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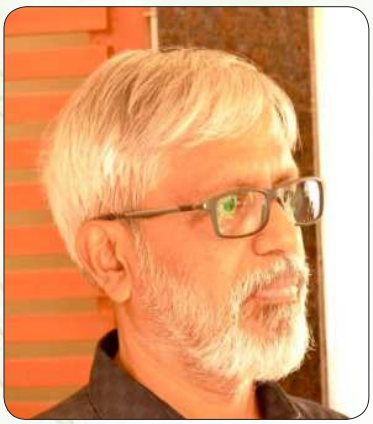

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Editor's Choice (Vol. 30)

PMHE @ 30

"30 Years….Old enough to look back and young enough to look forward"
-A wiseman's word

Three decades is certainly an important milestone in the progressive journey of an organisation or a publication. Hence we have every reason to be proud that our journal 'Pest Management in Horticultural Ecosystems (PMHE)' and its parent organisation 'Association for Advancement of Pest Management in Horticultural Ecosystems (AAPMHE)' have successfully completed 30 years of glorious journey and are moving ahead with more zeal and energy. Now 30th volume of PMHE is in your hands.

Conceptualized and executed way back in 1994 by the highly enthused and visionary Entomologists and Nematologists of IIHR that time, the journal PMHE had come a long way and today enjoys a wide patronage among plant protection fraternity across the country and overseas to. This would not have been possible but for the unstinted support and guidance of all Executive Council members of AAPMHE since its inception, the Editorial Advisory Board, researchers and scholars who had opted PMHE to publish their valuable research and reviewers.

PMHE has been a platform to eminent researchers as well as budding scholars to effectively disseminate their valuable research findings with high applied value in the field of pest management in horticultural crops. The journal has also been in forefront in reporting and spreading the awareness about the entry of serious invasive pests like *Tuta absoluta, Aleurodicus rugioperculatus*, *Spodoptera frugiperda*, *Thrips parvispinus* etc. The spectacular increase in the number of life members over the years reflects the growing patronization. Journal has made steady progress in terms of rating by NAAS to 5.14 and is being widely abstracted in all reputed Abstracting services. Aligning with the technological advancements and to achieve faster processing, journal has gone completely online shifting to the latest OJS platform.

The first Executive Council of AAPMHE with Dr. P. Parvatha Reddy as President deserves an applause for providing a strong foundation to the Society as well as the journal. At this juncture, I fondly recollect and acknowledge the unparalleled contributions of former Chief Editor, Dr. Abraham Verghese, who was instrumental in journal achieving phenomenal popularity and reach. The solid start given by the founder Chief Editor, Dr. P. L. Tandon is also highly appreciated and acknowledged. I place on record the constant support extended by the present Excuetive Counsil under the leadership of Dr. V. Sridhar that has immensly helped in PMHE's progress.

My special appreciation and thanks to Dr. S. Sriram, Dr. G. Sangeetha and Dr. M. C. Keerthi, Associate Editors and Ms. Jishna and Ms. Komala, Editorial Assistants for their consistent support and help in improving the quality and timely publishing of journal.

The valuable support from the Director and the Administration of ICAR-IIHR, crucial in the successful journey of AAPMHE is sincerely acknowledged. The financial assistance from the ICAR to publish the journal is gratefully acknowledged. The cooperation of M/s Shreya Printers and M/s Navbharat Enterprises (earlier printers) in DTP work and printing hard copies of the journal is gratefully appreciated. Wish PMHE reaches still greater heights with encouragement and support from all stakeholders of crop protection in Horticultural Ecosystems.

P. V. RAMI REDDY
Chief Editor

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REVIEW ARTICLE

Exploration of bioagents tolerant to agrochemicals for better management of plant diseases

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ABSTRACT: The exploration of bioagents that are compatible with agrochemicals is required for advancing sustainable agriculture by improving disease management while reducing reliance on chemical inputs. Integrating bioagents with agrochemicals offers several advantages, such as enhanced disease control, decreased agrochemical usage, and minimized environmental impact. Additionally, this approach mitigates resistance development in pathogens by providing a complementary layer of protection. Practical applications of this integration include the development of co-formulated products, optimization of application timing, and the use of advanced delivery systems to maintain bioagent efficacy. Successful integration relies on thorough monitoring, adherence to regulatory standards, and ongoing innovation in formulation and application strategies. Ultimately, this exploration contributes to more sustainable and resilient agricultural systems by synergizing the strengths of bioagents and agrochemicals.

Keywords: Biocontrol agents, compatibility, fungicide, pathogen, resistance and sustainable agriculture.

INTRODUCTION

Today, feeding the increasing population demands efficient and sustainable agricultural practices which is a challenging aspect. The farmers are relying heavily on synthetic agrochemicals to manage plant diseases and prevent losses. The extensive use of agrochemicals has led to emergence of resistant pathogens and has residual effect on environment and human health. In this context, biocontrol agents (BCAs) have an alternative to conventional fungicides. Although BCAs put forward potential for disease management, their effectiveness can be limited by uncontrollable environmental factors. Combining BCAs with agrochemicals is expected to reduce fungicide usage and minimize residues on harvested crops. This strategy not only lowers the amount of fungicide but also employs diverse modes of action to reduce pathogen resistance, thereby mitigating risk of resistance development in agriculture (Ons *et al.,* 2020).

Integration of BCAs with agrochemicals represents a significant advancement in modern agriculture, devised in modernizing disease management strategies while fostering environmental sustainability. With challenges such as fungicide resistance, environmental degradation, and need for efficient resource use, it is crucial to explore BCAs that can work synergistically with agrochemicals. BCAs provide complementary means for managing diseases. When combined effectively with agrochemicals, BCAs can enhance control efficacy, decrease reliance on chemical, and reduce negative environmental impacts and vital in optimizing disease management strategies and advancing integrated disease management (IDM) practices that balance productivity with ecological stewardship. Successful integration depends on the progress in developing innovative formulations, optimization of application methods, and adherence to regulatory standards, eventually leading to more sustainable and resilient agricultural systems (Wojtkowiak *et al.,* 2006; Ons *et al.,* 2020).

Importance and need

BCAs act with various modes of action to defend plants from various pathogens. They act indirectly through plant resistance induction or directly by parasitism, antibiosis, or competition for nutrients and space. Combining BCAs with fungicides has been shown to enable a reduction in dose or frequency of fungicide applications, contributing to more sustainable agricultural practices and supporting marketing strategies that emphasize low or zero-residue produce.

BCAs must be compatible with fungicides, as fungicides can potentially inhibit their growth. When incompatibility is there, application strategies such as temporal separation, alternation, or spatial separation can be employed, though such separation is challenging for antagonists that directly target pathogens. Research should be precisely focus on upgrading fungicide

resistant antagonists. Common biological resistance inducers combined with fungicides include *Trichoderma* sp. and *Bacillus* sp., offer long-lasting systemic effects that broaden disease control. While there is public concern about chemicals, those classified as Generally Recognized As Safe (GRAS) and chemical inducers of resistance are usually non-toxic to humans and the environment. Additionally, BCAs with long shelf-lives and stability offer significant advantages for supply chain and stock management.

BCAs compatible with agrochemicals

Understanding compatibility between BCAs and agrochemicals is essential in upgrading BCAs and integrate them in disease management. Combining of BCAs in field depends on how agrochemicals are employed for other diseases.

Trichoderma along with fludioxonil extensively improved control of *Fusarium* sp. and increased survival rates of coneflower seedlings in greenhouses (Wang *et al.*, 2005). Mancozeb was found extremely effectual in reducing *F. solani* mycelial growth and companionable with *T. harzianum* and *T. viride* at 0.05% and 0.1% concentrations (Singh and Varma, 2005). Integrating *T. asperellum* T8a with a low dose of captan provided greater *in vitro* growth inhibition of *Colletotrichum gloeosporioides* in mango (Peláez-Álvarez *et al*., 2016). Combining fungicides with BCAs like *Trichoderma* spp. can produce synergistic effects. Terrero *et al.* (2018) verified compatibility of *Trichoderma* spp. with azoxystrobin and copper hydroxide fungicides. Ruano *et al.* (2018) applied *Trichoderma* spp. with fluazinam to control *Rosellinia necatrix* in avocado and improved root rot control. Palmieri *et al.* (2022) showed combining *Papiliotrema terrestris* and *B. subtilis* with synthetic fungicides resulted in 95.5–97% control in field and 63–91% in postharvest, with zero fungicide residues in fruit.

T. reesei C2A and *T. harzianum* with mancozeb improved mycoparasitic activity against *F. oxysporum*(González *et al.*, 2020; Huilgol *et al.*, 2022). Similarly, *T. asperellum* growth was supported by COC and mancozeb at 500 ppm (Maheshwary *et al.*, 2020). Tolerance of *Trichoderma* strains to agrochemicals is attributed to a variety of factors, including changes in oxidoreductase and ABC transporter genes, contributing to resistance against dichlorvos, mancozeb, thiram, tebuconazole, and carbendazim (Hirpara *et al.,* 2018; Sun *et al.,* 2019; Hu *et al.,* 2016). Compatibility of *Trichoderma*to fungicides was attributed to its membrane pumps and detoxification mechanisms (Ruocco *et al.*, 2009).

Rhodotorula mucilaginosa (Lv316) compatible with carbendazim, dimethomorph, mandipropamid, and azoxystrobin reduced disease incidence (Uribe-Gutierrez *et al.,* 2022). *R. mucilaginosa* showed no sensitivity to dimethomorph and mandipropamidthat target cellulose synthesis in oomycete membranes, affecting cell wall structure and spore germination. Mandipropamid may inhibit cellulose synthase-like PiCesA3 (Blum *et al.,* 2010). As yeast with chitin-based cell walls, *R. mucilaginosa* is not impacted by these fungicides (Bahmed *et al.,* 2003). Notably, *R. mucilaginosa* Lv316 exhibited high compatibility with carbendazim, which disrupts spindle formation during cell division in fungi (Yang *et al.,* 2011), and possibly will also restrain respiratory and fermentative metabolism in yeasts (Chiba *et al*., 1987). Combining *Pseudomonas fluorescens* 1, with azoxystrobin was more effective against of *Botrytis cinerea*, *Colletotrichum capsici*, and *Leveillula taurica*. *P. fluorescens*-16 showed compatibility with propiconazole, tebuconazole, trifloxystrobin + tebuconazole, azoxystrobin, carbendazim, and carbendazim + mancozeb, based on its growth. It was rated as good at 100 ppm concentration and was highly compatible with both azoxystrobin and carbendazim + mancozeb combination (Anand *et al.* 2010).

Carbendazim tolerant *Trichoderma harzianum* formulations were developed and tested effectively the management of groundnut root rot (Jalali *et al.* 2012). In Haryana, India, combining *Pseudomonas fluorescens*, *Mesorhizobium cicero*, and *Trichoderma harzianum* with carboxin and thiram resulted in reduced wilt incidence along with highest seed germination, increased grain yield in chickpea (Dubey *et al.,* 2015). In rice field, combination of *T. harzianum*, *P. fluorescens*, and carbendazim was found to be more effective against *Magnaporthe oryzae* than individual applications (Jambhulkar *et al.,* 2018). Additionally, *Piriformospora indica*, a root endophytic fungus, not only suppresses *Colletotrichum gloeosporioides* but also promotes plant growth and was compatible with strobilurins, triazoles, carbendazim, and pencycuron. Especially, germination of chlamydospores was significantly higher with these fungicides (Amrutha *et al.,* 2024). The more examples are mentioned in the Table1.

Copper induced Resistance in BCA

Bacillus subtilis applied with copper hydroxide (HCu) as part of an integrated strategy for citrus canker with active ingredients like cupric compounds shows potential consequences. Specifically, alternating applications of *B. subtilis* QST 713 with HCu

significantly decreased incidence and severity of disease (Ibrahim *et al.,* 2016). In field, foliar applications of HCu achieved highest reduction in citrus canker incidence. A new copper sulfate formulation, Bioactive Copper (BioCu) includes amino acids and peptides and contains about 19% Cu (BAYER, 2016). The combination of *B. subtilis* and BioCu gave a 76% reduction in citrus canker under low disease incidence and up to 21.8% reduction under high disease incidence. Moreover, products with amino acids in their formulations have been shown to positively impact citrus tree height, leaf area, and both fresh and dry leaf mass (Mustafa and El-Shazly, 2015). Copper-based products can also trigger defense response in plants through post-formed biochemical factors. Treatment with BioCu, drastically amplified the *PR-2* gene 24 hours after application, with this elevated expression persisting for at least 7 days. This enhanced and prolonged gene activation in citrus trees treated with BioCu, compared to standard copper formulations, may be attributed to presence of complexing agents and amino acids in BioCu's formulation (Ramos *et al.,* 2022).

Table 1. List of compatible BCA with the agrochemicals

Biocontrol agent	Compatible agrochemical	Target disease	Reference
Fusarium oxysporum strain CS-20	Mefenoxam and mefenoxam + copper	Fusarium wilt of watermelon	Fravel *et al.* (2005)
Bacillus subtilis	Difenoconazole	Maydis leaf blight of corn (*Bipolaris maydis*)	Djaenuddin *et al.* (2021)
B. methylotrophicus TA-1	Fluopimomide	Gray mold in tomato	Ji *et al.* (2019)
F. solani	Thiophanate-methyl, fenhexamid, cyprodinil, boscalid and mancozeb	Fusarium crown and root rot disease in tomato.	Malandrakis *et al.* (2018)
Trichoderma asperellum	Mancozeb, Azoxystrobin, Cymoxinil+Mancozeb, Metalxyl+Mancozeb	*Pythium aphanidermatum, Pythium debaryanum, Sclerotium rolfsii* Sr1, *Sclerotium rolfsii* Sr3, *Fusarium oxysporum* f.sp. *lycopersici* and *Alternaria solani*	Manjunath *et al.* (2017)
Trichoderma spp.	Azoxystrobin and copper hydroxide	*Fusarium solani*	Terrero *et al.* (2018)
Trichoderma spp.	Fluazinam	*Rosellinia necatrix* in avocado	Ruano *et al.* (2018)
B. subtilis	Bioactive Copper (BioCu)	Citrus canker	Mustafa and El-Shazly, (2015)
T. aggressivum f. *europaeum*	Kresoxim-methyl, Pencycuron and Cymoxanil		Sánchez-Montesinos *et al.* (2021)
T. asperellum	Captan and Mancozeb	*F.solani*	Parraguirre Lezama *et al.* (2023)
T. asperelloides	Fosetyl Al, Amisulbrom and Cyflufenamid	Downy mildew and powdery mildew in grapes	Saha *et al.* (2023)

Piriformospora indica (Endophyte)	Strobilurins, strobilurins and triazoles, carbendazim and pencycuron	*Colletotrichum gloeospoiodes* causing anthracnos in yard long bean	Amrutha *et al.* (2024)
P. fluorescens 1	Propiconazole, tebuconazole, trifloxystrobin + tebuconazole, azoxystrobin, carbendazim, carbendazim + mancozeb	*Botrytis cinerea*, *Colletotrichum capsici*, and *Leveillula taurica*	Anand *et al.* (2010)
T. asperellum T8a	Captan	*Colletotrichum gloeosporioides* in mango	Peláez-Álvarez *et al*. (2016).
Rhodotorula mucilaginosa (Lv316)	Carbendazim, dimethomorph, mandipropamid, and azoxystrobin	Root rot	Uribe-Gutierrez *et al.* (2022)
B. subtilis QST 713	HCu	Citrus canker	Ibrahim *et al.* (2016)
Rhodosporidium kratochvilovae (Yeast)	Boscalid or cyprodinil	Blue mold caused by *Penicillium expansum*	Lima *et al.* (2011)
T. virens	Thiophanate-methyl	*Fusarium solani* and *Fusarium oxysporum* in dry bean	Abd-El-Khair *et al.* (2019)
B. megaterium	Carbendazim	*F. oxysporum* in tomato	Omar *et al.* (2006)
B. subtilis	Azoxystrobin	*Podosphaera xanthii* causing *Powdery mildew* on *zucchini*	Gilardi *et al.* (2008)
Combination of *P. fluorescens*, *Mesorhizobium cicero* and *T. harzianum*	Carboxin and thiram	*F. oxysporum* in chickpea	Dubey *et al.* (2015)
Clonostachys rosea	Prothioconazole	*F. graminearum and F. culmorum* in wheat and barley	Bengtsson, (2020)
T. asperellum	Copper oxychloride, Cymoxanil +Mancozeb, Mefenoxam+ Mancozeb and Cymoxanil +Famoxadone	Collar rot of elephant foot yam, tuber rot of cassava, stem and root rot of cassava, yam anthracnose and taro leaf blight	Veena *et al.* (2022)
B. subtilis	Tebuconazole	Rice false smut	Liu *et al.* (2023)
Clonostachys rosea	Fluxapyroxad and fluopyram a succinate dehydrogenase inhibitors (SDHI)	Tomato gray mold	Song *et al.* (2022)

Trichoderma asperellum SC012	Hymexazol	Fusarium wilt in cowpea	Zhang *et al.* (2021)
Yeasts and *Lactobacillus*	Mancozeb and Ridomil gold	Mango anthracnose caused by *Colletotrichum gloeosporoides*	Fenta and Kibret. (2021)
Trichoderma harzianum	Carbendazim	Fusarium wilt in carnation and marigold	Kumawat *et al.* (2019)

Multidrug resistant concept

Exploiting multidrug resistance (MDR) concept in BCAs provides a tactical improvement in IDM. By engineering or selecting BCAs with MDR traits, these agents be able to carry on and function effectively despite presence of multiple pesticides, fungicides, or herbicides. This competence helps in reliable disease control, even in face of chemical use that cab otherwise weaken efficiency. The MDR approach not only reduces need for excessive chemical use, promoting more sustainable agricultural practices, but also aids in managing resistance development in pathogens. By maintaining their biocontrol functions in chemically intensive environments, MDR BCAs contribute to more resilient and effective disease management systems, aligning with both productivity and environmental stewardship goals.

