infected whiteflies often declined as a result of the infected individuals' reduced ability to reproduce.

Data pertaining to defoliation by citrus butterfly and incidence of leaf miner are shown in table 2. From the table it is confirmed that the most effective treatment for defoliation by citrus butterfly was observed in thiamethoxam 25WG @ 0.6ml/L with 1.50 and 2.00 percent at 7 and 14 DAS of the 4th spray, respectively. With regards to biopesticides the *B. thuringiensis* NBAIR BTG4 @ 5.0ml/L (3.00% and 3.90%) was recorded with least defoliation followed by TB_5 (Azadirachtin 1500 ppm @ 2ml/L) (3.30% and 4.10%) at 7 and 14 DAS of the 4th spray. This parameter correlates with the number of citrus butterfly caterpillar per plant. Since the population of caterpillar reduces due to the effect of Bt the percentage of defoliation eventually reduces.

Regarding the leaf miner the most effective treatment was in chemical treatment thiamethoxam 25WG @ 0.6ml/L with 8.80 and 6.50 percent incidence respectively, at 7 and 14 DAS of 4th spray followed by the treatment Azadirachtin 1500 ppm @ 2ml/L *i.e.,* 11.40 and 11.70 percent incidence. Among all the treatments tested, thiomethoxam was found the most superior and this results are in agreement with Shinde *et al.* (2017) which evaluated different insecticides against citrus leaf miner in Nagpur Mandarin and reveals that thiomethoxam 25 WG (0.06%) recorded lowest leaf infestation percentage (5.47) and likewise Farmanullah and Gul (2005) evaluated six different insecticides for the control of citrus psylla and

reveals that the percent decrease in population of *D. citri* was highest in thiomethoxam 25 WG (72.20 %) in first spray and (83.54 %) in the 2nd spray. Similar outcomes regarding biopesticides are also noted by Kalita *et al.* (2015) and Abebe (2019) while using azadirachtin and neem oil, both of which have insect-repelling qualities. These materials coat plants with oil particles, which create a barrier that discourages female moths from choosing to lay their eggs. This reduces infestation and population. Moreover, Azadirachtin hinders insect larvae's ability to moult and develop, which keeps them from maturing and reproducing, which stops the pest population from growing.

The data in the table 3 depicts the occurrence of natural enemies mainly spiders present per seedlings. During 7 and 14 DAS of last spray application the maximum number of spiders was recorded in control treatment of water spray (2.10 and 1.90, respectively) followed by *B. thuringiensis* NBAIR BTG4@ 5.0 ml/L (1.66 and 1.51, respectively) and *M. anisopliae* NBAIR-Ma 35@ 5.0 ml/L (1.53 and 1.35, respectively). Considering the aforementioned findings during the investigation, it was noticed that the literature on this aspect was found negligible. However, increased activity of natural enemies, which often follows the pest building pattern under normal agro-ecosystems, can be related to the decreased occurrence of pests during the fourth spray. This study demonstrates that Bacillus thuringiensis NBAIR BTG4 (at a concentration of 2×10^8 cfu/ml) at 5.0ml/L, *Lecanicillium lecanii* Vl-8 (with 1×10^8 spores/ml) at 5.0ml/L, and Azadirachtin at 1500 ppm (applied at 2ml/L) were effective in reducing the populations of citrus butterfly caterpillars, whiteflies, and leaf miners, respectively.

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An annotated checklist of the genus *Popillia* (Coleoptera: Scarabaeidae) of India

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ABSTRACT: *Popillia* Dejean, 1821 is one of the small genera in the subfamily Rutelinae of Scarabaeidae family. (Coleoptera). The Specis of *Popillia* are economicaly important pest of several agricultural and horticultural crops. Though several authors though reported the occurrence of these species throughout the Oriental region, a comprehensive checklist of the Indian fauna is not in place. This paper provides a comprehensive checklist of 43 species of the genus *Popillia* of Indian fauna along with their synonyms, combinations and distribution.

Key words: Checklist, *Popillia,* India, Scarabaeidae

INTRODUCTION

Popillia Dejean, 1821 is a small genus of tribe Anomalini and subtribe Popilliina in the subfamily Rutelinae of Scarabaeidae which is represented by 322 species and 27 subspecies globally (Schoolmeesters 2021). Arrow (1917) gave an account on 42 species in Indian sub-continent in his Fauna volume of British India (part-2). The species of this genus are serious pests of agricultural and horticultural crops and are often found in great numbers in aggregation. They cause damage by feeding on leaves, flowers and fruits. The affected plants are left with only veins with complete skeletonization of the foliage. The adult beetles feeding on fruits of horticultural crops *viz.,* apple, peach, pear, plum (Sreedevi *et al*., 2019) results in heavy fruit drop thus causing serious economic losses. The larvae are subterranean feeders on roots of different horticultural plants and grasses in different ecosystems. Certain species of this genus feed extensively on roots of grasses thus becoming detrimental to golf courses, parks, lawns. Several authors though reported the occurrence of these beetles throughout the globe, yet a comprehensive checklist of the Indian fauna is still not available. We made an atempt to compile the annotated checklist as given below.

ANNOTATED CHECKLIST OF THE GENUS, *POPILLIA*

1. *Popillia acuta* Newman, 1838
Synonyms and Combinations: *Popillia nasuta* Newman, 1838
Distribution: Himachal Pradesh, Uttarakhand and Maharashtra (Arrow 1917; Chandra 2005; Chandra *et al.,* 2012a,b; Sreedevi *et al.,* 2017; Chandra *et al.,* 2018).

2. *Popillia adamas* Newman, 1838
Synonyms and Combinations: *Popillia adamas viridinitens* Kraatz, 1892
Distribution: West Bengal, Jharkhand (Mandar) and Maharashtra (Bombay, Bandra, Khandala, Khandesh). (Arrow 1917).

3. *Popillia amabilis* Arrow, 1913
Distribution: Assam, Manipur, Nagaland, Meghalaya and West Bengal. (Arrow, 1917;
Chatterjee and Biswas 2000b; Chatterjee 2004; Sarkar *et al.,* 2016; Gosh *et al.,* 2020).

4. *Popillia birmanica* Arrow, 1913
Distribution: Tripura and Assam. (Arrow 1917; Chatterjee and Biswas 2000a).

5. *Popillia chlorion* Newman, 1838
Distribution: Tamil Nadu (Madras, Nilgiris hills, Ooty) (Arrow 1917; Chandra 2009).

6. *Popillia clypealis* Ohaus, 1897
Distribution: Assam, Meghalaya (Khasi hills), Jammu & Kashmir, Himachal Pradesh (Shimla), Punjab and Uttarakhand. (Arrow 1917; Chatterjee and Biswas 2000b; Chandra 2005; Chandra *et al.,* 2018).

7. *Popillia complanata* Newman, 1838
Distribution: Maharashtra (Bombay), N. Karnataka, Tamil Nadu (Nilgiris hills, Nadhugani), Kerala (Malabar), (Arrow 1917; Chandra 2009).

8. *Popillia cupricollis* Hope, 1831
Synonyms and Combination*:*
Popillia hirta Lin, 1987
Popillia suturata Newman, 1841
Popillia caschmirensis Kollar& Redtenbacher, 1848

Popillia smaragdula Hope, 1831
Popillia formosa Hope, 1831
Popillia hilaris Burmeister, 1855
Distribution: Arunachal Pradesh, Meghalaya, Assam, Sikkim, Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Uttarakhand, Uttar Pradesh and West Bengal (Arrow 1917; Kacker 1966 ; Chatterjee and Biswas 1995; Chatterjee and Biswas 2000b; Chatterjee and Biswas 2003; Chandra 2005; Chatterjee 2010; Chandra *et al.,*2012a,b; Sreedevi *et al.,*2017; Chandra *et al.,*2018).

9. *Popillia cyanea* Hope, 1831
Synonyms and Combinations:
Popillia concolor Castelnau, 1840
Popillia cyanea somnulosa Newman, 1841
Popillia beryllina Hope, 1831
Distribution: Arunachal Pradesh, Assam, Nagaland, Sikkim, Meghalaya, Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Uttarakhand, Uttar Pradesh and West Bengal. (Arrow 1917; Kacker 1966; Chatterjee and Saha 1981; Chatterjee and Biswas 1995; Chatterjee and Biswas 2000b; Chandra 2005; Chatterjee 2010; Chandra *et al.,*2012a, b; Pathania *et al.,*2015; Sreedevi *et al.,*2017; Chandra *et al.,*2018; Gosh *et al*.,2020).

10. *Popillia difficilis* Newman, 1838
Distribution: India (Newman, 1838).

11. *Popillia eximia* Arrow, 1913
Distribution: Tamil Nadu (Madras, Nilgiris hills) (Arrow 1917; Chandra 2009).

12. *Popillia feae* Kraatz, 1892
Synonyms and Combinations; *Popillia semiaenea* Kraatz, 1892

Popillia semiaenea cupricollis Kraatz, 1892

Popillia semiaeneaaenea Kraatz, 1892

Popillia tesari Sabatinelli, 1984

Popillia simoni Kraatz, 1892
Distribution: Assam, West Bengal, Sikkim, Meghalaya, Himalaya. (Arrow 1917; Chatterjee and Biswas 2000b; Chatterjee and Biswas 2003; Chandra *et al.,* 2018)

13. *Popilla felix* Arrow, 1913
Distribution: Assam (Arrow 1917).

14. *Popillia gemma* Newman, 1838
Synonyms and Combinations: *Popillia nitidicollis* Gory, 1844

Popillia metallicollis Fairmaire, 1887

Distribution: Assam, Sikkim and Indian Himalaya. (Arrow 1917; Chandra *et al.,* 2018).

15. *Popillia girardi* Sabatinelli, 1994
Distribution: Tamil Nadu (Chennai) and Kerala (Sabatinelli, 1994, Chandra 2009).

16. *Popillia impressipyga* Ohaus, 1897
Distribution: Assam, Sikkim, Meghalaya and Indian Himalaya (Arrow 1917; Chatterjee and Biswas 2000b; Chandra *et al.,* 2018).

17. *Popillia kanarensis* Arrow, 1917
Distribution: Maharashtra (Arrow 1917).

18. *Popillia laevicollis* Kraatz, 1892
Distribution: Assam, Meghalaya, Sikkim, Indian Himalaya, Uttarakhand and West Bengal. (Arrow 1917; Chatterjee and Biswas 1995; Chatterjee and Biswas 2000b; Chatterjee 2010; Chandra *et al.,* 2012a, b; Chandra *et al.,* 2018).

19. *Popillia laevis* Burmeister, 1855
Synonyms and Combinations; *Popillia clara* Arrow, 1913
Distribution: Chhattisgarh, Tamil Nadu (Chennai, Nilgiris hills), Pondicherry and Kerala (Travancore) (Arrow 1917; Chandra 2009; Chandra and Gupta 2012a; Chandra *et al.,* 2018).

20. *Popillia laevistriata* Arrow, 1913
Distribution: Tripura and Assam. (Arrow 1917; Chatterjee and Biswas 2000a)

21. *Popillia lasiopyga* Lin, 1987
Distribution: Sikkim and Indian Himalaya (Chandra *et al.,* 2018)

22. *Popillia lucida* Newman, 1838
Distribution: Indian Himalaya, Uttarakhand, Tamil Nadu and Kerala. (Madras, Nilgiris hills), (Arrow 1917; Chandra 2009; Chatterjee 2010; Chandra *et al.,* 2012a; Chandra *et al.,* 2018).

23. *Popillia madrasicola* Machatschke 1913
Synonyms and Combinations: *Popillia propinqua* Arrow, 1913

Distribution: Tamil Nadu (Nilgiri Hills, Ootakamand), Kerala (Chandra 2009)

24. *Popillia macclellandi* Hope, 1845

Distribution: Arunachal Pradesh, Nagaland, Assam, Manipur, Meghalaya, Himachal Pradesh, Uttarakhand, and West Bengal. (Arrow 1917; Chatterjee and Biswas 2000b; Chandra and Gupta 2012b; Sarkar *et al.,* 2016; Sreedevi *et al.,* 2017; Chandra *et al.,* 2018; Gosh *et al.,* 2020).

25. *Popillia marginicollis* Hope, 1831
Synonyms and Combinations:
Popillia marginicollis purpuricollis Kraatz, 1892
Popillia marginicollis atrata Kraatz, 1892
Popillia marginicollis viridichlamys Benderitter, 1929
Distribution: Assam, Sikkim, Himachal Pradesh and Uttarakhand. (Arrow 1917; Biswas, Chatterjee and Sengupta 1999; Sreedevi *et al.,* 2017; Chandra *et al.,* 2018).

26. *Popillia minuta* Hope, 1831
Distribution: Sikkim and Indian Himalaya (Chandra *et al.,* 2018).

27. *Popillia nitida* Hope, 1831
Synonyms and Combinations: *Popillia trichiopyga* Lin, 1981
Distribution: Assam, Manipur, Meghalaya (Khasi Hills), Sikkim and West Bengal. (Arrow 1917; Kacker 1966; Chatterjee and Biswas 1995; Chatterjee and Biswas 2000b; Chatterjee 2004; Chandra *et al.,* 2018).

28. *Popillia nottrotti* Kraatz, 1892
Distribution: Arunachal Pradesh, Sikkim, Indian Himalaya and West Bengal. (Arrow 1917; Chatterjee and Biswas 1995; Chandra and Gupta 2012b; Chandra *et al.,* 2018).

29. *Popillia patkaina* Arrow, 1917
Distribution: Assam and Meghalaya (Patkhai hills) (Arrow 1917)

30. *Popillia patricia* Arrow, 1917
Synonyms and Combinations: *Popillia bothynoma* Ohaus, 1938
Distribution: Nagaland, Assam and Meghalaya (Khasi Hills and Jaintia Hills). (Arrow 1917; Chatterjee and Biswas 2000b; Gosh *et al.,* 2020).

31. *Popillia pilicollis* Kraatz, 1892
Distribution: Arunachal Pradesh, Assam, Sikkim, Indian Himalaya and West Bengal (Gopaldhara, Rungbong Valley, Kurseong, Mungphu), (Arrow 1917; Chatterjee and Biswas 1995; Chandra *et al.,* 2018).

32. *Popillia pilosa* Arrow, 1913
Distribution: Himachal Pradesh and Uttarakhand (Dehradun, Kumaon, Almora, Lansdowne, Garhwal, Ranikhet). (Arrow 1917; Chandra 2005; Chatterjee 2010; Chandra *et al.,* 2012ab; Pathania *et al.,* 2015).

33. *Popillia pulchripes* Arrow, 1913
Synonyms and Combinations: *Popillia complanata testaceipes* Ohaus, 1897
Distribution: Tamil Nadu (Madras, Nilgiris hills) (Arrow 1917; Chandra 2009).

34. *Popillia pulchra* Arrow, 1913
Distribution: Meghalaya (East Khasi Hills Dist.) (Chatterjee and Biswas 2000b).

35. *Popillia puncticollis* Kraatz, 1897
Distribution: Meghalaya (Jaintia Hills Dist.), Sikkim and Indian Himalaya (Arrow 1917; Chatterjee and Biswas 2000b; Chandra *et al.,* 2018).

36. *Popillia schizonycha* Arrow, 1913
Distribution: Karnataka (Bangalore) and Tamil Nadu (Madras, Nilgiris hills) (Arrow 1917; Chandra 2009).

37. *Popillia semicuprea* Kraatz, 1892
Synonyms and Combinations:
Popillia spediceipennis Lin, 1987
Popillia fallaciosasemicuprea Kraatz,
Distribution: Indian Himalaya and Sikkim (Chandra *et al.,* 2018)

38. *Popillia shillongensis* Sabatinelli, 1994
Distribution: Assam (Sabatinelli, 1994)

39. *Popillia sikkimensis* Lin, 1987
Synonyms and Combinations: *Popillia unicolor* Frey, 1975
Popillia virescens concolor Kraatz,
Distribution: Sikkim and Indian Himalaya (Chandra *et al.,* 2018)

40. *Popillia simlana* Arrow, 1913
Distribution: Himachal Pradesh and Punjab. (Arrow 1917; Chandra 2005; Chandra *et al.,* 2018).

41. *Popillia subquadrata* Kraatz, 1892
Distribution: Nagaland and Assam. (Arrow 1917; Gosh *et al.,* 2020).

42. *Popillia sulcata* Kollar& Redtenbacher, 1848
Synonyms and Combinations:
Popillia sulcata testaceipennis Kraatz, 1892
Popillia scutellaris Blanchard, 1850
Popillia sulcata cupripennis Kraatz, 1892
Distribution: Indian Himalaya, Jammu & Kashmir and Uttarakhand (Chatterjee 2010; Chandra *et al.,* 2012a; Chandra *et al.,* 2018).

43. *Popillia testaceipennis* Kraatz, 1892
Distribution: Meghalaya (East Khasi Hills, West Garo Hills) (Chatterjee and Biswas 2000).

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Seasonal incidence of key natural enemies of onion thrips, *Thrips tabaci* L. in relation to weather parameters in the *Terai* region of Uttarakhand, India

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ABSTRACT: *Thrips tabaci*, Lindeman (Thysanoptera: Thripidae) is the most serious pest on onion causing substantial yield losses. The present study aims to document the population dynamics of thrips and natural enemies in the Terai region of Uttarakhand, India. Observations were recorded during the crop growing seasons, *i.e.* April 2021 and April 2022 and the continued till crop harvest. Spiders, Coccinellids, and Orius bugs exhibited distinct activity peaks. Temperature, rainfall (2021), and sunshine hours (2022) positively influenced spider populations. Conversely, morning and evening relative humidity (RH) during 2022 had a negative impact on the spider population. Coccinellids were positively affected by maximum temperature (second year), minimum temperature, and first-year rainfall, while RH negatively influenced them. Orius bugs responded positively to temperature and sunshine hours but were negatively affected by morning and evening RH.

Keywords: Natural enemies, onion, seasonal incidence, thrips

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most significant vegetable crops of the Alliaceae family, originated in Central Asia (Brewster, 1994), and it is grown primarily for its bulb (Sani and Jaliya, 2009). It has been a good source of carbohydrates (11.0 g), proteins (1.2 g), fibre (0.6 g), water (86.8 g), and vitamins such as vitamin A (0.012 mg), vitamin C (11 mg), thiamine (0.08 mg), riboflavin (0.01 mg), and niacin (0.2 mg), as well as minerals such as phosphorus (39 mg), calcium (27 mg), sodium (1.0 mg), iron (0.7 mg), and potassium (157 mg) (Suresh, 2007). Several insect pests infest onion crops, including thrips, onion maggots, cutworms, tobacco caterpillars, etc. The most severe pest of onion is onion thrips, *Thrips tabaci*, Lindeman (Thysanoptera: Thripidae), which has a global distribution and is polyphagous, infesting a wide variety of crops and causing substantial yield losses (Trdan *et al*., 2005). Initially, yield loss ranged between 10-15% owing to insects and diseases, but *T. tabaci* incidence alone resulted in a 30-50% yield decline (Karar *et al*., 2014). While onion agro-ecosystems often host a diverse range of natural predators like spiders, coccinellids, Orius bugs, and green lacewings, the indiscriminate use of broad-spectrum insecticides poses threats to both onion pests and their natural enemies. This practice results in resistance issues and harms the environment, soil fertility, human health, and beneficial insects (Rueda and Shelton, 1995; Rola and Pingali, 1993; Antle and Pingali, 1994; Tjornhom *et al.,* 1997).

Recognizing the importance of preserving these natural enemies, many researchers advocate for their conservation to address pest problems such as onion thrips. A comprehensive understanding of the seasonal occurrence of natural enemies of *T. tabaci* is crucial for devising effective pest management strategies. This study aims to explore the seasonal incidence of key natural enemies of *T. tabaci* and their interactions with weather parameters.

MATERIALS AND METHODS

Field experiments were conducted during the winter seasons of 2020-21 and 2021-22 at the Vegetable Research Centre (VRC), GBPUA&T, Pantnagar, Uttarakhand using the onion variety "Agrifound Light Red" in a randomized block design (RBD) with four replications. Planting was donde on December 20 in 5×5 m plots, maintaining row-to-row and plant-to-plant intervals of 20 and 15 cm, respectively. Adhering to recommended agronomic practices for the region, the crop was diligently managed throughout its growth period. To assess *T. tabaci* predator populations, nymphal and adult stages were quantified. Weekly visual recordings were made on ten randomly selected and tagged plants from the inner rows of each plot, starting from transplanting until crop harvest. They were conducted in the early hours before 9:00 AM to coincide with the insects' least active period. To count predator populations accurately, leaves were gently turned to expose the full underside. Weather parameters (maximum and minimum temperature in °C, morning and evening relative humidity in %, rainfall in mm, sunshine hours in hrs, and wind velocity in km/hr) were recorded during different standard weeks

Table 1. Influence of weather factors on seasonal incidence of spiders, coccinellids and Orius bugs at weekly interval on onion during Rabi, 2020-21

Std. Week	Weather parameters							Population/plant		
	Temperature (^{0}C)		RH (%)		Rainfall (mm)	Sunshine Hrs	Wind velocity (km/hr)	Spiders	Coccinellids	Orius bug
	Max.	Min.	Mor.	Even.						
9	29.3	11.2	90	37	0.0	9.2	5.8	0.0	0.0	0.25
10	29.6	14.2	83	46	0.0	7.6	3.8	0.0	0.0	1.29
11	30.3	13.6	86	36	0.0	7.0	2.5	0.0	0.0	1.24
12	32.6	14.1	82	27	0.0	8.0	4.2	0.0	0.0	2.54
13	33.4	14.6	84	23	0.0	7.9	4.3	0.4	0.5	1.72
14	37.5	14.1	59	11	0.0	10.0	3.8	0.6	0.7	2.50
15	36.8	17.8	57	17	0.7	8.4	5.2	0.9	0.9	4.81
16	36.7	17.1	52	16	0.0	10.3	3.0	1.2	1.4	3.51
17	34.9	19.9	73	41	36.4	8.5	4.7	1.9	1.7	1.58
18	32.4	21.1	76	50	80.4	5.1	2.4	1.3	1.4	1.32

Std. - Standard; RH- Relative Humidity

Table 2. Influence of weather factors on seasonal incidence of spiders, coccinellids and Orius bugs at weekly interval on onion during Rabi, 2021-22

Std. Week	Weather parameters							Population/plant		
	Temperature (^{0}C)		RH (%)		Rainfall (mm)	Sunshine Hrs	Wind velocity(km/hr)	Spiders	Coccinellids	Orius bug
	Max.	Min.	Mor.	Even.						
11	23.7	9.8	86	45	0	8	6.3	0.0	0.0	1.0
12	24.7	10.0	93	48	2.2	8	3.1	0.0	0.0	1.5
13	27.5	11.3	92	48	0	7	3.4	0.0	0.0	2.1
14	30.7	16.6	90	49	0	7.1	1.9	0.0	0.0	1.9
15	33.9	17.1	84	40	0	8.8	2.4	0.8	0.8	2.4
16	34.0	15.9	80	31	0.0	9.0	4.6	0.9	0.7	2.0
17	37.1	20.9	65	31	2.6	8.3	5.1	1.2	1.2	2.9
18	38.2	19.1	63	19	0.0	9.0	3.5	1.8	1.4	5.4
19	38.0	19.0	54	19	0.0	10.3	4.0	2.2	1.9	3.0
20	35.2	23.3	67	43	5.0	8.5	4.1	1.7	1.5	1.50

Std- Standard; RH- Relative Humidity

throughout the cropping seasons. The relationship between these weather parameters and the incidence of *T. tabaci* predators on onion plants was determined through simple correlation analysis.

RESULTS AND DISCUSSION

The following are the findings and discussions from investigations on the seasonal incidence of *T. tabaci* natural enemies on the onion crop throughout both years:

Seasonal incidence of natural enemies

Spider

This study observed unspecified spider species on onion plants starting from the 13th standard week in the first year and the 15th standard week in the second year until crop harvest (Tables 1 and 2). The spider population exhibited a parallel increase with the thrips population, peaking at 1.9 spiders per plant in the last week of April (17th SW) during the first year and 2.20 spiders per plant in the second week of May (19th SW) in the second year. Subsequently, the spider population declined but persisted on the crop until harvest, ranging between 0.40 and 1.90 per plant in the first year and 0.80 and 2.20 per plant in the second year.

Coccinellids

Concerning coccinellids, three species (*Coccinella septempunctata* Linnaeus, *Menochilus sexmaculatus* Fabricius, and *Brumoides suturalis* Fabricius) were identified on onion crop from the first week of April (13th SW) in 2020-21 and the second week of April (15th SW) in 2021-22 (Tables 1 and 2). The coccinellid population gradually increased, reaching its maximum at 1.7 per plant in the last week of April (17th SW) in the first year and 1.9 per plant in the first week of May (19th SW) in the second year, persisting until crop harvest. Coccinellid population varied between 0.50 and 1.70 per plant in the first year and 0.80 and 1.90 per plant in the second year.

Orius bug

Throughout the crop cycle, the Orius bug exhibited activity against thrips in the onion cultivation area. Nymphs and adults of the Orius bug were first observed in the 9th standard week of the first year and the 11th standard week of the second year (Tables 1 and 2). In the first year, the bug population varied from 0.25 to 4.81 per plant, while in the second year, it ranged from 1.0 to 5.40 per plant. The Orius bug population steadily increased, reaching its peak at 4.81 per plant in the third week of April (15th SW) during the first year and 5.40 per plant in the last week of April (18th SW) in the second year, persisting until crop harvest. Seasonal weekly bug numbers are presented in Tables 1 and 2. This observation aligns with Wagan et al. (2014) findings, reporting spider populations between 0.20 and 4.91 per plant, coccinellid populations ranging from 0.73 to 2.95 per plant, and Orius bug populations recorded at 0.53 to 4.30 per plant, with the highest population (4.30 per plant) observed in the 11th week after transplanting.

Dhaka and Pareek (2007) documented spider populations in a cotton ecosystem ranging from 1.75 to 9.50 per 10 plants in the first year and 2.5 to 19.75 per 10 plants in the second year. Additionally, coccinellid populations varied between 2.25 and 21.00 per 10 plants in the first year and 4.75 to 19.75 per 10 plants in the second year. In a study on Bhut Jolokia, Begam et al. (2016) identified *Coccinella transversalis* and *Micraspis discolor* as the dominant predator species combating sucking pests, including thrips, throughout the cropping season. The highest coccinellid populations (1.86 and 1.80 per plant) were observed in March 2014 and 2015, respectively.

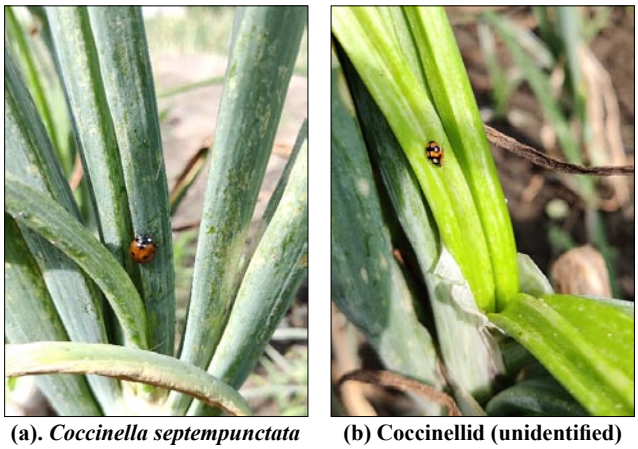
Correlation between seasonal incidence of natural enemies and weather parameters

Spider

Maximum temperature (r = 0.61 in the first year and r = 0.89 in the second year), minimum temperature (r = 0.88 in the first year and r = 0.81 in the second year), rainfall (r = 0.61 in the first year), and sunshine hours (r = 0.83 in the second year) exhibited a significant positive impact. However, morning relative humidity (r = -0.57 in the first year and r = -0.96 in the second year) and evening relative humidity (r = -0.84 in the second year only) had a significant negative influence on the spider population that preys on onion thrips (Table 3). Meanwhile, all other meteorological factors showed no discernible effect. These findings align with Dhaka and Pareek (2007) study, who observed a positive correlation between maximum and minimum temperatures and the spider population involved in controlling insect pests in cotton.

Coccinellids

The information presented in Table 3 reveals that maximum temperature (r = 0.65 in the first year, r = 0.89 in the second year), minimum temperature (r = 0.88 in the first year, r = 0.83 in the second year), and rainfall (r = 0.60 in the first year only) have a significantly positive impact on the population of Coccinellids. Conversely, morning relative humidity (r = -0.63 in the first year and r = -0.96 in the second year) and evening relative humidity (r = -0.81 in the second year only) significantly negatively influence. The coccinellid population remains



(a). *Coccinella septempunctata* (b) Coccinellid (unidentified)

(c) Coccinellid (unidentified) (d) Pirate bug *(Orius spp.)*

Fig 1. Coccinellids and Orius bugs feeding on *T. tabaci*

(e) (f)

(g) (h)

Fig 2. Fig. (e), (f), (g) and (h): Different kind of spiders feeding on *T. tabaci*

unaffected by other meteorological variables. This aligns with Dhaka and Pareek (2007) findings, who observed a negative significant effect of evening relative humidity on the coccinellid population combating insect pests in cotton. Additionally, Begam et al. (2016) reported a significant positive correlation between coccinellid predators and maximum temperature.

Orius bug

The results presented in Table 3 demonstrate that both maximum and minimum temperatures (r = 0.78 in the first year, r = 0.69 in the second year) and (r = 0.31 in the first year, r = 0.43 in the second year), respectively, along with sunshine hours (r= 0.43 in the first year and r=0.42 in the second year), significantly positively influenced the Orius bug population. Conversely, morning relative humidity (r = -0.82 in the first year and r = -0.59 in the second year) and evening relative humidity (r = -0.71 in the first year and r = -0.78 in the second year) had a significant negative impact. Other weather variables had minimal effects on the bug population. Notably, these data cannot be compared or discussed due to the absence of existing research on the influence of abiotic factors on the incidence of Orius bugs on onions and other crops.

Based on the above results, it may be concluded that, in the onion crop, the population of natural enemies like spiders, coccinellids, Orius bugs, etc., has been active since the appearance of thrips and has continued till harvesting of the crop, which can play a major role in keeping the onion thrips population below the economic threshold level (ETL). More extensive research is needed to determine the seasonal incidence of these natural enemies of key insects, such as onion thrips and other onion pests, as well as the effect of weather factors on their population dynamics, which will aid researchers and other scientists in developing safe and environmentally friendly integrated pest management strategies.

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Seasonal incidence of major insect pests and their natural enemies on Cauliflower (*Brassica oleracea* var. *botrytis*) in relation to weather parameters

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ABSTRACT: A field experiment was conducted to study the population dynamics of major insect pests and their natural enemies in cauli flower eco system in two consecutive cropping seasons *i.e.,* winter 2021-22 and 22-23 at experimental farm of Dr Rajendra Prasad Central Agricultural University, Pusa, Bihar. In cropping season 2021-22, the incidence was at its peak of the pests: Aphids (123.72), Diamondback Moth (12.83), Tobacco caterpillar (1.02), Leaf webber (2.83), and Flea beetle (2.56) per plant were recorded on 12thSMW, 11thSMW, 12thSMW, 9thSMW, and 12thSMW, respectively. Similarly, on 11thSMW, 12thSMW, 12thSMW, 10thSMW, and 12thSMW of cropping season 2022-23 maximum incidence of aphids (147.2), DBM (17.47), tobacco caterpillar (1.65), leaf webber (1.43) and flea beetle (2.53) per plant were recorded, respectively. Maximum population of *Cotesia plutellae* were 3.86 and 5.43*, Oomyzus* sp. were 1.01 and 1.21, *Aphidius colimani* 13.48 and 15.83, Coccinellids were 5.13 and 9.56, and Syrphids were 2.12 and 2.16 recorded during the cropping season 2021-22 and 2022-23, respectively. The correlation between population of insect pests and natural enemies in relation to weather parameters shown significant positive correlation with temperature (maximum and minimum), rainfall and sunshine while negative and significant correlation to relative humidity (morning - evening) during both the cropping season.The coefficient of determination (adjusted R^2) value and regression equations obtained after subjecting insect population data in multiple linear regression analysis in relation to weather parameters indicates significant influence of weather parameter on population build-up of insect pests and related natural enemies.