Techniques to develop agrochemical compatible BCA

Natural selection and breeding

The natural selection and breeding are important strategies in developing agrochemical compatible BCAs. Through the isolation and screening of naturally occurring strains with agrochemical exposure, identification of BCAs with inherent resistance traits can be exploited. The continuing exposure to agrochemicals in controlled surroundings promotes progression of tolerance, enabling assortment of robust isolates; however, once the selection pressure is withdrawn the tolerance to fungicide may come down. Hybridization of these robust isolates with other effective ones combines enviable traits, such as high biocontrol efficacy with agrochemical resistance. Recurrent selection and backcrossing improve these hybrids, escalating constancy and performance. Field trials make lawful effectiveness of these bred BCAs, ensuring compatibility with agrochemicals and their overall role in disease control.

Mutagenesis

The BCAs improvement for *Trichoderma* sp. holds substantial potential, with prime focus on developing fungicide tolerant mutants, along with improved hydrolytic enzyme production. Developing new molecules needs huge investment and modern agriculture will more and more depends on BCA. With climate change, there is increasing need for microbial pesticides that withstands abiotic stresses with improved biocontrol abilities. While genetic engineering offer opportunity of creating novel strains and likelihood of these GMOs overcome regulatory barriers and being approved for field use remains low. As a result, mutation will hold decisive role in developing superior strains of *Trichoderma* and facilitating their approval as feasible alternative to chemicals.

Several *Trichoderma* strains have been developed using mutagenesis to boost biocontrol properties. UV ray mutagenesis improved *Trichoderma* sp. mutants to carbendazim tolerance which strongly inhibits *Trichoderma* with remarkable variations compared to wild-type strains in appearance, growth habits, soil survival, antibiosis, and disease control efficacy (Papavizas and Lewis, 1983). *Trichoderma* sp. exposed to nitrosoguanidine and mutants selected on benomyl with superior rhizosphere colonization and biocontrol potential (Ahmad and Baker, 1988). Through a two-step mutagenesis progression through UV and gamma radiation, Mukherjee *et al.* (1999) developed stable benomyl tolerant mutants of *T. pseudokoningii* having better biocontrol capabilities compared to wild ones. Through gamma-ray, benomyl-resistant mutants with enhanced mycoparasitic activity of *T. virens* with distinct colony morphology, increased production of secondary metabolites such as the antimicrobial viridin, and improved disease control potential was done (Olejnikova *et al.* (2010).

Genome shuffling (GS)

Genome shuffling (GS) is a noteworthy progression in combinatorial engineering, first introduced by Stemmer group in 2002. This method includes *in vitro* homologous recombination of pool of preferred mutant genes through random fragmentation (Zhang *et al.,* 2002). Over traditional methods like mutagenesis and protoplast fusion, GS gives better competence for phenotypic improvement. The GS has accelerated strain upgrading processes through the recursive protoplast fusion between multiple parent strains, providing more hybrid strains. This approach allows for integration of advantageous traits from multiple parents, to achieve desired one in shorter period. Remarkably, two rounds of GS can bring about results that formerly necessary up to 20 years *via* classical improvement methods (Zhang *et al.,* 2002; Gong *et al.,* 2009).GS is multipurpose and not constrained to microbes with well characterized genetic backgrounds. It is a cost effective method without expensive facilities; a round of GS is comparable in cost to a cycle of protoplast fusion. The process is fairly uncomplicated and can be employed in most laboratories, which rely on protoplast fusion without classified as genetically modified (Zhang *et al.,* 2002). This peculiarity enables it to keep away from public concern frequently allied with GMOs (Gong *et al.,* 2009; Côrtes *et al.,* 2021).

Genome editing

CRISPR-Cas technology offers an influential tool for enhancing efficiency of BCAs by editing their genomes to get better resistance to specific fungicides. By employing CRISPR-Cas technology, researchers can bring in or boost resistance mechanisms within BCAs. Genome editing can be used to modify BCAs' metabolic pathways, allowing them to evade or counterbalance the fungicide's effects. CRISPR-Cas allows for defined genome editing, ensuring modifications are precise and do not upset other critical genes affecting BCAs role or compatibility with fungicides. Additionally, gene editing can design and construct synthetic metabolic pathways within BCAs to augment their ability to degrade or resist agrochemicals. The use of CRISPR in developing BCAs is subject to regulatory inquiry. Developing BCAs with resistance to fungicides must be cautiously managed to keep away from the potential for resistance development in pathogens as well.

Exploration of fungicide resistance mechanism

Fungicides are requisite for high value crops. Resistance to site specific fungicides has predominantly been associated to target and non-target site mechanisms. These mechanisms change the structure or expression, disturbing fungicide efficiency and resulting in diverse and varying resistance levels exploited in developing agrochemical compatible BCAs.

Alterations of target site

Mutations correspond to abrupt inheritable changes in DNA, impacting an organism's response to fungicides. Non-synonymous mutations, outcomes in amino acid substitutions at target site of a fungicide, repeatedly lessen binding affinity of fungicide to its target enzyme. The genetic mutations are decisive in development of fungicide resistance. Resistance to site specific fungicides like Quinone Outside Inhibitors (QoIs) can come up from a single point mutation in gene encoding target enzyme, imparts high-level resistance. QoI disrupts ATP production through disturbing electron transfer system, leading to demise of susceptible ones. However, mutations in cyt b alter binding site, preventing QoI from attaching to target protein and allows ATP synthesis in resistant ones (di Rago *et al.,* 1989; Chopra *et al.,* 2003).

On contrary, quantitative resistance have multiple genes providing resistance and typically develops progressively over time. This can occur with multisite fungicides or some single site fungicides. Fungi may develop quantitative resistance to demethylation inhibitors (DMIs) due to mutations in CYP51 gene, enhanced expression of target site with altered efflux pump action, or improved detoxification of fungicides (Hawkins and Fraaije, 2018). Reports frequently highlight point mutations at target sites linked to fungicide resistance. Organizations like Fungicide Resistance Action Committee (FRAC) and European and Mediterranean Plant Protection Organization (EPPO) make available comprehensive information on pathogen resistance to various fungicide classes, focusing chiefly on point mutations linked with QoIs, MBCs, DMIs, and SDHIs.

Target site overexpression

Resistance to DMIs and MBCs (methyl benzimidazole carbamates), is commonly allied with overexpression of target genes. For DMIs, resistance mechanisms

frequently entail with enhanced expression of sterol 14 alpha-demethylase gene CYP51. In *Zymoseptoria tritici*, high levels of resistance were associated with insertions in CYP51 gene promoter region (Lucas *et al.,* 2015), in *Cercospora beticola* of beet and *Monilinia fructicola* of peaches, attributed to overexpression of CYP51 gene (Luo and Schnabel, 2008) while in *Pyrenophora teres* on oats, specific mutation (F489L) in CYP51A gene provides overexpression and gives resistance to DMIs. This overexpression subjected to genetic rearrangements or mutations in promoter region. *Mycosphaerella fijiensis* in bananas comprises parallel resistance patterns with changes in PfCYP51 gene promoter region show the way to heightened DMI resistance (Leroux *et al.,* 2007). This mechanism of overexpression observed in *Aspergillus flavus*, *A. niger*, *A. parasiticus*, and *Pyricularia oryzae* (Yan *et al.,* 2011; Fan *et al.,* 2013) but *C. gloeosporioides* shows DMI resistance through both mutations and overexpression of CYP51. In MBCs, resistance in *C. acutatum* in grapes is associated with overexpression of β-tubulin gene CaTUB1, regulated by CaBEN1 (Wei *et al.,* 2020; Sun *et al.,* 2013; Nakaune and Nakano, 2007). Overexpression of target genes remains a critical factor in developing resistance mechanisms against various fungicide classes.

Non-target site mechanisms

These mechanisms allow fungi to resist fungicides without changing their interaction with target site. Key non-target site resistance mechanisms comprise drug efflux transporters, CYP51 paralogs, mitochondrial heteroplasmy, alternative respiration pathways, altered sterol metabolism, detoxification processes, stress response regulation, quantitative resistance effects, and transcription factors (TFs). Resistance to DMIs is predominantly diverse and often associated with a variety of non-target site mechanisms. Key non-target site mechanisms comprise overexpression of drug efflux pumps, improved detoxification, and CYP51 paralogs existence. A noteworthy terror with non-target site resistance is its ability to award cross-resistance to a variety of fungicides with varied modes of action. This occurs because resistance mechanism affected fungicide detoxification or transport pathways rather than fungicide's target protein. Thus, non-target site resistance might comprise a negligible impact on fungal fitness, allowing resistant populations to persist and spread even in absence of fungicides. This poses a confront for fungicide management and underscores necessitate for developing efficient IDM strategies (Dorigan *et al.,* 2023).

In developing agrochemical companionable BCAs, understanding non-target site resistance mechanisms might leveraged to add on efficacy and robustness of BCAs. By targeting or bypassing these resistance mechanisms, researchers can design BCAs that remain effective against resistant fungal populations. This approach not only improves disease control but also integrates BCAs more effectively into offered agricultural practices, contributing to IDM and reduced reliance on chemical fungicides.

CONCLUSION

In conclusion, looking at compatible BCAs with agrochemicals is imperative in advancing modern agriculture. As reliance on synthetic agrochemicals intensifies, the associated risks of resistance development, environmental impact, and human health concerns highlights urge for alternative or supplementary management strategies. Effectual integration requires an inclusive understanding of interactions between BCAs and agrochemicals to optimize their joint effectiveness while preventing downbeat impacts on BCAs performance. By exploring and developing compatible BCAs, expansion in disease management systems can be seen, delays resistance development, and promote sustainable agricultural practices. The research should also further focus on fungicide resistant antagonists and their bypass mechanism to exploit it in various ways. The Central Insecticide Board (CIB) and FRAC have to look into the concept and frame guidelines for the proper implementation. Thus, compatibility of BCAs with agrochemicals will help in IDM.

REFERENCES

Abd-El-Khair, H., Elshahawy, I. E. and Haggag, H. E. K. 2019. Field application of *Trichoderma* spp. combined with thiophanate-methyl for controlling *Fusarium solani* and *Fusarium oxysporum* in dry bean. *Bulletin of the National Research Centre*, **43:** 19.

Ahmad, J. S. and Baker, R. 1988. Rhizosphere competence of benomyl-tolerant mutants of *Trichoderma* spp. *Canadian Journal of Microbiology*, **34**(5): 694-696.

Amrutha, P., Jojy, E. T., Johnson, J. M., Sarada, S., Sajeena, A., Radhakrishnan, N. V. and Alex, S. 2024. Compatibility of fungal root endophyte *Piriformospora indica* with new generation fungicides. *International Journal of Plant & Soil Science*, **36**(6): 709–719.

Anand, T., Chandrasekaran, A., Kuttalam, S., Senthilraja, G. and Samiyappan, R. 2010. Integrated control of fruit rot and powdery mildew of chilli using the biocontrol agent *Pseudomonas fluorescens* and a chemical fungicide. *Biological Control*, **52**: 1–7.

Bahmed, K., Quile's, F., Bonaly, R. and Coulon, J. 2003. Fluorescence and infrared spectrometric study of cell walls from Candida, Kluyveromyces, Rhodotorula and Schizosaccharomyces yeasts in relation with their chemical composition. *Biomacromolecules*, **4**: 1763–1772.

BAYER. 2016. Dados técnicossobreBayfolanCobre. Enxoval de lançamento. 1–63.

Bengtsson, S. 2020. Combining biocontrol fungus *Clonostachys rosea* with chemical fungicides for integrated management of Fusarium foot/root rot.

Blum, M., Boehler, M., Randall, E., Young, V., Csukai, M., Kraus, S., Moulin, F., Scalliet, G., Avrova, A. O., Whisson, S. C. and Fonne-Pfister, R. 2010. Mandipropamid targets the cellulose synthase-like PiCesA3 to inhibit cell wall biosynthesis in the oomycete plant pathogen, *Phytophthora infestans*. *Molecular Plant Pathology*, **11:** 227–243.

Chiba, M., Bown, A. W. and Danic, D. 1987. Inhibition of yeast respiration and fermentation by benomyl, carbendazim, isocyanates, and other fungicidal chemicals. *Canadian Journal of Microbiology*, **33**: 157–161.

Chopra, I., O'Neill, A. J. and Miller, K. 2003. The role of mutators in the emergence of antibiotic-resistant bacteria. *Drug Resistance Updates*, **6:** 137–145. https://doi.org/10.1016/S1368-7646(03)00024-8

Côrtes, M. V. D. C. B., de Sousa Oliveira, M. I., Mateus, J. R., Seldin, L., Silva-Lobo, V. L. and Freire, D. M. G. 2021. A pipeline for the genetic improvement of a biological control agent enhances its potential for controlling soil-borne plant pathogens. *Biological Control*, **152:** 104460.

di Rago, J. P., Coppee, J. Y. and Colson, A. M. 1989. Molecular basis for resistance to myxothiazol, mucidin (strobilurin A), and stigmatellin: Cytochrome b inhibitors acting at the center of the mitochondrial ubiquinol-cytochrome c reductase in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry*, **264:** 14543–14548.

Djaenuddin, N., Sebayang, A., Nonci, N. and Muis, A. 2021. Compatibility of biocontrol agent formulas and synthetic fungicides in controlling maydis leaf blight on corn caused by *Bipolaris maydis*. In *IOP Conference Series: Earth and Environmental Science* (Vol. 911, No. 1, p. 012062). IOP Publishing.

Dorigan, A. F., Moreira, S. I., da Silva Costa Guimarães, S., Cruz□Magalhães, V. and Alves, E. 2023. Target and non□target site mechanisms of fungicide resistance and their implications for the management of crop pathogens. *Pest Management Science*, **79**(12): 4731-4753. https://doi.org/10.1002/ps.7408

Dubey, S. C., Singh, V., Priyanka, K., Upadhyay, B. K. and Singh, B. 2015. Combined application of fungal and bacterial bio-agents, together with fungicide and *Mesorhizobium* for integrated management of Fusarium wilt of chickpea. *BioControl*, **60**: 413–424.

Fan, J., Urban, M., Parker, J. E., Brewer, H. C., Kelly, S. L., Hammond-Kosack, K. E. 2013. Characterization of the sterol 14alpha-demethylases of *Fusarium graminearum* identifies a novel genus-specific CYP51 function. *New Phytologist*, 198: 821–835. https://doi.org/10.1111/nph.12123

Fenta, L. and Kibret, M. 2021. Fungicide tolerance of antagonists in the management of mango anthracnose caused by *Colletotrichum gloeosporoides*. *Journal of Biological Control*, **35**(4): 209–217.

Fravel, D. R., Deahl, K. L. and Stommel, J. R. 2005. Compatibility of the biocontrol fungus *Fusarium oxysporum* strain CS-20 with selected fungicides. *Biological Control*, **34**(2): 165–169.

Gilardi, G., Manker, D. C., Garibaldi, A. and Gullino, M. L. 2008. Efficacy of the biocontrol agents *Bacillus subtilis* and *Ampelomycesquisqualis* applied in combination with fungicides against powdery mildew of zucchini. *Journal of Plant Disease and Protection*, **115**: 208–213.

Gong, J., Zheng, H., Wu, Z., Chen, T. and Zhao, X. 2009. Genome shuffling: progress and applications for phenotype improvement. *Biotechnology Advances*, **27**(6): 996–1005.

Gonzalez, M. F., Magdama, F., Galarza, L., Sosa, D. and Romero, C. 2020. Evaluation of the sensitivity and synergistic effect of Trichoderma reesei and

Mancozeb to inhibit under in vitro conditions the growth of *Fusarium oxysporum*. *Communications in Integrative Biology*, **13:** 160–169.

Hawkins, N. J. and Fraaije, B. A. 2018. Fitness penalties in the evolution of fungicide resistance. *Annual Review of Phytopathology*, 56: 16.11–16.22. https://doi.org/10.1146/annurev-phyto-080417-050433

Hirpara, D. G. and Gajera, H. P. 2018. Molecular heterozygosity and genetic exploitations of *Trichoderma interfusants* enhancing tolerance to fungicides and mycoparasitism against *Sclerotium rolfsii*Sacc. *Infection, Genetics and Evolution*, **66:** 26–36.

Hu, X., Roberts, D. P., Xie, L., Yu, C., Li, Y., Qin, L. and Liao, X. 2016. Use of formulated *Trichoderma* sp. Tri-1 in combination with reduced rates of chemical pesticide for control of *Sclerotinia sclerotiorium* on oilseed rape. *Crop Protection*, **79:** 124–127.

Huilgol, S. N., Pratibha, M. P., Hegde, G. M. and Banu, H. 2022. Evaluation and compatibility of new fungicides with *Trichoderma harzianum* for managing the charcoal rot of soybean. *Pharma Innovation Journal*, **11:** 659–664.

Ibrahim, Y. E., Saleh, A. A., El Komy, M. H. and Al Saleh, M. A. 2016. *Bacillus subtilis* QST 713, copper hydroxide, and their tank mixes for control of bacterial citrus canker in Saudi Arabia. *Journal of Citrus Pathology*, **3**(1).

Jalali, S. K., Poorani, J., Murthy, K.S., Sriram, S., Desai, S., Verghese, A. 2014. Final report of NAIP sub-project 'Effect of abiotic stress on the natural enemies of crop pests: *Trichogramma*, *Chrysoperla*, *Trichoderma* and *Pseudomonas*, and mechanism of tolerance to these stresses', Project No. 4163.National Bureau of Agriculturally Important Insects, Bengaluru, India. p.83.

Jambhulkar, P. P., Sharma, P., Manokaran, R., Lakshman, D. K., Rokadia, P. and Jambhulkar, N. 2018. Assessing synergism of combined applications of *Trichoderma harzianum* and *Pseudomonas fluorescens* to control blast and bacterial leaf blight of rice. *European Journal of Plant Pathology*, **152:** 747–757.

Ji, X., Li, J., Meng, Z., Zhang, S., Dong, B. and Qiao, K. 2019. Synergistic effect of combined application of a new fungicide fluopimomide with a biocontrol agent *Bacillus methylotrophicus*TA-1 for management of gray mold in tomato. *Plant Disease*, **103**(8): 1991–1997.

Kumawat, M.K., Chandran, N.K. and Sriram, S. 2019. Evaluation of carbendazim tolerant strain of *Trichoderma harzianum* GJ16B for its efficacy in the management of carbendazim resistant *Fusarium solani* isolate causing marigold wilt. *Indian Phytopathology,* **72**(4): 675-680.

Leroux, P., Albertini, C., Gautier, A., Gredt, M. and Walker, A. S. 2007. Mutations in the CYP51 gene correlated with changes in sensitivity to sterol 14 alpha-demethylation inhibitors in field isolates of *Mycosphaerellagraminicola*. *Pest Management Science*, 63: 688–698. https://doi.org/10.1002/ps.1371

Liu, L., Zhao, K., Cai, L., Zhang, Y., Fu, Q. and Huang, S. 2023. Combination effects of tebuconazole with *Bacillus subtilis* to control rice false smut and the related synergistic mechanism. *Pest Management Science*, **79**(1): 234–243.

Lima, G., Castoria, R., De Curtis, F., Raiola, A., Ritieni, A. and De Cicco, V. 2011. Integrated control of blue mould using new fungicides and biocontrol yeasts lowers levels of fungicide residues and patulin contamination in apples. *Postharvest Biology and Technology*, **60**(2): 164–172.

Lucas, J. A., Hawkins, N. J. and Fraaije, B. A. 2015. The evolution of fungicide resistance. Advances in Applied Microbiology, 90: 29–92. https://doi.org/10.1016/bs.aambs.2015.01.002

Luo, C. and Schnabel, G. 2008. The cytochrome P450 Lanosterol 14-demethylase gene is a demethylation inhibitor fungicide resistance determinant in *Moniliniafructicola* field isolates from Georgia. *Applied and Environmental Microbiology*, 74: 359–366. https://doi.org/10.1128/AEM.02196-07

Maheshwary, N., Gangadhara-Naik, B., Amoghavarsha-Chittaragi, M., Naik, S. K. and Nandish, M. 2020. Compatibility of *Trichoderma asperellum* with fungicides. *Pharma Innovation Journal*, **9**: 136–140.

Malandrakis, A., Daskalaki, E. R., Skiada, V., Papadopoulou, K. K. and Kavroulakis, N. 2018. A *Fusarium solani* endophyte vs fungicides: Compatibility in a *Fusarium oxysporum* f. sp. *radicis-lycopersici*–tomato pathosystem. *Fungal Biology*, **122**(12): 1215–1221.

Manjunath, M., Singh, A., Tripathi, A. N., Prasanna, R., Rai, A. B. and Singh, B. 2017. Bioprospecting the fungicides compatible *Trichoderma asperellum* isolate effective against multiple plant pathogens in vitro. *Journal of Environmental Biology*, **38**(4): 553.

Mukherjee, P. K., Sherkhane, P. D. and Murthy, N. B. K. 1999. Induction of stable benomyl-tolerant phenotypic mutants of *Trichoderma pseudokoningii* MTCC 3011, and their evaluation for antagonistic and biocontrol potential.

Mustafa, N. S. and El-Shazly, S. M. 2015. Impact of some bio stimulant substances on growth performance of Washington navel orange trees. *Acta Horticulturae*, 1065: 1795–1800.

Nakaune, R. and Nakano, M. 2007. Benomyl resistance of *Colletotrichum acutatum* is caused by enhanced expression of β-tubulin 1 gene regulated by putative leucine zipper protein CaBEN1. *Fungal Genetics and Biology*, 44: 1324–1335. https://doi.org/10.1016/j.fgb.2007.04.002

Olejníková, P., Ondrušová, Z., Kryštofová, S. and Hudecová, D. 2010. Benomyl-resistant mutant strain of Trichoderma sp. with increased mycoparasitic activity. *Folia Microbiologica*, **55:** 102–104.

Omar, I., O'Neill, T. M. and Rossall, S. 2006. Biological control of Fusarium crown and root rot of tomato with antagonistic bacteria and integrated control when combined with the fungicide carbendazim. *Plant Pathology*, **55**: 92–99.

Ons, L., Bylemans, D., Thevissen, K. and Cammue, B. P. 2020. Combining biocontrol agents with chemical fungicides for integrated plant fungal disease control. *Microorganisms*, **8**(12): 1930.

Palmieri, D., Ianiri, G., Conte, T., Castoria, R., Lima, G. and De Curtis, F. 2022. Influence of biocontrol and integrated strategies and treatment timing on plum brown rot incidence and fungicide residues in fruits. *Agriculture*, **12**(10): 1656.

Papavizas, G. C. and Lewis, J. A. 1983. Physiological and biocontrol characteristics of stable mutants of *Trichoderma viride* resistant to MBC fungicides. *Phytopathology*, **73**: 407–411.

Parraguirre Lezama, C., Romero-Arenas, O., Valencia de Ita, M. D. L. A., Rivera, A., Sangerman Jarquín, D. M. and Huerta-Lara, M. 2023. In vitro study of the compatibility of four species of *Trichoderma* with three fungicides and their antagonistic activity against *Fusarium solani*. *Horticulturae*, **9**(8): 905.

Peláez-Álvarez, A., Santos-Villalobos, S. D. L., Yépez, E. A., Parra-Cota, F. I. and Reyes-Rodríguez, R. T. 2016. Synergistic effect of *Trichoderma asperelleum* T8A and captan 50® against *Colletotrichum gloeosporioides* (Penz.). Revista Mexicana de CienciasAgrícolas, **7**(6): 1401–1412.

Ramos, Y. G., Duin, I. M., da Silva, M. R. L. and Júnior, R. P. L. 2022. Bioactive copper and *Bacillus subtilis* for the control and resistance induction against citrus canker in sweet orange [*Citrus sinensis* (L.) Osbeck] orchard establishment. *Scientia Horticulturae*, 303: 111238.

Ruano-Rosa, D., Arjona-Girona, I. and López-Herrera, C. J. 2018. Integrated control of avocado white root rot combining low concentrations of fluazinam and *Trichoderma* spp. *Crop Protection*, **112**: 363–370.

Ruocco, M., Lanzuise, S., Vinale, F., Marra, R., Turrà, D., Woo, S. L. and Lorito, M. 2009. Identification of a new biocontrol gene in *Trichoderma atroviride*: The role of an ABC transporter membrane pump in the interaction with different plant-pathogenic fungi. *Molecular Plant-Microbe Interactions*, **22**: 291–301.

Saha, S., Pharate, S., Thosar, R. U. and Chavan, V. 2023. Compatibility of *Trichoderma asperelloides* with fungicides controlling downy mildew and powdery mildew in grapes. *Grape Insight*, 32–36.

Sánchez-Montesinos, B., Santos, M., Moreno-Gavíra, A., Marín-Rodulfo, T., Gea, F. J. and Diánez, F. 2021. Biological control of fungal diseases by *Trichoderma aggressivum* f. *europaeum* and its compatibility with fungicides. *Journal of Fungi*, **7**(8): 598.

Singh, G. and Varma, R. K. 2005. Compatibility of fungicides and neem products against *Fusarium solani*f.sp. *glycines* causing root rot of soybean and *Trichoderma* spp. *Journal of Mycopathological Research*, **43**: 211–214.

Song, J., Lei, T., Hao, X., Yuan, H., Sun, W. and Chen, S. 2022. Synergistic effects of *Clonostachys rosea*

isolates and succinate dehydrogenase inhibitors against fungal pathogens. *Journal of Fungi*, **8**: 134.

Sun, J., Zhang, T., Li, Y., Wang, X. and Chen, J. 2019. Functional characterization of the ABC transporter TaPdr2 in the tolerance of biocontrol fungus *Trichoderma atroviride* T23 to dichlorvos stress. *Biological Control*, **129**: 102–108. https://doi.org/10.1016/j.biocontrol.2018.11.003

Sun, X., Xu, Q., Ruan, R., Zhang, T., Zhu, C. and Li, H. 2013. PdMLE1, a specific and active transposon acts as a promoter and confers *Penicillium digitatum* with DMI resistance. *Environmental Microbiology*, **5:** 135–142. https://doi.org/10.1111/1462-2920.12080

Terrero-Yépez, P. I., Peñaherrera-Villafuerte, S. L., Solís-Hidalgo, Z. K., Vera-Coello, D. I., Navarret-Cedeño, J. B. and Herrera-Defaz, M. A. 2018. Compatibilidad*in vitro* de *Trichoderma* spp. con fungicidas de usocomúnen cacao (*Theobroma cacao* L.). *InvestigaciónAgraria*, **20:** 146–151.

Uribe-Gutiérrez, L., Moreno-Velandia, C. A. and Villamizar, L. F. 2022. Compatibility of a biopesticide based on the yeast *Rhodotorulamucilaginosa* (Lv316) with chemical fungicides used in blackberry crops. *BioControl*, **67**(1): 89–100.

Veena, S. S., Baby, A. and Karthikeyan, S. 2022. Study on compatibility of *Trichoderma asperellum* and fungicides for the development of environment-friendly and cost-effective disease management strategies. *Journal of Root Crops*, **48**(1 & 2): 35–40.

Wang, H., Chang, K. F., Hwang, S. F., Turnbull, G. D., Howard, R. J., Blade, S. F. and Callan, N. W. 2005. Fusarium root rot of coneflower seedlings and integrated control using *Trichoderma* and fungicides. *BioControl*, **50**: 317–329.

Wei, L., Chen, W., Zhao, W., Wang, J., Wang, B., Li, F. et al. 2020. Mutations and overexpression of CYP51 associated with DMI-resistance in *Colletotrichum gloeosporioides* from Chili. *Plant Disease*, 104: 668–676. https://doi.org/10.1094/PDIS-09-19-1912-RE

Wojtkowiak-Gębarowska, E. and Pietr, S. J. 2006. Colonization of roots and growth stimulation of cucumber by iprodione-resistant isolates of *Trichoderma* spp. applied alone and combined with fungicides. *Phytopathologia Polonica*, **41**: 51–64.

Yan, X., Ma, W. B., Li, Y., Wang, H., Que, Y. W., Ma, Z. H. et al. 2011. A sterol 14alpha-demethylase is required for conidiation, virulence and for mediating sensitivity to sterol demethylation inhibitors by the rice blast fungus *Magnaporthe oryzae*. *Fungal Genetics and Biology*, **48**: 144–153. https://doi.org/10.1016/j.fgb.2010.09.010

Yang, C., Hamel, C., Vujanovic, V. and Gan, Y. 2011. Fungicide: Modes of action and possible impact on nontarget microorganisms. ISRN Ecology, 2011: 130239. https://doi.org/10.5402/2011/130239

Zhang, C., Wang, W., Xue, M., Liu, Z., Zhang, Q., Hou, J., Xing, M., Wang, R. and Liu, T. 2021. The combination of a biocontrol agent *Trichoderma asperellum* SC012 and hymexazol reduces the effective fungicide dose to control Fusarium wilt in cowpea. *Journal of Fungi*, **7**: 685.