Keywords: Aphids, *Aphidius, colimani,* cauliflower, coccinellids, *Cotesia plutellae,* diamondback moth, weather parameters

INTRODUCTION

India is the second largest producer of fruit and vegetables with production of 108.34 and 212.91 million tonnes, respectively (2nd Advance Estimate for the year 2022-23 resealed by Ministry of Agriculture and Farmers Welfare). Cole crops are the most important group of vegetable grown in winter months in sub-tropical plains to temperate hilly regions of the country. Among them, cauliflower is most important crop, cultivated in 4.90 lakh hectares which produces 95.21 lakh metric ton with productivity of 19.42 metric ton per hectare (2022-23-2nd Advance Estimate, India state Agri). Major cauliflower growing states includes Uttar Pradesh, Orissa, Bihar, West Bengal, Assam, Karnataka, Maharashtra, Madhya Pradesh and Tamil Nadu. Bihar is the third largest producer of cauliflower with an area and production of 0.68 lakh hectares and 1.11metric ton, respectively (2022-23-2nd Advance Estimate, India state Agri).

Production of cauliflower is constrained by number of factors like fluctuating weather factors, lack of knowledge about improved varieties for different season, diseases and pest incidence, inappropriate their management techniques. Across the world, insect pests and diseases causing annually 40 per cent of total crop loss while 35 per cent in India (Annual Report-2020, FAO). Major insect pests causing potential damage to cauliflower in the referred region includes Diamondback moth, *Plutella xylostella*; Head borer, *Hellula undalis*; Leaf webber, *Crosidolomia binotalis*; Aphids, *Brevicoryne brassicae*; Cabbage butter fly, *Pieris rapae*, Tobacco caterpillar, *Spodoptera litura* and painted bugs, *Bagrada cruciferarum etc* (Sahu *et al.,* 2019). In India, diamondback moth is one of the limiting factors in successful production of good quality marketable cauliflower due to its higher damage potential ranging from 14 to 84 per cent (Abhijith *et al.,* 2019; Gautam *et al.,* 2018) causing 30 to 100 per cent of quality yield loss (Lingappa *et al.,* 2004). *Spodoptera litura* is a polyphagous pest reported on about 112 cultivated plants, also considered as a major pest on early crucifers. It is responsible to cause economic loss ranging 25 to cent per cent (Sahu *et al.,* 2020a). Aphids is also one of the serious pest among the sucking pests has potential to cause considerable damage to the crop by its presence and sucking plant juice resulting leaf yellowing and curling, plant stunting and wilting.

Natural enemies play vital role in regulation of population of arthropod pests. In cauliflower ecosystem, the predators like lady bird beetle, mantids, green lace wings, ants and syrphids are more common and abundant. Hymenopteran parasitoids *viz*., *Aphidius*sp, *Aphilinus*sp,

Encarsia spare nymphal parasitoid on aphids, *Cotesia plutellae* a larval parasitoid on DBM, *Oomyzus*sp a larval-pupal parasitoid on DBM and *Bracon* sp a larval parasitoid on *S. lituraetc*., are the potential biocontrol agents. Pagore *et al.* (2021) reported the significant parasitizing potential of biocontrol agent ranging from 10 per cent to 80 per cent based on their reproductive capacity, host preference and climatic factors.

As the world is moving towards sustainable agriculture production system with the objective of successful management of pest through eco-friendly, economically feasible and socially acceptable techniques. Integrated Pest Management (IPM) is an effective strategy to attain it. Chemical management of pests using insecticide may leads to various environmental and health hazards. The knowledge of the seasonal incidence of insect pests at different growth stages of cauliflower crop may be helpful in evolving proper management. For the sustainable management of pests under IPM, the knowledge on seasonal incidence, and its relation with metrological variables are the key attributes for early prediction of its incidence, identification of critical stage of pest and to schedule of management practices in advance. Keeping above facts in mind this insect pest management in cauliflower was planned and the experiment was conducted.

MATERIALS AND METHODS

Field experiments on population dynamics of major insect pests and their natural enemies in cauliflower ecosystem were carried out in two consecutive cropping seasons *i.e.,* winter 2021-22 and 22-23 in experimental farm of Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar. About thirty days old locally suitable and popular variety (Pusa snowball) cauliflower seedlings were transplanted in the prepared experimental plots in the evening hoursat 60 cm x 60 cm plant spacing to 10 x 5 m plots in second fortnight of December. Crop were grown by following standard package of practice recommended by Dr. RPCAU, Pusa, Bihar to get proper crop stand to get suitable yield except pest management practices which were applied as per the proposed experimental plan.

Observations were recorded to know the abundance of major insect pests and their natural enemies in cauliflower ecosystem since their appearance get on plants to till harvesting. To know the population of diamond back moth, tobacco caterpillar, leaf webber and aphid, absolute counting method were used on randomly selected and tagged 10 plants which were tagged after ten days of planting. Among the natural enemies, predators like coccinellids (grub+pupa+adult) and syrphids (only adult) were counted on per plot basis. Mummified aphids were observed which were parasitized by *Encarsia* sp. which was easily differentiable with healthy aphids, pupal cases of *Cotesia* sp protruding from the DBM larvae are prominent milky white color were recorded as observation (Gaikwad *et al.,* 2018; Bhagat *et al.,* 2018). The observations were recorded since inception of the pest till the harvesting.

The weather data *viz.*, maximum and minimum temperature, relative humidity, rainfall, wind velocity and bright sunshine hours was obtained from the university meteorological observatory of the cropping seasons. These meteorological observations were utilized to correlate with observed population fluctuations. Multiple linear regression analysis was carried to find out the influence of weather parameters on population dynamics of insect pest using SPSS software.

RESULTS AND DISCUSSION

The observations of current study revealed that the incidence of insect pests *viz*., aphids, diamond back moth (DBM), tobacco caterpillar, leaf webber, flea beetle and the natural enemies *viz*., *Cotesia plutellae, Oomyzus* sp., *Aphidius colimani*, Coccinellids and *Syrphid* spp. The observations recorded in both cropping seasons *i.e.,*2021-22 and 2022-23 were presented in Table- 1 and 2, respectively.

Status of insect pests during winter, 2021-22 and 2022-23

Diamondback moth: During cropping season 20221-22, initial incidence of DBM were recorded on 8th SMW with mean population of 0.92 larvae per plant which gradually increased and reached at its peak in 12th SMW (12.83 larvae per plant), there after population were gradually declined up to 3.68 larvae per plant at the harvesting of the crop (15th SMW). Similarly, during cropping season 2022-23, the incidence was recorded from 8th SMW (1.83 larvae plant) and observed till the harvest of the crop (15th SMW with 7.73 larvae/plant) with peak incidence of 17.74 larvae per plant in 12th SMW.

Aphid: During the cropping period 2021-22, first incidence was recorded on 6th SMW with 1.21 aphids per plant and it continue till the harvest (15th SMW) (23.84 per plant). The maximum incidence of 123.72 aphid per plant was observed on 12th SMW during winter 2021-22. Similar trend of aphids infestation were again observed in the consecutive cropping season i.e. 2022-23, with primary incidence of 5.30 aphids per plant in 6th SMW with peak incidence of 147.20 aphids per plant in 11th SMW. Crop was observed was observed till the harvest of the crop with steady decease in population up to 18.84 per plant(15th SMW).

Tobacco caterpillar: This pest was not serious during

Table 1. Seasonal incidence of major insect pests and their natural enemies in cauliflower ecosystem during late winter, 2021-22

SMW	Date	Crop stage	Mean Number of Insect pest and natural enemies per plant with their stage									
			Aphid	Diamond back moth	Tobacco caterpillar	Leaf Webber	Flea Beetle	*Cotesia plutellae*	*Oomyzus sp.*	*Aphidius colimani*	Coccinellids	*Syrphid* sp.
			Nymphs & adults	Larvae & pupae	Larvae	Larvae	Adult	Pupae	Parasitoid of DBM pupae	Mummified aphid	Grubs, pupae & adults	Larvae, pupae & adult
3	15-Jan-22	Vegetative stage 1-49 DAT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	22-Jan-22		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	29-Jan-22		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	05-Feb-22		1.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00
7	12-Feb-22		2.05	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.47	0.00
8	19-Feb-22	Curd Initiation and Development 50-78 DAT	12.67	0.92	0.00	1.92	0.35	0.00	0.00	0.00	0.81	0.75
9	26-Feb-22		25.82	2.86	0.00	2.83	0.73	0.09	0.00	1.06	1.25	1.14
10	05-Mar-22		68.93	5.32	0.00	2.32	1.26	0.35	0.00	4.89	3.34	1.67
11	12-Mar-22		110.73	12.83	0.02	1.02	2.06	1.94	0.04	7.82	4.22	2.12
12	19-Mar-22	Maturation and harvesting of curd 79-101 DAT	123.72	9.83	1.02	0.00	2.56	3.86	0.39	12.78	5.13	1.87
13	26-Mar-22		90.73	8.96	0.65	0.00	1.97	2.84	1.01	13.48	4.93	0.93
14	02-Apr-22		57.94	4.61	0.34	0.00	1.03	1.05	0.67	9.23	3.43	0.47
15	09-Apr-22		23.84	3.68	0.00	0.00	0.78	0.58	0.32	5.07	1.92	0.16

SMW-Standard meteorological week; DAT-Days after transplanting; DBM- Diamondback moth;

Table 2. Seasonal incidence of major insect pests and their natural enemies in cauliflower ecosystem during late winter, 2022-23

SMW	Date	Crop stage	Mean Number of Insect pests and natural enemies per plant with their stage									
			Aphid	Diamond back moth	Tobacco leaf eating caterpillar	Leaf Webber	Flea Beetle	*Cotesia plutellae*	*Oomyzus sp.*	*Aphidius colimani*	Coccinellids	Syrphid sp
			Nymphs & adults	Larvae & pupae	Larvae	Larvae	Adult	Pupae	Parasitoid DBM pupae	Mummified aphid	Grubs & adults	Larvae and pupae
3	15-Jan-23	Vegetative stage 1-49 DAT	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00
4	22- Jan-23		0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00
5	29- Jan-23		0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00
6	05-Feb-23		5.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.2	0.00
7	12-Feb-23		23.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.54	0.21
8	19-Feb-23		37.3	1.83	0.00	0.32	0.10	0.00	0.00	0.00	0.86	0.52
9	26-Feb-23	Curd Initiation 50-78 DAT	77.1	3.72	0.00	0.79	0.23	0.19	0.00	1.84	1.65	0.83
10	05-Mar-23		123.7	7.00	0.03	1.43	0.67	1.45	0.05	6.83	3.65	1.08
11	12-Mar-23		147.2	14.33	0.16	1.07	1.92	3.26	0.64	9.64	6.94	1.67
12	19-Mar-23	Maturation and harvesting of curd 79-101 DAT	128.83	17.47	1.65	0.25	2.53	5.43	0.89	15.83	9.56	2.16
13	26-Mar-23		80.42	13.82	1.47	0.00	3.04	4.96	1.01	13.94	8.43	1.48
14	02-Apr-23		43.56	11.73	0.56	0.00	1.93	2.63	0.71	8.93	6.93	0.95
15	09-Apr-23		18.84	7.73	0.00	0.00	0.78	1.85	0.00	3.85	4.93	0.43

SMW-Standard meteorological week; DAT-Days after transplanting; DBM- Diamondback moth;

the time of experimentation which was observed as minor population with its peak in 12th SMW (1.02 and 1.65 per plant) in both the year of experimentation, respectively. With population fluctuation from 11th to 14th SMW in both the cropping season.

Leaf webber: This pest was recorded from 7th SMW (0.22 larvae per plant) to 11th SMW (1.02 larvae per plant) in the cropping season 2021-22 with peak at 9th SMW (2.83 larvae per plant) while from 8th (0.32 larvae per plant) to 12th SMW (0.25 larvae per plant) in cropping season 2022-23 with peak at 10th SMW (1.43 larvae per plant).

Flea beetle: Incidence of flea beetle were recorded on cauliflower in both the year of experimentation 2021-22 and 2022-23. Flea beetle incidence was observed between 8th to 15th SMW on the crop with population ranging from 0.35 to peak 2.56 (12th SMW) adults per plant during cropping season 2021-22 thereafter population goes down at faster rate. Similarly, during cropping season 2022-23, incidence was varied between 0.10(8th SMW) to 3.04 (13th SMW) per plant thereafter population decreases very fast and at minimal level of 0.78 in 15th SMW *i.e.,* senescence stage of the crop.

Status of major natural enemies during cropping period 2021-22 and 2022-23

Coccinellids: The observations of coccinellids were taken at weekly intervals throughout the cropping season which includes grub, pupae and adults absolute population. Although we had recorded the population of coccinellids collectively comprising the species *viz., Coccinella septempunctata*, *Coccinella transversalis*, *Propylea dissecta*, *Micraspis yasumatsui, Menochilus sexmaculata* and *Brumoides suturalis* on cauliflower during cropping season 2021-22. Among the available species *C. septempuctata* was most abundant while *C. transversalis* were second abundant and *B. suturalis* was least. The observed data depicting that the first incidence of coccinellids on 6thSMW with 0.18 per plant which reached at its peak of 5.13 per plant in 12th SMW thereafter gradual decrease in population were observed because the crop was leading to maturity, so aphid population was also decreasing that is why the population of coccinellids decrease accordingly. Similarly in consecutive cropping season *i.e.,*2022-23 the same trends of infestation were recorded other than varying in numerical value.

Syrphids: Syrphids (hover fly) are the another most important natural enemies present in the crucifers' ecosystem. The abundance of syrphids are depends on the availability of a stable ecosystem near by the cropping area. More number of species and their abundance was observed during the course of investigation. Each and every individuals were not distinguished species wise but collectively all individuals belonging to syrphid groups are recorded collectively as syrphid adults which mainly comprises *Episyrphus balteatus, Syrphus* sp. *etc.* showed peak incidence of 2.12 and 2.16 per plant on 11th and 12th SMW of 2021-22 and 2022-23, respectively which was available from 8th (0.75, 0.21) to 15th SMW (0.16, 0.43).

Parasitoids: Hymenopteran parasitoids *viz.*, *Aphidius* sp., *Aphilinus* sp, *Encarsia* sp. are nymphal parasitoids on aphids, *Cotesia plutellae* a larval parasitoid on DBM, *Oomyzus* sp. a larval-pupal parasitoids on DBM and *Bracon*sp a larval parasitoid on S. *lituraetc.,* are the potential biocontrol agents which were available in the cauliflower ecosystem and it was recorded a potential natural enemy in the cropping system. The parasitoids *viz*., *Cotesia plutellae, Oomyzus* sp. larval parasitoids of DBM and *Aphidius colimani* (a natural enemies of aphid) were found major biocontrol agents with maximum population of 3.86 (12th SMW), 1.01 (13th SMW) and 13.48 (13th SMW) per plant during winter 2021-22; of 5.43 (12th SMW), 1.01 (13th SMW) and 15.83 (12th SMW) per plant during winter 2022-23, respectively.

Experiments revealed that the significantly lower incidence of insect pest were recorded in early growth stage of the crop (52st SMW) due to quick decrease in minimum temperature. Initial incidence of most of the insects were observed from the 5th SMW (February) onwards with gradual rise of temperature except aphids. In 12th SMW (March) peak incidence of insect pest were recorded after that the pest incidence were recorded gradual decrease because crop was leading to its maturity and simultaneously the population of natural enemies also multiplying with faster rate because of suitable growing temperature and abundance in the food. Similar trends of results were also recorded by Mane *et al*. (2021) the peak incidence of diamondback moth and tobacco caterpillar in the month of March in Bihar. Jakhar and Singh 2018 and Jhumar *et al.* (2020) also reported the significant incidence of diamondback moth, aphid, flea beetle and coccinellid predators on 9th to 12th SMW (March).

Correlation between population dynamics of insect pests and their natural enemies in cauliflower ecosystem with meteorological variables

The weather factors especially temperature, humidity, sunshine, wind speed and direction are affecting life events of all organisms but the smaller organisms including insects are greatly affected by the climatic variables. Many times, the weather fluctuations affect their life stages, their breeding and developments which are studied under the field of ecological-natural population management. Similarly, the insect pests affecting the crops are also affected by these climatic variables. We had studied these variations under the cauliflower ecosystem during cropping season 2021-22 and 2022-23,where weather factors were significantly affected the

population dynamism of insect pest and natural enemies and recorded on experimental crop which is placed in the Table 1 and 2 and Figure- 1 and 2, respectively.

Temperature

Maximum (14:00 hours LMT) (T_{max}): The impact of climatic parameters were studied on pest during cropping season 2021-22 and 2022-23, theT_{max} showed significant and positive correlation with aphids (r= 0.65 and 0.46), diamondback moth (r= 0.65 and 0.82), tobacco caterpillar (r= 0.35 and 0.57), leaf webber (r= 0.29 and 0.05) and flea beetle (r= 0.68 and 0.79), respectively. Similarly, the natural enemies *viz*., *Cotesia plutellae* (r= 0.48and 0.77), *Oomyzus* sp. (r= 0.47and 0.69), *Aphidius colimani* (r= 0.64and 0.76), Coccinellids (r = 0.72 and 0.86) and *Syrphid* sp. (r= 0.60 and 0.64) showed significant and positive correlation during corresponding years.The present finding also more or less showing similar trends as findings of Jakhar and Singh (2018) who reported positive relation of diamondback moth and *Coccinella septempunctata* with maximum temperature. Similar results were also reported by Gaikwad *et al.* (2018) with respect to diamondback moth, syrphids and mummified aphids, and Mishra *et al.* (2018) and Khan and Talukder (2017) with respect to tobacco caterpillar. Yadav and Agarwal (2018) reported that the positive and significant effect of maximum temperature on incidence of aphids and their natural enemies coccinellid predators *i.e., Coccinella septempunctata*, *Coccinella transversalis* and *Menochilus sexmaculata.*

Minimum (07:00 hours LMT) (T_{Min}): The impact of minimum temperature were also studied in relation to the associated arthropod fauna in cauliflower ecosystem revealed that the aphids shown correlation coefficient (r) of 0.73 and 0.16, diamondback moth with 0.72 and 0.64, tobacco caterpillar with 0.59 and 0.43, leaf webber with 0.00 and - 0.19 and flea beetle with 0.77 and 0.64 (r) value shown the significant and positive correlation to the minimum temperature during 2021-22 and 2022-23, respectively. The impact of minimum temperature was found highly significant on population buildup of aphid, diamondback moth, flea beetle in 2021-22, while not highly significant in consecutive year, this may be due to annual climatic variation which need further study to know its impact in different years and cause of variations. Likewise for the corresponding years, significant and positive correlation coefficient of 0.68 and 0.61, 0.69 and 0.54,0.82 and 0.57, 0.83 and 0.70, 0.53 and 0.37 were recorded on the natural enemies *viz*., *Cotesia plutellae, Oomyzus* sp., *Aphidius colimani*, Coccinellids and *Syrphid* spp. respectively in corresponding year of experimentation. The current results are more or less similar with the findings of Yadav and Agarwal (2018) in respect to aphids and their natural enemies in cauliflower ecosystem. Lal *et al.,* 2020 also found the positive relation of mean atmospheric temperature with diamondback moth, flea beetle and painted bugs. Similarly, Khan and Talukder (2017) reported positive and highly significant correlation between tobacco caterpillar and minimum temperature.

Relative Humidity

Morning (07:00 hours LMT) (%): A negative and non-significant correlation was observed with relative humidity and all major pests of cauliflower in both the cropping year including aphid(r= -0.38 and -0.40), diamondback moth (r= -0.43 and -0.73), tobacco caterpillar (r= -0.20 and -0.54), and flea beetle (r= -0.41 and -0.70) in respect to morning relative humidity (%) for the corresponding years *i.e.,* 2021-22 and 2022-23while in case of leaf webber we had observed positive and negative non-significant correlations in the corresponding years (r= 0.16 and -0.06).In cropping season 2021-22, the natural enemy population *viz*., *Cotesia plutellae, Aphidius colimani*, Coccinellids and *Syrphid* sp. were shown negative and non-significant correlation with the morning relative humidity with r value of -0.31, -0.55, -0.51 and -0.15, respectively while *Oomyzus* sp. have negative and significant correlation with morning relative humidity (-0.56). But in the consecutive cropping season, the natural enemies shown negative and highly significant correlation with *Cotesia plutellae* (-0.71)*,Aphidius colimani*(-0.73), Coccinellids *(*-0.78) but Oomyzus sp. having negative and significant correlation and *Syrphid* sp. (-0.55) having negative and non-significant correlation with morning relative humidity. These variations are due to change in weather factors between the corresponding cropping seasons. The similar results were also reported by Yadav and Agarwal (2018) that the morning relative humidity showed negative influence on the incidence of insects. The current results were also supported by the findings of Shigwan *et al.* (2022) and Mishra *et al.* (2018).

Evening (14:00 hours LMT) (%): In the cropping season 2021-22, the evening relative humidity (%)also shown negative and non-significant correlation in respect to insect pests *viz*., aphids (r= -0.28), diamondback moth (r= -0.35), tobacco caterpillar (r= -0.08), leaf webber (r= -0.39) and flea beetle (r= -0.31)and similar results were also observed in case of the natural enemies population *viz*., *Cotesia plutellae* (-0.06)*, Oomyzus* sp. (-0.28), *Aphidius colimani*(-0.29), Coccinellids (-0.38) and *Syrphid* sp. (-0.31).

In the cropping season 2022-23, the insect pest aphids (-0.72), diamondback moth (-0.84) and flea beetle (-0.73) have negative and highly significant correlation with evening relative humidity while tobacco caterpillar (-0.55) and leaf webber (-0.40) have negative and non-significant correlation with the evening relative humidity

	Aphid	*DBM*	*Tobacco caterpillar*	*Leaf webber*	*Flea Beetle*	*Cotesia plutellae*	*Oomyzus* sp.	*Aphidius colimani*	*Coccinellids*	*Syrphids*
T_{max}(^{O}C)	**0.65***	**0.65***	**0.35**	**0.29**	**0.68***	**0.48**	**0.47**	**0.64***	**0.72****	**0.60***
T_{min}(^{O}C)	**0.73****	**0.72****	**0.59***	**0.00**	**0.77****	**0.68***	**0.69****	**0.82****	**0.83****	**0.53**
RH_{morn}(%)	**-0.38**	**-0.43**	**-0.20**	**0.16**	**-0.41**	**-0.31**	**-0.56**	**-0.55**	**-0.51**	**-0.15**
RH_{eve}(%)	**-0.28**	**-0.35**	**0.08**	**-0.39**	**-0.31**	**-0.06**	**-0.28**	**-0.29**	**-0.38**	**-0.31**
Rainfall (mm)	**0.20**	**0.24**	**0.25**	**-0.27**	**0.29**	**0.31**	**0.25**	**0.33**	**0.25**	**0.04**
Bright sunshine (hr.)	**0.35**	**0.42**	**-0.05**	**0.37**	**0.36**	**0.12**	**0.33**	**0.35**	**0.45**	**0.36**

	Aphid	*DBM*	*Tobacco caterpillar*	*Leaf webber*	*Flea Beetle*	*Cotesia plutellae*	*Oomyzus* sp.	*Aphidius colimani*	*Coccinellids*	*Syrphids*
T_{max}(^{O}C)	**0.46**	**0.82****	**0.57***	**0.05**	**0.79****	**0.77****	**0.69****	**0.76****	**0.86****	**0.64***
T_{min}(^{O}C)	**0.16**	**0.64***	**0.43**	**-0.19**	**0.64***	**0.61***	**0.54***	**0.57***	**0.70****	**0.37**
RH_{morn}(%)	**-0.40**	**-0.73****	**-0.54**	**-0.06**	**-0.70****	**-0.71****	**-0.60***	**-0.73****	**-0.78****	**-0.55**
RH_{eve}(%)	**-0.72****	**-0.84****	**-0.55**	**-0.40**	**-0.73****	**-0.74****	**-0.64***	**-0.78****	**-0.83****	**-0.80****
Rainfall (mm)	**-0.22**	**-0.32**	**-0.18**	**-0.21**	**-0.27**	**-0.27**	**-0.22**	**-0.28**	**-0.32**	**-0.26**
Bright sunshine (hr.)	**0.82****	**0.82****	**0.53**	**0.55**	**0.72****	**0.71****	**0.63***	**0.76****	**0.79****	**0.86****

Scale

-1 0 1

T_{max} – Maximum Temperature;T_{min} - Minimum Temperature;RH_{eve} - Evening Relative Humidity;RH_{morn} - Morning Relative Humidity; **- Correlation is significant at the 0.01 level (2-tailed); *-Correlation is significant at the 0.05 level (2-tailed).

Fig- 1.Comparative map on Correlation co-efficient(r) between meteorological parameters and population of major cauliflower insect pest and their natural enemies whereas, A- during 2021-22; B-during 2022-23

in the same cropping year. The natural enemies *viz*., *Cotesia plutellae*(-0.74) ,*Aphidius colimani*(-0.78), Coccinellids(-0.83) and *Syrphid*sp(-0.80) were also shown negative and highly significant correlation with evening relative humidity while *Oomyzus* sp.,(-0.64) have negative and significant correlation.The current experimental finding sare also supporting the findings of Gaikwad *et al.,* 2018 that the evening relative humidity has significant negative impact on the incidence of diamondback moth, tobacco caterpillar, leaf webber, aphid and natural enemies (syrphid and mummified aphid).The similar findings are also reported by Bhagat *et al.* (2018), Jakhar and Singh, (2018) and Yadav and Agarwal, (2018).

Rainfall (mm)

A positive and non-significant correlation were observed with respect to aphids, diamondback moth, tobacco caterpillar and flea beetle with correlation (r) value of 0.20, 0.24, 0.25, and 0.29, respectively during cropping season 2021-22 except for leaf webber shown negative and non-significant correlation (-0.27). Similarly, the natural enemies *viz*., *Cotesia plutellae* (r= 0.31)*,Oomyzus* sp. (r= 0.25), *Aphidius colimani* (r= 0.33), Coccinellids (r= 0.25) and *Syrphid*sp (r= 0.04) was also observed positive and non-significant correlation with rainfall during the cropping season 2021-22.

In the cropping season 2022-23, all insect pest namely aphids (-0.22), diamondback moth (-0.32), tobacco caterpillar (-0.18), leaf webber (-0.21) and flea beetle (-0.27) and the natural enemies namely *Cotesia plutellae* (-0.27)*,Oomyzus* sp. (-0.22), *Aphidius colimani* (-0.28), Coccinellids (-0.32) and *Syrphid* sp. (-0.26) were shown negative and non-significant correlation with rainfall. Our findings are at par with Mishra *et al.,* (2018) and Kumar *et al.*(2023), who had also reported that the substantial decrease of population of diamondback moth, tobacco caterpillar, leaf webber and aphid occurrence with increasing rainfall. Rainfall affects negatively the percent parasitisation of aphids and incidence of syrphid predator (Gaikwad, 2018).

Bright sunshine (hrs.)

Sunshine hour shown positive but non-significant impact on all pests and natural enemies *viz*., aphids (0.35), diamondback moth (0.42), leaf webber (0.37) and flea beetle (0.36) in the cropping season 2021-22 while tobacco caterpillar (-0.05) shown negative and non-significant correlation. While in case of natural enemies also shown positive and non- significant correlation *viz*.,*Cotesia plutellae* (0.12)*, Aphidius colimani* (0.33), Coccinellids (0.35) and *Syrphid*sp (0.45) while *Oomyzus* sp. (0.36).

Whereas in 2022-23 positive and highly significant correlation were observed with aphids (0.82), diamondback moth (0.81) and flea beetle (0.72) while tobacco caterpillar (0.53) and leaf webber(0.55) have non-significant positive correlation. In same manner all natural enemies have positive and highly significant correlation with bright sunshine namely *Cotesia plutellae* (0.71)*, Aphidius colimani* (0.76), Coccinellids (0.79) and *Syrphid* sp. (0.86) while *Oomyzus* sp. (0.63) have significant and positive correlation.

Current findings are also had similar trends which was observed by Gaikwad *et al.,* (2018). who had reported the positive influence of the sunshine on the diamondback moth, tobacco caterpillar, leaf webber, aphidand natural enemies *i.e.,* syrphids and mummified aphids *etc*. Similarly, Yadav and Agarwal (2018) reported the positive and significant correlation of sunshine with respect to aphid and their predatory coccinellids *i.e., Coccinella septempunctata*, *Coccinella transversalis* and *Menochilus sexmaculata.*

Multilinear regression equation on seasonal incidence of insect pest and their natural enemies in relation to weather factors

During 2021-22 and 2022-23 our experiments showing populations were significantly influenced by the weather parameters (Table. 3) with coefficient of determination value (r^2) 0.79 and 0.69 for aphid, 0.77 and 0.85 for DBM, 0.49 and 0.78 for tobacco caterpillar, 0.70 and 0.45 for leaf webber and 0.84 and 0.76 for flea beetle, respectively for corresponding cropping seasons. Similarly, the values (r^2) for natural enemies *viz*., *Cotesia plutellae* (0.85 and 0.88)*, Oomyzus* sp. (0.68 and 0.69), *Aphidius colimani* (0.83 and 0.82), Coccinellids (0.73 and 0.89) and *Syrphid* sp. (0.83 and 0.82) are obtained showing these also influenced by the weather factors during respective years.

The experimental findings presented here showing significant effects of the weather parameters on the incidence of insect pests of cauliflower and their natural enemies in which the relatively higher temperature, lower relative humidity, reduced rainfall and optimum sunshine are more suitable for the establishment and development of insect pests. Similar trends of findings were also reported by Hemchandra and Singh (2007) revealing such conditions found to be favourable for the growth of the insect pest population. Marchioro and Foerster (2011) also observed the significance of temperature on development and survival of diamondback moth affecting the population dynamics. They also reported the decrease of development time of the immature stages, increase of survival and number of generations per year with increase in temperature within a threshold limit aiding in higher incidence of insect pests. In line with current findings, Soh *et al.* (2018). also reported

Table 3. Co-efficient of determination (r^2) between weather parameters and population of major cauliflower insect pest and their natural enemies during cropping season 2021-22 and 2022-23

	Regression Equation 2021-22	2022-23	r^2 (2021-22)	r^2 (2022-23)
Aphid	$Y=369.16+24.22X_1-30.39X_2-6.04X_3+1.00X_4-1.00X_5-6.21X_6$	$Y=-716.93+11.69X_1-5.13X_2+1.95X_3+3.88X_4+37.10X_5+21.15X_6$	0.79	0.69
DBM	$Y=1.604+2.69X_1-3.11X_2-0.37X_3+0.22X_4-0.124X_5-0.58X_6$	$Y=5.48+0.416X_1+0.005X_2-0.51X_3+0.43X_4+5.89X_5+2.22X_6$	0.77	0.85
Tobacco caterpillar	$Y=0.639+0.23X_1-0.26X_2 0.02X_3+0.003X_4-0.01X_5-0.15X_6$	$Y=0.016-0.14X_1+0.17X_2+0.02X_3-0.018X_4+0.71X_5+0.7X_6$	0.49	0.78
Leaf webber	$Y=10.72-0.70X_1+0.60X_2-0.04X_3-0.02X_4+0.04X_5+0.81X_6$	$Y=-8.59+0.16X_1-0.70X_2+0.04X_3+0.02X_4-0.19X_5+0.03X_6$	0.70	0.45
Flea Beetle	$Y=6.10+0.47X_1-0.57X_2-0.12X_3+0.03X_4-0.02X_5-0.08X_6$	$Y=-0.404-0.05X_1+0.11X_2-0.07X_3+0.08X_4+0.95X_5+0.51X_6$	0.84	0.76
Cotesia plutellae	$Y=-1.51+1.03X_1-1.16X_2-0.09X_3+0.06X_4-0.05X_5-0.50X_6$	$Y=0.202-0.04X_1+0.15X_2-0.12X_3+0.14X_4+2.06X_5+0.76X_6$	0.65	0.84
Oomyzus sp.	$Y=-3.55+0.22X_1-0.19X_2+0.001X_3+0.02X_4-0.01X_5-0.11X_6$	$Y=-0.108+0.002X_1+0.001X_2-0.04X_3+0.05X_4+0.39X_5+0.23X_6$	0.68	0.69
Aphidius colimani	$Y=17.60+3.30X_1-3.54X_2-0.57X_3+0.18X_4-0.14X_5-1.36X_6$	$Y=-17.19+0.31X_1+0.26X_2-0.29X_3+0.46X_4+5.12X_5+2.19X_6$	0.83	0.82
Coccinellids	$Y=16.81+0.93X_1-1.11X_2-0.28X_3+0.05X_4-0.02X_5-0.15X_6$	$Y=7.54+0.05X_1+0.16X_2-0.28X_3+0.21X_4+3.22X_5+1.25X_6$	0.85	0.88
Syrphids	$Y=9.53+0.11X_1-0.26X_2-0.10X_3+0.005X_4-0.004X_5+0.16X_6$	$Y=-4.41+0.03X_1+0.04X_2+0.02X_3+0.01X_4+0.78X_5+0.20X_6$	0.83	0.82

Whereas, X_1- Maximum Temperature; X_2- Minimum Temperature; X3- Morning Relative Humidity; X_4- Evening Relative Humidity; X_5-Rainfall and X_6-Sunshine Hour; $r^{2=}$ Co-efficient of determination

significant effects of temperature on *Brevicoryne brassicae* population. Relative humidity influences the biological and behavioural changes in insects. Under heat stress condition, low humidity reduces insect survival (Bubliy *et al.,* 2012). Rainfall also acts as a signal for diapausing insects to resume their life processes. Egg and neonate stages are highly susceptible to the rainfall causes the physical damage, dislodge from the plant and disrupting movement to feeding sites and affects the oviposition sites. It also creates congenial microclimate suitable for entomopathogenic organisms thereby affecting the survival (Rahman *et al.,* 2017). Insects shows characteristic photoperiodism which profoundly influences the geographical distribution, seasonal biology, growth, form, metabolism and behaviour of the insect. Sunshine influence directly and indirectly the insect pest dynamics through regulation of temperature and relative humidity in addition to photoperiodism (Beck, 1980).