Zhang, Y. X., Perry, K., Vinci, V. A., Powell, K., Stemmer, W. P. and del Cardayré, S. B. 2002. Genome shuffling leads to rapid phenotypic improvement in bacteria. *Nature*, **415**(6872): 644–646.

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Diversity of chalcidid wasps (Hymenoptera: Chalcididae) in natural and man-made agroecosystems of Chhattisgarh, India

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ABSTRACT: The present study was aimed to document and compare the diversity of chalcidid wasps (Hymenoptera: Chalcididae) in the natural ecosystems and man-made agroecosystems of Chhattisgarh, India. Sweep net and yellow pan traps were used for sampling and a total of 354 individuals belonging to 70 species, 11 genera and four subfamilies of Chalcididae were collected in the last five years i.e. between 2019 – 2023 and studied. About 156 individuals were reported from the collections of 2019 – 2020 that belonged to 42 species of 10 genera under four subfamilies from the two different ecosystems whereas 198 specimens belonging to 56 species, nine genera and 4 subfamilies were recorded from Chhattisgarh's two different ecosystems in 2021 – 2023. The natural ecosystems of Chhattisgarh stood out as the rich Chalcididae diverse areas throughout the study periods.

KEYWORDS: Agroecosystems, natural ecosystems, Chalcididae, diversity

INTRODUCTION

India is considered as a megadiverse nation because of its vast diversity of flora and fauna (Roy and Roy, 2015). It is based at the triad junction of the Indo-Malayan, Afrotropical and Palearctic realms because of which the rich biological diversity has been encouraged (Newton and Dale 2001). India has 10 biogeography zones including twenty-four provinces of biota (Rodgers and Panwar 1990; Rodgers *et al*. 2000) and four biodiversity hotspots (Myers *et al.* 2000). Chhattisgarh is one such state which comes in the Chota Nagpur province of Deccan Peninsula biogeography zone. The state possesses a rich biodiversity with around 44% of its area under forest cover. Three different agroclimatic zones namely the Northern Hills zone (28.0%), the Chhattisgarh Plains zone (51.0%) and the Bastar Plateaus zone (21.0%) are found in Chhattisgarh (Anonymous, 2019). Very few studies have been conducted to study and document any arthropod (insect) fauna of Chhattisgarh.

Chalcidoidea is a tremendous diverse superfamily of Apocrita suborder (Hymenoptera) comprising of 23 families (Heraty *et al*., 2013; Askew and Mifsud, 2016). Members of the family Chalcididae are easily noticeable due to the presence of numerous teeth on the ventral edge of their enlarged hind femora, strong punctation of the thorax and a sharp posterior carina bordering the gena (Narendran and van Achterberg, 2016). Members of Chalcididae often parasitize on various other insects, mainly in their pupae (Narendran and van Achterberg, 2016). The family currently includes 87 genera and 1,464 species placed in five subfamilies namely Chalcidinae, Dirhininae, Epitraninae, Haltichellinae and Smicromorphinae. Although taxonomic studies on Indian Chalcididae (Narendran, 1989) are available but such an enormous study to document and compare Chalcididae diversity with respect to its natural ecosystem and agroecosystem habitats in Chhattisgarh state has not been done before.

MATERIALS AND METHODS

Extensive surveys were undertaken and the last five years i.e. from 2019 – 2023 were considered to document chalcidid wasps from the three different agro-climatic zones of Chhattisgarh state. Rigorous collections were done from different agriculture college farms, KVKs, wildlife sanctuaries and National Park, etc. Permission to collect samples was accorded from the Fig. 1. Office of the Principal Chief Conservator of Forests (Wildlife Management & Bio-diversity Conservation cum-Chief Wildlife Warden) Chhattisgarh via letter no. 4369 and 806, dated 30/11/2021 and 17/02/2023, respectively.

Sweep net and yellow pan traps were used to collect the samples. The sampling was done by laying 100 Moericke traps at each site generally for a period of one week. The obtained samples were transferred to a container having 70% alcohol. After being killed, the chalcidid wasps were brought to lab; curated, labelled and secured in fumigated insect boxes. Specimens were later on examined under a Leica MZ16A stereo – zoom microscope and identified up to the species level using

Joseph *et al*. (1973), Bouček and Narendran (1981), Narendran (1989), Narendran & van Achterberg (2016) and others. The identified specimens have been deposited in the National Insect Museum (NIM) of ICAR- National Bureau of Agricultural Insect Resources, Bangalore, India.

Statistical analysis

Alpha or species diversity of each site was estimated using the following ecological indices: -

- **Simpson's Diversity Index (SDI) = 1-D** where D = Simpson's Index.
- **Simpson's Index (D) = Σn (n-1) / N(N-1)** where n = number of a species' individuals and N = total number of all species' individuals.

Simpson's Diversity Index is given by subtracting the value of Simpson's index from 1. The index value varies from 0 to 1, with 1 representing infinite diversity and 0 representing no diversity, respectively. SDI is a diversity measure which takes into consideration both the number of species present and the relative abundance of each species (Simpson, 1949).

- **Shannon-Wiener index (H') = – ΣPi ln (Pi)** where Pi = S / N; S = number of a species' individuals, N = total number of all species' individuals, ln = logarithm to base e. The greater the value of H', the higher the diversity (Shannon and Wiener, 1949).
- **Margalef index α = (S – 1) / ln (N)** where S = total number of species, N = total number of all species' individuals (Margalef, 1958).
- **Pielou's Evenness Index E1 = H' / ln (S)** where H' = Shannon-Wiener diversity index, S = total number of species in the sample (Pielou, 1966). As species richness and evenness increase, diversity also increases (Magurran, 1988).

RESULTS AND DISCUSSION

A total of 354 specimens belonging to eleven genera under four subfamilies and 70 species were examined during the study period. The chalcidid wasps belonged to Chalcidinae, Dirhininae, Epitraninae and Haltichellinae subfamilies. The subfamily Haltichellinae (n = 226, 64.0%) with 41 species under 7 genera was the most abundant followed by subfamily Dirhininae (n = 69, 19.0%) subfamily Chalcidinae (n = 46, 13.0%) with 16 species in one genus and Epitraninae (n = 13, 4.0%) represented by 5 species under one genus. In the year 2019-2020, the genera *Antrocephalus* Kirby, *Brachymeria* Westwood, *Dirhinus* Dalman, *Haltichella* Spinola, *Hockeria* Walker, *Kriechbaumerella* Dalla Torre and *Psilochalcis* Kieffer were recorded from both the ecosystems and can be considered as the generalists (Table 1). In the year 2019 – 2020, Chalcidid wasp's genus *Brachymeria* Westwood, *Dirhinus* Dalman and *Kriechbaumerella* Dalla Torre were the most speciose with 4 species each in the natural ecosystems;

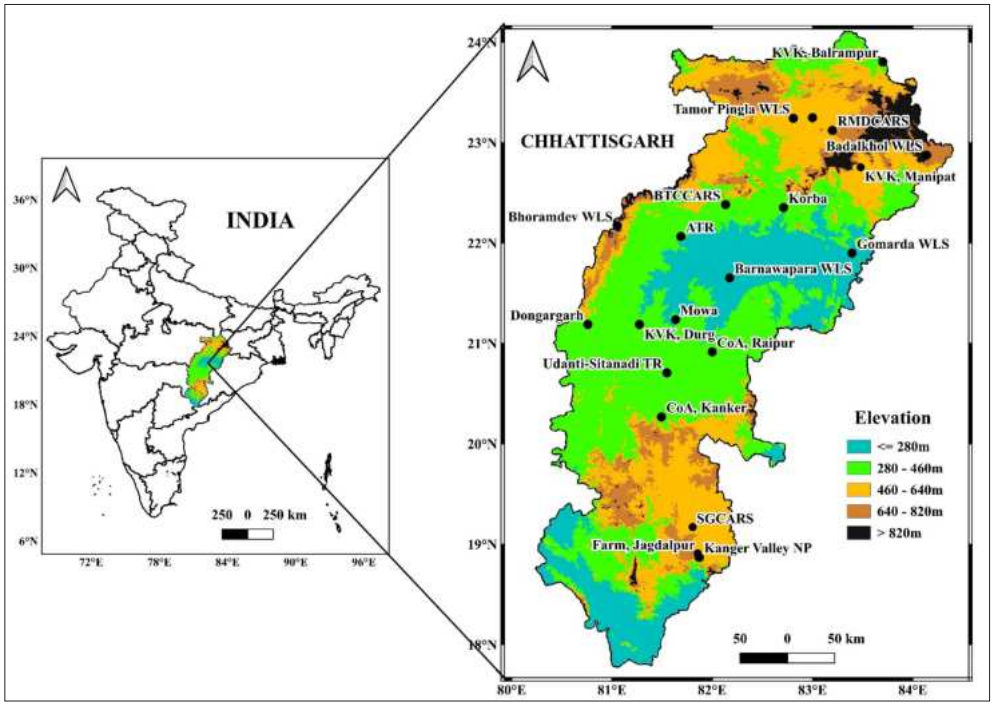

Fig.1. Map showing sites of sample collections

Table 1. Abundance, richness and relative frequency of chalcidid wasps (Hymenoptera: Chalcididae) species collected in natural and man-made agroecosystems of Chhattisgarh, India

S. No.	Species	2019 – 20						2021 – 23					
		Natural ecosystem		Agro -ecosystem		Total		Natural ecosystem		Agro -ecosystem		Total	
		Abundance	Relative frequency (%)	Abundance	Relative frequency (%)	Abundance	Relative frequency (%)	Abundance	Relative frequency (%)	Abundance	Relative frequency (%)	Abundance	Relative frequency (%)
1.	*Brachymeria apicornis* Cameron, 1911	0	0.00	0	0.00	0	0.00	1	1.52	4	3.05	5	2.54
2.	*Brachymeria banksi* Ashmead, 1905	0	0.00	4	3.70	4	2.55	0	0.00	2	1.53	2	1.02
3.	*Brachymeria bengalensis* Cameron, 1897	0	0.00	1	0.93	1	0.64	1	1.52	1	0.76	2	1.01
4.	*Brachymeria burksi* Chhotani, 1966	2	4.08	0	0.00	2	1.27	0	0.00	0	0.00	0	0.00
5.	*Brachymeria euploea* Westwood, 1837	6	12.24	1	0.93	7	4.46	1	1.52	3	2.27	4	2.02
6.	*Brachymeria fulvitarsis* Cameron, 1906	0	0.00	1	0.93	1	0.64	0	0.00	1	0.76	1	0.51
7.	*Brachymeria hattoriae* Habu, 1961	1	2.04	0	0.00	1	0.64	0	0.00	0	0.00	0	0.00
8.	*Brachymeria jambolana* Gahan, 1942	1	2.04	1	0.93	2	1.27	2	3.03	0	0.00	2	1.02

9.	*Brachymeria jayaraji* Joseph, Narendran and Joy, 1972	0	0.00	1	0.93	1	0.64	0	0.00	0	0.00	0	0.00
10.	*Brachymeria lugubris* Walker, 1871	0	0.00	4	3.70	4	2.55	0	0.00	0	0.00	0	0.00
11.	*Brachymeria manjerica* Narendran, 1989	0	0.00	1	0.93	1	0.64	0	0.00	0	0.00	0	0.00
12.	*Brachymeria megaspila* Cameron, 1907	0	0.00	0	0.00	0	0.00	0	0.00	2	1.53	2	1.02
13.	*Brachymeria nephantidis* Gahan, 1930	0	0.00	1	0.93	1	0.64	0	0.00	1	0.76	1	0.51
14..	*Brachymeria podagrica* Fabricius, 1787	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	1	0.51
15.	*Brachymeria rufescens* Cameron, 1906	0	0.00	0	0.00	0	0.00	0	0.00	1	0.76	1	0.51
16.	*Brachymeria wiebsina* Joseph, Narendran and Joy, 1972	0	0.00	0	0.00	0	0.00	0	0.00	1	0.76	1	0.51
17.	*Dirhinus anthracia* Walker, 1846	0	0.00	3	2.78	3	1.91	3	4.55	15	11.45	18	9.14
18.	*Dirhinus auratus* Ashmead, 1905	7	14.29	8	7.41	15	9.55	0	0.00	8	6.11	8	4.06
19.	*Dirhinus bakeri* Crawford, 1915	0	0.00	0	0.00	0	0.00	0	0.00	1	0.76	1	0.51

20.	*Dirhinus banksi* Rohwer, 1923	0	0.00	0	0.00	0	0.00	0	0.00	1	0.76	1	0.51
21.	*Dirhinus claviger* Bouček and Narendran, 1981	1	2.04	2	1.85	3	1.91	0	0.00	2	1.53	2	1.02
22.	*Dirhinus deplanatus* Bouček and Narendran, 1981	1	2.04	0	0.00	1	0.64	0	0.00	0	0.00	0	0.00
23.	*Dirhinus himalayanus* Westwood, 1836	7	14.29	6	5.56	13	8.28	0	0.00	2	1.53	2	1.02
24.	*Eniacomorpha madagascariensis* Masi, 1947	1	2.04	1	0.93	2	1.27	0	0.00	0	0.00	0	0.00
25.	*Epitranus albipennis* Walker, 1874	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	1	0.51
26.	*Epitranus crassicornis* Bouček, 1982	1	2.04	0	0.00	1	0.64	0	0.00	0	0.00	0	0.00
27.	*Epitranus elongatulus* Motschulsky, 1863	0	0.00	0	0.00	0	0.00	0	0.00	1	0.76	1	0.51
28.	*Epitranus erythrogaster* Cameron, 1888	0	0.00	0	0.00	0	0.00	3	4.55	6	4.58	9	4.57
29.	*Epitranus indicus* Husain and Agarwal, 1982	0	0.00	0	0.00	0	0.00	0	0.00	1	0.76	1	0.51
30.	*Antrocephalus cariniaspis* Cameron, 1911	0	0.00	0	0.00	0	0.00	0	0.00	1	0.76	1	0.51

31.	*Antrocephalus cariniceps* Cameron, 1911	4	8.16	16	14.81	20	12.74	1	1.52	10	7.63	11	5.58
32.	*Antrocephalus dividens* Walker, 1860	0	0.00	0	0.00	0	0.00	0	0.00	3	2.29	3	1.52
33.	*Antrocephalus hakonensis* Ashmead, 1904	0	0.00	0	0.00	0	0.00	0	0.00	3	2.29	3	1.52
34.	*Antrocephalus indicus* Husain and Agarwal, 1982	0	0.00	1	0.93	1	0.64	0	0.00	0	0.00	0	0.00
35.	*Antrocephalus japonicus* Masi, 1936	0	0.00	5	4.63	5	3.18	1	1.52	5	3.82	6	3.05
36.	*Antrocephalus mitys* Walker, 1846	0	0.00	0	0.00	0	0.00	0	0.00	1	0.76	1	0.51
37.	*Antrocephalus nasutus* Holmgren, 1868	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	1	0.51
38.	*Antrocephalus niger* Masi, 1929	0	0.00	1	0.93	1	0.64	2	3.03	7	5.34	9	4.57
39.	*Antrocephalus peechiensis* Narendran, 1989	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	1	0.51
40.	*Antrocephalus phaeospilus* Waterston, 1922	0	0.00	1	0.93	1	0.64	5	7.58	2	1.53	7	3.55
41.	*Antrocephalus sepyra* Walker, 1846	3	6.12	9	8.33	12	7.64	0	0.00	1	0.76	1	0.51

42.	*Antrocephalus validicornis* Holmgren, 1868	0	0.00	6	5.56	6	3.82	10	15.15	8	6.11	18	9.14
43.	*Haltichella clavicornis* Ashmead, 1904	0	0.00	1	0.93	1	0.64	1	1.52	0	0.00	1	0.51
44.	*Haltichella delhensis* Roy and Farooqi, 1984	0	0.00	1	0.93	1	0.64	0	0.00	2	1.53	2	1.02
45.	*Haltichella luzonica* Narendran, 1989	2	4.08	7	6.48	9	5.73	4	6.06	4	3.05	8	4.06
46.	*Haltichella macrocera* Waterston, 1922	0	0.00	0	0.00	0	0.00	2	3.03	0	0.00	2	1.02
47.	*Hockeria ammioshimensis* Habu, 1960	0	0.00	0	0.00	0	0.00	0	0.00	1	0.76	1	0.51
48.	*Hockeria anupama* Narendran, 1989	0	0.00	1	0.93	1	0.64	0	0.00	0	0.00	0	0.00
49.	*Hockeria assamensis* Narendran, 1989	2	4.08	5	4.63	7	4.46	3	4.55	3	2.29	6	3.05
50.	*Hockeria atra* Masi, 1929	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	1	0.51
51.	*Hockeria bifasciata* Walker, 1834	0	0.00	1	0.93	1	0.64	0	0.00	0	0.00	0	0.00
52.	*Hockeria guptai* Narendran, 1989	0	0.00	0	0.00	0	0.00	0	0.00	1	0.76	1	0.51
53.	*Hockeria hayati* Narendran, 1989	0	0.00	0	0.00	0	0.00	0	0.00	3	2.29	3	1.52

54.	*Hockeria menoni* Narendran, 1986	0	0.00	0	0.00	0	0.00	0	0.00	1	0.76	1	0.51
55.	*Hockeria polycarinata* Narendran, 1989	0	0.00	0	0.00	0	0.00	2	3.03	1	0.76	3	1.52
56.	*Hockeria tristis* Strand, 1911	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	1	0.51
57.	*Kriechbaumerella gibsoni* Narendran, 1989	1	2.04	0	0.00	1	0.64	0	0.00	0	0.00	0	0.00
58.	*Kriechbaumerella kraussi* Narendran, 1989	1	2.04	4	3.70	5	3.18	1	1.52	1	0.76	2	1.02
59.	*Kriechbaumerella nepalensis* Narendran, 1989	0	0.00	1	0.93	1	0.64	2	3.03	5	3.82	7	3.55
60.	*Kriechbaumerella ornatipennis* Cameron, 1902	0	0.00	3	2.78	3	1.91	3	4.55	2	1.53	5	2.54
61.	*Kriechbaumerella pulvinata* Masi, 1932	4	8.16	3	2.78	7	4.46	0	0.00	4	3.05	4	2.03
62.	*Kriechbaumerella rufimanus* Walker, 1860	2	4.08	2	1.85	4	2.55	2	3.03	5	3.82	7	3.55
63.	*Kriechbaumerella titusi* Narendran, 1989	0	0.00	2	1.85	2	1.27	0	0.00	0	0.00	0	0.00
64.	*Lasiochalcidia dargelasii* Latreille, 1805	1	2.04	0	0.00	1	0.64	0	0.00	0	0.00	0	0.00
65.	*Proconura caryobori* Hanna, 1934	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	1	0.51

66.	*Psilochalcis adhara* Narendran, 1989	0	0.00	0	0.00	0	0.00	1	1.49	0	0.00	1	0.51
67.	*Psilochalcis carinigena* Cameron, 1907	0	0.00	1	0.93	1	0.64	1	1.52	4	3.05	5	2.54
68.	*Psilochalcis crasssicornis* Masi, 1929	0	0.00	0	0.00	0	0.00	1	1.52	0	0.00	1	0.51
69.	*Psilochalcis soudanensis* Steffan, 1951	0	0.00	1	0.93	1	0.64	5	7.58	0	0.00	5	2.54
70.	*Psilochalcis subarmata* Foerster, 1855	1	2.04	1	0.93	2	1.27	1	1.52	1	0.76	2	1.02
Total individuals		**49**		**108**		**157**		**66**		**132**		**198**	
Species richness		**20**		**36**		**42**		**32**		**43**		**56**	

Brachymeria Westwood was the most speciose with 9 species in the agroecosystems. In the year 2021 – 2023, *Antrocephalus* Kirby was the most speciose with 7 species in the natural ecosystems and 10 species in the agroecosystems. *Dirhinus himalayanus* Westwood and *Dirhinus auratus* Ashmead were the most abundant species in natural ecosystems (n = 7; 14.29%) whereas *Antrocephalus cariniceps* Cameron was the most abundant species in agroecosystems, (n = 16; 14.81%) in the year 2019 – 2020. *Antrocephalus validicornis* Holmgren was the most abundant species in natural ecosystems (n = 10; 15.15%) whereas *Dirhinus anthracia* Walker was the most abundant species in agroecosystems (n = 15; 11.45%) in the year 2021 – 2023 (Table 1).

Out of 354 examined specimens, 156 specimens were reported from the collections of 2019 – 2020 that belonged to 42 species of 10 genera under 4 subfamilies from the two different ecosystems whereas 198 specimens belonging to 56 species, 9 genera and 4 subfamilies were recorded from Chhattisgarh's two different ecosystems in 2021 – 2023. In 2019 – 2020, comparatively higher species diversity and low abundance of Chalcididae was observed in the natural ecosystems (H' = 2.71, α = 4.88) whereas agroecosystems (H' = 1.71, α = 7.48) had low species diversity and higher abundance. In the natural ecosystems, comparatively higher species diversity and low abundance (H' = 0.78, α = 7.40) was observed than the agroecosystems (H' = 0.63, α = 8.62) that had low species diversity and higher abundance in 2021 – 2023 collections. Chalcididae species was comparatively more abundant and evenly distributed in natural ecosystems (SDI = 0.94, E1 = 0.90) than agroecosystems (SDI = 0.95, E1 = 0.48) that was less abundant and unevenly distributed in 2019 – 2020. During 2021 – 2023, Chalcididae species was comparatively more abundant and evenly distributed in natural ecosystems (SDI = 0.96, E1 = 0.22) and less abundant and unevenly distributed in agroecosystems (SDI = 0.96, E1 = 0.17).

CONCLUSION

Because of the many strata between the ground and canopy, the structural variability of forest stands provides a vast number of ecological niches that support species diversity and structural richness has a significant impact on forest biodiversity. Similar research on Chalcididae from the state has also demonstrated that, in comparison to natural ecosystems, chalcidid wasps diversity is often lower in agroecosystems (Alisha et al. 2020). This study emphasizes the importance of the forest ecosystems for chalcidid wasps as well as the lack of studies on Chalcididae documentation across different ecosystem and habitats in the Central region of India. Further studies should be encouraged in the country to document and improve our knowledge of Chalcididae diversity and distribution. More data and information on chalcidid wasps will enable us to use them as potential biological control agents in Integrated Pest Management strategies for agroecosystems.

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REFERENCES

Alisha, S., Gupta, A. and Rana, D. K. 2020. Diversity of chalcidids (Hymenoptera: Chalcididae) from different agro-climatic zones of Chhattisgarh, India. Journal of Biological Control, 34(2), 101–108.

Anonymous 2019. Training Manual. Climate Resilient Agriculture in Chhattisgarh. Action on Climate Today (ACT).

Askew, R. and Mifsud, D. 2016. A preliminary check-list of the Chalcidoidea (Hymenoptera) of the Maltese Islands. Bulletin of the Entomological Society of Malta, **8:** 47–72. https://doi.org/10.17387/bullentsocmalta.2016.04

Heraty, J. M., Burks, R. A., Cruaud, A., Gibson, G. A., Liljeblad, J., Munro, J., Rasplus, J. Y., Delvare, G., Jansta, P., Gumovsky, A. and Huber, J. 2013. A phylogenetic analysis of the mega diverse Chalcidoidea (Hymenoptera). *Cladistics*, **29**(5): pp. 466-542. https://doi.org/10.1111/cla.12006

Jaccard, P. 1912. The distribution of the flora in the alpine zone. *New Phytol,* **11:** 37–50. https://doi.org/10.1111/j.1469-8137.1912.tb05611.x

Magurran, E. A. 1988. Ecological diversity and its measurement. Croom Helm, Australia, 215 pp. https://doi.org/10.1007/978-94-015-7358-0

Margale, F. R. 1958. Temporal succession and spatial heterogeneity in phytoplankton, pp. 323-347. In: Perspectives in Marine Biology. University of California Press, Berkeley. https://doi.org/10.1525/9780520350281-024

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. and Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature*, **403**(6772):pp.853-858.

Narendran, T. C. 1989. Oriental Chalcididae (Hymenoptera: Chalcidoidea). Zoological Monograph. *Department of Zoology*, University of Calicut, Kerala, 1–441.

Narendran, T. C. and C van Achterberg 2016. Revision of the family Chalcididae (Hymenoptera: Chalcidoidea) from Vietnam, with the description of 13 new species. *Zookeys,* **576:** 1–202. https://doi.org/10.3897/zookeys.576.8177

Newton, I. and Dale, L. 2001. A comparative analysis of the avifaunas of different zoogeographical regions. *J. Zool,* **254**(2): 207–218.

Pielou, E. C. 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. **13:**131– 144. https://doi.org/10.1016/0022-5193(66)90013-0

Rodgers, W. A. and Panwar, H. S. 1990. A biogeographical classification for conservation planning. Wildlife Institute of India, Dehradun.

Rodgers, W. A., Panwar, H. S. and Mathur, V. B. 2000. Biogeographical classification of India. In: Wildlife protected area network in India: a review (executive summary). Wildlife Institute of India, Dehradun. 49pp.

Roy, A. and Roy, P. S. 2015. Biodiversity information in India: Status and future scope. In: Biodiversity in tropical ecosystem. Today and Tomorrow's Printers and Publishers, New Delhi.

Shannon, C. E. and Wiener, W. 1949. The Mathematical Theory of Communication. Urbana, University of Illinois Press, 177 pp.

Simpson, E. H. 1949. Measurement of species diversity. *Nature,* **163:**688. https://doi.org/10.1038/163688a0

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Morphological description and predatory potential of two *Chelisoches* species of earwigs on arecanut inflorescence caterpillar, *Thirathaba* sp. from South India

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ABSTRACT: Earwigs (Dermaptera) are omnivorous insects distributed worldwide. Their ecological role in agricultural cropping ecosystem is not fully understood. The present study explores the ecological role of two black earwig species in arecanut ecosystem. In the present study, two species of earwigs were collected and found predating on arecanut inflorescence caterpillar, *Thirathaba* sp. (Lepidoptera: Pyralidae) for the first time from India. Further, survey was conducted in the major arecanut growing regions of Karnataka from 2021-23 to explore the diversity of earwigs. Results revealed the occurrence of two black earwig species *viz*., *Chelisoches brevipennis* and *C. morio* (Chelisochidae). Among them *C. brevipennis* is a new record from south India. The species were morphologically identified and an illustrated identification key to both the species was provided. Further study confirmed the predatory role of earwigs on arecanut inflorescence caterpillar implying a significant potential for use in biocontrol. The current study reported two species of earwigs *C. brevipennis* and *C. morio* from arecanut ecosystems which were observed as efficient predators on arecanut inflorescence caterpillar.

Keywords: Biocontrol, dermaptera, diversity, India, palms

INTRODUCTION

Earwigs are a moderately diversified group of insects which comprise approximately 1,900 species distributed mainly in tropical and subtropical parts of the world (Hopkins *et al*., 2018). The previous taxonomic study by Srivastava (2013) reported 284 species from India. Earwigs are omnivorous insects that may be considered as helpful organisms within agro ecosystems (Van Huis, 1981; Jones *et al.,* 1988; Gravena and Da Cunha, 1991; Mariani *et al*., 1996). The beneficial actions of earwigs in many crops of economical relevance have been described previously by Buxton (1974) and Canellas *et al*. (2005). *Chelisoches* is an important genus of the family Chelisochidae belongs to the order Dermaptera. *Chelisoches* includes only two species in India *viz*., *C. brevipennis* and *C. morio*. Owing to their ecological and behavioural observations in agricultural ecosystem they are considered as the important predators of insect pests (Zhong *et al*., 2016; Li *et al*., 2011). The adults of these two species have been reported to predate on different stages of coconut leaf beetle, *Brontispa longissima* in Thailand and Philippines (Chomphukhieo *et al*., 2008). The extensive work of Srivastava (2013) mainly concentrated on taxonomy of Dermaptera and there was no publishised information about the ecological role of earwigs in different crops. Karthik *et al*. (2022) recently reported one new species from sugarcane crop which shows the importance of taxonomy of Dermaptera in India. There is a need to study earwig species distribution, status, and role in agricultural and horticultural cropping systems (Karthik and Kalleshwaraswamy, 2023; Kamimura *et al.,* 2022). The present study emphasizes the species composition and ecological role of earwigs as predators in arecanut cropping ecosystem.

MATERIALS AND METHODS

Survey was done in the major arecanut growing regions of Karnataka covering Chitradurga, Shivamogga, Mysore, Chikkamangalore and Davanagere from 2021 to 2023. From all the surveyed regions, the earwig samples were collected in 70% ethanol and brought to laboratory. For the morphological identification, the specimen was examined under a Stemi 508 stereozoom microscope (Carl Zeiss Microscopy GmbH, Jena, Germany). Photographs of the habitus and external body parts were taken under an M205C stereozoom microscope attached with a DFC450 camera (Leica, Wetzlar, Germany). The male genitalia were removed by gently lifting the penultimate abdominal sternite, pulling out from the genital chamber with forceps, and cutting at the site of attachment to the ejaculatory ducts. The genitalia were processed by submersion in 5% KOH for two days for clearing tissues and mounted on a glass slide with glycerol. Photographs of dissected genitalia were taken an M205C stereozoom microscope attached with a DFC450 camera. The terminology of Kamimura (2014) was adopted to describe male genital structures

of the species collected. The species were identified by using keys developed by Srivastava (2013) and a revised key with illustrations and additional morphometric measurements were provided for easy identification. The additional taxonomic characters with digital images were provided for quick and reliable identification of earwigs.

Arecanut inflorescence infested with inflorescence caterpillar stages such as larvae, pupae including earwigs were brought to laboratory and separated manually. Larvae were reared in plastic containers (15 cm x 8 cm) covered with muslin cloth and provided with fresh inflorescence pieces for feeding. The pupae were placed individually in glass tubes (15 cm x 1.5 cm) covered with a cotton plug till the emergence of adult moth. Once the moths are emerged, they are morphologically identified up to the genus level. The voucher specimens were deposited at Insect Systematics Laboratory, Department of Entomology, College of Agriculture, Shivamogga.

In order to assess the predatory potential, early instar larvae of *Thirthaba* sp. were kept in insect breeding dish (Himedia, TCP030- 90 × 40 mm dia) and the active adults of *Chelisoches* species were released into insect breeding dish to test their predatory efficiency of earwigs. Initially the earwigs were collected from the field were pre-starved for 3 to 4 days. After four days, one adult earwig (n=10) was released into insect breeding dish (Himedia, TCP030- 90 × 40 mm dia) containing five early instar larvae of inflorescence caterpillar. Then, after 48 hours of release predation of earwigs on *Thirthaba* sp. was confirmed by counting the number of larvae remained in insect breeding dish (Himedia, TCP030- 90 × 40 mm dia).