CONCLUSION

From the present findings we can conclude that the infestation of major insect pests (Aphid and Diamondback moth) started from the first fortnight of the February and reached at its peak in the month of early March in both years of experimentation. The natural enemies shown similar trend in which incidence was in parallel with the population of their host pest. The correlation between population of insect pest and their natural enemies against weather parameters showing significant positive correlation with temperature (maximum and minimum), rainfall and sunshine while negative correlation with relative humidity (morning - evening). Multiple linear regression analysis is also indicating that the significant impact on the population dynamics of the insects by weather factors. These findings will help the farmers to plan comprehensive management schedule to supress the developing pest population and reduce the loss caused by them. Although the findings of two different experiments couldn't be compared exactly as in physical and chemical science but the trends of variations in their population could be compared in biological sciences may be recognised as parameters which affecting the life processes either positively or negatively. These types of experimental findings need their validation in corresponding environmental condition before executing at the mass level.

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Modified QuEChERS technique and analysis methodology using UHPLC for residue analysis of thiacloprid and emamectin benzoate in chilli

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ABSTRACT: The presence of insecticide residues in chilli fruits limits the export and poses a health risk to consumers. Thus, it is necessary to develop quick and effective methods for conducting residue analysis of commonly used insecticides. The present research validates a modified QuEChERS technique for analysing thiacloprid and emamectin benzoate residues in chilli using UHPLC. The proposed methods recovered thiacloprid and emamectin benzoate by 80.33-88% and 79.33-96%, respectively. LOQ for both the insecticides was 0.01 mg/kg. Matrix effect values were within an acceptable range of -20 to 20%. The developed methodology can be used for periodical monitoring of the residue levels of the target insecticides in chilli.

Keywords: Chilli, emamectin benzoate, Quechers technique, thiacloprid, UHPLC.

INTRODUCTION

Chilli (*Capsicum annum* L.) is a major commercial spice crop cultivated in India. With a production of 4363.17 thousand metric tonnes, chilli is grown over 410.9 thousand hectares in the country (Anonymous, 2022a), making India one of the largest chilli producer, consumer and exporter in the world. Being a culinary ingredient of year-round availability, yield loss in chilli often goes unnoticed by the common man but is a matter of concern in the farmers' field. The incidence of insect pests is one of the major causes of reduced yield. Out of all the insect pests, sucking insects, especially thrips (*Scirtothrips dorsalis* Hood), attack alone can cause up to 50 to 90 per cent yield losses (Reddy and Reddy, 1999). In hopes of combatting pest attacks, chilli crop is repeatedly subjected to different insecticidal sprays. However, food contamination from pesticide residue has turned into an alarming case of human health risk. Not only the consumers, but the grower himself is in danger of the toxicants. Besides this, tonnes of Indian chilli exports are being rejected in the international market because our chilli samples still contain pesticide residue (Reddy *et al*. 2007). Therefore, it is necessary to develop methods to quickly and effectively estimate commonly used insecticides in chilli crop. With the need to use effective and safe insecticides two chemicals-thiacloprid and emamectin benzoate have been recently recommended by the Central Insecticides Board and Registration Committee (CIB&RC) to be used for the control of chilli thrips (Anonymous, 2022b).

Thiacloprid is a highly active, broad-spectrum novel insecticide belonging to the neonicotinoid group of systemic insecticides. It is an acute stomach and contact poison. It acts agonistically against the nicotinic acetylcholine receptor (Elbert *et al.,* 2000). Emamectin benzoate, a highly selective novel macrocyclic lactone insecticide (Ishaaya *et al*., 2002), is derived from avermectin B1. It is a non-systemic or semi-systemic chemical pesticide with an oral mode of entry and some contact action. It inhibits muscle contraction, which causes chlorine ions to flow continuously in the H-Glutamate and GABA receptor sites (Fanigliulo and Sacchetti, 2008). Residue analysis of thiacloprid in chilli has been previously done by Kumar *et al*. (2018) using methanol as a solvent. Similarly, Parmar *et al*. (2012) studied the persistence of thiacloprid in chilli and used a different approach for the extraction and cleanup of the sample. The dissipation kinetics of emamectin benzoate residue in chilli has been studied by Bhattacharyya *et al*. (2017) using a modified QuEChERS method with variation in the instrument method. The novelty of the current experiment lies in the methodology described, which is quick, suitable, gives satisfactory results and can be further used for residue studies of the two insecticides in chilli crop.

MATERIALS AND METHODS

Chemicals and reagents

Certified reference materials of thiacloprid (purity 99.9%) and emamectin benzoate (purity 99.3%) were purchased from Dr. Erhenstrofer, India. HPLC grade acetonitrile (ACN) and HPLC grade water were obtained from Merck Life Science Pvt. Ltd. Sodium chloride (NaCl), anhydrous sodium sulphate (Na_2SO_4), anhydrous magnesium sulphate ($MgSO_4$) and graphitic carbon black were all bought from HiMedia Laboratories Pvt. Ltd. Primary Secondary Amine (PSA) was obtained from Agilent Technologies.

Preparation of standard solutions

Standard stock solutions of thiacloprid and emamectin benzoate of 400 µg/mL concentration were prepared from their CRMs concerning HPLC grade acetonitrile. The two stock solutions were subjected to serial dilution to prepare working standards of different concentrations. Both stock solutions and working solutions were stored in the refrigerator until further use.

Instrumentation

Residue estimation of thiacloprid and emamectin benzoate in chilli fruit sample extracts was done through Dionex Ultimate 3000 Ultra High Performance Liquid Chromatography (UHPLC) from Thermo Fisher Scientific Inc. A C18 column with 150 mm length and 3 mm diameter was used for stationary phase. The detection of insecticide was done by a Photodiode Array Detector (PDA). For estimation of thiacloprid, the instrument method was set with a mobile phase of ACN: HPLC water in 80: 20 ratio, flow rate of 3 mL/min, temperature 35ºC and UV wavelength of 256 nm for a run time of 10 minutes. For estimation of emamectin benzoate, residues extract samples were run in a mobile phase of ACN: HPLC water in a 70: 30 ratio at a flow rate of 2 mL/min, with a temperature of 35ºC and UV wavelength of 260 nm. The total run time for estimation was 15 minutes.

Sample collection

Chilli fruit samples were collected from the AICRP vegetable farm, Dr. Rajendra Prasad Central Agricultural University, Pusa. 200 grams of green chillies were plucked from the plots, which were free of any insecticide treatment. Samples were collected in polythene bags, properly labelled and brought to the Sample Processing Laboratory and Pesticide Residue Analysis Laboratory of the Department of Entomology, Post Graduate College of Agriculture, RPCAU, Pusa.

Sample preparation

Chilli samples were prepared for analysis by following a "Quick, Easy, Cheap, Effective, Rugged and Safe" (QuEChERS)" technique with slight modifications. First step in QuEChERS technique is extraction of insecticide from sample. Chilli sample from each polythene bag was cut and macerated in a mixer grinder for homogenization of the sample. In an analytical balance, 15 g of this macerated chilli was weighed and transferred into clean and labelled 50 mL centrifugal tubes. Chilli samples in the tubes were fortified with the required amount of insecticide (thiacloprid or emamectin benzoate) and kept in the refrigerator for 30 minutes to one hour. Next, 30 mL HPLC grade acetonitrile and 10 g anhydrous sodium chloride were added to the tubes. The tubes were first shaken vigorously by hand, then vortexed (Spinerex) for 1 to 3 mins and then shaken on rotospin (Tarson®) at 50 rpm for 5 mins. After 5 mins the tubes were centrifuged at 3000 rpm for 5 mins. From the centrifuged tubes 16 mL of supernatant was carefully pipetted out into another clean and labelled 50 mL polypropylene centrifugal tube.

Clean-up of the extracted sample began with the addition of 10 g of anhydrous sodium sulphate. This was followed by cleanup of the sample through "dispersive solid phase extraction (DSPE)". 0.15 ± 0.01 g primary secondary amine (PSA) sorbent, 0.90 ± 0.01 g anhydrous magnesium sulphate and 0.05 ± 0.01 g graphitic carbon black was weighed into a clean and labelled 15 mL DSPE tube. To this tube, 6 mL aliquot was pipetted from the 16 mL extract, shaken thoroughly by hand and vortexed. The tubes were centrifuged at 3000 rpm for 5 mins and finally 3 mL supernatant was pipetted into separate 15 mL glass tubes. This terminal 3 mL volume was labelled properly and stored in a refrigerator until its analysis. For final quantification, 1 mL from the terminal volume was pipetted into a small glass vial and placed in the sampler compartment of the UHPLC.

METHOD VALIDATION

The quantitative determination of thiacloprid and emamectin benzoate residues in chilli samples was validated as per the instructions of bio-analytical method recommendations mentioned in the SANCO guidelines.

Selectivity

Selectivity of the method was obtained by comparing the peaks of the chromatograms from running the blank ACN samples with the peaks obtained from the chromatograms of standard solutions. Selectivity is determined to distinguish the peaks of the analyte from those of the matrix.

Linearity

A linear relationship was generated from the calibration curves of different concentrations (0.05, 0.1, 0.5, 1 and 2 ug/mL) of thiacloprid and emamectin benzoate against their area.

LOD and LOQ

Limit of Detection (LOD) refers to the minimum quantity of pesticide residue that can be detected by the analytical method. Meanwhile, the Limit of Quantification (LOQ) is the minimum amount of pesticide residue that can be quantified or measured by the instrument accurately and precisely. To determine, LOD and LOQ values, chilli

samples were spiked with different concentrations of thiacloprid and emamectin benzoate. The baseline of the chromatogram from the unfortified blank was magnified to obtain the noise response of the instrument. The noise response was converted to a concentration estimate from the known concentration of spiked extract.

Matrix effect

A matrix-match study was conducted to eliminate the matrix effect produced by various compounds. Matrix-matched calibration curves were obtained by spiking the matrix extracts with working standards of the insecticide at five different concentrations- 2, 1, 0.5, 0.1 and 0.05 mg/L. The area obtained from this set was compared with the area of standards prepared in ACN. The matrix effect percentage was calculated by the formula-

$$ME\% = \frac{\text{Peak area of standard in matrix} - \text{Peak area of standard in solvent}}{\text{Peak area of standard in solvent}} \times 100$$

Accuracy and precision

Recovery studies were carried out to determine the accuracy and precision of the methodology. For precision calculation, chilli fruit samples were spiked with three concentrations of the standard *viz*., at LOQ, 5 times of LOQ and 10 times of LOQ. Three replications were made for each spike level. RSD values from these replications were used to evaluate the repeatability and reproducibility of the methodology. Intraday precision or instrumental repeatability was obtained from RSD_r value from standard deviation of recovery studies resulting from running the three replicates on the same day, using the same methodology, same operational conditions, and same operator and in the same laboratory. Interday precision or reproducibility was obtained from RSD_R value by analysing different batches of chilli extract and reagents by different analysts on three consecutive days.

RESULTS AND DISCUSSION

Selectivity

Upon observation of peaks in the blank samples, no significant peak was detected at the insecticide retention time. Thus, the methodology was determined to be selective for thiacloprid and emamectin benzoate without interference.

Linearity

Linearity curves of both the insecticides resulted in R^2 value greater than 0.99 (Figure 1), which implied that the prepared standards and detector used were fit and further residue analysis of chilli samples for thiacloprid and emamectin benzoate could proceed with their use.

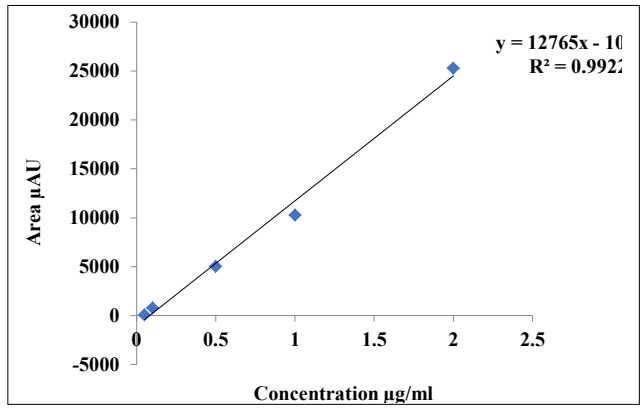

Fig. 1a

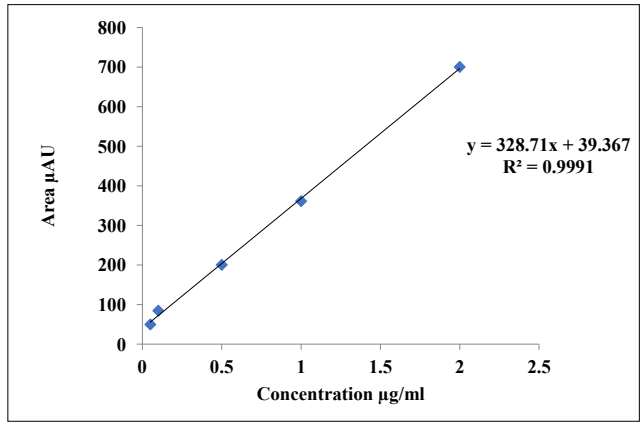

Fig. 1b

Fig 1. Linearity curve of (a) thiacloprid standards (b) emamectin benzoate standards

LOQ and LOD

Full scale deflection in both thiacloprid and emamectin benzoate was achieved with 1 ng of their standards. Chilli samples underwent processing to result in the terminal volume of 3 ml. The terminal volume was concentrated up to 1.5 ml from where 10 µL of sample was injected into a vial in UHPLC observe maximum load of samples that could be analysed by the machine without the occurrence of any interference peak in the area of the compound getting estimated. The limit of quantification (LOQ) for both thiacloprid and emamectin benzoate was quantified at 0.01 mg/kg, and the limit of detection (LOD) was 0.003 mg/kg.

Matrix effect

The linearity curve of the matrix matched the standards of thiacloprid, giving the determination coefficient (R^2) value 0.9877 and linearity equation y = 1207.1x + 117.25. Similarly, the linearity curve of matrix matched standards of emamectin benzoate gave the determination coefficient (R^2) value 0.9972 and linearity equation y

Table 1. Matrix effect of thiacloprid and emamectin benzoate standards in chilli fruits

Spiking level	ME % Thiacloprid	ME % Emamectin benzoate
2	-17.25	-17.40
1	-16.40	-12.25
0.5	-11.37	-12.49
0.1	-18.01	-17.95
0.05	-17.05	-18.23

Table 2. Recovery (%) of thiacloprid and emamectin benzoate from spiked chilli samples with respect to matrix matched standard

Substrate	Spiked Level (mg/kg)	Thiacloprid		Emamectin benzoate	
		Recovered (%) *Mean ± SD	RSD_r	Amount Recovered *Mean ± SD	RSD_r
Chilli	0.25	98.67 ± 6.79	6.89	109.33 ± 16.44	15.03
	0.05	99.33 ± 2.49	2.51	90 ± 3.26	3.63
	0.01	95.33 ± 3.39	3.56	116.67 ± 9.42	8.08

Table 3. Recovery (%) of thiacloprid and emamectin benzoate from spiked chilli samples

Substrate	Spiked Level (mg/kg)	Thiacloprid		Emamectin benzoate	
		Amount Recovered *Mean ± SD	RSD_r	Amount Recovered *Mean ± SD	RSD_r
Chilli	0.25	88.00 ± 5.35	6.08	89.33 ± 8.22	9.20
	0.05	81.33 ± 4.11	5.05	79.33 ± 2.49	3.14
	0.01	80.33 ± 4.03	5.01	96.00±2.83	2.95

*Mean of six replications
SD = "Standard Deviation"
RSDr = "Relative Standard Deviation" (Repeatability)

Table 4. Reproducibility for thiacloprid and emamectin benzoate at 0.01 mg/ kg

Substrate	Day	Thiacloprid			Emamectin benzoate		
		Amount recovered (%)	Standard deviation (%)	RSD_R (%)	Amount recovered (%)	Standard deviation (%)	RSD_R (%)
Chilli	1	113.33	12.47	5.99	92	13.95	10.47
	2	97.33	0.94		93.67	12.71	
	3	80.33	4.03		96	2.83	

RSD_R= "Relative Standard Deviation" (reproducibility)

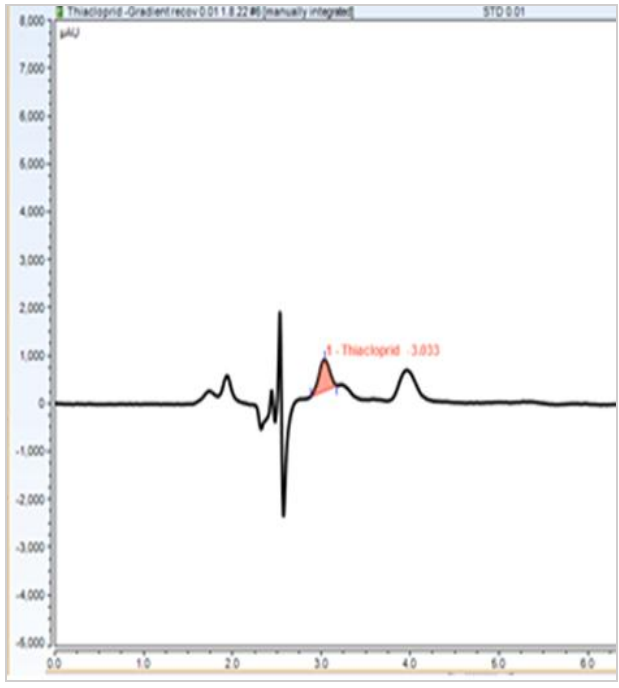

Fig. 3a

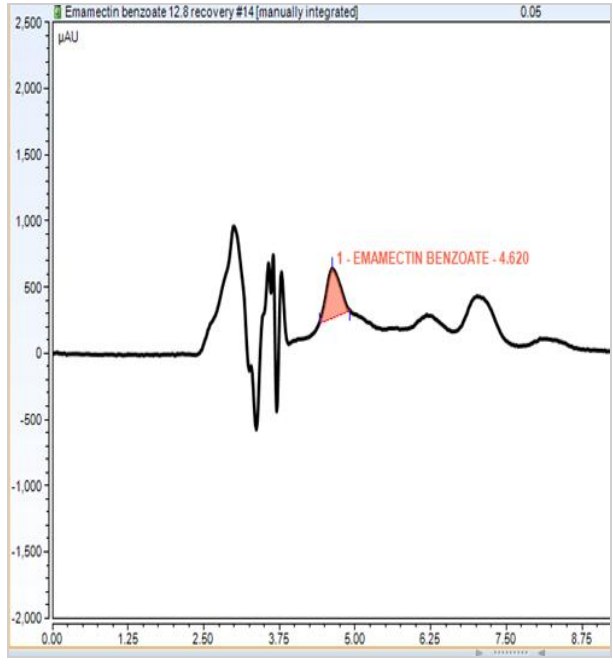

Fig. 3b

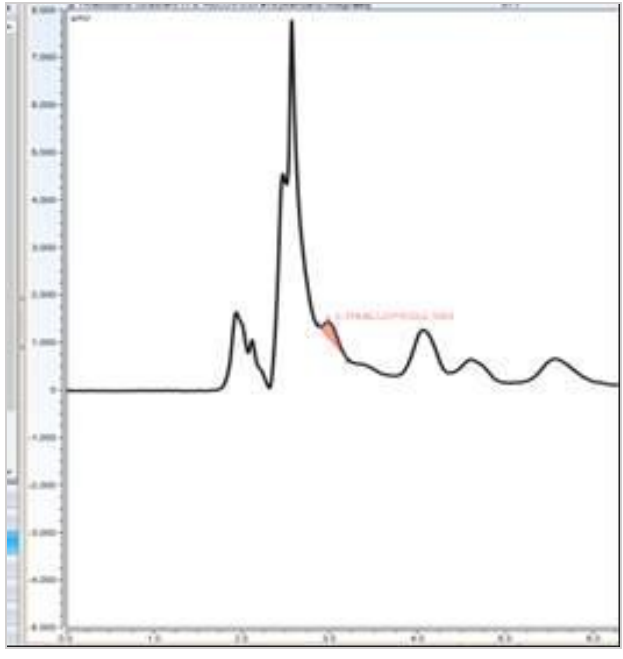

Fig. 3c

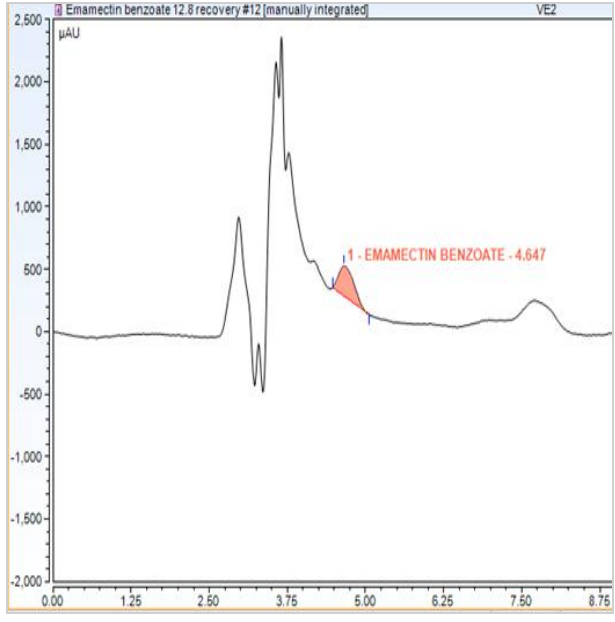

Fig. 3d

Fig. 3. UHPLC Chromatograms of (a) thiacloprid standard (0.01 µg/ml) (b) chilli spiked with thiacloprid (0.01 mg/kg) (c) emamectin benzoate standard (0.01 µg/ml) (d) chilli spiked with emamectin benzoate (0.01 mg/kg)

= 284.19x + 19.411 (Fig. 2). For the matrix matched standards of thiacloprid and emamectin benzoate in chilli fruits ME% at all concentrations was found within the acceptable range of -20% to 20% (Table 1). The recovery percentage of thiacloprid and emamectin benzoate from the spiked chilli sample with respect to matrix matched standard is given in Table 2.

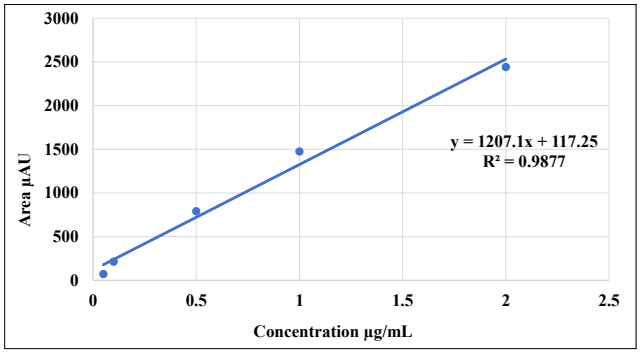

Fig. 2a

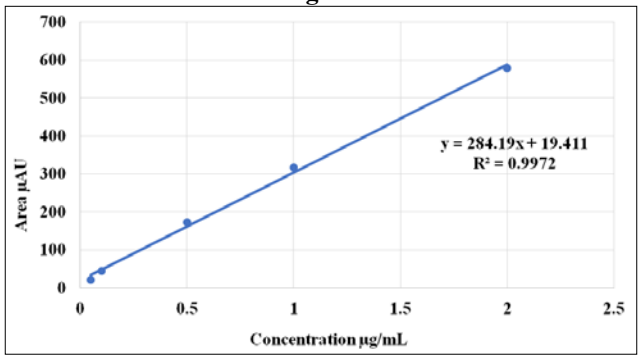

Fig. 2b

Fig 2. Linearity curve of matrix-matched standards of (a) thiacloprid (b) emamectin benzoate

Accuracy and precision

Chromatograms of thiacloprid and emamectin benzoate standards at 0.01 mg/kg and chilli samples spiked with 0.01 mg/kg of the insecticides are presented in Fig. 3. The instrumental repeatability (RSD_r) or intraday precision for thiacloprid in chilli at 0.25, 0.05 and 0.01 µg/ml corresponded to 6.08, 5.05 and 5.01%, respectively. The intraday precision (RSD_r) for emamectin benzoate was obtained at 9.2, 3.14 and 2.95% for the concentrations 0.25, 0.05 and 0.01 µg/ml concentrations, respectively (Table 3). Interday precision (between-batch recoveries) and reproducibility (RSD_R) were examined for thiacloprid and emamectin benzoate residue in chilli at 0.01 mg/kg. The reproducibility of thiacloprid and emamectin benzoate from the separate batches of chilli samples was obtained as 5.99 % and 10.47 %, correspondingly (Table 4). For both the insecticides, the values were obtained within 15 per cent at a concentration of 0.01 mg/kg.

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Evaluation of biopesticides and chemicals for the management of sucking pests of curry leaf in Thiruvananthapuram, Kerala

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ABSTRACT: A field investigation was carried out to evaluate the efficacy of certain biopesticides and chemical insecticides against the major sucking pests of curry leaf, *Murraya koenigii* (L.) Spreng. in the Neyyattinkara region of Thiruvananthapuram district in Kerala, India during September 2022. Among the treatments evaluated, neem garlic soap formulation- KAU Raksha and talc-based formulation of *Lecanicilium lecanii* were proved effective in reducing the psyllids, *Diaphorina citri* population by 65 and 58.17 per cent. Further, Horticultural Mineral Oil and talc-based formulations of *L. lecanii* were effective in reducing the mite *Schizotetranychus baltazari* population by 39.18 and 33.44 per cent, respectively. Whitefly, *Aleuroclava complex* was effectively managed by talc-based formulation of *L. lecanii* with a per cent efficacy of 75.23, which was on par with chemical management. Among the chemical insecticides, chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC reduced psyllid, mite and whitefly populations by 89.91, 49.76 and 76.63 per cent, respectively, over untreated control. The study highlighted the effectiveness of some biopesticides, which can be utilized for the organic pest management of sucking pests of curry leaf.

Keywords: Biopesticides, curry leaf, mite, *Murraya koenigii*, psyllid, whitefly

INTRODUCTION

Curry leaf, *Murraya koenigii* (L.) Spreng. is a native of India and is utilized for medicinal and culinary purposes. The major cultivators of curry leaves in India are Tamil Nadu, Karnataka and Andhra Pradesh. It is also a highly export-oriented crop, India being its largest exporter globally, followed by Sri Lanka and Kenya (Volza's India Export Data, 2023). In recent years, the curry leaf has progressed from an underexploited crop to a commercial commodity in the country. However, the infestation of pests and pathogens is a major constraint to the successful production of healthy curry leaves. Tara and Sharma (2010) have reported that despite curry leaves possessing insecticidal properties, they are attacked by several insect pests, which decreases the plant's economic value. They also observed that the plants are attacked mainly by insects from the order Hemiptera, followed by Lepidoptera and Coleoptera. Significant variations in weather parameters have also caused exotic pest invasion in curry leaves. These problems have resulted in commercial curry leaf farmers taking up pesticide sprays to obtain a remunerative price and increased yield, thus deteriorating the quality of the leaves (Mathrubhumi, 2020). Approximately twenty pests attacking curry leaves were recorded in a one-year survey conducted at twenty homesteads in Thiruvananthapuram district, with the major ones being lemon butterflies (*Papilio demoleus* L. and *P. polytes* L.), citrus psyllid (*Diaphorina citri* Kuwayama), citrus green mite (*Schizotetranychus baltazari* Rimando), curry leaf tortoise beetle (*Silana farinosa* (Boheman)), citrus leaf roller (*Psorosticha zizyphi* (Stainton)), scale insects (*Pinnaspis strachani* (Cooley) and *Icerya aegyptiaca* (Douglas), blackflies (*Aleurocanthus terminaliae* Dubey & Sundararaj, *Aleurolobus orientalis* David & Jesudasan) and whiteflies (*Aleuroclava complex* Singh). Sucking pests were the major insect pests, with the predominant ones being *D. citri, S. baltazari* and *A. complex*. They sucked the vital fluids from the leaves and tender parts of the plant and caused drying and wilting of the growing points and a sick appearance of the leaves. The damage caused by these pests on curry leaves was so evident that it led to a decrease in the value of this crop in the markets. Therefore, a study was conducted in a farmer's field using some selected biopesticides and chemicals to evaluate their performance in controlling these sucking pests that damaged curry leaves.

MATERIALS AND METHODS

Evaluation of bipesticides, botanicals, mineral oils

Table 1: Effect of treatments on the population and extent of damage by psyllids

Treatments	Mean number of psyllids per five cm apical twig					Damage (%)	
	Pre-count	3 DAS*	5 DAS	7 DAS	14 DAS	Pre count	14 DAS
Talc based formulation of *B. bassiana* NBAIR Bb 5 @ 20 g L^{-1}	14.58 (3.82)	12.00 (3.46)^{dc}	5.78 (2.40)^{bc}	6.06 (2.46)^{bc}	8.17 (2.86)^{d}	30.06 (33.15)	20.00 (26.43)^{c}
Talc based formulation of *L. lecanii* NBAIR Vl 8 @ 20 g L^{-1}	12.36 (3.51)	11.28 (3.36)^{bcd}	5.25 (2.29)^{ab}	5.42 (2.33)^{bc}	5.89 (2.43)^{c}	31.12 (33.88)	18.89 (25.54)^{bc}
Talc 2% @ 20 g L^{-1}	13.03 (3.61)	12.53 (3.54)^{de}	10.33 (3.21)^{e}	9.50 (3.08)^{d}	10.64 (3.26)^{e}	35.19 (36.24)	34.31 (35.82)^{d}
Neem garlic soap formulation-KAU Raksha @ 10 g L^{-1}	11.33 (3.37)	10.61 (3.26)^{bc}	6.56 (2.55)^{cd}	5.19 (2.25)^{b}	4.92 (2.21)^{c}	31.88 (34.32)	23.22 (28.78)^{cd}
Horticultural Mineral Oil HMO @ 25 mL L^{-1}	13.61 (3.68)	10.06 (3.17)^{b}	7.19 (2.68)^{d}	7.33 (2.71)^{cd}	8.22 (2.87)^{d}	31.86 (34.30)	24.53 (29.37)^{cd}
Chlorantraniliprole 8.8 % w/w+ Thiamethoxam 17.5 % w/w SC @ 150 g a.i ha^{-1}	13.89 (3.72)	6.47 (2.54)^{a}	4.61 (2.14)^{a}	1.97 (1.39)^{a}	1.42 (1.19)^{a}	30.01 (33.14)	10.01 (17.79)^{a}
Quinalphos 25 EC @ 250 g a.i ha^{-1}	12.81 (3.58)	6.97 (2.64)^{a}	4.50 (2.12)^{a}	2.22 (1.47)^{a}	2.53 (1.58)^{b}	33.88 (35.49)	9.99 (18.30)^{ab}
Untreated	14.33 (3.78)	14.25 (3.77)^{e}	15.06 (3.88)^{f}	13.08 (3.62)^{e}	14.08 (3.75)^{f}	32.95 (34.99)	47.86 (43.77)^{e}
CD (0.05)	(NS)	(0.239)	(0.249)	(0.398)	(0.232)	(NS)	(7.720)

Mean of three replications; * DAS – Days After Spraying; Value in the parenthesis – Square root transformed values (Population); Arcsine transformed values (Percent damage)

Table 2: Effect of treatments on the population and extent of damage by mites

Treatments	Mean number of mites per leaf					Damage (%)	
	Pre-count	3 DAS	5 DAS	7 DAS	14 DAS	Pre count	14 DAS
Talc based formulation of B. *bassiana* NBAIR Bb 5 @ 20 g L^{-1}	80.61 (8.98)	74.85 (8.65)^{cd}	73.65 (8.58)^{d}	70.45 (8.39)^{d}	70.27 (8.38)^{d}	34.45 (35.86)	25.91 (30.57)^{cd}
Talc based formulation of *L. lecanii* NBAIR Vl 8 @ 20 g L^{-1}	82.92 (9.11)	71.45 (8.45)^{bcd}	62.92 (7.93)^{c}	60.39 (7.77)^{c}	56.01 (7.48)^{c}	31.12 (33.72)	19.06 (25.84)^{abc}
Talc 2% @ 20 g L^{-1}	82.69 (9.09)	78.70 (8.87)^{de}	77.35 (8.80)^{d}	76.16 (8.73)^{e}	76.91 (8.77)^{e}	35.01 (36.20)	32.25 (34.59)^{d}
Neem garlic soap formulation-KAU Raksha @ 10 g L^{-1}	82.33 (9.07)	70.74 (8.41)^{bc}	64.77 (8.05)^{c}	61.45 (7.84)^{c}	54.08 (7.35)^{bc}	28.89 (32.46)	25.42 (30.17)^{cd}
Horticultural Mineral Oil HMO @ 25 mL L^{-1}	83.19 (9.12)	68.90 (8.30)^{bc}	60.68 (7.79)^{c}	55.42 (7.44)^{b}	51.18 (7.15)^{b}	33.82 (35.48)	21.45 (27.36)^{bc}
Chlorantraniliprole 8.8 % w/w+ Thiamethoxam 17.5 % w/w SC @ 150 g a.i ha^{-1}	81.13 (9.01)	64.75 (8.03)^{ab}	49.46 (7.03)^{b}	47.85 (6.92)^{a}	42.28 (6.50)^{a}	27.78 (31.75)	14.83 (22.61)^{ab}
Quinalphos 25 % EC @ 250 g a.i ha^{-1}	80.74 (8.99)	58.96 (7.68)^{a}	43.33 (6.58)^{a}	47.46 (6.89)^{a}	41.06 (6.41)^{a}	27.78 (31.77)	14.09 (21.92)^{a}
Untreated	82.44 (9.08)	85.11 (9.23)^{e}	82.75 (9.10)^{e}	81.31 (9.02)^{f}	84.15 (9.17)^{f}	30.56 (33.55)	33.38 (35.27)^{d}
CD (0.05)	(NS)	(0.423)	(0.258)	(0.271)	(0.246)	(NS)	(5.420)

Mean of three replications; * DAS – Days After Spraying; Value in the parenthesis – Square root transformed values (Population); Arcsine transformed values (Percent damage)

and new molecules of insecticides was carried out at a farmer's field in Neyyattinkara (8.3999° N, 77.1061° E) of Thiruvananthapuram district, Kerala, against the pests *D. citri, S. baltazari* and *A. complex*. Curative treatments were given when the plants had a 10% pest incidence. There were eight gtreaments including a control (Table 1) in a RBD with three replications. The pre-count was taken one day before spraying. The number of pests in each treatment was counted by appropriate methods, and the mean population was calculated. Psyllids were counted visually by tagging tender twigs, and the mean number of psyllids in a 5 cm apical twig was recorded from tagged branches on four sides of each tree. Mites and whiteflies were counted visually from the undersurface of leaves, and the mean was calculated. The percentage damage of each treatment was also worked out by recording the number of leaves damaged out of twenty randomly selected leaves from each replication. Post-treatment counts were made on the 3rd, 5th, 7th and 14th days after spraying.

Statistical analysis

Data obtained from each experiment were transformed as required and subjected to analysis of variance using WASP 2.0 software (Jangam and Wadekar, 2019).

RESULTS AND DISCUSSION

Effect of treatments on the population and extent of damage by psyllids, *D. citri*

The observations recorded on psyllids three days after spraying indicated that treatments chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC and quinalphos'25 % EC were statistically at par, with a mean population of 6.47 and 6.97 psyllids per five cm twig, respectively (Table 1). Among the non-insecticidal treatments, the treatment Horticultural Mineral Oil which recorded a mean population of 10.06, was found on par with the treatments neem garlic soap formulation-KAU Raksha (10.61) and talc based formulation of *L. lecanii* NBAIR Vl 8 (11.28). Sharma (2008) also tested the bio-efficacy of different insecticides where thiamethoxam (0.008%) and quinalphos (0.075%) showed significantly high reduction (91 to 100 per cent) of citrus psylla nymphs. The greater residual effect of thiamethoxam was reported by Arora and Sharma (2011) compared to imidacloprid and acetamiprid against psyllids in kinnow. Seven days after spraying, the treatments quinalphos;25 % EC (2.22) and chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC (1.97) were found on par with each other, and both these treatments were found superior to the treatment talc formulation of *L. lecanii* NBAIR Vl 8 (5.42). After fourteen days of spraying, observations indicated that the treatment chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC was the best in reducing the psyllid population (1.42 /plant). Among the biopesticides, neem garlic soap formulation-KAU Raksha (4.92/ plant) and talc based formulation of *L. lecanii* NBAIR Vl 8 (5.89/ plant) were also on par. Rao and Shivankar (2011) have a different account where they found neem soap @ five gL^{-1}, Pongamia soap @ five gL^{-1}, neem oil @ 6.76 ml L-1 and azadirachtin (10000 ppm) @ 3.65 ml L-1 as most effective than *B. thuringiensis*, *V. lecanii* and sweet flag against second instar nymphs of *D. citri* at 15 days after application.

Effect of treatments on the population and extent of damage by *S. baltazari*

The effect of various treatments on the population of *S. baltazari,* 3 days after spraying, revealed that the statistically superior treatment was quinalphos 25 % EC which recorded a mean population of 58.96 mites/leaf followed by the treatment chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC which recorded a mean of 64.75 mites/leaf (Table 2). After five days of spraying, the lowest population of mites was recorded on plants treated with treatment quinalphos 25 % EC (43.33) followed by on plants sprayed with chlorantraniliprole.8.8 % w/w + thiamethoxam 17.5 % w/w SC (49.46). Seven days after spraying, among the rational treatments, the effective one was Horticultural Mineral Oil (55.42 mites/leaf). Still, it was found inferior to the treatments quinalphos 25 % EC (47.46 mites/leaf) and chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC (47.85 mites/ leaf). Ramanna (2009) reported that, among organic insecticides evaluated, the minimum number of mites (0.83 mites/leaf) was recorded in *V. lecanii* (2 g L-1). Still, it was found inferior to the ashwagandha insecticide dicofol (2.5 ml L-1). Observations after fourteen days of spraying indicated that the treatments quinalphos 25 % EC and chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC were effective and on par in reducing the mite population with a mean count of 41.06 and 42.28, respectively followed by treatment Horticultural Mineral Oil (51.18) and neem garlic soap formulation-KAU Raksha (54.08) among the biorational treatments. Yadav (2018) also stated that in okra, HMO at 2.5% (84.00 per

Table 3: Effect of treatments on the population and extent of damage by whiteflies

Treatments	Mean number of whiteflies per leaf					Damage (%)	
	Pre-count	3 DAS	5 DAS	7 DAS	14 DAS	Pre count	14 DAS
Talc based formulation of *B. bassiana* NBAIR Bb 5 @ 20 g L^{-1}	2.67 (1.63)	1.64 (1.27)^{c}	1.06 (1.01)^{b}	1.11 (1.05)^{c}	1.03 (1.01)^{c}	34.81 (36.15)	16.67 (23.39)^{ab}
Talc based formulation of *L. lecanii* NBAIR Vl 8 @ 20 g L^{-1}	2.22 (1.49)	1.39 (1.18)^{bc}	0.58 (0.76)^{a}	0.50 (0.70)^{ab}	0.53 (0.72)^{ab}	31.67 (34.14)	17.23 (24.01)^{ab}
Talc 2% @ 20 g L^{-1}	2.06 (1.43)	1.72 (1.30)^{c}	1.72 (1.31)^{cd}	1.69 (1.30)^{d}	1.14 (1.06)^{c}	31.15 (33.83)	30.56 (33.37)^{e}
Neem garlic soap formulation-KAU Raksha @ 10 g L^{-1}	2.89 (1.69)	1.08 (1.04)^{b}	0.53 (0.72)^{a}	0.72 (0.84)^{b}	0.97 (0.98)^{c}	32.31 (34.51)	25.56 (30.17)^{bc}
Horticultural Mineral Oil HMO @ 25 mL L^{-1}	2.61 (1.61)	1.19 (1.09)^{bc}	1.28 (1.12)^{bc}	1.14 (1.06)^{c}	1.19 (1.09)^{c}	29.60 (32.76)	18.39 (25.35)^{b}
Chlorantraniliprole 8.8 % w/w+ Thiamethoxam 17.5 % w/w SC @ 150 g a.i ha^{-1}	2.78 (1.67)	0.61 (0.77)^{a}	0.31 (0.55)^{a}	0.50 (0.71)^{ab}	0.50 (0.69)^{a}	27.75 (31.73)	8.89 (16.53)^{a}
Quinalphos 25 EC @ 250 g a.i ha^{-1}	2.25 (1.50)	0.44 (0.66)^{a}	0.53 (0.72)^{a}	0.42 (0.64)^{a}	0.92 (0.94)^{bc}	27.78 (31.77)	9.53 (17.44)^{a}
Untreated	2.56 (1.60)	2.72 (1.65)^{d}	2.39 (1.54)^{d}	2.22 (1.49)^{e}	2.14 (1.46)^{d}	36.29 (36.98)	46.89 (43.21)^{d}
CD (0.05)	(NS)	(0.212)	(0.244)	(0.145)	(0.240)	(NS)	(7.600)

Mean of three replications; * DAS – Days After Spraying; Value in the parenthesis – Square root transformed values (Population); Arcsine transformed values (Percent damage)

Table 4: Percentage reduction in population and damage of pests over untreated control, 14 DAS

Treatments	Psyllids		Mites		Whiteflies	
	Population	Damage	Population	Damage	Population	Damage
Talc based formulation of *B. bassiana* NBAIR Bb 5 @ 20 g L^{-1}	41.97	58.21	16.49	22.38	51.87	64.44
Talc based formulation of *L. lecanii* NBAIR Vl 8 @ 20 g L^{-1}	58.17	60.53	33.44	42.37	75.23	63.23
Talc 2% @ 20 g L^{-1}	24.43	28.31	8.60	3.34	46.73	34.83
Neem garlic soap formulation-KAU Raksha @ 10 g L^{-1}	65.06	51.48	35.73	23.85	54.67	45.49
Horticultural Mineral Oil HMO @ 25 mL L^{-1}	41.62	48.75	39.18	35.74	44.39	60.78
Chlorantraniliprole 8.8 % w/w+ Thiamethoxam 17.5 % w/w SC @ 150 g a.i ha^{-1}	89.91	79.08	49.76	55.57	76.63	81.04
Quinalphos 25 EC @ 250 g a.i ha^{-1}	82.03	79.12	51.20	57.79	57.00	79.68

cent) and neem oil at 2.0 % (81.33 per cent) were on par with each other in mite mortality, but the superior treatment was HMO at 3.0% (92.00 per cent).

Effect of treatments on the population and extent of damage by *A. complex*

Three days after spraying, treatments quinalphos 25 % EC and chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC were on par in reducing the population of *A. complex* with a mean population of 0.44 and 0.61 whiteflies/leaf (Table 3). Patil (2016) obtained similar results using another neonicotinoid, imidacloprid (0.005%), against *A. woglumi*, demonstrating that it was the most effective in reducing the blackfly population by up to 76.77% 14 days after spraying. Jadhav *et al.* (2018) also reported that thiamethoxam 25 WG @ 25 g a.i ha-1 was the most effective treatment (6.31 whitefly/plant) at 14 DAS against whitefly in brinjal. After five days of spraying, lowest population of whiteflies was recorded on plants treated with chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC (0.31), which was also on par with treatments quinalphos 25 % EC (0.53), neem garlic soap formulation- KAU Raksha (0.53) and talc based formulation of *L. lecanii* NBAIR Vl 8 (0.58). Fourteen days after spraying, chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC recorded a minimum number of whiteflies (0.50 /leaf), which was followed by talc based formulation of *L. lecanii* NBAIR Vl 8 (0.53), superior among the rationals.

All biorational treatments *viz.*, talc based formulation of *B. bassiana* NBAIR Bb 5, talc based formulation of *L. lecanii* NBAIR Vl 8, talc 2%, neem garlic soap formulation-KAU Raksha, and Horticultural Mineral Oil displayed a significant reduction in the population and extent of damage caused by the above mentioned pests over untreated control. Against *D. citri*, treatments of neem garlic soap formulation-KAU Raksha and *L. lecanii* NBAIR Vl 8 showed 65.06 and 58.17% reduction in population and 51.48 and 60.53% reduction in damage after 14 days of spray (Table 4). Against spider mites, effective ones were Horticultural Mineral Oil and neem garlic soap formulation-KAU Raksha, which showed 39.18 and 35.73 per cent reductions in mite population at the end of the observation schedule. At the same time, in reducing the extent of damage, the talc-based formulation of *L. lecanii* NBAIR Vl 8 and Horticultural Mineral Oil was superior, with 42.37 and 35.74 per cent reduction, respectively. Treatments effectively controlled whitefly, A. complex chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC and talc based formulation of *L. lecanii* NBAIR Vl 8, which reduced the population and extent of damage caused by whiteflies by 76.63, 75.23 and 81.04 and 63.23 per cent, respectively. They were also comparable with each other in reducing the whitefly population. Since synthetic pesticides are not an option in curry leaves, this result seems promising as an advantage over chemicals in reducing whiteflies in curry leaves. Also, when comparing the percentage reduction in the population of whiteflies from 3 to 14 DAS, talc based formulation of *L. lecanii* NBAIR Vl 8 caused a reduction of 61.87 per cent with respect to chlorantraniliprole 8.8 % w/w + thiamethoxam 17.5 % w/w SC which only caused 18.03 per cent reduction. This sheds light on the sustainability of mycoinsecticides compared to chemical insecticides in pest management.

Based on our evaluation results, it can be concluded that for managing sucking pests infesting curry leaves, talc based formulation of *L. lecanii* NBAIR Vl 8 @ 20 g L-1, neem garlic soap formulation-KAU Raksha @ 10 g L-1 or Horticultural Mineral Oil HMO @ 25 mL L-1 can be recommended as non-chemical, eco-friendly, and safe options for farmers.

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Diversity and morphometrics of fruit flies (Diptera: Tephritidae) in South Gujarat, India

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ABSTRACT: An extensive survey was conducted to find out the distribution of different fruit fly species in the Navsari district of Gujarat from January to December, 2022. Among different fruit fly species, *Bactrocera* spp., are widely distributed and caused significant damage to horticultural crops. Four fruit fly species *viz*., *Bactrocera dorsalis* (Hendel)*, B. zonata* (Saunders)*, B. correcta* (Bezzi), and *B. cucurbitae* (Coquillett), were recorded from six talukas, namely Navsari, Jalalpore, Gandevi, Chikhli, Khergam and Vansda. Among all species, *B. dorsalis* (53.87%) was the most abundant followed by *B. zonata* (19.92%) and *B. correcta* (2.91%). Whereas, *B. cucurbitae* was the most common in cucurbits with relative abundance of 23.30 per cent. The activity of fruit flies was observed throughout the year. However, the peak fruit fly population was observed between April to July and gradually decreased from September to December. Higher diversity index was recorded in Gandevi, followed by Navsari and Jalalpore. *Bactrocera dorsalis* was larger than *B. zonata* and *B. correcta*. However, female flies were bigger than their male counterparts among all fruit fly species.

Keywords: Abundance, *Bactrocera*, fruit flies, Gujarat, diversity

INTRODUCTION

Tephritidae is one of the largest, most diversified and fascinating acalyptrate families of Diptera and include fruit flies, which are serious pests of fruits and vegetables. The greatest diversity of fruit flies has been noticed in Oriental region followed by tropical and subtropical regions. Due to their habit of strutting around, vibrating their wings and flaunting their intricate wings and body markings, these flies are sometimes referred to as "peacock flies (Jena, 2023). In courting and agonistic displays, their intricate body and wing patterns act as visual releasers (Kapoor, 1993). According to David and Ramani (2011), 325 species of fruit flies belonging to 243 families and 79 genera are found in India. The tribe Dacini, with the genus *Bactrocera*, is important in India. From an economic point of view, *B. cucurbitae*, *B. dorsalis*, and *B. zonata,* are the most common pest species, while *B. correcta*, *B. diversa* (Coquillett) and *B. latifrons* (Hendel) are still restricted to specific areas (Kapoor, 2005). The activity of fruit flies observed throughout the year with peak activity coinciding with the maturity and harvesting of fruits (Jena *et al.*, 2022b, 2022c; Jena, 2023). The management of fruit flies is difficult as the most damaging stage is the larva which remains unexposed to the pesticides (Jena *et al.,* 2022a, 2022d). Even if insecticides are used repeatedly, consumers may be exposed to potential health risks. South Gujarat is the leading producer of fruits and vegetables in Gujarat. The agri-export zone for fruits and vegetables includes three districts: Surat, Navsari, and Valsad. Damage intensity was as high as 30 per cent in mango and sapota and 20-40 per cent in cucurbitaceous crops (Patel *et al.,* 2021). Furthermore, morphometric analysis is a useful technique in detecting morphological differences among organisms to distinguish closely related species including fruit flies, justify synonymies, demonstrate morphological variation along altitudinal or geographical gradients and propose new species (Adsavakulchai *et al.*, 1999). Due to their wide climate tolerance, polyphagous nature, high reproductive potential, multivoltine character and a great capacity for dissemination of fruit flies management is challenging. However, sanitation in conjunction with the use of lures, traps, and baits, proved to be the most effective methods of controlling fruit flies. These traps are highly specific, inexpensive, and environmentally friendly (Sureshbabu and Viraktamath, 2003). In this view, we have undertaken an extensive survey in the Navsari district of South Gujarat from January to December, 2022 to profile the distribution, diversity, species composition and species richness of fruit flies.

MATERIALS AND METHODS

The survey was carried out to find out the diversity of fruit fly species in six talukas of Navsari district (Navsari, Jalalpore, Gandevi, Chikhli, Vansda and Khergam) from January to December, 2022. During the peak season of

fruit fly infestation, the infested samples of fruits and vegetables were collected and methyl eugenol and Cue-lure based attractant-insecticide traps were also installed. Three locations were identified in each taluka based on the abundance of fruit orchards and vegetable farms. Two traps were installed in each location and hung vertically, 6 ft. above the ground level on tree branches. A total of six traps were installed in each taluka and trapped fruit fly samples were collected at monthly intervals and lures were changed at every 45 day interval to maintain the efficacy of the trapping system. The infested samples were brought to the laboratory and kept until the adult emergence. The fruit flies caught in the attractant traps were also brought to the laboratory and identified based on morphological description given by White and Elson-Harris (1992) and Drew and Raghu (2002). The total number of samples captured from each taluka and each month was averaged. Then, relative abundance (Relative abundance = (Number of individuals of one species/Number of individuals of all species) × 100) of each species and location was estimated. The species diversity of fruit flies of each taluka was quantified based on the samples collected during the study. Shannon-Wiener index (*H'*) (Shannon, 1948), species evenness (*J'*) (Pielou, 1966) and species richness (S) were also calculated. Furthermore, for making morphometric studies on adult mango fruit flies, collected specimens from traps (methyl-eugenol) and rearing cages were used. The species wise morphometric measurements of various body parts were recorded under Stereoscopic Trinocular Microscope fitted with a Brand Catcam-130 Camera (Make: Olympus SZ-61).

RESULTS AND DISCUSSION

During the survey, four fruit fly species namely, *B. dorsalis, B. zonata, B. correcta* and *B. cucurbitae* were recorded from Navsari district. Out of four species, three fruit fly species *viz. B. cucurbitae, B. dorsalis* and *B. zonata* were reported as major species. Whereas, one species *i.e., B. correcta* was reported as minor species. Adult males of *B. dorsalis, B. zonata* and *B. correcta* were found trapped in the methyl eugenol-based attractant traps while, *B. cucurbitae* males were trapped in cue-lure traps (Table 1). All flies collected belong to the subfamily Dacinae and tribe Dacini.

Table 1. Fruit fly diversity in different locations of Navsari district, Gujarat, India

Study sites	Locations	No. of traps	Species recorded	Host	Attractant trap
Navsari	3	6	*B. dorsalis*	Mango, Sapota, Banana, Guava, Jamun, Papaya	Methyl eugenol
			B. zonata	Mango, Sapota	
			B. correcta	Mango, Guava	
			B. cucurbitae	Cucurbits	Cue-lure
Jalalpore	3	6	*B. dorsalis*	Mango, Sapota, Banana, Guava, Jamun, Papaya	Methyl eugenol
			B. zonata	Mango, Sapota	
			B. correcta	Mango, Guava	
			B. cucurbitae	Cucurbits	Cue-lure
Gandevi	3	6	*B. dorsalis*	Mango, Sapota, Guava, Banana	Methyl eugenol
			B. zonata	Mango	
			B. correcta	Guava, Mango	
			B. cucurbitae	Cucurbits	Cue-lure
Chikhli	3	6	*B. dorsalis*	Mango, Sapota	Methyl eugenol
			B. zonata	Mango, Sapota	
			B. cucurbitae	Cucurbits	Cue-lure
Vansada	3	6	*B. dorsalis*	Mango, Sapota	Methyl eugenol
			B. zonata	Mango, Sapota	
			B. cucurbitae	Cucurbits	Cue-lure
Khergam	3	6	*B. dorsalis*	Mango, Sapota	Methyl eugenol
			B. zonata	Mango, Sapota	
			B. cucurbitae	Cucurbits	Cue-lure

The highest number of male fruit flies were collected during May, 2022 with an average of 356.75, 289.00, and 265.75 male fruit flies/trap in Gandevi, Jalalpore and Navsari talukas, respectively. A similar trend was observed in Khergam and Vansda with an average of 208.33 and 197.00 male fruit flies/trap, respectively. Whereas, in Chikhli, the fruit fly population was high, with an average of 203.50 male fruit flies/trap in June (Fig. 1). The peak population was recorded from May to July, 2022 in all the talukas as this was the time for mango and sapota fruiting coinciding with the cucurbit crops *viz.,* cucumber, little gourd, pointed gourd *etc.* A more or less similar finding was made by Nandre and Shukla (2014), who reported the maximum activity of fruit flies (172.10 flies/trap) during March to August and lower during December and January (11.10 to 21.30 flies/trap) in sapota orchard at Gandevi, South Gujarat. While, Das et al. (2017) noticed that during 4th week of April, the mean fruit fly population was 10.67 male fruit flies/trap/week in the case of *B. cucurbitae*.

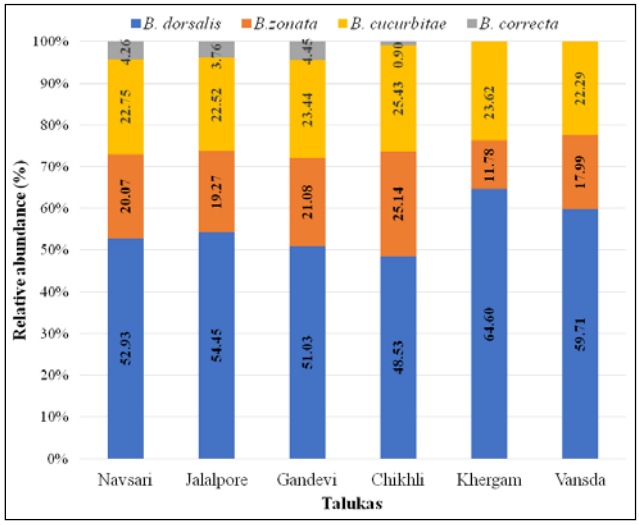


Fig. 1. Average fruit fly samples captured during January-December, 2022 in different Talukas of Navsari district in Gujarat. The value represents average numbers of fruit flies trapped per trap from each taluka and month (N=12).

In the present study, *B. dorsalis* (53.87%) was the most abundant in all the talukas, followed by *B. zonata* (19.92%) and *B. correcta* (2.91%) in fruit crops. Whereas, *B. cucurbitae* was the most common in cucurbits with a relative abundance of 23.30 per cent (Fig. 2). The present findings were in close affinity with earlier work of Nandre and Shukla (2012), who recorded three species of fruit flies *viz., B. dorsalis*, *B. zonata* and *B. correcta* in sapota orchards at Navsari using methyl eugenol traps with *B. dorsalis* being the most predominant species (65.14%), followed by *B. zonata* (32.87%), and *B. correcta* (1.97%). Also, Bisane (2017) investigated the population diversity and cyclicity of fruit fly, *Bactrocera spp.* using a modified "Nauroji Stonehouse" fruit fly trap and revealed that *B. dorsalis* was the dominating species with more than 95% population over *B. correcta* and *B. zonata*. Borah (1998) reported 39.10 per cent fruit damage by *B. cucurbitae* to the cucumber crop grown during *kharif* season, whereas on the *rabi* and summer season crops, the damage was 77.60 and 20.30 per cent, respectively.

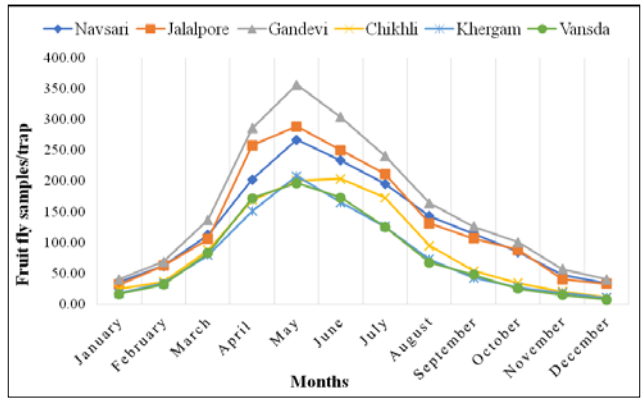


Fig. 2. Relative abundance (%) of fruit fly species observed during January-December, 2022 in different Talukas of Navsari district in Gujarat. The value represents abundance (%) of fruit fly species from total fruit fly samples collected from each Taluka for 12 months

The diversity indices and richness of fruit flies in each taluka were estimated. Shannon diversity index was maximum in Gandevi (1.15) followed by Navsari (1.13), Jalalpore (1.11), and Chikhli taluka (1.09), respectively. However, diversity index was minimum for Vansda (0.92) and Khergam (0.87), respectively (Table 2). Species evenness was found high in Vansda (0.84) followed by Gandevi (0.83) and Navsari (0.82). However, Jalalpore and Khergam showed similar results (0.80) and species evenness was lowest in Chikhli (0.79), respectively.

Morphometric studies on mango fruit flies

1. Bactrocera dorsalis

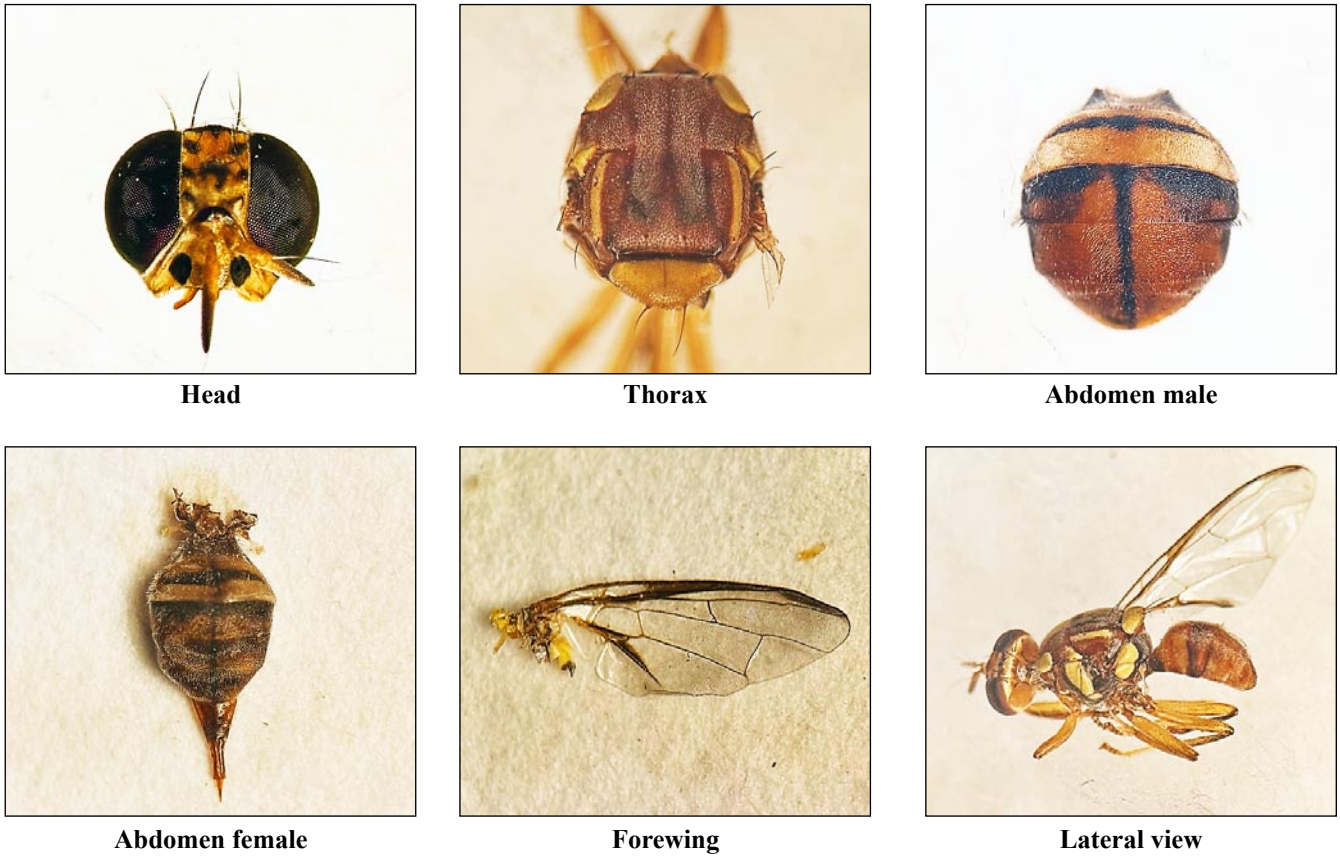
Description: Face yellowish with two separate medium-sized circular black spots. Scutum black with red-brown areas of varying shapes and sizes. Two broad parallel sided lateral postsutural vittae ends at intera-alar setae, medial postsutural vittae absent. Scutellum entirely yellow coloured except for narrow basal band and two apical setae. Wing with a distinct brown costal band continuous from cell Sc to wing apex, confluent with vein R_{2+3}, crossveins r-m and dm-cu not covered by any markings. Abdomen predominantly orange red with prominent T shaped mark. Male with pecten

Table 2. Diversity indices of fruit fly population in different talukas of Navsari district

Taluka	Shannon diversity index (H')	Species evenness (J')	Species richness (S)
Navsari	1.13	0.82	4
Jalalpore	1.11	0.80	4
Gandevi	1.15	0.83	4
Chikhli	1.09	0.79	4
Khergam	0.87	0.80	3
Vansda	0.92	0.84	3

on third abdominal tergum whereas female adults had a tapering ovipositor for laying eggs in host fruits. All femora yellow with rows of long setae on fore femora and without any fuscous/dark marking (Plate 1). These findings were similar to the description given by Drew and Raghu (2002), White and Elson-Harris (1992) and Narayanan and Batra (1960).

The morphometric studies on mango fruit flies revealed that adult female fruit flies were bigger in size as compared to male fruit flies in all the three species *viz., B. dorsalis, B. zonata* and *B. correcta* infesting mango. The average body length for male and female of *B. dorsalis* was 6.28 ± 0.55 and 7.55 ± 0.80 mm, respectively. Whereas, the average body width (wing expanse) was 12.30 ± 0.97 and 13.11 ± 0.80 mm for male and female fruit flies, respectively (Table 3). The above findings on body length and width of *B. dorsalis* were in close conformity with Jena et al. (2022d), who revealed that the length and width (wing expanse) of male adult varied from 4.91 to 7.23 mm and 10.10 to 12.65 mm, while female adult was found to vary from 6.70 to 8.98 mm and 12.20 to 16.50 mm, respectively.