RESULTS AND DISCUSSION

From arecanut inflorescence infested with caterpillar, a total of 42 earwigs were collected. Out of them, 12 were males and 21 were females and nine were nymphs. Based on the keys of Srivastava (2013), they were identified as two species of *Chelisoches viz*., *C. brevipennis* and *C. morio*. It appears that they co-existed as both the species were found in a single infested inflorescence (Fig 1e). During the survey (2021-2022) to different arecanut growing regions of Karnataka two species of black earwigs were collected and identified as *C. morio* and *C. brevipennis*. In India little published information available on the ecological role of earwigs

Fig.1. Habitus of earwigs in arecanut ecosystems; a) Earwigs within unopened infloresence; b) Earwigs with in spadix; c) Half decayed infloresencce; d) and e) Ants and earwigs on infloresence; f) Earwig feeding on *Thirathaba* larvae

in different cropping ecosystem. The present study highlights the predatory role of black earwigs on arecanut inflorescence caterpillar. Different species of earwigs are the efficient predators of many lepidopteran insects (Schlinger *et al*., 1959). Chomphukhieo *et al*. (2008) observed the predation of *C. morio* on coconut leaf beetle, *B. longissima*

Taxonomy

Order Dermaptera de Geer, 1773
Infraorder Epidermaptera Engel, 2003
Parvorder Eteodermaptera Engel, 2003
Nanorder EudermapteraVerhoeff, 1902
Family Chelisochidae Verhoeff, 1902

1. *Chelisoches brevipennis* Borelli, 1923

Diagnosis

Body dark black in colour (Fig 2a), measures 14.16 mm length without forceps. Head triangular, frons moderately and occiput distinctly raised, median suture deep, dividing occiput into two halves, measures 1.84 mm length and 2.00 mm width (Table 1). Eyes distinct, shorter than the post-ocular length. Antennae 19-segmentd or more first segment stout, slightly expanded apically, shorter than the distance between antennal bases; second short, about as long as broad; third about twice as long as broad; fourth subclavate, slightly shorter than third; fifth and sixth segments subclavate, remaining gradually increasing in length (Fig 2b). Pronotum slightly broad, measures 1.69 mm length and 1.84 mm width (Table 1) anterior margin convex, lateral margin straight and posteriorly widened, hind margin and angles rounded; differentiated prozona and metazona. Sternal region of body depressed (Fig 2d); well developed elytra, sparsely punctate. Wings little projecting beyond elytra (Fig 2c), tegmen measures 4.31 mm length and 3.08 mm width (Table 1). Legs typical, hind tarsi with first segment about as long as third segment, on underside covered with thick pubescence (Fig 2e). Abdomen apically widened, punctate, convex tergites, lateral folds on third weakly and on fourth distinctly marked. Penultimate sternite rounded with little emargination in middle (Fig 1f). Ultimate tergite transverse and measures about 2.77 mm width (Table 1), disc faintly punctate, convex, tumid above the bases of forceps, in between little depressed with two pairs of compressed tubercles, their inner pair larger and contiguous, laterally above the bases of

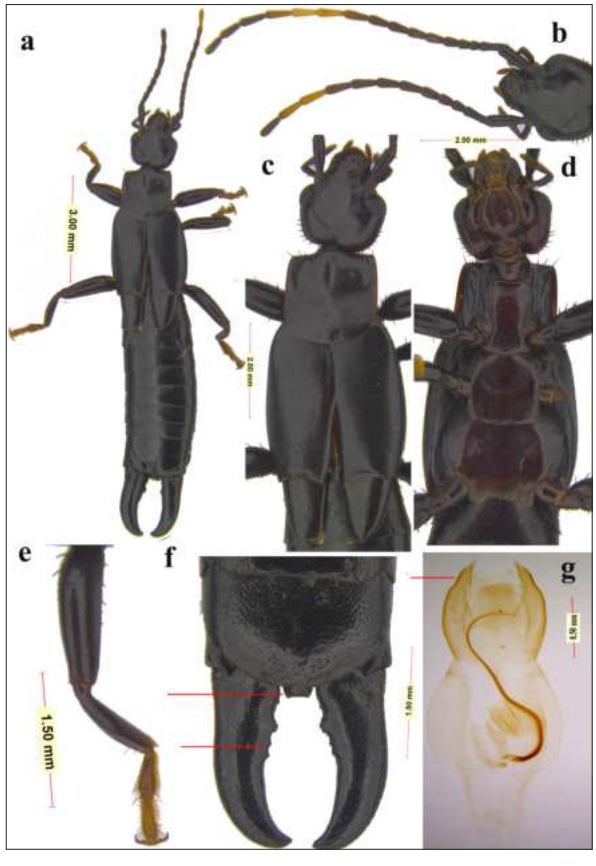

Fig.2. *Chelisoches brevipennis* Borelli, 1923; a) Habitus; b) Antenna; c) Pronotum, tegmina and wings; d) Thoracic sterna e) Right foreleg; f) Penultimate sternite and forceps; g) Genitalia

Table 1. Morphometric measurements of *C. brevipennis* and *C. morio*

C. brevipennis (Male)		*C. morio* (Male)	
Length	**Measurement in mm**	**Length**	**Measurement in mm**
Body without forceps	14.16	Body without forceps	15.09
Head	1.84	Head	1.69
Pronotum	1.69	Pronotum	2.00
Tegmen	4.31	Tegmen	4.92
Forceps	2.46	Forceps	4.15
Width		**Width**	
Head	2.00	Head	2.00
Pronotum	1.84	Pronotum	2.15
Tegmen	3.08	Tegmen	3.23
Ultimate tergite	2.77	Ultimate tergite	2.80

forceps oblique and hind margin trisinuate. Slanting pygidium, with bilobes and narrowed apically. Forceps branches (Fig 2f) depressed, stout, straight, apices gently incurved, inner margin of forceps armed with blunt tooth, but posterior one smaller, branches comparatively longer, less stout, variable internal armature with minute teeth in two thirds of base followed by another larger one in apical one third. Forceps measures about 2.46 mm length (Table 1). Genitalia with parameres slightly enlarged externally in middle and with a slight emargination before spex (Fig 2g); virga tubular, short, without accessory plates at base.

Material examined: 1. INDIA, Karnataka, Davanagere, Channagiri, 14°1'36"N, 75°54'52"E, 636m, 29.vi.2022, Coll. Karthik, C. M., ex. Arecanut. 2. INDIA, Karnataka, Shivamogga, 13°35'22"N, 75°17'58"E, 680m, 22.vii.2022, coll. Karthik, C. M., ex. Arecanut). 3. INDIA, Karnataka, Chikkamangalore, Koppa, 13°32'48"N, 75°24'7"E, 724m, 1.x.2021, Coll. Karthik, C. M., ex. Arecanut.

2. *Chelisoches morio* (Fabricius, 1775)

Diagnosis

Stout body measures 6.46 mm length without forceps (Table 1). Black colour with intermediate shades (Fig 3a); two pre apical antennal segments yellow and tarsi brownish. Head slightly convex, triangular with obsolete sutures, hind margin emarginated, measures 0.07 mm length and 0.09 mm width (Table 2). Eyes slightly shorter than post-ocular length. Antennae 21-segmented, first stout, about as long as the distance between antennal bases, little expanded apically; second segment short, about as long as broad; third segment long and gently expanded apically; fourth segment shorter than preceding one, subclavate; fifth slightly longer than the fifth, subclavate, remaining segments length gradually increasing, each segment gently expanded apically (Fig 3b). Pronotum about as long as broad, somewhat widened posteriorly, rounded hind margin, median sulcus distinct (Fig 3c), measures 2.00 mm length and 2.15 mm width (Table 1); convex prozona and depressed metazona. Depressed ventral side of the body (Fig 3d). Well developed elytra and wings, tegmen measures 4.92 mm length and 3.23 mm width (Table 1). Underside of tarsi covered with golden pubescence (Fig 3e). Elongated abdomen, lateral margin gently widened in middle, tergites slightly convex, finely punctate, hind margin of tergites with a row of compressed tubercles, lateral folds on third tergite weakly and on fourth marked distinctly. Penultimate sternite with rounded posterior margin and slight emargination in middle (Fig 3f). Ultimate tergite transverse, disc slightly convex, sloping backwards, low folds above the forceps base and between a pair of compressed tubercles, contiguous, inner pair smaller, outer pair larger on inner margin of folds above the bases of forceps, trisinuate hind margin, hind margin on lateral side oblique, ultimate tergite measures about 2.77 mm width (Table 1). Pygidium declivitous, hind margin truncate or emarginated slightly. Forceps with branches stout measures 4.15 mm length (Table 1), elongated, depressed, gradually tapering and incurving at tip, deplanate internally in basal half, followed by one

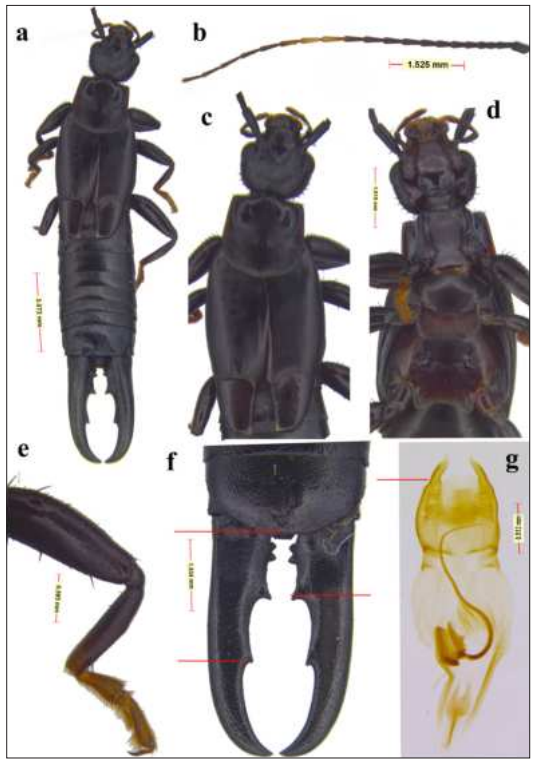

Fig.3. *Chelisoches morio* (Fabricius, 1775); a) Habitus; b) Left antenna; c) Pronotum, tegmina and wings; d) Thoracic sterna e) Right foreleg; f) Penultimate sternite and forceps; g) Genitalia

Table 2. Predatory efficiency of earwig, *C. brevipennis* and *C. morio* on larvae of *Thirathaba* sp.

Predatory potential of *C. brevipennis*						
Particulars	**Mean no. of larvae**	**Standard deviation**	**variance**	**t value**	**df**	**P- value**
Pre count	5.00	0.00	0.00	3.25	9	< 0.01
Post count	1.60	0.69	0.48			
Predatory potential of *C. morio*						
Pre count	5.00	0.00	0.00	3.25	9	< 0.01
Post count	2.10	0.73	0.54			

(SD- Standard deviation, df – Degrees of freedom,*Mean of ten observations)

or two teeth; short, internal margin with one or two teeth at base and minute teeth in middle, internal position of teeth variable. Genitalia with parameres narrow (Fig3g), external dilation in middle slight; thick, short tubular virga.

Material examined: 1. INDIA, Karnataka, Mysore, Hunsur, 12° 18' 3.39" N 76° 17' 18.45" E, 792m, 29.vi.2022, Coll. Karthik, C. M., ex. Arecanut.

Bio ecology

The earwig specimens were collected from *Tirathaba* sp. infested inflorescence of arecanut. They were known to breed inside the arecanut inflorescence due to their concealed habitat and availability of enough moisture within the unopened spadix, possibly taking advantage of protective and cool environmental condition.

Key to *Chelisoches* species known from India (Modified from Srivastava, 2013)

1(2). Genitalia with parameres slightly emarginated near apex (Fig 2g), forceps having blunt teeth at middle; posterior part of pygidium with distinct notch (Fig 2f) ..... *C. brevipennis*

2(1). Parameres not emarginated near apex (Fig 3g), forceps having internal armatures in basal half with internal margin crenulate; posterior part of pygidium truncated (Fig3f) ..... *C. morio*

Predatory potential of black earwig *C. brevipennis* on inflorescence caterpillar

Earwigs were collected from the inflorescence which is at maturity stage (Fig 1a and 1b). Due to attack by the *Tirathaba* sp. inflorescence was in half decayed condition (Fig 1c) with actively moving earwigs inside and holding *Tirathaba* sp. larvae with forceps. So the predation of *C. brevipennis* and *C. morio* on larvae of arecanut inflorescence caterpillar, *Tirathaba* sp. (Fig 1f) was studied in laboratory condition. The results indicated that, there was a significant difference in the number of larvae released into the insect breeding dish to the number of larvae remained in the insect breeding dish after predator release. The number of larvae released into insect breeding dish before predator release was (5.00±0.00) but after 48 hours of predator *C. brevipennis* activity the larval population had been reduced (1.60±0.69) indicating effective predation of *C. brevipennis* on *Tirathaba* sp. [t(9) = 3.25] (Table 2).

Predatory potential of black earwig *C. morio* on inflorescence caterpillar

Similarly, same results were obtained in case of *C. morio* which actively predated significant number of *Tirathaba* larvae (2.10±0.73) in the insect breeding dish to the released larvae into insect breeding dish (5.00±0.00) before predator release (Table 2). Earlier, Zhong *et al*. (2016) reported predatory role of *C. morio* on larval stage of *Tirathaba rufivena,* a pest of palms in Southeast Asia and China. These black earwigs are robust and larger in size and attack the inflorescence caterpillar with forceps, holding the larvae and starts feeding on it. These preliminary results indicated that, *C. morio* and *C. brevipennis* adults were able to predate on early instars larvae of *Tirathaba* sp. Previous studies by Zhong *et al*. (2016) confirmed the preference of *C. morio* on younger and smaller larvae, but that they had poor ability to feed on the later instar larvae. This difference may be due to the fact that later instar larvae are able to spin silken webs which could restrict black earwig activity. In majority of opened arecanut inflorescence we noticed the activity of ants and earwigs within the sheath (Fig 1d &e). Most probably ants are attracted to the sugary exudates from the inflorescence. The ecological role of ants and possible relation either with inflorescence caterpillar and earwigs need to be studied. Naranjo-Guevara *et al*. (2017) recent studies reported that some herbivore induced plant volatiles attract some predatory earwigs. In the present study earwigs may attracted towards arecanut inflorescence due to herbivore induced plant volatiles released due to damage by inflorescence caterpillar. Further studies should focus on this tropic interactions and their significance in different crop ecosystems. It has been reported that *C. morio* predates red palm weevil eggs and young larvae (Abraham and Kurian, 1974) and hence here in this there is a need of observation for establishing relationship in arecanut ecosystem. Similarly, *C. morio* is an important predator feeds on eggs and different stages of *Brontispa*. It was commonly associated with *B. longissima* in majority of plantations and complements *Tetrastichus brontispae* and other biocontrol agents (Li *et al*., 2011). The management of *Tirathaba* sp. by insecticide spray is often cumbersome because larvae occur within the concealed spathe and inaccessible parts of the plant. Hence, it is imperative to look for alternative pest management strategies. These natural enemies are potential candidates to successfully check the pest population. In future, these two potential biocontrol agents *viz*., *C. morio* and *C. brevipennis* could efficiently be utilized for suppressing *Tirathaba* sp. and further strengthens the biocontrol research in arecanut pest management.

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REFERENCES

Abraham, V. A. and Kurian, C. 1974. *Chelisoches morio* F. (Forficulidae: Dermaptera), a predator on eggs and early instar grubs of the red palm weevil, *Rhynchophorus ferrugineus* F. (Curculionidae:Coleoptera). *Journal of Plantation Crops,* **1**: 47–152.

Borelli. 1923. Bollettino deimusei di zoologia edanatomia comparatadella R. Università. *Torino*, **38**: 13-12.

Buxton, J. H. 1974. The biology of the European earwig, *Forficula auricularia* L. with reference to its predatory activities on the damson-hop aphid, *Phorodom humili* (Schrank). PhD Thesis Wye College University of London.

Canellas, N., Pinol, J. and Espadaler, X. 2005. Las tijeretas (Dermaptera, Forficulidae) en el control del pulgon en citricos. *Boletin Sanidad Vegetal y Plagas,* **31**: 61-169.