Head **Thorax** **Abdomen male**

Abdomen female **Forewing** **Lateral view**

Plate 1: *Bactrocera dorsalis*

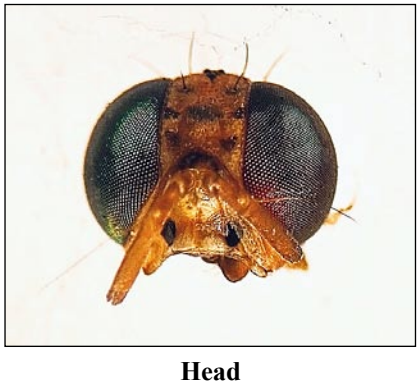
Table 3. Morphometrics of *B. dorsalis*

Body characters	Adult male (mm)			Adult female (mm)		
	Min.	Max.	Avg. ± SD	Min.	Max.	Avg. ± SD
Body length	5.30	7.31	6.28 ± 0.55	6.47	8.96	7.55 ± 0.80
Body width	10.45	13.66	12.30 ± 0.97	12.07	14.63	13.11 ± 0.80
Fore leg length	4.17	5.26	4.76 ± 0.33	4.56	5.27	4.89 ± 0.22
Mid leg length	5.65	6.68	6.22 ± 0.28	5.95	6.89	6.45 ± 0.30
Hind leg length	5.02	5.93	5.36 ± 0.27	5.23	6.07	5.64 ± 0.28

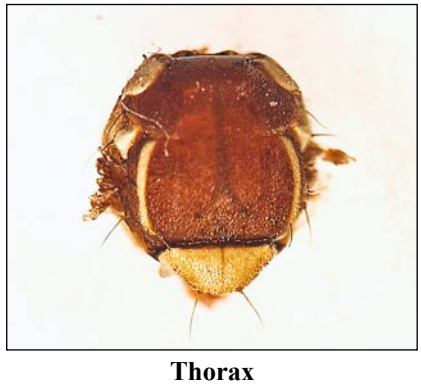
2. *Bactrocera zonata*

Description: Face fulvous with two medium sized circulars to oval black spots in antennal furrow. Scutum orange brown to red-brown with a pale fuscous pattern posteriorly with lateral yellow or orange postsutural stripes, scutellum entirely pale yellow coloured except for a narrow dark red-brown basal band and with two apical scutellar setae. Wing lacks a complete costal band (reduced to an isolated apical spot), cubital stripe absent but with a very tiny pale fuscous tint in the cell cup. Abdomen orange brown with dark hind margins on terga 3 -5 and dark posterolateral margins on terga 4 and 5, spots on tergum 5 dark reddish brown. Male with pecten on third abdominal tergum. Legs fulvous and mid tibiae having an apical black spur. The hindwings are modified into halteres. Male consists of rounded abdomen with pecten and female with pointed ovipositor. Aristate type of antennae were present. Females were bigger than males in size (Plate 2). These findings were similar to the description given by Drew and Raghu (2002), White and Elson-Harris (1992). Also, Jena and Patel (2022a) revealed that the abdominal end of male was rounded with pecten while, it was developed in to pointed ovipositor in case of female.

The average body length for male and female of *B. zonata* was 4.75 ± 0.29 and 6.09± 0.32 mm, respectively, whereas the average body width (wing expanse) was 10.65 ± 0.99 and 11.81 ± 0.82 mm for male and female fruit flies, respectively (Table 4). The above findings were more or less similar to Jena and Patel (2022a) who found that the length and width (wing expanse) of male adult varied from 4.20 to 5.10 mm and 8.42 to 11.40 mm, while female adult was found to vary from 5.32 to 6.21 mm and 10.40 to 12.60 mm, respectively.



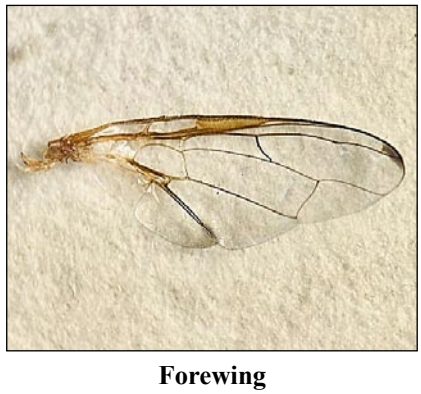
Head



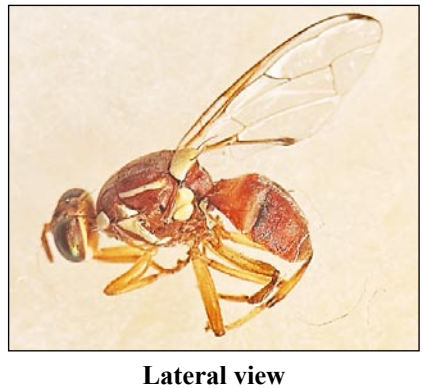
Thorax

Abdomen male

Abdomen female



Forewing



Lateral view

Plate 2: *Bactrocera zonata*

Table 4. Morphometrics of *B. zonata*

Body characters	Adult male (mm)			Adult female (mm)		
	Min.	Max.	Avg. ± SD	Min.	Max.	Avg. ± SD
Body length	4.25	5.24	4.75 ± 0.29	5.73	6.82	6.09 ± 0.32
Body width	8.86	11.95	10.65 ± 0.99	10.33	12.95	11.81 ± 0.82
Fore leg length	3.96	4.61	4.21 ± 0.22	4.11	4.93	4.47 ± 0.23
Mid leg length	5.15	6.07	5.65 ± 0.32	5.45	6.10	5.79 ± 0.22
Hind leg length	4.65	5.35	5.06 ± 0.19	4.45	5.50	**1.11** 0.28

Bactrocera correcta

Description: Face fulvous with small black longitudinally oval to circular spots on antennal furrow meeting in the middle just above the mouth opening. Scutum predominantly black, anterior supra-alar setae present, two yellow lateral postsutural vittae ending beyond intra alar setae, prescutellar setae present. Scutellum completely yellow with narrow basal black band and two apical scutellar setae. Legs fulvous, hind tibiae with keel like process on posterodorsal surface near apex. Wings with a reduced pattern, coastal band from cell Sc to r_1, confluent with R_{2+3}, broken in cell r_{2+3}, leaving an apical dark spot in cell r_{2+3} and r_{4+5} cubital streak contained with cell cup, base of cell br without my microtrichia. Abdomen dark brown with a median longitudinal dark band on terga 3 -5. Male with pecten on third abdominal tergum (Plate 3). These findings were similar to description given by Drew and Raghu (2002), White and Elson-Harris (1992).

For *B. correcta* the average body length for male and female fruit flies was 4.71 ± 0.27 and 5.61 ± 0.35 mm, respectively, whereas the average body width was 10.08 ± 0.92 and 11.54 ± 0.77 mm for male and female fruit flies, respectively (Table 5). Weems and Fasulo (2004) depicted that *B. correcta* was approximately 5.40 mm in length.

Table 5. Morphometrics of *B. correcta*

Body characters	Adult male (mm)			Adult female (mm)		
	Min.	Max.	Avg. ± SD	Min.	Max.	Avg. ± SD
Body length	4.30	5.10	4.71 ± 0.27	5.25	6.35	5.61 ± 0.35
Body width	8.20	11.50	10.08 ± 0.92	10.11	12.45	11.54 ± 0.77
Fore leg length	3.65	4.45	4.01 ± 0.23	4.05	4.65	4.35 ± 0.19
Mid leg length	4.90	5.98	5.41 ± 0.33	5.24	6.01	5.67 ± 0.23
Hind leg length	4.28	5.20	4.89 ± 0.28	4.12	5.35	4.91 ± 0.4

Note: Observations for body length and width were recorded at 0.67X while, for legs observation were recorded at 1.5X.

CONCLUSION

Four species (*B. dorsalis, B. zonata, B. correcta* and *B. cucurbitae*) of fruit flies were recorded from different talukas of the Navsari district. *Bactrocera dorsalis* was the most abundant species, followed by *B. zonata* and *B. correcta* in all Talukas in fruit crops. Whereas, *B. cucurbitae* was the most dominant in the cucurbit crop ecosystem. *Bactrocera dorsalis* was larger than *B. zonata* and *B. correcta*. However, female flies were bigger than their male counterparts among all fruit fly species. Forecasting and analyzing the geographic distribution of the fruit fly population will make it easier to conduct suitable control methods to decrease the pest population under field conditions.

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Head

Thorax

Abdomen male

Abdomen female

Forewing

Lateral view

Plate 3: ***Bactrocera. correcta***

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Biology of false spider mite, *Raoiella macfarlanei* on *Syzygium cumini* and *Syzygium jambos*: A comparative study of development, behavior, and impact on host plants

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Abstract: A study investigated the traits and life cycle of the pest mite *Raoiella macfarlanei* on two host plants, *Syzygium cumini* and *Syzygium jambos*, at two temperatures. Results revealed distinct developmental patterns: females and males matured faster on *S. cumini* at both 30°C (21.70 and 18.93 days) and room temperature (26.88 and 25.71 days) compared to *S. jambos* (30°C: 24.16 and 21.04 days; room temperature: 29.98 and 28.25 days). Additionally, mated females had shorter preoviposition and oviposition periods on *S. cumini* at room temperature (4.47 and 35 days) and 30°C (3.92 and 27.10 days) than on *S. jambos* (5.53 and 35.40 days at room temperature; 4.16 and 28.00 days at 30°C). Net Reproductive Rate and Mean Generation Time were higher on *S. jambos* (room temperature: 22.11 and 53.39; 30°C: 19.55 and 41.50) compared to *S. cumini* (room temperature: 21.00 and 48.80; 30°C: 18.30 and 38.20). These findings deepen our understanding of *R. macfarlanei's* biology and life cycle in different environments, offering insights into its impact on agricultural ecosystems.

Key words: Biology, demography, host plants, life history studies, *Raoiella macfarlanei*

INTRODUCTION

The Tenuipalpidae, a mite family (Acari) crucial in economics and agriculture, ranges from 190 to 330 micrometers in size. Often mistaken for spider mites, they're distinct for not spinning webs, earning them the name "false spider mites." Dorsoventrally flattened and slow-moving, they vary in color and have a global presence. Exclusive plant feeders, they cause significant damage to a variety of crops, feeding on leaf undersides, midribs, veins, stalks, and even forming galls on host plants (Ghai and Shenhmar, 1984; Sadana, 1985). Feeding activities of mites harm plants by damaging the epidermis and depleting leaves of chlorophyll and nutrients, affecting photosynthesis, growth, and yield (Goyal and Sadana, 1983). Infestation signs include stippling, scars, bronzing, a silvery appearance, and rusty brown or tannish scales (Goyal *et al.,* 1984). Punctures may lead to secondary infections. Mites reduce leaf area and water intake, causing wilting, leaf death, and defoliation, ultimately leading to plant devitalization and death. Tenuipalpidae members have stylet-like chelicerae and a simple palpus without a thumb-claw process. They have three pairs of setae on the propodosoma and 7 to 13 pairs on the hysterosoma. The metapodosoma may have one to several pairs of ventral setae, and the ventral and genital plates may or may not be distinct. They possess claw-like or pad-like true claws with a pad-like empodium bearing tenent hairs. Identifications rely on factors like the number and position of hysterosomal setae and the presence of striations and reticulations on the dorsal and ventral hysterosomal surfaces (Sadana, 1997). The Tenuipalpidae family has 891 described species in 34 genera globally, found across various zoogeographic regions (Mesa *et al*., 2009). In India, Gupta and Mandal (2014) identified 102 species from 15 genera, including 20 species from seven genera in Karnataka.

The false spider mite, *R. macfarlanei* Pritchard and Baker, was first found in 1974 on *Jambosa vulgaris* in Karnataka, India, and later in Kerala and Gujarat. It infests *Syzygium* spp., causing leaf desapping, yellowing, browning, and leaf dryness. They are mainly found on the lower surface of leaves along the midrib, piercing plant tissue and sucking sap. Heavy infestation leads to yellowing and brown patches on the leaves, with older leaves being more affected. Mite populations peak from January to April, persisting throughout the year (Nageshachandra, 1980).

Despite their economic importance, studies on the Tenuipalpidae family are scarce in India and specifically in Karnataka. This study aims to explore the false spider mite fauna associated with fruit plants in Bengaluru and its neighboring regions, focusing on the biological aspects and life parameters of *R. macfarlanei* in relation to different host plants and temperature conditions, offering a deeper understanding of the dynamics of this species in agricultural ecosystems.

MATERIAL AND METHODS

This section covers the methods used for collecting, preserving, extracting, processing, describing, illustrating,

and measuring mite specimens. It also addresses research on the developmental biology, reproduction, and life table parameters of *R. macfarlanei* on *Syzygium* spp. The studies were conducted at the Acarology unit of the Department of Agricultural Entomology, UAS, GKVK, Bengaluru.

Collection, purification and maintenance of mite culture in the laboratory

Leaves infested with *R. macfarlanei* from *Syzygium* trees (*S. cumini* and *S. jambos*) were collected from hostel premises, GKVK and transported to the lab. Thirty random mating pairs of mites, each consisting of single female deutonymph and one to two male mites, were selected from these samples. These pairs were placed on separate 2.5 cm × 2.5 cm leaf sections within 6" Petri plates on moist cotton wads for colonization. One female and one male mite from each section were slide-mounted in Hoyer's medium for taxonomic identification. After identification, the leaf sections with *R. macfarlanei* mites were combined to form a pure starter culture, maintained on host leaves (*Syzygium* spp.) in separate polyethylene trays (25×20×5 cm) with wet foam. To support mite culture multiplication, leaf sections were kept turgid and replaced with fresh ones as needed due to deterioration or loss of color.

Comparative developmental biology of *R. macfarlanei* on two different host plants

The developmental biology study of *R. macfarlanei* occurred in controlled lab conditions, examining two temperature settings: room temperature (21-22°C, 83-88% RH) and a higher temperature of 30°C (74-79% RH). The experiment used two host plants, *S. cumini* and *S. jambos*. In the initial phase, 30 eggs laid on *S. cumini* leaves were collected within a 2 to 4-hour window. These eggs were individually transferred to 30 separate small leaf sections (2.5 x 2.5cm) of *S. cumini*, placed on damp cotton wads inside 6-inch Petri dishes. Two sets of eggs were used: one set developed at room temperature, while the other was placed in a BOD incubator at 30°C.

A parallel procedure was applied to *S. jambos*, involving 30 eggs under two temperature conditions (room temperature and 30°C). Special care was taken to maintain cotton wad moisture through periodic wetting with clean water. Mite development on leaf sections was observed at 3-hour intervals using a stereo zoom microscope, enabling tracking of stages such as incubation, egg hatching, and durations of various immature stages (larva, quiescent I, protonymph, quiescent II, deutonymph, and quiescent III) until adult mite emergence. The resulting adult mites' gender was also recorded. If leaf sections dried or deteriorated, immature mites were transferred to fresh ones for continued observations.

Reproduction and demography of *R. macfarlanei* on *Syzygium* spp.

A similar procedure was employed for *S. jambos*, using 30 eggs for each temperature condition (room temperature and 30°C). Ensuring the cotton wad's moisture, periodic wetting with clean water was meticulously maintained. Mite development on leaf sections was observed at 3-hour intervals through a stereo zoom microscope. This tracking covered stages like incubation, egg hatching, and durations of larva, quiescent I, protonymph, quiescent II, deutonymph, and quiescent III, leading to the emergence of adult mites. The resulting adult mites' sex was also recorded. If leaf sections dried or deteriorated, immature mites were moved to fresh sections, and observations continued.

The objective was to compare the influenmce of host plants on various reproduction attributes, such as the preoviposition period, oviposition period, post-oviposition period, fecundity, and sex ratio. Additionally, we calculated various demographic characteristics or life table parameters, including Mean Generation Time (T), Doubling Time (DT), Finite Rate of Increase (λ), Net Reproduction Rate (R_o), Gross Reproductive Rate (GRR), and Intrinsic Rate of Natural Increase (rm) using the method recommended by Birch (1948).

RESULTS AND DISCUSSION

A thorough investigation into the developmental biology of *R. macfarlanei* was conducted under laboratory conditions, specifically examining two *Syzygium* species and two temperature settings. The study revealed significant variations in developmental timelines, reproduction, and demographic parameters based on gender, host plants, and temperature conditions.

Biology of *R. macfarlanei*

At room temperature (21-22 °C & RH 83-88%)

On *S. cumini*, *R. macfarlanei* female required 218.88, 81.26, 28.52, and 91.17 hours for egg, larval, protonymphal, and duetonymphal stages, respectively. In comparison, the corresponding stages in males took 218.88, 78.00, 89.00, and 97.33 hours. The female's development from egg to adult was significantly longer (26.88 days) compared to the male on *S. cumini*. On *S. jambos*, the female needed 244.0, 116.75, 78.75, and 105.66 hours for the same stages, while the male required 244.00, 52.00, 84.00, and 80.00 hours. The

female's development from egg to adult (29.98 days) was significantly longer than the male (28.25 days) on *S. jambos*. *R. macfarlanei* female completed development in 26.88 days on *S. cumini* and 29.98 days on *S. jambos*, a significant difference. The male also developed faster on *S. cumini* (25.71 days) compared to *S. jambos* (28.25 days), with statistical significance (Table 1).

No literature is available on the biology of *R. macfarlanei* on any of the hosts and the results are discussed in the light of the studies conducted on other related species. Therefore, our study provides valuable insights into the developmental timelines of *R. macfarlanei*, which had not been previously documented in the literature. We compared our findings with related mite species' studies, such as *Raoiella indica*. Nageshachandra (1980) studied the biology of *R. indica* on coconut in the laboratory at ambient temperature ranging from 23.90 to 25.7°C and relative humidity averaging 59.85 per cent. The total developmental period was about 24.50 days and Moutia (1958) studied the life cycle of *R. indica* which occupied 18.00 to 26.00 days with an average of 22.00 days at 24.2°C in February-March and 30.00 to 36.00 days with an average of 33.00 days at 17.9°C in July and August. The work of Zaher *et al*. (1969) on *R. indica* with the host, date palm in Egypt yielded similar results, indicating the consistency of certain mite characteristics across species.

At 30°C (RH 74-79%)

The data on *R. macfarlanei* development in the laboratory on *Syzygium* spp. at 30°C was presented in the Table 2. On *S. cumini*, females required 114.00, 69.39, 88.07, and 89.00 hours for egg, larval, protonymphal, and duetonymphal stages, respectively, while males took 114.00, 52.50, 60.00, and 69.00 hours for the corresponding stages. Females on *S. cumini* had a significantly longer development time (21.70 days) compared to males (18.93 days). On *S. jambos*, females needed 168.00, 76.66, 91.55, and 95.51 hours for the mentioned stages, while males took 168.00, 70.50, 79.50,

Table 1. Development of *Raoiella macfarlanei* on *Syzygium* spp. under laboratory conditions (Temperature: 21-22 °C and Relative Humidity: 83 to 88%)

Developmental Stage	Mean duration of development (hours) on			
	Syzygium cumini		*Syzygium jambos*	
	Female (n = 23)	Male (n = 3)	Female (n = 24)	Male (n = 3)
Egg	218.88±00	218.88±00	244.00±00	244.00±00
Larva	81.26±0.84	78.00±3.00	116.75±2.05	111.00±1.73
Quiescent I	53.86±2.64	51.00±6.00	57.25±4.84	52.00±2.00
Protonymph	78.52±2.35	89.00±2.64	78.75±5.53	84.00±9.16
Quiescent II	48.56±3.28	32.00±5.29	57.36±3.53	62.00±8.54
Deutonymph	91.17±3.47	97.33±1.33	105.66±5.61	80.00±6.55
Quiescent III	73.08±3.28	51.00±3.00	60.87±2.92	45.00±6.24
Total (hours)	645.33±21.87	617.21±23.49	719.65±25.21	678±25.91
T test	Sig.		Sig.	
Development (egg to adult)	26.88 days	25.71 days	29.98 days	28.25 days

n= no. of individuals observed, Sig= significant

and 74.00 hours. *R. macfarlanei* females on *S. jambos* required significantly more time (24.16 days) than males (21.04 days) for development (Table 2).

Development from egg to adult took 21.70 days for females on *S. cumini* compared to 24.16 days on *S. jambos*. For males, the corresponding times were 18.93 days on *S. cumini* and 21.04 days on *S. jambos*, with statistically significant differences (Table 2). At 30°C, *R. macfarlanei* completed development faster than at room temperature on both hosts. On *S. cumini*, total development at 30°C was 21.70 days for females and 18.93 days for males, compared to 26.88 days and 25.71 days at room temperature. On *S. jambos*, at 30°C, development took 24.16 days for females and 21.04 days for males, while at room temperature, it was 29.98 days and 28.25 days, respectively (Table 1 & 2).

No literature is available on the reproductive parameters of *R. macfarlanei* and the results are discussed based on information available on related species. Thus, our study provides a foundation for understanding the reproductive parameters of *R. macfarlanei*, which had not been previously documented and are in accordance with the results of Zaher *et al.* (1969) who reported the preoviposition period (3.30 days) and fecundity (28.10 eggs) per female on *R. indica*. In a laboratory study by Moutia (1958) on an average survival period with a recorded of 27.00 days for mated females of *R. indica* which is almost equal to the duration recorded at 30°C in the present study. These findings offer valuable insights for pest management and ecological studies. However, the present findings contradict the results of Nageshachandra (1980) who studied the preoviposition, oviposition and post oviposition periods of *R. indica* on coconut and recorded 2.07, 40.10 and 6.50 days for the respective durations. The deviation with respect to various durations found in this study may be due to change in the host as well as the mite species.

Life table parameters of *R. macfarlanei*

Reproduction: This mite species exhibits both sexual and asexual reproduction. Unmated females' eggs exclusively yield males, while mated females produce both males and females. Reproduction parameters on two *Syzygium* host species at room temperature and 30°C are detailed in Tables 3.

Mating: Upon reaching sexual maturity after the final moult, both males and females actively sought mates. The male, upon emergence, searched for a female deutonymph, sometimes engaging in courtship during this stage. When encountering a quiescent female

Table 2. Development of *Raoiella macfarlanei* on *Syzygium* spp. under laboratory conditions (Temperature: 30 °C and Relative Humidity: 74 to 79%)

Developmental Stage	Mean duration of development (hours)			
	Syzygium cumini		*Syzygium jambos*	
	Female (n = 28)	Male (n = 2)	Female (n = 27)	Male (n = 2)
Egg	144±00	144±00	168.00±00	168.00±00
Larva	69.39±0.85	52.5±1.50	76.66±1.23	70.5±7.5
Quiescent I	40.87±0.84	34.5±4.5	49.66±1.26	41.00±2.00
Protonymph	88.07±2.11	60.00±3.00	91.55±1.90	79.5±1.5
Quiescent II	45.75±1.68	52.5±1.50	51.66±1.65	37.5±1.5
Deutonymph	89.00±2.12	69.00±00	95.51±1.78	74.00±1.00
Quiescent III	43.92±1.35	42.00±3.00	47.00±1.58	34.50±1.50
Total (hours)	520.8±13.93	454.5±13.84	580.04±16.9	505±17.48
T test	Sig.		Sig.	
Development (egg to adult)	21.70 days	18.93 days	24.16 days	21.04 days

n = no. of individuals observed, Sig = significant

Table 3. Reproduction parameters of *Raoiella macfarlanei* on *Syzygium* spp. at two different temperatures under laboratory conditions

Temperature and Relative humidity	*Syzygium spp.*	Reproduction parameters									
		Pre-oviposition period (days)	Oviposition period (days)	Post-oviposition period (days)	Longevity of mated female (days)	Longevity of male (days)	Unmated female *(n = 30)*	Mated female *(n =30)*			
							Mean no. of eggs/ female	Mean no. of eggs/ female	Mean no. of female offsprings/ female	Mean no. of male offsprings/ female	Sex ratio of the progeny (♂:♀)
21-22°C and 83 -88%	*S. cumini*	4.76	35.00	5.04	44.80	21.62	16.23	23.92	21.76	2.16	1:10.07
	S. jambos	5.53	35.38	5.07	45.98	23.87	17.36	24.23	22.03	2.19	1:10.05
	T test	*	*	NS	NS	*	*	NS	NS	NS	NS
30°C and 74 -79%	*S. cumini*	3.92	27.05	3.84	34.81	15.36	14.16	19.52	17.72	1.80	1:9.84
	S. jambos	4.16	28.00	3.92	36.08	16.82	16.32	21.56	19.64	1.92	1:10.22
	T test	*	*	NS	NS	NS	*	NS	NS	NS	NS

*N.B.: Unmated females produced only male offsprings; n= number observed, * significant @ 5%*

deutonymph, the male settled close and waited for its transition to adulthood. Courtship involved the male holding the female's last pair of legs with its first pair, and they moved together for several hours to two days. As the female approached the moult, the male became more active, positioning itself beneath the female and attempting to mate by holding the hysterosoma with its two anterior pairs of legs and bending its opisthosoma in a 'C' shape.

Preoviposition: During preoviposition, females began feeding immediately after mating and before initiating egg laying. The time lapse between adult emergence and the first egg deposition varied, ranging from 4.76 to 5.53 days at 21-22°C on *S. cumini* and *S. jambos*. At 30°C on both hosts, this period was shorter, ranging from 3.92 to 4.16 days. These differences were statistically significant.

Oviposition: During oviposition, females selected a suitable spot along the leaflet midrib for egg deposition. At room temperature, oviposition took 35.00 days on *S. cumini* and 35.38 days on *S. jambos*. At 30°C, the oviposition period was shorter, lasting 27.05 days on *S. cumini* and 28.00 days on *S. jambos*. These variations were statistically significant.

Fecundity: Fecundity in *R. macfarlanei* varied based on host plant and rearing temperature. Mated females exhibited higher egg-laying at room temperature (23.92 and 24.23 eggs/female on *S. cumini* and *S. jambos*, respectively) compared to 30°C (19.52 and 21.56 eggs/female on *S. cumini* and *S. jambos*, respectively). Unmated females laid fewer eggs at 30°C (14.16 and 16.32 eggs/female on *S. cumini* and *S. jambos*, respectively) than at 21-22°C (16.23 and 17.36 eggs/female on *S. cumini* and *S. jambos*, respectively).

Post-oviposition period: After the adult females stopped laying the eggs, adults lived for 5.04 and 5.07 days at room temperature on *S. cumini* and *S. jambos,* respectively and this period was higher compared to 3.84 and 3.92 days recorded at 30°C on the respective hosts.

Longevity of mated female: The total life span of adult female after emergence from the deutonymph was longer, 44.80 and 45.98 days at 21-22°C on *S. cumini* and *S. jambos,* respectively, compared to 34.81 and 36.08 days recorded at 30°C on the respective hosts. However the differences were statistically non significant (Table 3).

Longevity of male: The total active period of adult male after emergence from deutonymph was 21.62 and 23.87 days on *S. cumini* and *S. jambos,* respectively at room temperature as against 15.36 and 16.82 days recorded on the corresponding hosts at 30°C.

Demography of *R. macfarlanei* on *Syzygium* spp.

Demographic parameters, including mean generation time (T), gross reproductive rate (GRR), net reproductive rate (R0), doubling time (DT), finite rate of increase (λ), and intrinsic rate of natural increase (rm), were derived from the age-specific life table of *R. macfarlanei* on two *Syzygium* species at room temperature (21-22°C) and 30°C (Table 4). Demographic parameters of *R. macfarlanei* on *Syzygium* hosts at room temperature (21-22°C) & 83-89% RH were obtained from 30 mated females. On *S. jambos* and *S. cumini, R. macfarlanei*

Table 4. Demography of *Raoiella macfarlanei* on *Syzygium* spp. at two different temperatures under laboratory conditions

Temperature and Relative humidity	*Syzygium* spp.	Demographic parameters (n=30)					
		Mean Generation Time (T) (days)	Doubling time (Days) (DT)	Net Reproduction Rate (R_o)	Gross reproductive rate (GRR)	Finite Rate of Increase (λ)	Intrinsic Rate of Natural Increase (r_m)
21-22°C and 83 - 88%	*S. cumini*	48.80	11.17	21.03	21.82	1.063	0.062
	S. jambos	53.39	11.95	22.11	22.51	1.059	0.057
30°C And 74 - 79%	*S. cumini*	38.17	9.10	18.29	18.44	1.079	0.076
	S. jambos	41.51	9.67	19.55	19.68	1.071	0.071

n= number observed

showed a mean generation time (T) of 53.39 days and 48.8 days, Ro of 22.11 and 21.03, and similar λ and rm values (1.063, 0.062; 1.059, 0.057) for both hosts. However, doubling time was slightly higher on *S. jambos* (11.95 days) than on *S. cumini* (11.17 days).

At 30°C, demographic parameters on *Syzygium* hosts revealed T of 41.51 days and 38.17 days, Ro of 19.55 and 18.29 for *S. jambos* and *S. cumini*, respectively. Similar λ and rm values (Table 4) were observed on both hosts, but doubling time was relatively higher on *S. jambos* (9.67 days) compared to *S. cumini* (9.10 days). No attempt has been made on the age specific life table parameter of *R. macfarlanei*. To the best of our knowledge, this is the first study to provide insights into age-specific life table parameters for *R. macfarlanei*. However, Teodoro and Reis (2006) studied the reproductive success of *B. phoenicis* on citrus fruits and coffee leaves and reported that the intrinsic rate of the population increase (r_m) was 0.128 and 0.090 females/female/day on citrus fruits and coffee leaves, respectively. Comparisons with related species, such as *B. phoenicis*, underscore the variability in population growth rates within mite species and across different host plants.

Our findings indicate that *R. macfarlanei* is adaptable, demonstrating the capacity to feed, survive, and develop on two species of *Syzygium* at both room temperature (21-22°C) and 30°C. The development period from egg to adult was influenced by the host species and the rearing temperature. The choice of host plant appears to impact the mite's reproductive performance and developmental timeline, with *S. jambos* yielding a higher net reproductive rate (R_o) and a longer mean generation time (T).

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Diversity of entomopathogenic fungi across Agro-climatic zones of Karnataka, India

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Abstract: Entomopathogenic fungi are the most versatile and potential biocontrol agents, due to their adaptability, mode of entry, persistent nature and wide host range. The present study aimed to isolate and identify the native entomopathogenic fungi for the biocontrol of major sucking insect pests of floriculture. Eighty-one fungal isolates were isolated from 55 soil samples and 26 infected insect cadavers were collected from the eastern and southern dry zones of Karnataka, India. Serial dilution and plating techniques, and insect bait techniques were followed. The insecticidal activity of fungal isolates against aphids, mites, 2nd instar larvae of thrips and whitefly nymphs was determined by leaf disc bioassay under *in vitro* conditions. The results of the experiment revealed that among 81 fungal isolates, sixteen (19.25 %) exhibited insecticidal activity against test insects and further, a distribution study revealed that 50 per cent of EPF isolates were belonging to genera *Beauveria* and *Metarhizium* (25% each), 18.75% *Aspergillus,* 12.50% were *Lecanicillium* spp. and 13% were *Paecilomyces* spp. (6.25%) and *Hirsutella* spp. (6.25 %) respectively. It was concluded that the recovery rate of EPF is lower with 19.5% and the dominant genera of EPFs are *Beauveria* and *Metarhizium*. This show EPFs with broader host rage will have more chances of survival and the same criteria can be used for the selection of efficient EPFs.

Key words – *Beauveria,* biocontrol, bioassay, insect bait method, sucking pests.

INTRODUCTION

In modern agriculture, there is a decline in global crop losses due to various insect pests from 41.1 % during 1988-90 to 32.1 % during 2001-03 (Dhaliwal *et al*., 2015) due to the intensive use of chemical pesticides (approximately, 2.5 million tons of pesticides are applying annually, Sharma et al. 2019) despite the alarming problems like the development of resistance and resurgence of sucking pests (Vandoorn and Vos, 2013), residual toxic effects on humans, insect parasites, predators, animals and also the use will increase the cost of production. Because of these problems, it is largely felt that, it is necessary to find an alternative, sustainable and eco-friendly pest management technique i.e. biological control or biocontrol.

Entomopathogenic fungi (EPF) are potentially the most versatile biological control agents, due to their wide host range that often results in natural epizootics. These fungi have certain advantages in pest control programs over other insect pathogens because they infect all stages of insect pests, they directly infect insect pests through cuticle as other agents need ingestion hence these can even infect sucking and piercing pests also (Hajek and Leger, 1994; Mantzoukas *et al*., 2022) mass production techniques are simpler, easier and cheaper compared to the other microbial agents and their persistence nature. Over 1000 species of fungi belonging to 100 genera are known to be pathogenic to insects and many of these have great significance in pest management (Rabindra & Ramanujam, 2007). The most important and extensively studied fungal pathogens are *Lecanicillium lecanii, Beauveria* spp., *Metarhizium* spp., *Nomuraea rileyi*, *Paecilomyces* spp. *Aschersonia* spp. (Ascomycota: Hypocreales) and *Hirsutella* spp. (Wraight *et al*., 2007; Lacey et al. 2008; Kachhawa, 2017; Shaurub, 2023). *Lecanicillium* and *Beauveria* are used to combat different sucking pests under both greenhouses as well as field conditions but the success of biological control depends on environmental conditions like high relative humidity, moderate temperatures and soil organic matter (Namasivayam *et al*., 2015; Abdul Qayyum *et al*., 2021). Several researchers studied and evaluated the different entomopathogenic fungi for the biocontrol of various insect pests in agriculture, horticulture and forestry (Lacey et al. 2008) providing the most satisfying results and pieces of evidence. Despite considerable research on this topic in India, little information exists on the biocontrol of sucking pests of flower crops and screening of local fungal isolates for virulence characteristics is of predominant importance for the success of biocontrol strategies towards major insect pests (Faria and Wraight,

2001). Hence, the objective of the present study was to isolate and identify virulent native EPF strains from the soil as well as insect cadaver samples collected from different regions of south Karnataka, India.

MATERIALS AND METHODS

Sample collection

A systemic survey was conducted to collect soil and mummified insect samples from different flower growing locations of two agro-climatic zones (Eastern dry zone and Southern dry zone) of Karnataka, India. Soil samples were stored at 4±1°C whereas the insect cadavers samples were used within 24hr after collection for the isolation of entomopathogenic fungi.

Isolation of entomopathogenic fungi from insect cadavers

The insect cadavers were surface sterilized with 1 per cent sodium hypochlorite for one minute and then rinsed 3 times in sterilized water. The excess moisture from the surface-sterilized cadavers was removed by using tissue paper and transferred to a culture plate containing potato dextrose agar medium (PDA). The plates were incubated at 25±1 °C under dark conditions for the growth and development of fungi. After 5 days of incubation, the different fungal colonies were selected and purified by subculturing on PDA. Slants of each culture was prepared from purified fungal isolate and stored at 4±1°C for further studies (Reji Rani *et al*., 2015).

Isolation of entomopathogenic fungi from soil samples.

Serial dilution and plating method: Ten grams of soil from each soil sample was taken in a conical flask containing 100 ml sterile water saline and serially diluted up to 10^{-4}. Spread plating was done by transferring 0.1ml of aliquate from 10^{-2}, 10^{-3} and 10^{-4} dilutions on PDA and on selective media for *Metarhizium* spp. and *Beauveria* spp. given by Vestergaard and Eilenberg, 2000, and Meyling and Eilenberg, 2006 respectively. The Petri plates were incubated at 25±1 °C for the growth and development of fungi for 10 days. The colonies showing different characters on plates were picked up and transferred to PDA slants for further study.

Insect bait method: Insect-associating fungi were isolated from soil samples by using 'the insect bait method given by Zimmermann, 1986; Lui et al. 2021, with little modifications. Three 2nd instar *Spodoptera* larvae were released into a plastic box containing 100gm moistened soil sample and the boxes were incubated at 25±1°C for two weeks under the dark condition to promote the infection of pathogenic fungi. The larvae were examined at 7 and 14 days after incubation and dead larvae were surface sterilized with 1 per cent sodium hypochlorite for one min and then rinsed trice with sterile distilled water. After removing the free water from the larval surface, they were transferred onto PDA plates and incubated at 25±1°C for the growth and development of fungi for 10 days.

Insecticidal activity of fungal isolates

To screen the entomopathogens from non-entomopathogenic fungal isolates, preliminary screening was conducted using the leaf dip bioassay method as described by Sain et al. (2019) with few modifications. The experiment was carried out by cutting healthy gerbera leaves into 8 cm diameter leaf discs and surface sterilizing them with 70 per cent alcohol. The surface sterilized leaf discs were immersed in fungal spore suspension for 10s and the control was maintained by dipping the leaf discs in sterile distilled water. Further, all the leaf discs were air-dried and transferred onto sterile Petri plates containing filter paper to maintain humidity during incubation. Approximately Twenty laboratory-reared aphids, mites, second instar larvae of thrips and nymphs of whitefly were transferred onto the treated and control leaf discs by using sterile camel brush. The plates were kept for incubation at 25±1 ºC for 5 days. After incubation leaf discs were observed for mycosis of test insects. The isolates successfully caused mycosis were confirmed as entomopathogenic fungi and selected for future study.

Generic identification of entomopathogenic fungal isolates

Initial characterization of entomopathogenic fungal isolates was done by using the Atlas of entomopathogenic fungi (Robert et al. 1988). Macroscopic colony characteristics like colour, growth pattern, shape and elevation of the colony were observed on PDA plates. Microscopic observation of isolates was done by fungal slide culture technique (Rosana et al. 2014) with lactophenol blue staining. The slides were observed for the arrangement of conidia, phialide and type of mycelium under a Labomed iVu3000 binocular light microscope at 400X magnification.

RESULTS AND DISCUSSION

Different techniques were employed to isolate entomopathogenic fungi from both soil as well as from insect cadaver samples *viz*., serial dilution and plating technique on selective and non-selective media, insect bait technique and directly placing insect cadavers

on potato dextrose agar plates. Previously, Jaber *et al*. (2016) isolated 42 entomogenous fungal isolates from 17 different arthropod cadavers collected from pesticide-free areas. Similarly, Gurlek *et al.* (2018) from walnut fields in Turkey, Mar et al. 2012 isolated from Chiang Mai, Thailand isolated EPF. In the present study, total of eighty-one fungal isolates were isolated from 26 insect cadavers and 55 soil samples and coded serially as ENPF. Most of the isolates were isolated from soil samples and few isolates were isolated from insect cadavers. The isolates were identified as *Aspergillus*, *Penicillium, Metarhizium, Beauveria* spp. *Trichoderma*, *Fusarium*, *Paecilomyces* and *Hirsutella* spp. based on macro and microscopic observation.

The results of bioassay studies exhibited that, all the fungal isolates from the soil as well as from insect cadavers may not be entomopathogenic because soil harbors different microbial communities including fungi, among them some will be opportunistic pathogens, some will be saprophytes and merely some are true entomogenous fungi. Among eighty-one fungal isolates, sixteen isolates like ENPF-3, ENPF-6, ENPF-8, ENPF-9, ENPF-16, ENPF-24, ENPF-32, ENPF-33, ENPF-41, ENPF-48, ENPF-53, ENPF-58, ENPF-60, ENPF-67, ENPF-68 and ENPF-79 were successful in exhibiting insecticidal activity against test insect pests (**Table 1**). The majority of the isolates shown insecticidal activity was isolated from either insect cadavers or from insect bait method.

Table 1: Insecticidal activity of entomopathogenic fungal isolates against sucking pest

Isolate code	Insecticidal activity against test insects			
	Aphids	Thrips	Mites	whitefly
ENPF-3	+	+	-	+
ENPF-6	+	-	-	-
ENPF-8	-	-	+	-
ENPF-9	+	-	-	-
ENPF-16	+	+	-	+
ENPF-24	+	+	-	+
ENPF-26	+	-	-	-
ENPF-33	+	-	-	-
ENPF-41	+	+	-	+
ENPF-48	+	+	-	-
ENPF-53	+	-	-	-
ENPF-58	-	-	+	-
ENPF-60	+	+	-	+
ENPF-67	+	-	-	-
ENPF-68	+	+	-	-
ENPF-79	+	-	-	-

Note: +: Positive -: Negative

Initial characterization of entomopathogenic fungal isolates was carried out by referring to the Atlas of entomopathogenic fungi (Robert et al.1988). From the observations, the isolates were identified at the generic level as *Metarhizium* spp. (ENPF-6, ENPF-9, ENPF-60 and ENPF-68)*, Beauveria* spp. (ENPF-3, ENPF-16, ENPF-48 and ENPF-67)*, Aspergillus* spp. (ENPF-26, ENPF-33 and ENPF-53), *Lecanicillium* spp. (ENPF-24 and ENPF-41)*, Isaria* (formally known as *Paecilomyces* spp.) (ENPF-8) and *Hirsutella* sp. (ENPF-58).

Morphologically, all the *Metarhizium* isolates exhibited typical characteristics of *Metarhizium* like fast growth, green to light green colony. Whereas each isolate was slightly different concerning the size of the conidia which were between 4.1-5.7 µm. All the *Beauveria* isolates were white or creamy white colonies with dense or dispersed growth patterns and conidia were found in dense with round or spherical in shape and the size varied between 1.5 to 3.5 µm. *Aspergillus* isolates exhibited different shades of colony colour and

Table 2: Generic identification of entomopathogenic fungal isolates based on Macroscopic and Microscopic characteristics

Isolate Code	Macroscopic characteristics				Microscopic characteristics				Other key characters	Probable genera
	Growth pattern	Colour	Shape	Elevation	hyphae	Colour of conidia	Length of conidia (µm)	Shape of conidia		
ENPF-3	Disperse and slow growing	White	Round	Raised	Hyaline and septate	Hyaline to creamy spores	1.6-3.2	Globose or ovoid	Conidia found in dense clusters or whorls with characteristic denticulate rachis	*Beauveria*
ENPF-6	Uniform and fast-growing	Dark green	Circular	Flattened	Septate	Green	4.5-5.7	Long, cylindrical with round edges	Conidia arranged in parallel chains	*Metarhizium*
ENPF-8	Uniform and fast-growing	Pinkish white	Round	Flattened	Hyaline and septate	Pinkish white	3.5-4.0	Ovoid or round	Conidiophore smooth and colorless; long slender divergent phialides; conidial chains are often long	*Isaria*
ENPF-9	Uniform and fast-growing	Light or yellowish green	Circular	Flattened	Septate	Green or dull green	4.1-5.4	Long, cylindrical with round edges	Conidia arranged in parallel chains	*Metarhizium*
ENPF-16	Dense and slow growing	White	Round	Raised	Hyaline and septate	White to creamy spores	1.5-3.5	Globose or ovoid	Conidia found in dense clusters or whorls with characteristic denticulate rachis	*Beauveria*
ENPF-24	Dense and fast growing	White	Circular	Flattened with raised mycelium	Hyaline and septet	Hyaline to creamy spores	1.6-3.0	Spherical to ovoid	++	*Lecanicillium*
ENPF-26	Uniform and fast growing	Dark green	Circular	Flattened with raised mycelium	Septate	Light green	1.8-3.5	Spherical to ovoid with think wall	Short conidiophores with long conidial chains	*Aspergillus*
ENPF-33	Uniform and fast-growing	Dark green	Circular	Flattened with raised mycelium	Septate	Light green	1.6-3.2	Spherical to ovoid with think wall	Short conidiophores with long conidial chains	*Aspergillus*
ENPF-41	Dense and fast-growing	White	Circular	Flattened with raised mycelium	Hyaline and septet	Hyaline to creamy spores	1.5-3.2	Spherical to ovoid	++	*Lecanicillium*

Isolate Code	Macroscopic characteristics				Microscopic characteristics				Other key characters	Probable genera
	Growth pattern	Colour	Shape	Elevation	hyphae	Colour of conidia	Length of conidia (µm)	Shape of conidia		
ENPF-48	Dense and slow growing	White	Round	Raised	Hyaline and septate	White to creamy spores	1.5-3.5	Globose or ovoid	Conidia found in dense clusters or whorls with characteristic denticulate rachis	*Beauveria*
ENPF-53	Uniform and fast-growing	Dark green	Circular	Flattened with raised mycelium	Septate	Light green	1.7-3.3	Spherical to ovoid with think wall	Short conidiophores with long conidial chains	*Aspergillus*
ENPF-58	Uniform and slow growing	Brown	Round	Flattened	Septate	Brown	2.5-5.0	Boat-shaped (naviculoid) to cylindrical	Conidia borne in chains	*Hirsutella*
ENPF-60	Dense and slow growing	White	Round	Raised	Hyaline and septate	White to creamy spores	1.5-3.5	Globose or ovoid	Conidia found in dense clusters or whorls with characteristic denticulate rachis	*Beauveria*
ENPF-67	Uniform and fast-growing	Light or yellowish green	Circular	Flattened	Septate	Green or dull green	4.3-5.5	Long, cylindrical with round edges	Conidia arranged in parallel chains	*Metarhizium*
ENPF-68	Uniform and fast-growing	Dark green	Circular	Flattened	Septate	Green	4.5-5.7	Long, cylindrical with round edges	Conidia arranged in parallel chains	*Metarhizium*
ENPF-79	Uniform and fast-growing	Dark green	Circular	Flattened with raised mycelium	Septate	Light green	1.7-3.3	Spherical to ovoid with think wall	Short conidiophores with long conidial chains	*Aspergillus*

Note: ENPF – Isolate code

varied conidia sizes (Fig. 2). *Paecilomyces* produced pinkish-coloured, dense colonies with ovoid or round conidia. Colony characteristics of both the isolates of *Lecanicillium* have not varied much, the dense, white, fast-growing colony and spherical to ovoid conidia. *Hirsutella* produced a typical brown-coloured, thick colony with white strips and conidia that were boat-shaped or naviculoid. A detailed description of macroscopic and microscopic characteristics of EPF is given in table-2. In the present study, it was observed that the recovery rate of entomogenous fungi was 19.5 per cent, in India it varies between 15-38 per cent from the various surveys conducted by research workers all over the country and it is true with other countries.

Kassam *et al*. (2022), studied the morphology of *Metarhizium* sp. which were isolated from Migratory locust, *Locusta migratoria* L. and reported that the length of the conidia of the different *M. anisopliae* isolates varied from 6.1 to 7.4 µm and the width ranged from 2.2 um to 3.1µm. Similarly, Varela and Mornles, (1996), carried out the characterization of some *B. bassiana* isolates and reported that the colony colour of different isolates showed wide variations between white and yellow. In conidial morphology, they observed three sizes and two types of conidial shapes <2.5 µm (globose), 2.6-3.75 µm (globose) and 4.0-5.0×2.5-3.0 µm (Ellipsoidal).

Distributions of entomopathogenic fungi were studied based on the number of isolates exhibited insecticidal activity against sucking pests used in the study. Among 81 fungal isolates, sixteen isolates (19.25 %) were shown positive for pathogenicity against test insects. Among *Beauveria.* spp. (25 %), *Metarhizium* spp. (25%), *Aspergillus* spp. (18.75%), *Lecanicillium* spp. (12.50 %), *Paecilomyces* spp. (6.25%) and *Hirsutella* spp. (6.25 %) were identified (Fig. 1). The results of distribution explain that the fungi which were having a broader host range and adaptability will survive the most under natural conditions. Previously, Franco *et al.* (2011), were examined 142 soil samples from different states of Mexico for the isolation of entomopathogenic fungi using the insect bait method (*Galleria mellonella* L.). Around 23 per cent of samples were shown positive for the presence of entomopathogenic fungi in that 12 % (17 isolates) were *B. bassiana,* 1 % (2 isolates) were *M. anisopliae* and 10 % (14 isolates) were *Isaria fumosorosea.* This was further confirmed by González-Baca *et al*. (2019); Chen *et al*. (2021); Qayyum *et al*. (2021).

In the present study, it was observed that the recovery rate of entomogenous fungi was 19.5 per cent, in India it varies between 15-38 per cent from the various surveys conducted by research workers all over the country (Reji Rani *et al*., 2015; Maryam *et al*., 2014) and it is even true with other parts of the globe (Franco *et al*., 2011 from Mexico, Jaber *et al*., 2016 from Mexico, Niu et al. 2019 from Brazil, Gurlek *et al*. 2018 from Turkey, Maryam *et al*., 2014 from Iran). The low recovery rate from the various surveys conducted worldwide due to low soil organic matter content, high pesticide usage and influence of environmental factors *viz*., high temperature and low humidity were affecting the existence and survival of entomopathogenic fungi in soil (Márquez-Gutiérrez *et al*., 2022; Namasivayam *et al*., 2015; Abdul Qayyum *et al*., 2021). Since these fungi are heterotopic, they use soil as a habitat and organic matter as a source of nutrients for long-term persistence when crops are not present in the field. Meyling et al. in 2011, in a study on the diversity and distribution of entomopathogenic fungi revealed that the population of entomopathogenic fungi was significantly higher in the field under organic cropping systems than in the fields of the conventional cropping system. This was further confirmed by Uzman et al. 2019; Afandhi *et al*. (2022). There is a considerable effect of farming systems, field margins, land-use type and bait-insect on the occurrence of insect pathogenic fungi in soils (Klingen *et al*., 2002; Fernández-Bravo *et al*., 2021).

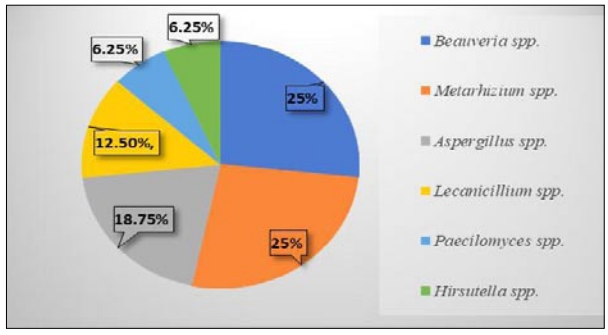

Fig 1: Diversity of entomopathogenic fungal isolates in soils of two agroclimatic zones of Karnataka, India.

CONCLUSION

In conclusion, the isolation and screening of efficient biocontrol agents plays an important role in the sustainable management of pests in agriculture. The native isolates will perform better due to adaptability in comparison to the isolates isolated from other locations having varied environmental, soil conditions and other factors. The presence of entomopathogenic fungal isolates will be largely affected by the use of agrochemicals and the low organic content of the soil as evidenced by the low recovery rate during the study.

Conflict of Interest

All the authors of the manuscript declare that they do not have any conflict of interest.

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Management of American serpentine leaf miner, *Liriomyza trifolii* (Burgess) in tomato under protected cultivation

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Abstract: The serpentine leaf miner, *Liriomyza trifolii*, is one of the most destructive invasive pests introduced to India from the American subcontinent during the nineteenth century. For ecofriendly management of *L.trifolii*the experiment was conducted at horticulture farm, College of Horticulture, S. D. Agricultural University, Jagudan (Mehsana), Gujarat in the *rabi* season of 2020-21 and 2021-22. Nine botanical insecticides were tested and among that neem seed kernel extract at 5% was significantly superior and recorded the lowest damage (9.80%) which was statistically at par with tobacco decoction at 2% (10.52%) and azadirachtin at 1500 ppm (11.13%). Similarly, based on the number of mines per three compound leaves, neem seed kernel extract at 5% demonstrated superior efficacy by recording the lowest number of mines (10.59 mines/3 compound leaves). The application of neem seed kernel extract at 5% resulted in the highest tomato fruit yield, harvesting 449.16 q/ha, a result on par with azadirachtin at 1500 ppm (445.83 q/ha) and tobacco decoction at 2% (436.00 q/ha). The incorporation of biopesticides into the management strategy presents a promising avenue for sustainable and environmentally friendly tomato production in protected cultivation systems.

Keywords: *Liriomyza trifoli,* tomato, NSKE, azadirachtin, tobacco decoction

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a major and widely cultivated vegetable crop in India, making substantial contributions to the agricultural sector. Cultivated extensively across diverse agro-climatic regions, tomatoes are a staple ingredient in Indian cuisine, fulfilling significant nutritional needs. In India, tomato cultivation spans over an area of 8,43,000 hectares, yielding a production of 2,06,94,000 metric tons with a productivity rate of 24.55 metric tons per hectare during the 2022-23 period (Anonymous, 2023a). Specifically in Gujarat, it is cultivated across 67,874 hectares, producing 19,22,220 metric tons with a productivity rate of 28.32 metric tons per hectare during the same period (Anonymous, 2023b). Tomato cultivation faces challenges from various insect pests, including the fruit borer, *Helicoverpa armigera*, *Bemisia tabaci*, *Aphis gossypii*, the leaf-eating caterpillar *Spodoptera litura*, *Thrips tabaci*, the American serpentine leaf miner, *Liriomyza trifoli*, and the two-spotted spider mite, *Tetranychus urticae* (Misra, 2010; Dodiya *et al.,* 2023). Of particular concern is the invasive pest *Liriomyza trifoli*, which was inadvertently introduced into India from the American subcontinent alongside chrysanthemum cuttings (Parella, 1987). Initially recorded on 55 plant species, its host range expanded to approximately 79 species, including pulses, oilseeds, vegetables, green manures, fodder, and fiber crops (Viraktamath *et al.,* 1993; Srinivasan *et al*., 1995). Larval feeding by *L. trifoli* diminishes plant marketability due to aesthetic damage and reduces photosynthetic capacity, affecting plant vigor, growth, and yield (Khateeb et al., 2006). In efforts to mitigate these challenges, researchers have explored the use of bio-insecticides such as ethanolic extracts from neem and petroleum ether extracts from jatropha seeds targeting the egg and larval stages of *T. absoluta* (Kona *et al.,* 2014). *Liriomyza trifoli* infestations are particularly detrimental as they result in extensive mining in the spongy mesophyll of foliage and petioles (Parrella *et al*., 1985), with peak infestation levels recorded by Satti (1997) at 6.9-8.6 infested leaves per 50 leaves. Recognizing the demand for environmentally friendly and sustainable pest management practices, biopesticides emerge as a targeted and eco-conscious approach to pest control (Dodiya et al., 2023). As the call for pesticide-free and sustainable agricultural methods grows louder, the findings of this research offer significant promise for tomato growers, especially those engaged in protected cultivation. By highlighting the efficacy of biopesticides in managing *trifoli*, this study contributes to ongoing discussions on environmentally conscious pest control strategies, promoting a balanced and resilient agricultural ecosystem.

MATERIALS AND METHODS

To identify effective and cost-efficient botanical insecticides for managing *L. trifolii* infestations in tomatoes under protected cultivation, the study tested the following botanicals: Neem Seed Kernel Extract (NSKE), Jatropha oil, Pongamia oil, Neem oil, Citronella oil, Custard apple leaf extract, Azadirachtin, Garlic bulb extract, and Tobacco decoction. The experiment was

Table 1. Bio-efficacy of botanicals against leaf miner in tomato (2020-21)

Tr. No.	Treatments	Damage leaves (%) at indicated days after spray													Pooled over periods and sprays
		1st spray					2nd spray				3rd spay				
		Before spray	3 DAS	7 DAS	10 DAS	Pooled	3 DAS	7 DAS	10 DAS	Pooled	3 DAS	7 DAS	10 DAS	Pooled	
T_1	NSKE 5%	5.00 (24.50)	3.79^{a} (13.86)	3.37^{a} (10.86)	3.87^{a} (14.48)	3.68^{a} (13.04)	3.28^{a} (10.26)	3.07^{a} (8.92)	3.52^{a} (11.89)	3.29^{a} (10.32)	3.22^{a} (9.87)	2.82^{a} (7.45)	2.51^{a} (5.80)	2.85^{a} (7.62)	3.28^{a} (10.26)
T_2	Jatropha oil 1%	4.91 (23.61)	4.63^{c} (20.94)	4.52^{b} (19.93)	4.84^{cd} (22.93)	4.67^{b} (21.31)	4.45^{b} (19.30)	4.25^{c} (17.56)	4.63^{bc} (20.94)	4.44^{b} (19.21)	4.41^{c} (18.95)	4.06^{b} (15.98)	3.81^{c} (14.02)	4.09^{b} (16.23)	4.41^{c} (18.95)
T_3	Pongamia oil 1%	4.87 (23.22)	4.55^{c} (20.20)	4.44^{b} (19.21)	4.76^{cd} (22.16)	4.58^{b} (20.48)	4.36^{b} (18.51)	4.16^{c} (16.81)	4.55^{bc} (20.20)	4.36^{b} (18.51)	4.31^{c} (18.08)	3.95^{b} (15.10)	3.69^{c} (13.12)	3.98^{b} (15.34)	4.31^{c} (18.08)
T_4	Neem oil 0.5%	4.90 (23.51)	4.59^{c} (20.57)	4.48^{b} (19.57)	4.80^{cd} (22.54)	4.62^{b} (20.84)	4.40^{b} (18.86)	4.21^{c} (17.22)	4.59^{bc} (20.57)	4.40^{b} (18.86)	4.36^{c} (18.51)	4.00^{b} (15.50)	3.75^{c} (13.56)	4.04^{b} (15.82)	4.36^{c} (18.51)
T_5	Citronella oil 1%	4.86 (23.12)	4.31^{abc} (18.08)	4.19^{b} (17.06)	4.53^{bc} (20.02)	4.34^{b} (18.34)	4.10^{b} (16.31)	3.90^{bc} (14.71)	4.30^{ab} (17.99)	4.10^{b} (16.31)	4.06^{bc} (15.98)	3.68^{b} (13.04)	3.40^{bc} (11.06)	3.71^{b} (13.26)	4.06^{b} (15.98)
T_6	Custard apple leaf extract 10%	4.88 (23.31)	4.56^{c} (20.29)	4.45^{b} (19.30)	4.77^{cd} (22.25)	4.59^{b} (20.57)	4.37^{b} (18.60)	4.17^{c} (16.89)	4.56^{bc} (20.29)	4.36^{b} (18.51)	4.33^{c} (18.25)	3.97^{b} (15.26)	3.71^{c} (13.26)	4.00^{b} (15.5)	4.33c (18.25)
T_7	Azadirachtin 1500 ppm	4.50 (19.75)	3.95^{ab} (15.10)	3.54^{a} (12.03)	4.08^{ab} (16.15)	3.86^{a} (14.40)	3.45^{a} (11.40)	3.24^{ab} (10.00)	3.62^{a} (12.60)	3.44^{a} (11.33)	3.43^{ab} (11.26)	3.04^{a} (8.74)	2.81^{ab} (7.40)	3.09^{a} (9.05)	3.47^{a} (11.54)
T_8	Garlic bulb extract 5%	4.77 (22.25)	4.53^{bc} (20.02)	4.41^{b} (18.95)	4.84^{cd} (22.93)	4.59^{b} (20.57)	4.44^{b} (19.21)	4.22^{c} (17.31)	4.63^{bc} (20.94)	4.43^{b} (19.12)	4.41^{c} (18.95)	4.05^{b} (15.90)	3.80^{c} (13.94)	4.08^{b} (16.15)	4.37^{c} (18.60)
T_9	Tobacco decoction2%	4.54 (20.11)	3.81^{a} (14.02)	3.38^{a} (10.92)	3.94^{ab} (15.02)	3.71^{a} (13.26)	3.33^{a} (10.59)	3.17^{ab} (9.55)	3.59^{a} (12.39)	3.36^{a} (10.79)	3.41^{ab} (11.13)	2.99^{a} (8.44)	2.80^{ab} (7.34)	3.07^{a} (8.92)	3.39^{a} (10.99)
T_{10}	Untreated control	4.89 (23.41)	5.26^{d} (27.17)	5.36^{c} (28.23)	5.38^{d} (28.44)	5.33^{c} (27.91)	5.40^{c} (28.66)	5.40^{d} (28.66)	5.39^{c} (28.55)	5.40^{c} (28.66)	5.40d (28.66)	5.42^{c} (28.88)	5.41^{d} (28.77)	5.41^{c} (28.77)	5.39^{d} (28.55)
S. Em. ± T		0.24	0.18	0.20	0.19	0.11	0.20	0.23	0.25	0.13	0.21	0.20	0.19	0.12	0.068
P		-	-	-	-	0.06	-	-	-	0.07	-	-	-	0.06	0.037
S		-	-	-	-	-	-	-	-	-	-	-	-	-	0.037
T x P		-	-	-	-	0.19	-	-	-	0.23	-	-	-	0.20	0.119
T x S		-	-	-	-	-	-	-	-	-	-	-	-	-	0.119
P x S		-	-	-	-	-	-	-	-	-	-	-	-	-	0.065
T x P x S		-	-	-	-	-	-	-	-	-	-	-	-	-	0.206
C.D. at 5% T		NS	0.54	0.59	0.56	0.26	0.59	0.67	0.72	0.31	0.63	0.59	0.56	0.28	0.160
C.V. (%)		8.48	7.17	8.19	7.21	7.51	8.40	9.90	9.75	9.38	8.89	9.10	9.27	9.09	8.65

Note: 1. Figures outside the parentheses are $\sqrt{X + 0.5}$ transformed values and those inside the parentheses are retransformed values
2. Treatment means followed by the same letter are not significantly different by Duncan's New Multiple Range Test (DNMRT) at 5% level of significance

Table 2. Bio-efficacy of botanicals against leaf miner in tomato (2021-22)

Tr. No.	Treatments	Damage leaves (%) at indicated days after spray													Pooled over periods and sprays
		1st spray					2nd spray				3rd spay				
		Before spray	3 DAS	7 DAS	10 DAS	Pooled	3 DAS	7 DAS	10 DAS	Pooled	3 DAS	7 DAS	10 DAS	Pooled	
T_1	NSKE 5%	4.9 (23.51)	3.60^{a} (12.46)	3.34^{a} (10.66)	3.65^{a} (12.82)	3.53^{a} (11.96)	3.12^{a} (9.23)	2.91^{a} (7.97)	3.32^{a} (10.52)	3.12^{a} (9.23)	3.18^{a} (9.61)	2.71^{a} (6.84)	2.46^{a} (5.55)	2.78^{a} (7.23)	3.14^{a} (9.36)
T_2	Jatropha oil 1%	4.82 (22.73)	4.53^{cd} (20.02)	4.49^{b} (19.66)	4.71^{cd} (21.68)	4.58^{b} (20.48)	4.34^{c} (18.34)	4.13^{b} (16.56)	4.49^{c} (19.66)	4.32^{b} (18.16)	4.30^{c} (17.99)	4.02^{b} (15.66)	3.77^{b} (13.71)	4.03^{c} (15.74)	4.31^{d} (18.08)
T_3	Pongamia oil 1%	4.77 (22.25)	4.44^{c} (19.21)	4.40^{b} (18.86)	4.62^{cd} (20.84)	4.49^{b} (19.66)	4.25^{c} (17.56)	4.04^{b} (15.82)	4.40^{c} (18.86)	4.23^{b} (17.39)	4.19^{c} (17.06)	3.91^{b} (14.79)	3.65^{b} (12.82)	3.91^{bc} (14.79)	4.21^{d} (17.22)
T_4	Neem oil 0.5%	4.84 (22.93)	4.48^{cd} (19.57)	4.45^{b} (19.3)	4.66^{cd} (21.22)	4.53^{b} (20.02)	4.29^{c} (17.9)	4.09^{b} (16.23)	4.44^{c} (19.21)	4.28^{b} (17.82)	4.25^{c} (17.56)	3.96^{b} (15.18)	3.71^{b} (13.26)	3.97^{bc} (15.26)	4.26^{d} (17.65)
T_5	Citronella oil 1%	4.76 (22.16)	4.19^{abc} (17.06)	4.15^{b} (16.72)	4.38^{bc} (18.68)	4.24^{b} (17.48)	$3.99b^{c}$ (15.42)	3.77^{b} (13.71)	$4.15b^{c}$ (16.72)	3.97^{b} (15.26)	$3.94b^{c}$ (15.02)	3.63^{b} (12.68)	3.36^{b} (10.79)	3.64^{b} (12.75)	3.95^{c} (15.10)
T_6	Custard apple leaf extract 10%	4.78 (22.35)	4.46^{c} (19.39)	4.42^{b} (19.04)	4.63^{cd} (20.94)	4.50^{b} (19.75)	4.26^{c} (17.65)	4.06^{b} (15.98)	4.41^{c} (18.95)	4.24^{b} (17.48)	4.22^{c} (17.31)	3.93^{b} (14.94)	3.67^{b} (12.97)	3.94^{bc} (15.02)	4.23^{d} (17.39)
T_7	Azadirachtin 1500 ppm	4.45 (19.30)	3.78^{ab} (13.79)	3.51^{a} (11.82)	3.87^{ab} (14.48)	3.72^{a} (13.34)	$3.39a^{b}$ (10.99)	3.08^{a} (8.99)	3.46^{ab} (11.47)	3.31^{a} (10.46)	3.29^{ab} (10.32)	2.95^{a} (8.20)	2.77^{a} (7.17)	3.00^{a} (8.50)	3.35^{b} (10.72)
T_8	Garlic bulb extract 5%	4.72 (21.78)	4.42^{bc} (19.04)	4.38^{b} (18.68)	4.70^{cd} (21.59)	4.50^{b} (19.75)	4.34^{c} (18.34)	4.11^{b} (16.39)	4.48^{c} (19.57)	4.31^{b} (18.08)	4.30^{c} (17.99)	4.02^{b} (15.66)	3.76^{b} (13.64)	4.02^{c} (15.66)	4.28^{d} (17.82)
T_9	Tobacco decoction2%	4.47 (19.48)	3.68^{a} (13.04)	3.38^{a} (10.92)	3.75^{ab} (13.56)	3.60^{a} (12.46)	3.18^{a} (9.61)	3.04^{a} (8.74)	3.41^{a} (11.13)	3.21^{a} (9.80)	3.26^{ab} (10.13)	2.87^{a} (7.74)	2.75^{a} (7.06)	2.97^{a} (8.32)	3.26^{ab} (10.13)
T_{10}	Untreated control	4.83 (22.83)	5.16^{d} (26.13)	5.20^{e} (26.54)	5.26d (27.17)	5.21^{e} (26.64)	5.29^{d} (27.48)	5.31^{e} (27.70)	5.28^{d} (27.38)	5.29^{e} (27.48)	5.31^{d} (27.70)	5.33^{e} (27.91)	5.38^{e} (28.44)	5.34^{d} (28.02)	5.28^{e} (27.38)
S. Em. ±	T	0.23	0.20	0.18	0.20	0.11	0.22	0.22	0.23	0.13	0.22	0.18	0.17	0.11	0.067
	P	-	-	-	-	0.06	-	-	-	0.07	-	-	-	0.06	0.037
	S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.037
	T x P	-	-	-	-	0.19	-	-	-	0.23	-	-	-	0.19	0.117
	T x S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.117
	P x S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.064
	T x P x S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.203
C.D. at 5%	T	NS	0.59	0.52	0.59	0.26	0.66	0.65	0.68	0.31	0.64	0.53	0.50	0.26	0.188
C.V. (%)		8.29	8.04	7.35	7.84	7.76	9.60	9.85	9.59	9.68	9.30	8.39	8.35	8.74	8.73