Chomphukhieo, N., Suksen, K., Uraichuen, S. and Suasa-ard, W. 2008. Biology and feeding capacity of *Chelisoches morio* (Fabricius) (Dermaptera: Chelisochidae) against *Brontispa longissima* Gestro. In: Proceedings of the 46th Kasetsart University annual conference, Kasetsart. 149–154.

Costa, J. T. 2006. The other insect societies. Harvard University Press Cambridge, pp.767.

De Geer, C. 1773. Memoires pour servira I' Histoire des. *Insects*, **3**: 460-504.

Engel, M. S. 2003. The earwigs of Kansas, with a key to genera north of Mexico (Insects: Dermaptera). *Transaction of the Kansas Academy of Science,* **106**(4): 115–123.

Fabricius, J. C. 1775. Systema Entomologicae. Munksgaard, Copenhagen.

Gravena, S. and Da Cunha, J. 1991. Predation of cotton leaf worm first instar larvae, *Alabama argillacea* (Lepidoptera: Noctuidae). *Entomophaga*, **36**(4): 481-491.

Günther, K. and Herter, K. 1974. Ordnung Dermaptera (Ohrwürmer). Helmcke J.G., D Starck, H Wermuth (eds.). *Kükenthal's Handbuch der Zoologiea*, **2**(4): 58-162.

Haas, F. 2018. Biodiversity of dermaptera. *Insect biodiversity: science and society*, **2**:pp.315-334.

Hopkins, H., Maehr, M. D., Haas, F. and Deem, L. S. 2018. Dermaptera Species File. Version 5.0/5.0. http://dermaptera.speciesfile.org [Accessed on 2024-2-10].

Jones, R., Gislstrap, F. and Andrews, K. 1988. Biology and life table for the predaceous earwig, *Doru taeniatum* (Dermaptera:Forficulidae). *Entomophaga*, **33**(1): 43-54.

Kamimura, Y., Karthik, C. M., & Kalleshwaraswamy, C. M. (2023). Time to Restart: Study of the Dermaptera of the Indian Subcontinent. *Indian Journal of Entomology*, **85**(4): 1213–1220.

Karthik, C. M. and Kalleshwaraswamy, C. M. 2023. An annotated checklist of earwigs (Dermaptera) of South India with two new records from India. *Zootaxa*, **5330**(4): 561-585.

Karthik, C. M., Kamimura, Y. and Kalleshwaraswamy, C. M. 2022. A new species of *Diplatys* (Insecta, Dermaptera, Diplatyidae) earwig from the Western Ghats of India. *ZooKeys*, **1088**: 53-64.

Li, C. X., Huang, S. C., Ma, Z. L., Qin, W. Q., Yu, F. Y. and Zhang, Z. H. 2011. Predatory functional response of black earwig, *Chelisoches morio* to coconut leaf beetle, *Brontispa longissima*. *Journal of Fruit Science,* **28**(2): 353–357.

Lv, B. Q., Zhu, W. J., Jin, Q. A., Wen, H. B., Peng, Z. Q. and Du, Y. Z. 2012. Biology of *Chelisoches morio* as an enemy of *Brontispa longissima*. *Chinese Journal of Applied Entomology,* **49**(5): 1268–1273.

Mariani, R., Vera, L. and Virla, E. 1996. Aportes al conocimiento de Dorulineare (Eschs, 1822) (Dermaptera, Forficulidae), uninsecto de importancia agronomica en el Noroeste Argentino. *CIRPON Revista de investigacion*, **4**(1): 13-18.

Naranjo-Guevara, N., Penaflor, M. F. G., Cabezas-Guerrero, M. F. and Bento, J. M. S. 2017. Nocturnal herbivore-induced plant volatiles attract the generalist predatory earwig *Doru luteipes* Scudder. *Science of Nature,* **104**: 1-11.

Renz, D. C. F. and Kevan, D. K. 1991. Dermaptera. Naumann ID *et al*. (eds) The Insects of Australia, 2nd ed. Cornell University Press, Ithaca, N.Y, pp. 360-368.

Schlinge, E. I., Van den Boscb, R. and Dietrick, E. J. 1959. Biological notes on the predaceous earwig, *Labidura riparia* (Pallas), a recent immigrant to California (Dermaptera: Labiduridae). *Journal of Economic Entomol*ogy, **52**: 247-253.

Srivastava, G. K. 2013. Fauna of India and the Adjacent Countries, Dermaptera Part III: Apachyoidea and Forficuloidea. Zoological Survey of India, Kolkata, 268 pp.

Van Huis, A. 1981. Integrated pest management in the small farmer´s maize crop in Nicaragua. *Mededelingen Landbouwhogeschool Wageningen*, **81**(6): 93-100.

Verhoeff, K. W. 1902. Uber Dermapteren. *Zoologischer Anzeiger*, **25**(665): 181-208.

Zhong, B., Lv, C. and Qin, W. 2016. Preliminary study on biology and feeding capacity of *Chelisoches morio* (Fabricius) (Dermaptera: Chelisochidae) on *Tirathaba rufivena* (Walker). *Springer plus*, **5**(1): 1-4.

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Facultative bacterial diversity associated with silverleaf whitefly, *Bemisia tabaci* (Gennadius) on tomato crop

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ABSTRACT: The adults and nymphs of *Bemisia tabaci* (Gennadius) were collected on tomato crop from different locations during 2021-2023. Bacterial colonies were isolated from adults and nymphs of *B. tabaci* using spread-plate technique and identified through 16srRNA sequencing. Nymphs showed high (55%) abundance of bacteria than adults (45%). 63.64% of the bacterial population in the nymphs belong to the phylum Bacillota followed by pseudomonadota (36.36%). In adults, Bacillota found dominant (100%). The class bacilli were dominant in both nymphs and adults (63.64 and 100% respectively). In the nymphal stage Bacillales was dominant order (54.55%). Similarly, in adults also Bacillales was found dominant (77.79%). Bacillaceae was abundant in nymphs (45.45%) and in adults same family accounted for 66.67%. The genus Bacillus was dominant in both nymphs (45.45%) and adults (55.55%). The species, *B. licheniformis*, *B. pumilus*, *B. safensis* and *Staphylococcus saprophyticus* were found common between adults and nymphs.

Keywords: 16srRNA, *Bacillus, Bemisia tabaci,* Facultative bacteria

INTRODUCTION

Whitefly (Hemiptera: Aleyrodidae) is one of the most economically important groups of pests with global distribution and very wide range of host plants (Kanakala and Ghanim, 2019). It causes damage in an active way by acting as vector for various plant viruses (*Begomovirus*, *Crini* virus, *Clostero* virus *etc*.) and passively by encouraging sooty mould deposits on plants through honeydew secretion (Head and Savinelli, 2008). Later, the sooty mould formed by the honeydew secreted by them leads to the closing of stomata as a result the gas exchange by the plants will be interrupted and leads to poor development of plants. So far, 440 species of whiteflies under 63 genera are known from India and few of them are economically important (Sundararaj *et al*., 2018). More than 320 plant species belonging to 225 genera and 73 families in India have been recorded as hosts of whiteflies (Sundararaj and Pushpa, 2011).

The most commonly known whitefly is the silverleaf whitefly, *Bemisia tabaci* (Gennadius), which is originated in Central Asia and invaded all over the world. The major host crops of this pests are field crops (Green gram, Soybean, Blackgram, etc.), vegetables (Tomato, Chilli, Bhendi, Brinjal, Beans, Gourds, etc.), flower crops (Chrysanthemum, Jasmine, Marigold etc.) and commercial crops (Cotton, Tobacco, Jute etc.) and infestation on plantation crops is rarely seen. Currently there are 40 cryptic species (morphologically indistinguishable but genetically distinct in biological characteristic species) has been recorded in *B. tabaci*. The Middle East–Asia Minor 1 (MEAM1) and Mediterranean (MED) complexes (previously known as B biotype and Q biotype, respectively) are considered as the most invasive species with broad host range of plants. New world 1 (NW 1, A biotype) is also reported in some parts of the world (De-Barro *et al*., 2011).

One of the factors for successful establishment of *B. tabaci* is its nutritional flexibility. The two main functions of these endosymbionts of sap sucking insects are; those which are beneficial to the insect under specific ecological conditions and those which play a role in metabolic activities of the insect. (Gosalbes *et al*., 2010). Along with this, the microbes inside the insects plays major role in their survival, development, reproduction, fecundity, viral transmission and resistance against the various chemicals. About 99 per cent of symbiotic bacteria are non-culturable under laboratory conditions (Amann *et al.*, 1995) but advances in molecular biology have outstandingly improved the culture-independent techniques to study microorganisms, all praises to PCR amplification of bacterial genes straight from environmental samples, pursued by direct sequencing of PCR products. Different gene targets like 16S, 23S, GroEL *etc*., have been used to identify bacteria. Several studies have used PCR techniques to identify the different endosymbionts like *Portieraaleyrodidarum* (Primary), *Wolbachia*, *Rickettsia, Arsenophonus, Cardinium, Hamiltonella, Fritschea*, *Bacillus*, *Staphylococcus*, *Enterococcus* (Secondary) in *B. tabaci*. The current study is giving special emphasis on the diversity of facultative bacteria in the nymphs and adults of *B. tabaci* on tomato.

MATERIALS AND METHODS

The whiteflies and nymphs collected on tomato from different locations were starved for 3 h and surface sterilized with 70 per cent ethanol for 1 minute followed by 0.1 per cent sodium hypochlorite for 1 minute and then rinsed with sterile distilled water for 2 to 3 times to remove the external microbes and wax.

Serial dilution and plating

The surface sterilized adults were crushed in a sterilized 1.5 ml micro-centrifuge tube using a sterilized micro pestle with 1 ml of phosphate buffer saline (PBS) solution (pH 7.4). Prior to that, micro-centrifuge tubes were labelled with date, host and location. The homogenized samples were centrifuged at 2000 RPM for 10 minutes. Then 100 µl of the homogenized mixture was added to micro centrifuge tubes containing 900 µl of sterile distilled water and serial dilution of samples was made up to 10^{-7} dilutions. 100 µl of aliquot of all the dilutions were plated on both 1M of nutrient agar media and spread using a sterilized glass spreader. Then, Petri plates were incubated at 28 ^{0}C for 24 to 48 h in bio-oxygen demand (BOD) incubator. Further, plates were observed for microbial growth after every 24 hours.

Purification and storage of colonies of bacteria

Representative colony from each colonies showing similar morphology were selected and pure culture was obtained by sub-culturing it in the same media. The pure cultures were added to autoclaved nutrient broth in sterilized test tubes along with respective labels and incubated at 28 □ for 24 h in BOD until the clear nutrient broth turn into turbid by the multiplication of bacterial cells.

Bacterial genomic DNA isolation and quantification

Bacterial culture grown in a nutrient broth was used for genomic DNA isolation by following sucrose buffer method. 1.5 ml bacterial culture was transferred to a sterilized micro centrifuge tube with respective label and centrifuged at 1000 rpm for 3 minutes to get a pellet. Later, supernatant was discarded and pellet was retained. It was repeated with a 1.5 ml culture to collect the sufficient amount of pellet. The pellet was re-suspended into 400 µl sucrose buffer (consists of 1M Tris, 0.5M EDTA and 10 per cent sucrose) and subjected to vortex (SPINIX) to dissolve the pellet. Then, 32µl lysozyme was added and incubated for 10 min at 60□ in hot water bath. 140 µl of freshly prepared 10 per cent sodium dodecyl sulphate (SDS) was added along with 5 µl of protease. Later, 240 µl of NaCl (5M) and freshly prepared 10 per cent CTAB was added and incubated for 10 min at 60 □. It was followed by addition of 500 µl chloroform: isoamyl alcohol (24:1) and mixed well by inverting the tube until the phase is mixed completely. The mixture was centrifuged at 12000 rpm in a micro centrifuge (SPINWIN MC03) for 10 min. Upper aqueous phase was transferred to a new labelled tube and 50µl of 3M sodium acetate (ice cold) was added and mixed well. Then 300 µl isopropanol (ice cold) was added and gently mixed to precipitate DNA and the sample was incubated overnight at -20□.

The sample was spun at 12000 rpm for 15 min on the next day, to pellet down DNA and 1ml of 70 per cent ethanol was added to the pellet and spinning was done at 12000 rpm for 10 min (twice). Then the supernatant was discarded and the pellet was allowed for air dry. After complete drying, the DNA pellet was re-suspended in 30 µl of protease, DNase, RNase, free water (GeNei™) followed by 2 µl of RNase treatment and incubation at 60 □ in water bath stored at -20 □ until use (Takakura and Nishio, 2012). The concentration of isolated DNA was quantified by using nanodrop.

Quality and quantity check of genomic DNA

Quality of genomic DNA was checked by 0.8 per cent (0.8g in 100 ml) of agarose which was dissolved in 100 ml of 1X TAE buffer in microwave oven and 5 µl EtBr was added after cooling. This mixture was poured into a pre-set template used with appropriate comb kept on the template, to make wells and the gel was allowed for solidification for 45 minutes. After that, 2µl of DNA was loaded with 2 µl of loading dye (6X Cresol-red DNA loading dye). Electrophoresis was carried at 80 V for 45 min. The genomic DNA was visualized on UV transilluminator (Bio-Rad, USA) and documented using gel documentation system (GelDoc Go).

The amplification of 16s rRNA was carried out by using the universal primer (Forward-5'AGAGTTTGATCCTGGCTCAG3' and Reverse-5' ACGGCTACCTTGTTACGACTT-3'). The stocks of primers were prepared as per the instructions given and prepared a working primers by adding 0.1 ml of stock in 0.9 ml double distilled water, further stored at -20□. Polymerase chain reactions were performed with 25 µl of PCR mixture in PCR system (ProFlex) with an initial denaturation at 94 °C for 3 minutes, followed by 35 cycles each consisting of denaturation for 1 minute at 94°C, annealing for 45 seconds at 59°C with an extension for 1.5 minute at 72°C followed by final extension for 10 minutes at 72 °C and kept hold at 4 ^{0}C for infinite time.The amplified PCR products were

Table 1. Bacterial diversity in the nymphs of the *Bemisia tabaci* collected on tomato crop

Phylum	Class	Order	Family	Species
Bacillota	Bacilli	Bacillales	*Bacillaceae*	*Bacillus licheniformis*
				Bacillus subtilis
				Bacillus cereus
				Bacillus safensis
				Bacillus pumilus
			Staphylococcaceae	*Staphylococcussaprophyticus*
		Lactobacillales	Enterococcaceae	*Enterococcus casseliflavus*
		Enterobacteriales	Enterobacteriaceae	*Klebsiella variicola*
				Atlantibactersubterranea
	Gamma-Proteobacteria		Morganellaceae	*Proteus penneri*
		Pseudomonadales	Pseudomonadaceae	*Pseudomonas putida*
Pseudomonadota				

Table 2. Bacterial diversity in the adults of the *Bemisiatabaci* collected on tomato crop

Phylum	Class	Order	Family	Adults (9)
Bacillota	Bacilli	Bacillales	*Bacillaceae*	*Bacillus safensis*
				Bacillus aerius
				Bacillus pumilus
				B. licheniformis
				Heyndrickxia oleronia *Bacillus velezensis*
			Staphylococcaceae	*Staphylococcus saprophyticus*
		Lactobacillales	Enterococcaceae	*Enterococcus gallinarum* *Enterococcus mundtii*

sent for nucleotide sequencing to Eurofins Genomics India Pvt. Ltd. Bangalore. The obtained DNA sequences corresponding to the 16S rRNA gene was confirmed using BLAST search in NCBI. The obtained forward and reverse sequences were aligned together using the NCBI alignment tool to obtain a contig sequence.