Note: 1. Figures outside the parentheses are $\sqrt{X + 0.5}$ transformed values and those inside the parentheses are retransformed values
2. Treatment means followed by the same letter are not significantly different by Duncan's New Multiple Range Test (DNMRT) at 5% level of significance

Table 3. Bio-efficacy of botanicals against leaf miner in tomato (2020-21)

Tr. No.	Treatments	No. of mines/ 3 compound leaves at indicated days after spray													Pooled over periods and sprays
		1st spray					2nd spray				3rd spay				
		Before spray	3 DAS	7 DAS	10 DAS	Pooled	3 DAS	7 DAS	10 DAS	Pooled	3 DAS	7 DAS	10 DAS	Pooled	
T_1	NSKE 5%	4.28 (17.82)	3.56^{a} (12.17)	3.37^{a} (10.86)	3.64^{a} (12.75)	3.52^{a} (11.89)	3.54^{a} (12.03)	3.39^{a} (10.99)	3.56^{a} (12.17)	3.50^{a} (11.75)	3.41^{a} (11.13)	3.22^{a} (9.87)	2.89^{a} (7.85)	3.17^{a} (9.55)	3.40^{a} (11.06)
T_2	Jatropha oil 1%	4.13 (16.56)	4.13^{bc} (16.56)	4.05^{bcd} (15.90)	4.25^{bcd} (17.56)	4.14^{b} (16.64)	4.22^{cd} (17.31)	4.13^{c} (16.56)	4.23^{cde} (17.39)	4.19^{b} (17.06)	4.10^{bcdef} (16.31)	3.97^{c} (15.26)	3.71^{b} (13.26)	3.92^{b} (14.87)	4.09^{b} (16.23)
T_3	Pongamia oil 1%	4.39 (18.77)	4.20^{cd} (17.14)	4.13^{de} (16.56)	4.32^{cd} (18.16)	4.22^{b} (17.31)	4.29^{cd} (17.90)	4.21^{cd} (17.22)	4.31^{de} (18.08)	4.27^{b} (17.73)	4.18^{ef} (16.97)	4.11^{c} (16.39)	3.86^{b} (14.40)	4.05^{b} (15.90)	4.18^{b} (16.97)
T_4	Neem oil 0.5%	4.09 (16.23)	4.12^{bc} (16.47)	4.04^{bcd} (15.82)	4.24^{bcd} (17.48)	4.13^{b} (16.56)	4.22^{cd} (17.31)	4.13^{c} (16.56)	4.23^{cde} (17.39)	4.19^{b} (17.06)	4.10^{bcdef} (16.31)	4.04^{c} (15.82)	3.79^{b} (13.86)	3.97^{b} (15.26)	4.10^{b} (16.31)
T_5	Citronella oil 1%	4.25 (17.56)	4.06^{abc} (15.98)	3.98^{abcd} (15.34)	4.18^{abc} (16.97)	4.0^{b} (16.06)	4.15^{bc} (16.72)	4.06^{bc} (15.98)	4.17^{bcd} (16.89)	4.13^{b} (16.56)	4.04^{abcde} (15.82)	3.87^{bc} (14.48)	3.61^{b} (12.53)	3.84^{b} (14.25)	4.01^{b} (15.58)
T_6	Custard apple leaf extract 10%	4.39 (18.77)	4.16^{cd} (16.81)	4.07^{bcde} (16.06)	4.28^{cd} (17.82)	4.17^{b} (16.89)	4.24^{cd} (17.48)	4.15^{c} (16.72)	4.26^{de} (17.65)	4.22^{b} (17.31)	4.13^{def} (16.56)	3.96^{c} (15.18)	3.70^{b} (13.19)	3.93^{b} (14.94)	4.11^{b} (16.39)
T_7	Azadirachtin 1500 ppm	3.97 (15.26)	3.57^{ab} (12.24)	3.43^{abc} (11.26)	3.67^{ab} (12.97)	3.56^{a} (12.17)	3.58^{ab} (12.32)	3.43^{ab} (11.26)	3.62^{abc} (12.60)	3.54^{a} (12.03)	3.47^{abcde} (11.54)	3.28^{ab} (10.26)	2.97^{a} (8.32)	3.24^{a} (10.00)	3.45^{a} (11.40)
T_8	Garlic bulb extract 5%	4.40 (18.86)	4.17^{cd} (16.89)	4.10^{cde} (16.31)	4.29^{cd} (17.90)	4.19^{b} (17.06)	4.23^{cd} (17.39)	4.15^{c} (16.72)	4.25^{cde} (17.56)	4.21^{b} (17.22)	4.12^{def} (16.47)	3.96^{c} (15.18)	3.70^{b} (13.19)	3.92^{b} (14.87)	4.11^{b} (16.39)
T_9	Tobacco decoction2%	4.17 (16.89)	3.57^{ab} (12.24)	3.42^{ab} (11.20)	3.64^{a} (12.75)	3.54^{a} (12.03)	3.56^{ab} (12.17)	3.40^{a} (11.06)	3.60^{ab} (12.46)	3.52^{a} (11.89)	3.45^{abcd} (11.40)	3.26^{a} (10.13)	2.96^{a} (8.26)	3.22^{a} (9.87)	3.43^{a} (11.26)
T_{10}	Untreated control	4.35 (18.42)	4.72^{d} (21.78)	4.74^{e} (21.97)	4.82^{d} (22.73)	4.76^{c} (22.16)	4.83^{d} (22.83)	4.84^{d} (22.93)	4.85^{e} (23.02)	4.84^{c} (22.93)	4.81^{f} (22.64)	4.80^{d} (22.54)	4.82^{c} (22.73)	4.81^{c} (22.64)	4.81^{c} (22.54)
S. Em. ±	T	0.22	0.17	0.20	0.18	0.11	0.19	0.20	0.19	0.11	0.21	0.19	0.16	0.11	0.063
	P	-	-	-	-	0.06	-	-	-	0.06	-	-	-	0.06	0.034
	S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.034
	T x P	-	-	-	-	0.18	-	-	-	0.20	-	-	-	0.19	0.109
	T x S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.109
	P x S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.059
	T x P x S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.189
C.D. at 5%	T	NS	0.50	0.59	0.53	0.25	0.58	0.60	0.57	0.27	0.61	0.56	0.48	0.26	0.147
C.V. (%)		8.98	7.33	8.79	7.46	7.87	8.26	8.76	8.14	8.39	8.95	8.56	7.85	8.51	8.25

Note: 1. Figures outside the parentheses are $\sqrt{\square + 0.5}$ transformed values and those inside the parentheses are retransformed values
2. Treatment means followed by the same letter are not significantly different by Duncan's New Multiple Range Test (DNMRT) at 5% level of significance

Table 4. Bio-efficacy of botanicals against leaf miner in tomato (2021-22)

Tr. No.	Treatments	No. of mines/ 3 compound leaves at indicated days after spray													
		1st spray					2nd spray				3rd spay				Pooled over periods and sprays
		Before spray	3 DAS	7 DAS	10 DAS	Pooled	3 DAS	7 DAS	10 DAS	Pooled	3 DAS	7 DAS	10 DAS	Pooled	
T_1	NSKE 5%	4.25 (17.56)	3.50^{ab} (11.75)	3.29^{a} (10.32)	3.55^{a} (12.10)	3.45^{a} (11.40)	3.33^{a} (10.59)	3.19^{a} (9.68)	3.38^{a} (10.92)	3.30^{a} (10.39)	3.23^{a} (9.93)	3.06^{a} (8.86)	2.78^{a} (7.23)	3.02^{a} (8.62)	3.23^{a} (9.93)
T_2	Jatropha oil 1%	4.09 (16.23)	4.07^{bc} (16.06)	3.97^{abc} (15.26)	4.18^{bcd} (16.97)	4.07^{b} (16.06)	4.03^{b} (15.74)	3.93^{b} (14.94)	4.09^{c} (16.23)	4.01^{b} (15.58)	3.93^{c} (14.94)	3.84^{c} (14.25)	3.61^{b} (12.53)	3.79^{b} (13.86)	3.93^{c} (14.94)
T_3	Pongamia oil 1%	4.36 (18.51)	4.16^{bc} (16.81)	4.05^{cd} (15.90)	4.24^{cd} (17.48)	4.15^{b} (16.72)	4.12^{b} (16.47)	4.01^{b} (15.58)	4.16^{c} (16.81)	4.10^{b} (16.31)	4.03^{c} (15.74)	3.99^{c} (15.42)	3.78^{b} (13.79)	3.94^{b} (15.02)	4.03^{c} (15.74)
T_4	Neem oil 0.5%	4.06 (15.98)	4.11^{bc} (16.39)	3.96^{abc} (15.18)	4.17^{bcd} (16.89)	4.08^{b} (16.15)	4.03^{b} (15.74)	3.93^{b} (14.94)	4.09^{c} (16.23)	4.02^{b} (15.66)	3.94^{c} (15.02)	3.91^{c} (14.79)	3.70^{b} (13.19)	3.85^{b} (14.32)	3.94^{c} (15.02)
T_5	Citronella oil 1%	4.22 (17.31)	3.40^{a} (11.06)	3.90^{abc} (14.71)	4.10^{abc} (16.31)	3.99^{b} (15.42)	3.96^{ab} (15.18)	3.86^{b} (14.40)	4.03b^{c} (15.74)	3.95^{b} (15.10)	3.88^{bc} (14.55)	3.73^{bc} (13.41)	3.52^{b} (11.89)	3.71^{b} (13.26)	3.88^{bc} (14.55)
T_6	Custard apple leaf extract 10%	4.35 (18.42)	4.11^{bc} (16.39)	3.99^{bc} (15.42)	4.21^{bcd} (17.22)	4.10^{b} (16.31)	4.08^{b} (16.15)	3.95^{b} (15.10)	4.12^{c} (16.47)	4.05^{b} (15.90)	3.97^{c} (15.26)	3.84^{c} (14.25)	3.62^{b} (12.60)	3.81^{b} (14.02)	3.97^{c} (15.26)
T_7	Azadirachtin 1500 ppm	3.94 (15.02)	3.56^{ab} (12.17)	3.34^{abc} (10.66)	3.58^{ab} (12.32)	3.49^{a} (11.68)	3.35^{a} (10.72)	3.19^{a} (9.68)	3.50^{ab} (11.75)	3.35^{a} (10.72)	3.29^{ab} (10.32)	3.13^{ab} (9.30)	2.87^{a} (7.74)	3.09^{a} (9.05)	3.29^{ab} (10.32)
T_8	Garlic bulb extract 5%	4.37 (18.60)	4.12^{bc} (16.47)	4.02^{bcd} (15.66)	4.21^{bcd} (17.22)	4.12^{b} (16.47)	4.07^{b} (16.06)	3.94^{b} (15.02)	4.11^{c} (16.39)	4.04^{b} (15.82)	3.96^{c} (15.18)	3.83^{c} (14.17)	3.61^{b} (12.53)	3.80^{b} (13.94)	3.96^{c} (15.18)
T_9	Tobacco decoction2%	4.14 (16.64)	3.51^{ab} (11.82)	3.32^{ab} (10.52)	3.54^{a} (12.03)	3.46^{a} (11.47)	3.35^{a} (10.72)	3.20^{a} (9.74)	3.46^{a} (11.47)	3.34^{a} (10.66)	3.26^{a} (10.13)	3.11^{ab} (9.17)	2.84^{a} (7.57)	3.07^{a} (8.92)	3.26^{a} (10.13)
T_{10}	Untreated control	4.35 (18.42)	4.65^{c} (21.12)	4.71^{d} (21.68)	4.80^{d} (22.54)	4.72^{c} (21.78)	4.85^{c} (23.02)	4.86^{c} (23.12)	4.91^{d} (23.61)	4.88^{c} (23.31)	4.94^{d} (23.90)	4.96^{d} (24.10)	4.97^{c} (24.20)	4.96c (24.10)	4.94^{d} (23.90)
S. Em. ±	T	0.17	0.20	0.21	0.19	0.12	0.20	0.18	0.17	0.11	0.19	0.21	0.16	0.11	0.0633
	P	-	-	-	-	0.06	-	-	-	0.06	-	-	-	0.06	0.0347
	S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0347
	T x P	-	-	-	-	0.20	-	-	-	0.19	-	-	-	0.19	0.1098
	T x S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1098
	P x S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0601
	T x P x S	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1901
C.D. at 5%	T	NS	0.58	0.61	0.57	0.27	0.60	0.53	0.51	0.25	0.55	0.63	0.46	0.25	0.176
C.V. (%)		7.06	8.51	9.28	8.25	8.67	8.96	8.16	7.50	8.22	8.39	9.88	7.66	8.73	8.54

Note: 1. Figures outside the parentheses are $\sqrt{\square + 0.5}$ transformed values and those inside the parentheses are retransformed values
2. Treatment means followed by the same letter are not significantly different by Duncan's New Multiple Range Test (DNMRT) at 5% level of significance

conducted during the rabi seasons of 2020 and 2021 at the horticulture farm of the College of Horticulture, S. D. Agricultural University, Jagudan (Mehsana), Gujarat, utilizing a completely randomized design with three replications and ten treatments. Tomato plants (Pant polyhouse hybrid tomato 2) were planted with a plot size of 128 m^2 at 60 cm × 45 cm spacing. All botanicals were prepared following standard protocols. Three applications of botanical insecticides were administered at 10-day intervals, with the first spray applied at the initiation of the pest population. Observations were recorded before spraying and 3, 7, and 10 days after each application. Marketable fruit yield was assessed for all treatments at each harvest. To evaluate the economic viability of the various treatments compared to tomatoes infested by *L. trifolii*, the Incremental Cost Benefit Ratio (ICBR) was calculated.

RESULTS AND DISCUSSION

Based on damage leaves (%)

The results from the *rabi* season of 2020-21 are summarized in Table 1, indicating significant differences among various biopesticide treatments. Plants treated with neem seed kernel extract at 5% exhibited the lowest damage percentage (10.26%) and outperformed all other treatments. Additionally, it showed comparable efficacy to tobacco decoction at 2% (10.99%) and azadirachtin at 1500 ppm (11.54%). Citronella oil at 1% (15.98%) was identified as the next most effective treatment, while significantly higher infestation percentages were observed with pongamia oil at 1% (18.08%), custard apple leaf extract at 10% (18.25%), neem oil at 0.5% (18.51%), garlic bulb extract at 5% (18.60%), and jatropha oil at 1% (18.95%).

In the *rabi* season of 2021-22, presented in Table 2, plants treated with neem seed kernel extract at 5% exhibited the lowest damage percentage (9.36%), statistically comparable to tobacco decoction at 2% (10.13%) and azadirachtin at 1500 ppm (10.72%). Citronella oil at 1% (15.10%) emerged as the next most effective treatment. However, significantly higher infestation percentages were recorded for Pongamia oil at 1% (17.22%), custard apple leaf extract at 10% (17.39%), neem oil at 0.5% (17.65%), garlic bulb extract at 5% (17.82%), and jatropha oil at 1% (18.08%).

Based on no. of mines

During the *rabi* season of 2020-21 (Table 3), the plant treated with Neem Seed Kernel Extract at 5% exhibited the lowest damage, recording 11.06 mines per 3 compound leaves, surpassing all other treatments. It performed comparably to tobacco decoction at 2% (11.26%) and azadirachtin at 1500 ppm (11.40 mines / 3 compound leaves). Following this, citronella oil at 1% (15.58 mines/ 3 compound leaves) emerged as the next effective treatment, trailed by jatropha oil at 1% (16.23 mines/ 3 compound leaves), neem oil at 0.5% (16.31 mines/ 3 compound leaves), garlic bulb extract at 5% (16.39 mines/ 3 compound leaves), custard apple leaf extract at 10% (16.39 mines/ 3 compound leaves), and pongamia oil at 1% (16.97 mines/ 3 compound leaves).

The pooled data over periods and spray for the *rabi* season of 2021-22 (Table 4) reaffirmed the superiority of Neem Seed Kernel Extract at 5%, with the lowest damage recorded at 10.13 mines per 3 compound leaves, surpassing all other treatments. Again, it performed comparably to tobacco decoction at 2% (10.32%) and azadirachtin at 1500 ppm (10.46 mines/ 3 compound leaves). Citronella oil at 1% (14.63 mines/ 3 compound leaves) emerged as the next effective treatment, followed by jatropha oil at 1% (15.18 mines/ 3 compound leaves), neem oil at 0.5% (15.34 mines/ 3 compound leaves), garlic bulb extract at 5% (15.34 mines/ 3 compound leaves), custard apple leaf extract at 10% (15.42 mines/ 3 compound leaves), and pongamia oil at 1% (15.98 mines/ 3 compound leaves). Previous studies have highlighted the efficacy of neem-based treatments in controlling leaf miner populations in various crops, corroborating our findings (Fagoonee and Toory, 1983; Suradkar and Ukey, 2014; Dodiya and Barad, 2022; Mohan and Anitha, 2017; Barde and Shrivastava, 2017). Other biopesticides such as tobacco decoction, azadirachtin, and certain plant extracts have demonstrated effectiveness against similar pests, supporting the present study's outcomes.

Yield

Table 5 presents the data on tomato fruit yield across various botanical treatments and control plots. Remarkably, the highest tomato fruit yield (449.16 q/ha) was obtained from plots treated with neem seed kernel extract at 5%, followed closely by azadirachtin at 1500 ppm (445.83 q/ha) and tobacco decoction at 2% (436.00 q/ha). Citronella oil at 1% also showed promising results, yielding 385.33 q/ha and outperforming the untreated control (285.83 q/ha). Regarding net realization (Table 5), the highest returns were observed in plants treated with neem seed kernel extract at 5% (₹326660), followed by azadirachtin at 1500 ppm (₹320000) and tobacco decoction at 2% (₹300340) and in terms of Profit-Cost Benefit Ratio (PCBR), tobacco decoction at 2% exhibited the highest ratio (1:86.06), followed by neem seed kernel extract at 5% (1:64.01).

CONCLUSION

Neem seed kernel extract at 5% recorded the

Table 5. Economics of botanicals evaluated against leaf miner, *L. trifolii* infesting tomato (Pooled: *Rabi,* 2020-21 and 2021-22)

Tr. No.	Treatment	Conc. (%)	Total cost of treatment (₹)	Yield (q/ha.)	Gross realization (Rs./ha.)	Net realization (Rs./ha.)	Net gain (Rs./ ha.)	PCBR
T_1	NSKE 5%	5%	5025	449.16	898320	326660	321635	1: 64.01
T_2	Jatropha oil 1%	1%	3450	314.66	629320	57660	54210	1: 15.71
T_3	Pongamia oil 1%	1%	3600	319.66	639320	67660	64060	1: 17.79
T_4	Neem oil 0.5%	0.5%	3000	314.66	629320	57660	54660	1: 18.22
T_5	Citronella oil 1%	1%	22620	385.33	770660	199000	176380	1: 7.80
T_6	Custard apple leaf extract 10%	10%	3150	307.00	614000	42340	39190	1: 12.44
T_7	Azadirachtin 1500 ppm	-	5700	445.83	891660	320000	314300	1: 55.14
T_8	Garlic bulb extract 5%	5%	5775	320.16	640320	68660	62885	1: 10.89
T_9	Tobacco decoction2%	2%	3450	436.00	872000	300340	296890	1: 86.06
T_{10}	Untreated control	- - - -	-	285.83	571660	0	-	-

NSKE = ₹25/kg Jatropha oil= ₹90/L Pongamia oil = ₹100/L Neem oil = ₹120/L. Citronella oil = ₹1368/L. Azadirachtin = ₹600/L.

Garlic = ₹35/kg Tobacco dust= ₹10/kg Labour cost = ₹1050/- (Extract preparation: 3 labour) and Rs. 700/- (2 labour/ha for one spray)

Tomato price = ₹20/kg

lowest damage (9.80%) and it was statistically at par with tobacco decoction at 2% (10.52%) followed by azadirachtin at 1500 ppm (11.13%). Citronella oil at 1% (15.50%) stood the next effective treatment. Similar trend of the treatment effect was noticed for the character no. of mines per three compound leaves. Significantly maximum (449.16 q/ha) tomato fruit yield was harvested from the plots treated with neem seed kernel extract 5% followed by azadirachtin 1500 ppm (445.83 q/ha) and tobacco decoction2% (436.00 q/ha). The highest PCBR was recorded in the treatment of Tobacco decoction 2% (1: 86.06) followed by NSKE 5% (1: 64.01).Ultimately, the integration of biopesticides into the management paradigm offers a pathway towards sustainable and eco-friendly tomato production under protected cultivation.

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Prevalence of seedling diseases of chilli in North-Eastern Karnataka

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ABSTRACT: Chilli is an important commercial crop affected by many pathogens causing pre-emergence and post-emergence damping-off, die-back and wilt to an extent of 62 per cent in seedling stage. Survey was conducted in North-Eastern Karnataka to assess the incidence and severity of seedling diseases of chilli and the results revealed that the maximum incidence was noticed in Ballari district with 32.07 per cent and during the survey the major diseases such as damping-off caused by *Fusarium oxysporum* and die-back caused by *Colletotrichum capsici* were noticed.

Keywords: Chilli, damping-off, dieback, seedling diseases.

INTRODUCTION

Chilli is one of the important commercial and universal crops cultivated in different parts of the world for both green and red chillies. The largest producer of chilli in the world is India, accounts an area of 802 m. ha., with the production and productivity of 1836 m. t. and 2.1 m. t/ha, respectively. India also stands second among world's chilli export contributing for improving Indian economy. The major chilli growing states in India are Tamil Nadu, Maharashtra, Karnataka, Nagaland, Telangana, Andhra Pradesh, West Bengal and parts of Madhya Pradesh contributing 86 per cent of total chilli cultivating area in the country and 90 per cent of the total Indian produce (Anon., 2023). In both nurseries and fields chilli seedlings are affected by many soil borne and seed borne fungal pathogens, killing 62 per cent of seedlings and accounting 90 per cent of plant deaths. The amount of damage caused to seedlings depends on the associated fungi, soil moisture and soil temperature and other factors rather than the particular species of plant concerned. Normally, however, cool wet soils favour the development of the disease. The disease is responsible for poor germination as well as poor stand of seedling in the nursery bed and often the infected seedlings carry the pathogen to the main field where transplanting is done (Pagoch *et al*., 2015). A successful survey will helps to know the severity and incidence of seedling diseases of chilli. Also it enables us to locate the endemic regions of the disease and provides an idea for establishing disease free seedlings to develop area wise integrated management and regulatory measures. Hence survey was conducted to assess the incidence and prevalence of seedling diseases of chilli.

MATERIALS AND METHODS

Survey

An intensive random roving survey was carried out to assess the severity and present status of seedling diseases of chilli under both nursery and field conditions in different chilli growing districts of North Eastern Karnataka *viz*., Raichur, Ballari and Koppal during 2022-23. The chilli fields and nurseries were randomly selected and in each district two-three taluks and in each taluk two-three villages were covered and in each village farmer fields and nurseries were selected. In each field and nurseries, observations were made randomly on the seedling diseases of chilli *viz*., damping-off, die-back and wilt based on the symptoms. Samples were collected, properly labelled and stored for further studies. Disease severity was assessed based on the severity rating scale given for damping-off (0- healthy plant, 1- root tip necrosis, 2- softening of stem, 3- dead seedling and 4- dead seed, given by Saba *et al.* (2022)) and die-back (0- 0 %, 1- <5 %, 2- 6-10 %, 3- 11-25 %, 4- 26-40 %, 5- 41-60 % and 6- >60 % area infected, given by Kwee *et al*. (1987)).

Per cent disease incidence was calculated using the formula given by Wheeler (1969).

$$\text{Per cent disease incidence} = \frac{\text{Number of seedlings infected}}{\text{Total number of seedlings observed}} \times 100$$

Per cent disease index (PDI) was calculated by using the formula given by Wheeler (1969).

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of numerical ratings}}{\text{Total number of plants scored} \times \text{Maximum scale}} \times 100$$

The symptomatic chilli seedlings showing wire stem, damping-off, die-back and necrosis symptoms were collected from surveyed districts of North-Eastern Karnataka and the causal agents were isolated from these infected parts by standard tissue isolation technique and the dominant pathogens associated with damping-off were *Fusarium oxysporum* and *Macrophomina phaseolina* whereae chilli die-back associated with *Colletotrichum capsici*.

RESULTS AND DISCUSSION

The maximum disease incidence and severity was recorded in Ballari district, where the per cent disease incidence and severity were ranged from 14.08 to 61.00 per cent and 33.16 to 53.33 per cent, respectively with mean per cent disease incidence of 32.07 per cent and severity of 40.99 per cent followed by Raichur district with incidence and severity ranging from 3.67 to 54.00 per cent and 28.82 to 56.66 per cent with an average incidence and severity of 29.70 and 40.97 per cent, respectively. In Koppal district, the per cent incidence and severity were ranged from 9.80 to 38 per cent and 23.02 to 43.33 per cent with an average of 21.77 per cent and 35.46 per cent, respectively. In Koppal, the maximum incidence and severity of 38 and 43.33 per cent was recorded in Hatti village and minimum was recorded in Kadur village. In Ballari district, the maximum incidence (61 %) and severity (53.33 %) of die-back was recorded in Siruguppa village, whereas minimum was noticed in Ballari (14.08 and 33.16 %). However in Raichur district, the maximum die-back incidence and severity (54.00 & 56.66 %) was noticed in Hunsihalhuda village, whereas damping-off incidence was maximum in Navilgudda village (30.00 %) and severity was maximum in Chandrabanda (41.26 %) (Table 1).

The survey results evidenced that the occurrence of seedling diseases of chilli differs significantly from one location to another. This variation might be attributed to the diverse interplay of weather parameters, including temperature, relative humidity and rainfall as well as the specific cultivars cultivated and type of soil in each area.

Table 1. Status of seedling diseases of chilli across major districts of North-Eastern Karnataka

District	Taluk	Village	Field/ Nursery	Variety	Method of sowing	Crop stage (days)	Diseases noticed	Disease incidence (%)	Disease severitynm (%)
Raichur	Raichur	Hosur	Field	Old 50	Direct seeded	35	Die-back	48.00	40.66
		Gonhal	Field	Super 10	Direct seeded	20	Die-back	38.00	40.00
		Hunsihalhuda	Field	Old 50	Direct seeded	17	Die-back	54.00	56.66
		Marchathal	Field	Byadagi	Direct seeded	30	Die-back	46.00	47.33
		Chandrabanda	Nursery	HPH 2043	Raised in protrays	28	Damping-off	17.43	41.26
		Kalmala	Field	Super 10	Direct seeded	22	Die-back	33.00	36.66
		Taluk mean						**39.41**	**43.76**
	Devadurga	Gabbur	Nursery	HPH 2043	Raised in protrays	36	Damping-off	4.89	30.01
			Nursery	HPH 2043	Raised in protrays	39	Damping-off	19.40	31.54
		Navilgudda	Field	1080	Direct seeded	25	Damping-off	30.00	35.55
							Die-back	42.00	65.00
		Devadurga	Nursery	HPH 5531	Raised in protrays	38	Damping-off	3.67	28.82
		Taluk mean						**19.99**	**38.18**
		District mean						**29.70**	**40.97**

	Gangavathi	Budugumpa	Nursery	Sukhino	Raised in protrays	30	Damping-off	18.37	25.48
		Kadur	Nursery	HPH 5531	Raised in protrays	30	Damping-off	9.80	23.02
				Taluk mean				**14.08**	**24.25**
	Kanakagiri	Kanakagiri	Nursery	VNR145	Raised in protrays	27	Damping-off	16.12	38.87
		Gouripura	Nursery	BASF 1080	Raised in protrays	30	Damping-off	12.85	37.63
Koppal				**Taluk mean**				**14.49**	**38.25**
	Kushtagi	Gunnal	Field	Avenger plus	Transplanted	42	Damping-off	20.00	32.50
		Kushtagi	Nursery	Saritha 074	Raised in protrays	25	Damping-off	21.02	39.54
				Taluk mean				**20.51**	**36.02**
	Koppal	Hatti	Field	VNR145	Direct seeded	50	Damping-off	38.00	43.33
				Taluk mean				**38.00**	**43.33**
				District mean				**21.77**	**35.46**
	Ballari	Ulavathi	Field	Avenger plus	Transplanted	28	Damping-off	40.00	45.00
		Ballari	Nursery	Avenger plus	Raised in protrays	24	Damping-off	14.08	33.16
				Taluk mean				**27.04**	**39.08**
Ballari	Siruguppa	Bagwadi	Nursery	Old 50	Raised in protrays	25	Damping-off	17.34	41.83
		Devalapura	Field	RJ33	Transplanted	34	Damping-off	40.00	34.50
							Die-back	30.00	40.00
		Siruguppa	Field	Old 50	Direct seeded	40	Die-back	61.00	53.33
				Taluk mean				**37.09**	**42.92**
				District mean				**32.07**	**40.99**

Moreover, the presence of varying pathogenic strains within the fungus also contributes to this observed diversity.

As per the findings of the survey, die-back was noticed only in the field conditions whereas, damping-off was observed in both nursery and field conditions. In nurseries, damping-off was majorly observed and variety Old 50 was found to be more susceptible with maximum severity of 56.66 while minimum severity was observed with HPH 5531 hybrid (23.02 %).

Damping-off disease manifested in the seedlings until 40 days of post sowing, later the incidence reduced as the age advances. In contrast, die-back symptoms were observed even beyond the 40 days old seedling and the disease predominantly thrived in black soil with flooded fields. Die-back was not recorded in Koppal district while damping-off was recorded in severe form and this might be due to improper spacing, watering in nurseries, rainfall, relative humidity might have played the role in causing damping-off.

Mougy *et al*. (2011) revealed that the surveyed nurseries at early stages showed highest records of damping-off in vegetable crops under protected condition. Ali *et al.* (2019) isolated different fungal pathogens from soil collected from infected chilli fields and found that

Damping-off **Die-back**

Fig. 1. Symptoms of damping-off and die-back

maximum number of pathogens were found in the month of October, later there was a decline in the number of pathogens in the month of March and April. Majeed *et al.* (2018) carried out a survey for assessing the prevalence of damping off disease in chilli and found mean incidence varying between 16.07 and 29.00 per cent, also reported that highest incidence was due to excessive moisture in soil and lesser maintenance of fields. Results were also similar to the findings of Hajong *et al*. (2023), they reported that, Khanapara village had recorded the highest disease incidence at 24.25 percent followed by Pimpari Deshmukh with 23.00 per cent and Selu with 21.25 percent, compared to other localities where they revealed that frequent watering of seedlings or their exposure to waterlogged conditions for extended periods increased the likelihood of damping-off in tomato seedlings and also reported that, disease was caused by the planting of susceptible tomato cultivars in heavy black soil.

CONCLUSION

Damping-off and die-back of chilli have become major constraints in the nursery as well as in the field conditions. Analysis of survey data revealed that the disease has significantly increased in Northern Karnataka in terms of its distribution and intensity, might be because of continuous cultivation of chilli over the years with susceptible varieties, direct seed sowing without maintaining the proper plant to plant and row to row spacing. The farmers are also practicing the drill sowing method for chilli varieties in the plain land without making any ridges and furrows. This may lead to more plant population, poor light penetration with poor ventilation. During a heavy rainfall situation, the sloppy and undulated fields without ridges and furrows result in water logging conditions and chilli crops become more vulnerable to pathogen infection. Hence, it can be concluded from the present study that crop rotation with non-host crops and maintaining proper plant population with spacing may check the disease intensity.

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Tetranychus gloveri (Acari: Tetranychidae): an emerging threat to tissue culture banana plantlets in nurseries

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ABSTRACT: For many decades, mites are posing threat to agricultural and horticultural industries. Recently mite infestation in Horticultural nurseries has become serious problem in Kerala, India. Purposive sampling surveys were conducted in the Horticultural nurseries of Thrissur and Ernakulam districts to record the incidence of mite pests on nursery plants. Study of morphological characters like setae and aedeagus confirmed the mite species infesting TC banana plants is *Tetranychus gloveri* Banks.

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Keywords: Field identification, tissue culture banana, taxonomic characters, *Tetranychus gloveri*

INTRODUCTION

Banana (*Musa* spp.), belonging to the family Musaceae is an important commercially grown fruit crop in tropical and sub tropical regions of the world. It is also commonly referred as "apple of the paradise"(Subba *et al*., 2023).The Nendran (AAB group), also called a French plantation is one of the leading banana cultivars in Kerala due to its wide acceptance and special social economic importance in the state. In India, an average of 30.47 million tonnes of bananas are produced annually in an area of 8,60,000 acres (FAOSTAT, 2017). During 2018-19, total production of 4.24 lakh tonnes was reported from an area of 52,898.61 ha in Kerala (GoK, 2019). In recent times, micro propagation of the planting material using tissue culture is gaining importance to meet the high demand for banana suckers in planting season. But the cultivation of the plantlets for acclimatization in hardening unit is often threatened by the high incidence of pests and diseases (Agustin *et al*., 2022). Mites (Acari) are a diverse group of arthropods that have been destroying and devastating the agricultural industry for many decades. They also occupy a wide range of habitats and a wide range of hosts including vegetable and ornamental crops (Al-Atawi, 2011)

MATERIALS AND METHODS

Purposive sampling surveys were conducted in the Horticultural nurseries of Thrissur and Ernakulam districts during January 2021 to march 2023 to record the incidence of mite pests on nursery plants. During the survey, symptoms of speckling, yellowing and drying of leaves were recorded in TC banana plants from four nurseries at Kannara (10^0 32' 52"N 76 ^{0}19'12"E), Vytilla ($9^0$55'37"N $76^0$19'11"E), Vellanikkara ($10^0$32'37"N $76^0$17'01"E) and Oddakkali ($10^0$49'53"N $76^0$40'35"E). On examination using a hand lens (5 X), large number of different stages of spider mites, moulted skin and fine webbing were noticed on the lower surface of the leaves. The affected leaves were excised and collected in polythene bags, secured with rubber bands, labelled and brought to the laboratory for further examination.

In the laboratory, on observation of the leaf samples under a stereo zoom microscope (30X) large number of scarlet red adult female mites, creamy white nymphs, adult males, and translucent white spherical eggs were found. The male and female mites were picked then slide mounted on Hoyer's medium for morphological characterisation and species determination. Morphological characters namely chaetotaxy, structure of empodium on the leg of

Fig. 1. Symptoms of mite infestation on banana plantlets A. TC plantlets in hardening unit B. Whitish speckles on leafC. Yellowing of leaves D. Adult female mites

female mite were studied for identification at genus level and for species identification, shape of male genitalia were studied using Radical RXLr- 4 phase contrast microscope with image analyser.

RESULTS AND DISCUSSION

In the nursery**,** mite colonises the TC banana plants on the lower surface of the leaves, and feed the sap leading to development of white speckles on the upper surface (Fig.1). Later, the leaf turns yellow, followed by bronzing and drying (Fig.1). Severely infested plantlets succumbed to mite infestation.

The study of morphological characters revealed that the mite species infesting TC banana plants is *Tetranychus gloveri* Banks. The key taxonomic characters of *T. glover* is furnished below.

Female: Tarsus I with two sets of duplex setae well separated, empodium of legs split distally (Fig.2 A and B).

Male: Aedeagus bent dorsad, knob much longer than the width of the neck, approximately half as long as dorsal margin of the shaft, knob axis sub parallel with dorsal margin of shaft, anterior projection of knob broadly rounded and posterior narrow and acute (Fig.2 C).

Tetranychus gloveri is is a significant pest of several crops in different countries (Jeppson *et al.,* 1975; Bolland *et al*., 1998). This species is wide spread in the Pacific and Americas, which may represent its natural range. Its highly polyphagous nature and high reproductive capacity enables the species to become a serious pest on agricultural crops, especially in green houses (Takafuji *et al*., 1996). Recently, it has been reported as an invasive pest in Kerala infesting a wide host range of 35 host plants in 24 plant families *viz*., Malvaceae, Cucurbitaceae, Fabaceae, Amaranthaceae, Rutaceae, Solanaceae, Musaceae, Moraceae, Anacardiaceae, Caricaceae, Adoxaceae, Rosaceae, Compositae, Gentianaceae, Convolvulaceae, Balsaminaceae, Orchidaceae, Asparagaceae, Goodeniaceae, Apocyanaceae, Euphorbiaceae, Oxalidaceae, Lamiaceae and Pontederiaceae (Bhaskar *et al*., 2022).

Tetranychus gloveri was first described by Banks on *Gossypium* from United States of America. Later, Pritchard and Baker (1955) synonymised *T. gloveri* and *T. tumidus* Banks. But, Boudreaux (1958) separated the two species during which, the taxa originally called *T. gloveri* became *T. tumidus* and vice-versa. However, Boudreaux (1979) rectified this misidentification later. In India, it was first reported from Thrissur district from a commercial horticultural nursery as *Tetranychus okinawanus* (Zeity *et al*., 2016). *Tetranychus okinawanus* was first reported on *Pueraria lobata* from Okinawa Islands of Japan by Ehara (1960). Recently, Sharkey *et al.,* (2022) synonymized *T. okinawanus* with *T. gloveri* based on morphological and molecular data.

The morphological identification of *T. gloveri* collected from Florida showed that only a single morphological character, the length of the solenidion on tarsus III, was used to separate *T. okinawanus* from *T. gloveri*. Using types of both species, Sharkey et al., (2022). reassessed the character and found no basis for the treatment of *T. okinawanus* as a distinct species. Further, molecular characterisation of COI and ITS1/ ITS2 sequences of *T. gloveri* were either highly similar or identical to GenBank sequences of *T.okinawanus* from Japan. Hence *T. okinawanus* was treated as a junior synonymn of *T. gloveri.*

In this study, severe infestation of *T. gloveri* was recorded on TC banana plants from four horticultural nurseries in Thrissur and Ernakulam district. This species was earlier reported on banana in plantations from Kerala (Arunima *et al*., 2018). As *T. gloveri* has high fecundity, developmental rate and polyphagous nature, the mite species can build up at a faster rate and may spread to other nursery plants. As many nurseries adopt chemical control as a strategy for mite management,

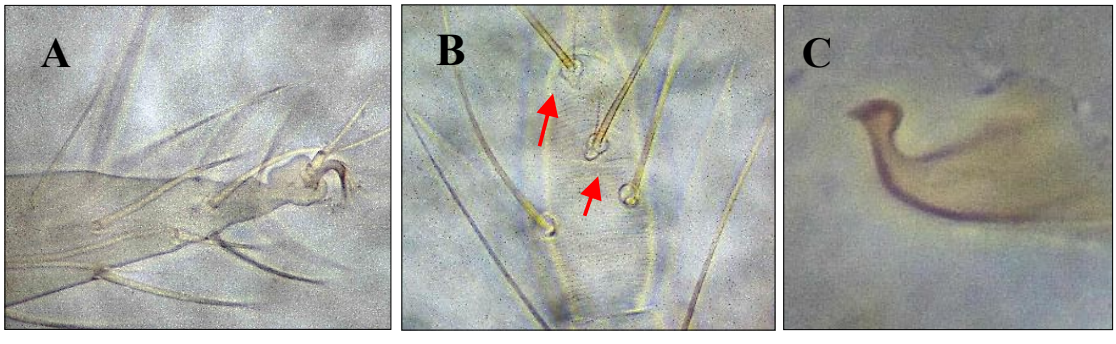

Fig. 2. *Tetranychus gloveri* Banks female A. empodium of legs B. Duplex setae on Tarsus I; Male C. Aedeagus

mite populations may develop resistance mechanisms which may lead to control failures. Thus, early detection, identification of mite species and employing integrated management tactics at initial stages is essential to reduce the risk of losing plantlets and their carry over to the main field.

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RESEARCH NOTE

A modified fungicide based media for the isolation of *Phytophthora cinnomomi* Rands. causing avocado root rot

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ABSTRACT: The commonly chosen medium for isolating Phytophthora is the P10VP medium, which contains pimaricin (10 mg), vancomycin (220 mg), and PCNB (pentachloronitrobenzene) (125 mg a.i.). Pimaricin has been a longstanding ingredient in the P10VP medium for *Phytophthora* isolation. In this research, we designed a modified isolation medium with propiconazole as the primary component for isolating *Phytophthora* from infected plant samples. This modified medium was tested and validated for various Phytophthora species affecting different crops.

Keywords: *Phytophthora cinnamomi*, Pimaricin, Propiconazole

Phytophthora cinnamomi Rands, an Oomycete, is known for causing severe root rot and dieback in various agricultural and forestry host plants, including avocado, chestnut, macadamia, oak, peach, and pineapple (Hardham and Blackman 2018; Madhu et al., 2023). The infection primarily targets feeder roots, leading to their decay. Isolating this pathogen from feeder roots is a challenging task due to the presence of numerous saprophytes in decayed feeder roots. Standard media are unsuitable for *P. cinnamomi* isolation as they quickly lead to contamination by other fungi. Therefore, the use of selective media containing P10VP (pimiricin 10 mg, vancomycin 220 mg, PCNB (pentachloronitrobenzene) 125 mg a.i.) or PARP-V8 (20 g agar, 200 ml filtered V8 broth, 800 mL deionized water, 5 mg pimiricin, 10 mg rifampicin, 250 mg ampicillin, and 125 mg a.i. PCNB) has proven effective for the selective isolation of Phytophthora (Tsao and Ocana, 1969).

However, the drawback of these media lies in the use of pimaricin, which is highly photosensitive (Gutteridge et al., 1953). Inoculated plates must be kept dark to prevent photo inactivation-related potency loss. Tsao and Ocana (1969) noted that prolonged storage of the medium in the dark and at low temperatures results in the loss of antibiotic action. Additionally, distinct strains of *Phytophthora* exhibit sensitivity to pimaricin. While pimaricin does not hinder mycelial growth, it strongly or completely inhibits the germination of chlamydospores, sporangia, and zoospores in many tested species (Jeffers and Martin 1986). Against this background, the present study aims to develop a fungicide-based medium as an alternative to the antibiotic pimaricin for isolating *Phytophthora* from infected plant samples. Attempts were made to isolate *Phytophthora* infecting avocados using Corn Meal Agar (CMA) supplemented with specific active ingredients. The composition of CMA per litre included Pr1VRP (propiconazole 25% EC 1ml, vancomycin chloride 200mg, rifampicin 10mg, PCNB 100mg) and Pr2VRP (propiconazole 25% EC 2mL, vancomycin chloride 200mg, rifampicin 10mg, PCNB 100mg), which were added after autoclaving. Infected avocado feeder roots displaying symptoms of dark, bristly roots were selected for organism isolation. The collected feeder roots were cleaned in sterile distilled water, air-dried, and then inoculated for isolation. Surface sterilization using alcohol or other chemicals was avoided to prevent occasional inhibition of *Phytophthora* growth. After seven days of incubation at 22°C, plates were examined for mycelia development and chlamydospore production.

Phytophthora cinnamomi produces coralloid hyphae with grape-like clusters of chlamydospores that grow laterally on the hyphae (Hardham and Blackman 2018). Colonies displaying cottony growth at the tip of growing mycelium were selected and subcultured to obtain a pure culture of the organism (Fig 1). The CMA medium containing propiconazole did not inhibit the mycelial growth and chlamydospore formation of *Phytophthora* in the culture medium. However, at a propiconazole concentration of 2 ml/L, the growth of saprophytic

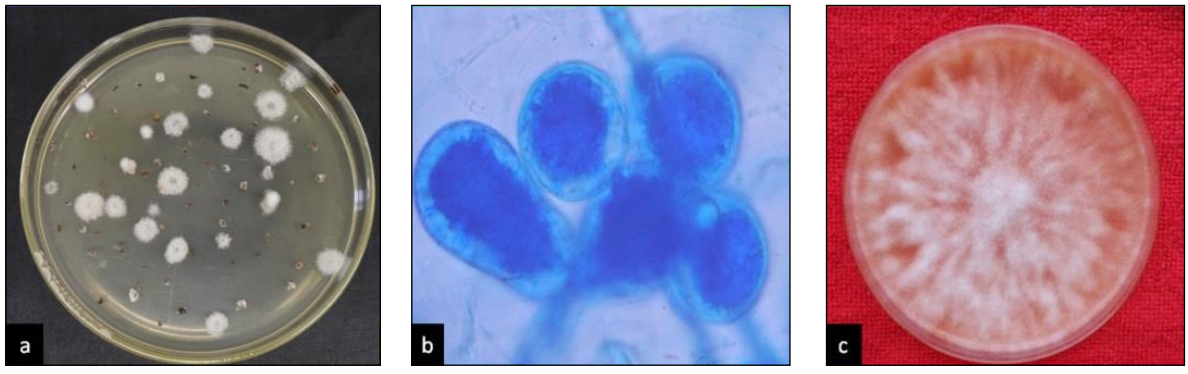

Fig 1. *Phytophthora cinnomomi* a. Whitish mycelial growth of *P. cinnomomi* after avocado root inoculated on Pr2VRP medium b. Chlamydospores on Pr2VRP medium c. Ten days old culture on PDA media

fungi was completely inhibited, successfully recovering Phytophthora. The antibacterial chemicals rifampicin + vancomycin chloride proved more effective than rifampicin alone at inhibiting bacterial development in the culture medium. After one week of incubation at 25°C, no bacterial colonies were observed on any inoculated plates with Pr1VRP or Pr2VRP. Pr2VRP emerged as the most effective chemical for isolating *P. cinnomomi* from infected feeder root samples. This medium, however, has not been tested for the isolation of *Phytophthora* from soil samples. Pr2VRP medium has also been standardized to isolate Phytophthora infecting black pepper roots, cacao pods, guava fruits, and papaya roots. Propiconazole, a triazole-based fungicide used to combat true fungal diseases, acts by inhibiting ergosterol biosynthesis, which is crucial for fungal cell wall formation. However, Propiconazole does not inhibit the growth of Oomycetes such as *Phytophthora* and *Pythium*, as their cell walls contain cellulose, and ergosterol is not a major sterol in the cell membrane. Fungicides targeting chitin and ergosterol synthesis are generally ineffective against Oomycetes (Gad *et al.,* 2014). Using Propiconazole for Phytophthora isolation is cost-effective, readily available in the market, and can serve as an alternative to pimaricin.

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RESEARCH NOTE

First report of *Coccus viridis* (Green) as a pest of dragon fruit in West Bengal

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ABSTRACT: A field study was conducted to document the insect pests of dragon fruit at Bidhan Chandra Krishi Viswavidyalaya, Mandouri, West Bengal, India during 2022 and 2023. The infestation of *Coccus viridis* was observed in pink and white-fleshed fruits during June to October. The population of nymphs and adults varied from 5 to 72 /fruit during the study period. The pest was found in close association with red ants and mealy bugs. However, the infestation was found confined to fruits only. Rusty specks formed on fruits and severely infested fruits remained underdeveloped and small. This is the first report of *C. viridis* infestation on dragon fruit from West Bengal, India.

Keywords: *Coccus viridis*, dragon fruit, mealy bug, red ant

Dragon fruit (*Hylocerus undatus*) has been introduced as a new crop in India, especially in dry and low rainfed areas (Nangare *et al*., 2020). The crop is now widely cultivated in India, which is currently one of the rising properties of commercially cultivated fruit. The urban consumers of India are now nutritionally aware and willing to try natural products for their ever increasing ailments like diabetes, cholesterol, and other stress related diseases (Nangare *et al*., 2020). Hence, dragon fruit has such potential because of its Vitamin C, phosphorus and calcium richness, which attracts the farming community to cultivate the crop. On the other hand, there is also a need to shift traditional orchards into diversified orchard with new and exotic fruits to fulfill the fruit basket of our nation. Hence, dragon fruit is a crop with amazing health benefits that needs to draw much attention from growers in India. Like other fruit crops, dragon fruit is also infested by insect pests and diseases. However, there is very limited study on diversity of insect pests infesting dragon fruit in India.With this background, a study was done to investigate the diversity of insect pest of dragon fruit in West Bengal during 2022 and 2023.

A fixed plot survey was conducted at the fruit research station of ICAR-All India Coordinated Research Project on Fruits, Mandouri, Bidhan Chandra Krishi Viswavidyalaya during 2022 and 2023to record the diversity and incidence of insect pests of dragon fruit. The location of the experimental plot was 22.48° N and 88.42° E, altitude 9.75 m AMSL (Above Mean Sea Level).During the study period, a scale insect was found to attack the fruits of dragon fruit. Hence, to identify insect species, live scale insect samples along with fruit were collected from the experimental field and put in polypropylene bag of 50 micron thicknessand 39cm x 9cm size. The collected samples were then immediately brought to the laboratory of Agricultural Entomology, BCKV, Mohanpur, for identification and subsequently identified as *Coccus viridis* (Green) (Coccidae: Hemiptera)by the expert. During the survey, the incidence of scale insect on dragon fruit, time of occurrence of the pest, pattern of damage *etc*. were recorded.

Coccus viridis is polyphagous and a major biotic constrain of a wide range of important crop plants like *arabica* and *robusta* coffee, citrus, tea, mango, cassava and guava (Le Pelley, 1968). In the present study, it has been found to attack dragon fruit. The attack was noticed in the developing to the ripening stage of the fruit. Interestingly, no pest population was observed on the stem of the plant. There was a strong association of red ant and *C. viridis* throughout the study period. In addition to scale infestation, mealy bug infestation was also noticed in the experimental field. *Coccus viridis* used to occur in tropical regions of the world and thought to be originated from Brazil. Presently it has been distributed in countries like Asia, Africa, America, Europe, Oceania and Australia. In India the pest has been reported from Assam, Bihar, Karnataka, Tamil Nadu and Kerala (EPPO global database).

The adult scale insect was dome shaped, pale with blackish internal markings that were found visible through the chitinous body wall. The adults and nymphs

were lack of antennae, wings and legs (Fig. 3 and 4). The crawlers were without scale found at the ventral side of female (Fig. 1). During this study, crawlers were observed moving freely on the host surface. Later, the crawlers moulted into nymphs by developing scales over their body, where they concealed themselves and at this point they fixed themselves at a particular spot of the spike (Fig. 2 &5), later continued to feed and multiply on developing fruits. They congregate in line through the inner and outer margin of the dragon fruit spike (Fig. 6) with a population of 5 to 29 insects per fruit spike depending on the length of the spike. The incidence of insects were recorded during June and remain active in the field till September-October or until the fruits remained in the field. However, maximum pest incidence was observed during June-July and another peak during first week of September. A positive relationship observed between a number of fruits and pest incidence. Red ants and mealy bugs were associated with the mature unripe fruit to ripe fruit. The red ant was found feeding on honeydew and acting as transmitter of the pests (Fig. 7 & 8). Due to continuous sucking of the peel surface, rusty specks developed on spike and peel of the infested fruit (Fig. 9), fruit, reduced its size and remained stunted with

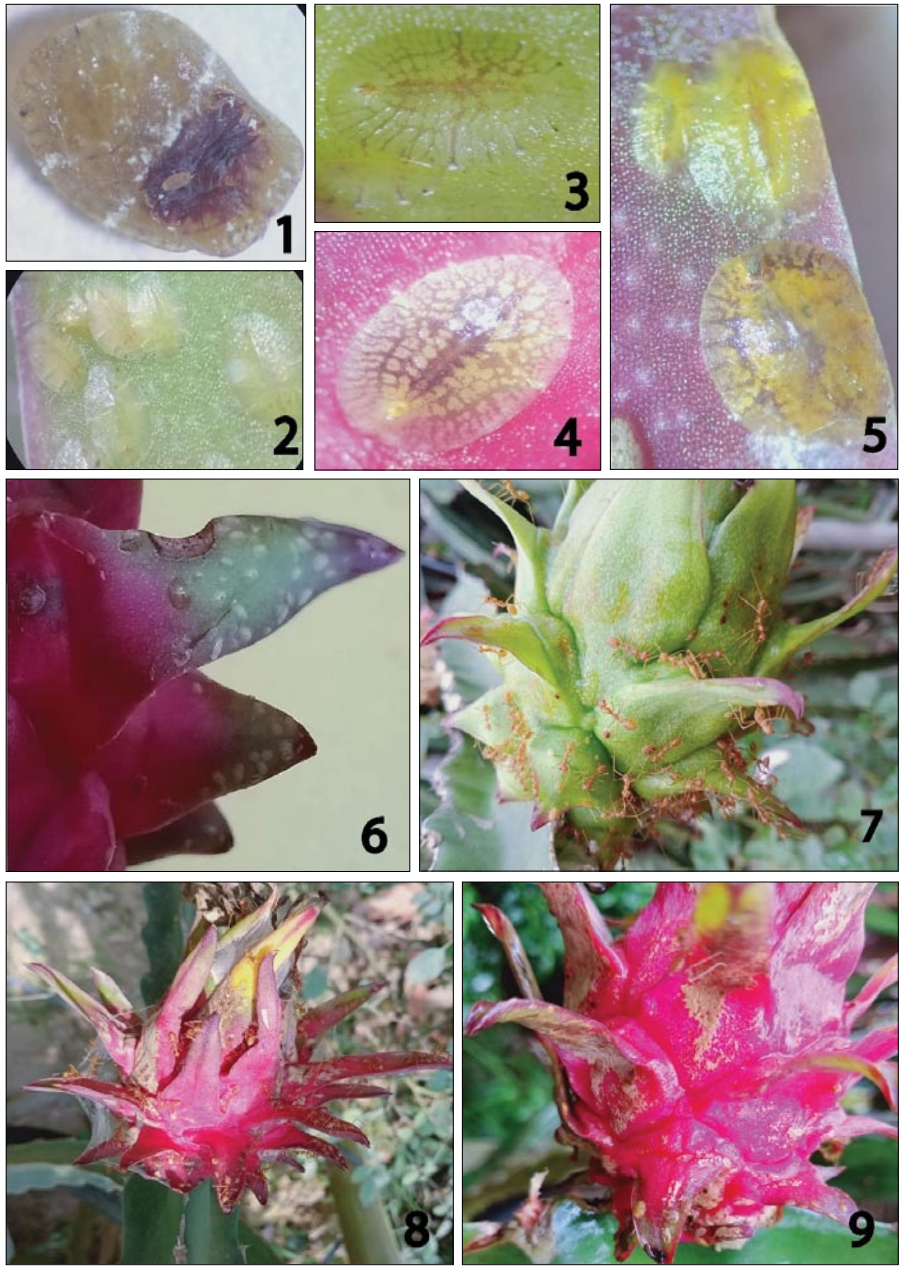

Fig. 1-9. *Coccus viridis* (Green) on dragon fruit; 1– Ventral part of female with crawler; 2 & 5 – Colony of scale; 3 – Nymph; 4 – Adult; 6 – Colony of scale on fruit spike; 7 – Scales in association with red ant on developing fruit; 8 & 9 – Damaged fruit

an unpleasant appearance. Ultimately, the infested fruits lost market quality as well as price. However, no fruit dropping was observed during the course of study.

Carrillo *et al*. (2021) reported the presence of soft scale insect, *Philephedra tuberculosa* Nakahara and Gill (Hemiptera: Coccidae) for the first time on dragon fruit in south Florida. They also reported a species of hard scale, *Diaspis echinocacti* (Bouche) (Hemiptera: Diaspididae) infesting the stems of pitaya or dragon fruit plants. However, there is no record of incidence of *C. viridis* on dragon fruit though the species is polyphagous. In India pests like mealy bugs, aphids and termites have been found damaging the dragon fruit, as reported by Nangare *et al*. (2020) and in the present study, the crop has been recorded as a new host of *Coccus viridis*.

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RESEARCH NOTE

Evaluation of different cereal and millet based media for large-scale production of entomopathogenic fungus, *Metarhizium anisopliae* (Metchnikoff) Sorokin

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ABSTRACT: Six different media consisting of rice, bajra, sorghum, maize, wheat, and foxtail millet, were assessed for their potential in the mass-scale cultivation of the entomopathogenic fungus, *Metarhizium anisopliae*. Among these grains, crushed rice with 1% yeast extract demonstrated the highest spore count (27.56 x 10^8 conidia/g), on the 25th day for the fungus. This was followed by crushed bajra with 1% yeast extract (22.89 x 10^8 conidia/g), crushed sorghum with 1% yeast extract (12.54 x 10^8 conidia/g), and crushed maize with 1% yeast extract (7.57 x 10^8 conidia/g). In contrast, Crushed fox tail millet with 1% yeast extract yielded the lowest conidial production on the 25th day (5.86 x 10^8 conidia/g). Conidial production showed an increasing trend from the 6th to the 25th day and remained relatively stable thereafter across all treatments. This study underscores the potential use of alternative nutrient sources derived from various agricultural products for the large-scale cultivation of entomopathogenic fungus *M. anisopliae*. Such findings hold promise for biopesticide development and sustainable pest control practices.

Keywords: Fungus, maize, millet, rice, sorghum, yeast

Entomopathogenic fungi are highly versatile biological control agents, owing to their broad host range, often leading to natural epizootics. One appealing aspect of these fungi is their mode of infectivity, primarily through contact and penetration (Nadeau *et al.,* 1996). This fungal group comprises a diverse assemblage of more than 100 genera, encompassing around 750 species known to infect various insect hosts. Many of these species hold significant promise in pest management strategies. Notably, key fungal pathogens in this context include *Metarhizium spp*., *Beauveria spp*., *Nomuraea rileyi*, *Verticillium lecanii*, and *Hirsutella* spp. The paramount requirement for promoting the widespread use of any microbial bioagent is the cost-effective production of inoculums suitable for field-level application. While Sabouraud's maltose agar with yeast extract (SMAY) is a globally accepted standard medium that facilitates maximum growth of *M. anisopliae*, it is associated with high production costs, necessitating the exploration of alternative mass multiplication media to reduce expenses. Numerous efforts have been undertaken to propagate the fungus using semi-synthetic media and solid substrates. Notably, *M. anisopliae* has been successfully mass-produced using crushed sorghum with the addition of 1% yeast extract (Mamta *et al.* 2011). Carrot and rice was identified as the most economical and well-suited media for the large-scale multiplication of the green muscardine fungus (Gopalakrishnan and Mohan, 2000). Recognizing the significance of producing inoculums at a competitive cost, various substrates have been explored for the mass multiplication of *M. anisopliae*, with the aim of achieving cost-efficiency and adaptability using locally available indigenous substrates.

The fungal isolate used in this study was obtained from Sugarcane breeding institute (SBI), Coimbatore, Tamil Nadu. The culture was originally isolated from field collected *M. anisopliae* infected white grub on sugarcane crops in experimental plot.

Mycelial discs of *M. anisopliae* were inoculated in SMAY broth supplemented with 1% yeast extract and incubated at 26ºC for 48 h with shaking at 180 rev min-1. The fungal spores were harvested in 25 ml of sterilized distilled water (SDW) containing 0.05% Tween 20 (Polyoxyethylene sorbitan monolaurate) and the spore count of this stock suspension was estimated with an improved Neubaur haemocytometer. The number of spores was adjusted to 1x10^8 spores/ml and this suspension was used as inoculums for further experiments.

Six food grains *viz.,* Sorghum, bajra, foxtail millet, maize, rice and wheat with 1 per cent yeast extract were assessed for their suitability as substrates for mass production of *M. anisopliae*. To each of food grains, sterile distilled water was added in order to bring the moisture content to50 per cent. After thorough mixing, the bottles were plugged with cotton and autoclaved at 15psi and 121°C for 30 minutes. Circular agar discs of

5 mm diameter were taken from the 8ht day old fungal culture grown on SMAY plates. One disc was inoculated to each bottle and mixed with it to disperse the inoculums. The bottles were incubated in BOD incubator at 25±1°C and RH of 85±1%. Four replications were maintained for each treatment. The spores were harvested from 6th day onwards at definite intervals of upto 25 days by sampling 1 g of the digested material. The spore suspension of each sample was made by dispersing the inoculums in 10ml sterile water blank with one drop of 0.02 per cent Tween-80, serially diluted and the spore count estimated using a haemocytometer. Different food grains were evaluated for their suitability to support the conidial production of the mycopathogens based on the time taken to initiate mycelial growth, sporulation and conidial yield at 6, 8, 15, 20 and 25 days after inoculation (DAI)

In general, the mycelial growth and conidial production exhibited an upward trend with an increase in the number of days after inoculation (DAI). By the 25th DAI, the mycelial growth almost entirely covered the surface of the media. Notably, the source of grain significantly influenced the conidial yield at all observed intervals. *M. anisopliae* displayed notably higher growth on crushed rice supplemented with 1% yeast extract, producing a remarkable (27.56 x 10^8 conidia per gram). This was followed by crushed bajra with 1% yeast extract, yielding 22.89 x 10^8 conidia per gram, and crushed sorghum with 1% yeast extract, which produced 12.54 x 10^8 conidia per gram. Crushed maize with 1% yeast extract showed a moderate yield (7.57 x 10^8 conidia per gram) followed by Crushed wheat with 1% yeast extract (6.18 x 10^8 conidia per gram). On the other hand, foxtail millet with 1% yeast extract was the least productive, generating (5.86 x 10^8 conidia per gram). The conidial production exhibited a notable increase (Table 1.) from the 6th to the 25th day, after which it remained relatively constant across all treatment groups, highlighting their limited suitability as substrates for fungal productivity. The peak conidial load was reached after 25 DAI across all tested treatments.

Table 1. Showing the conidial yield of *M. anisopliae* at 6th, 8th, 15th, 20th and 25th day intervals on different agricultural products

	Number of spores x 10^8/g (Days After Inoculation)				
Treatment	**6 DAI**	**8 DAI**	**15 DAI**	**20 DAI**	**25 DAI**
(Crushed rice +1% Yeast extract)	21.35	24.05	25.85	26.32	27.56
(Crushed bajra +1% Yeast extract)	14.25	16.66	18.86	20.26	22.89
(Crushed sorghum +1% Yeast extract)	8.37	9.75	10.26	11.66	12.54
(Crushed maize +1% Yeast extract)	3.66	4.06	5.15	6.79	7.57
(Crushed wheat +1% Yeast extract)	2.07	3.05	4.19	5.56	6.18
(Crushed fox tail millet +1% Yeast extract)	2.90	3.10	3.85	4.56	5.86

Grains are not only cost-effective and readily available but also serve as excellent substrates for the growth and multiplication of microorganisms on a large scale. They offer a substantial surface area and serve as nutritious media for mass-scale cultivation of various microorganisms. Rice, in particular, contains a higher proportion of starch and amylase. Starch hydrolysis in rice leads to the release of glucose and maltose, primarily depending on the enzymatic actions involved (Preen *et al.,* 1985). Maltose, generated through starch hydrolysis by enzymes present in the fungus, plays a crucial role in inducing sporulation, a process (Coudron *et al.,* 1985). Furthermore, rice has consistently demonstrated its suitability as a substrate for the rapid and efficient mass multiplication of *N. rileyi* (Gopalakrishnan and Mohan, 2000 and Lingappa *et al.,* 2002).

The results of the present study align with these findings, confirming the effectiveness of rice as a natural medium for mass multiplication. This natural medium can be applied in future assessments of mass-scale cultivation *M. anisopliae* and other related fungi with potential applications in biological control. To achieve cost-effectiveness and high concentrations of viable fungal spores and to make the most of the abundant agricultural waste produced in fields; this study proposes exploring alternative nutrient sources for mass-scale cultivation of *M. anisopliae*. The results obtained in this study suggest the viability of using various grains for the mass-scale cultivation of entomopathogenic fungi. Further work is required to optimize the media, particularly with regard to substrate moisture content, to enhance the process.

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CONTENTS 29 (2)

RESEARCH ARTICLES

Continued on back inside cover

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