RESULTS AND DISCUSSION

Twenty bacterial isolates were recorded from nymphs and adults of *B. tabaci*, in which nymphs had more number of isolates (11 species) compared to adults (9 species) (Table 1 and 2). The bacterial abundance was observed to be high (55 per cent) in nymphs compared to adults (45 per cent) (Fig. 1).

Phylum level

The bacteria isolated from *B. tabaci* which are collected on tomato grouped into two phyla i.e. Bacillota and Pseudomonadota. 63.64 per cent of the bacterial population in the nymphs belong to the phylum Bacillota and 36.36 per cent belongs to Pseudomonadota. Whereas, in adults, all the obtained bacterial colonies (100 per cent) belong to the phylum Bacillota (Fig. 1).

Class level

The class Bacilli found dominant (63.64 per cent) in the nymphs of *B. tabaci* followed by Gamma proteobacteria which accounted for 36.36 per cent. In

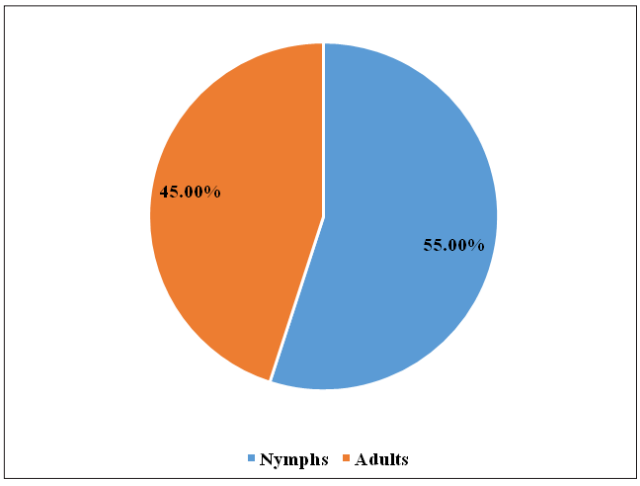

Fig. 1. Bacterial abundance of nymphs and adults of *B. tabaci*

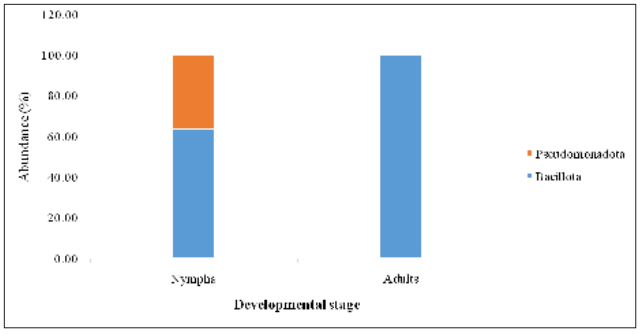

Fig. 2. Bacterial abundance of nymphs and adults of *B. tabaci* at phylum level

the adults of *B. tabaci,* all the bacterial colonies (100 per cent) were belong to the class Bacilli (Fig. 2).

Order level

The bacterial population of *B. tabaci* collected from tomato were classified into four orders (Bacillales, Lactobacillales, Enterobacteriales and Pseudomonadales). The order Bacillales was found to be the most abundant (54.55 per cent) in the nymphs of B. tabaci followed by Enterobacteriales (27.27 per cent). Both Lactobacillales and Pseudomonadales were found least abundant (9.09 per cent each). Whereas, in adults, only two bacterial orders were recorded. Among them, Bacillales was dominant (77.78 per cent) over Lactobacillales which accounted for only 22.22 per cent (Fig. 3).

Family level

The nymphal stage recorded more number (six families) of bacterial families than the adult stage (three families).In nymphs, majority of the bacterial population was belong to the family, Bacillaceae (45.45

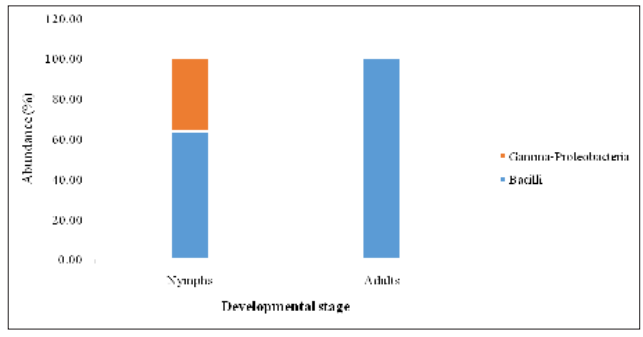

Fig. 3. Bacterial abundance of nymphs and adults of *B. tabaci* at class level

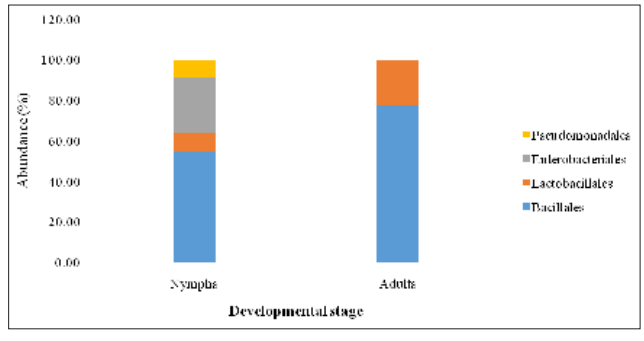

Fig. 4. Bacterial abundance of nymphs and adults of *B. tabaci* at order level

per cent) followed by Enterobacteriaceae (18.18 per cent) and remaining all four families showed equal abundance (9.09 per cent). In case of adults, among the three families, Bacillaceae accounted for 66.67 per cent followed by Enterococcaceae (22.22 per cent) and least abundant family was staphylococcaceae with 11.11 per cent abundance.

Genus level

The bacterial population in the nymphal stage of B. tabaci distributed under seven different genera. In which, Bacillus was the predominant genus (45.45 per cent) and remaining six genera accounts for 9.09 per cent each. Whereas, in adults, only four bacterial genera were recorded, among them Bacillus was the dominant genus (55.55 per cent) followed by Enterococcus (22.22 per cent). The genera *Klebsiella, Atlantibacter, Proteus Pseudomonas,* were found only in nymphal stage, whereas, the genus *Heyndrickxia* was confined only to the adult stage (Fig. 5).

Species level

In the nymphal stage of *B. tabaci*, 11 species of bacteria were recorded whereas, in adults nine bacterial species were recorded. Among them, the bacteria *Bacillus licheniformis*, *B. pumilus*, *B. safensis* and

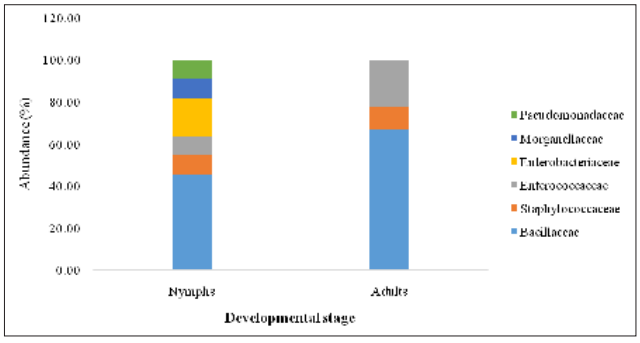

Fig. 5. Bacterial abundance of nymphs and adults of *B. tabaci* at family level

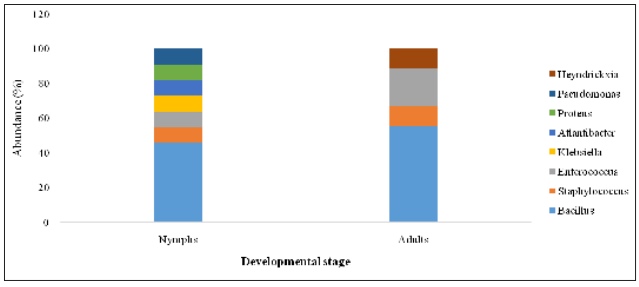

Fig. 6. Bacterial abundance of nymphs and adults of *B. tabaci* at genus level

Staphylococcus saprophyticus were found common in nymphs and adults (Table 1 and 2).

The endosymbionts of cryptic species of *B. tabaci* was determined by Marubayashi *et al*. (2014) through sequencing of 16SrRNA gene. The results of the present study are in accordance with the study of El *et al*. (2022) and Li *et al*. (2023) who determined the diversity of facultative bacteria *Bacillus*, *Staphylococcus*, *Enterobacater*, *Paracoccus*, *Acinetobacter* through plating techniques. Similarly, twenty different bacterial genera, including 31 species belong to Actinobacteria, 'alpha'-, 'beta'-, 'gamma'- Proteobacteria, and Firmicutes were isolated by Indira gandhi *et al*. (2010) from both the B and Q biotypes of *B. tabaci* and among them, *Bacillus*, *Kocuria*, *Moraxellla*, *Micrococcus*, *Sphingomonas* and *Staphylococcus* were common. Moreover, B biotype was associated with *Acinetobacter*, *Deinococcus*, *Modestobacter*, *Microbacterium*, and *Pseudomonas*, whereas, Q biotype was associated with *Arthrobacter*, *Bradyrhizobium*, *Janibacter, Morganella*, *Naxibacter* and *Streptomyces*. Host plants have a great influence on the gut microbial diversity in host insects (Jones *et al*., 2019). The genera Bacillus (30%), Acinetobacter (10%) and Exiguobacterium (10%) were observed by using culture-dependent method by Saranya *et al*. (2022) in the rugose spiralling whitefly, *A. rugioperculatus* reared on coconut plants whereas, Bacillus (81%), Lysinibacillus (11%), Arthrobacter (4%) and Pseudomonas (4%) from banana plants. Similarly, Pujar *et al*. (2023) recorded the dominance of the phylum Bacillota, class Bacilli, order Bacillales, family Bacillaceae and genus Bacillus in *A. Rugioperculatus* collected from four different hosts.

The residing bacteria (not all) perform various functions *viz*., *Bacillus* sp. and *Staphylococcus* sp. produce amylase enzyme which helps in production of medium-length sugars from derived sucrose and increase the stickiness of honeydew (Davidson *et al*., 2000). In *A. rugioperculatus*, along with honeydew secretion, *Bacillus* sp. helps in lactose fermentation and siderophore production. In case of termites it involves in cellulose digestion (Choi *et al*., 2015) and it helps in detoxification of profenophos and chlorpyrifos in *Paracoccusmarginatus* (Krishnamoorthy *et al*., 2020).

The symbiotic relationship between the insects and bacteria caused them to be successful creatures on the earth. Though, all the facultative microbes residing in the insects doesn't have any role (called opportunistic microbes) in the insects, some of the microbes are directly or indirectly involving in theirsurvival, reproduction and development of the insects.

REFERENCES

Amann, R. I., Ludwig, W. and Schleifer, K. H. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiology Reviews,* **59**(1): 143-169.

Choi, M. Y., Ahn, J. H., Song, J., Kim, S. H., Bae, J. W. and Weon, H. Y. 2015. Analysis of gut bacterial diversity and exploration of cellulose-degrading bacteria in xylophagous insects. *Korean Journal of Microbiology,* **51**(3): 209-220.

Davidson, E. W., Rosell, R. C. and Hendrix, D. L. 2000. Culturable bacteria associated with the whitefly, *Bemisiaargentifolii* (Homoptera: Aleyrodidae). *Florida Entomologist,* **6**:159-169.

De Barro, P. J., Liu, S. S., Boykin, L. M. and Dinsdale, A. B. 2011. *Bemisiatabaci*: a statement of species status. *Annual Review of Entomology,* **56**: 1-19.

El Hamss, H., Maruthi, M. N., Ally, H. M., Omongo, C. A., Wang, H. L., Van Brunschot, S., Colvin, J. and Delatte, H. 2022. Spatio-temporal changes in endosymbiont diversity and composition in the African cassava whitefly, Bemisia tabaci SSA1. *Frontiers in Microbiology*, **13:** p.986226.

Gosalbes, M. J., Latorre, A., Lamelas, A. and Moya, A. 2010. Genomics of intracellular symbionts in insects. *International Journal of Medical Microbiology,* **300**(5): 271-278.

Head, G. and Savinelli, C. 2008. Adapting insect resistance management programs to local needs. Insect resistance management: biology, economics and prediction, p.305.

Indira Gandhi, P., Yoon, C., Yang, J. O., Cho, S., Sa, T. M., Kim, G. H. 2010. Microbial communities in the developmental stages of B and Q biotypes of sweet potato whitefly, *Bemisiatabaci* (Hemiptera: Aleyrodidae). *Applied Biological Chemistry,* **53**(5): 605-17.

Jones, A. G., Mason, C. J., Felton, G. W. and Hoover, K. 2019. Host plant and population source drive diversity of microbial gut communities in two polyphagous insects. *Scientific Reports,* **9**(1): 1 - 11.

Kanakala, S. and Ghanim, M. 2019. Global genetic diversity and geographical distribution of *Bemisiatabaci* and its bacterial endosymbionts. *PLoS ONE,* **14**: e0213946.

Krishnamoorthy, R., ArulJose, P., Janahiraman, V., Indira Gandhi, P., Gandhi Gracy, R., Jalali, S. K., Senthil Kumar, M., Malathi, V. And Anandham, R. 2020. Function and insecticidal activity of bacteria associated with papaya mealybug, *Paracoccusmarginatus* Williams & Granara de Willink (Hemiptera: Pseudococcidae). *Biocontrol. Science Technol,* **30**(8): 762–778.

Li, S. N., Zhang, C., Li, F., Ren, N. Q. And Ho, S. H. 2023. Recent advances of algae-bacteria consortia in aquatic remediation. *Critical Reviews in Environmental Science and Technology,* **53**(3): 315-339.

Marubayashi, J. M., Kliot, A., Yuki, V. A., Rezende, J. A. M., Krause-Sakate, R., Pavan, M. A. and Ghanim, M. 2014. Diversity and localization of bacterial endosymbionts from whitefly species collected in Brazil. *PloS one,* **9**(9): 108363.

Pujar, K., Naik, D. J. and Shivanna, B. 2023. Study on comparative diversity of bacterial endosymbionts in invasive rugose spiralling whitefly, *Aleurodicusrugioperculatus* Martin on different hosts. *Mysore Journal of Agricultural Sciences,* **57**(2).

Saranya, M., Kennedy, J. S. and Anandham, R. 2022. Functional characterization of cultivable gut bacterial communities associated with rugose spiralling whitefly, *Aleurodicusrugioperculatus* Martin. *3 Biotech,* **12**(1): 14.

Sundararaj, R. and Pushpa, R. 2011. In B. V. David (Ed.), the whitefly or Mealywing bugs: Bioecology, Host specificity and management. Germany: Lambert Academic Publishing: 20-57.

Sundararaj, R., Amuthavalli, T. and Vimala, D. 2018. Invasion and establishment of the solanum whitefly *Aleurothrixustrachoides* (Back) (Hemiptera: Aleyrodidae) in South India. *Current Science,* **115**(1): 29-31.

Takakura, K. I. and Nishio, T. 2012. Safer DNA extraction from plant tissues using sucrose buffer and glass fiber filter. *Plant Research,* **125**(6): 805-807.

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Insights into the biochemical basis of ovipositional preference of *Earias vittella* (Fabricius) in *Abelmoschus* species

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ABSTRACT: The present study investigated the ovipositional preference of okra shoot and fruit borer, *Earias vittella* (Fabricius) (Order: Lepidoptera; Family: Noctuidae) in relation to host plant biochemical traits independently and also through combining with host plant morphological traits in different wild and cultivated *Abelmoschus* species. Correlation studies revealed a significant positive relationship between the number of eggs laid by *E. vittella* to total protein (P, r = 0.17) and a significant negative relationship with total phenols (TP, r = -0.57), total flavonoids (TF, r = -0.38), total sugar (TS, r = -0.57) and total reducing sugars (TRS, r = -0.64). Path co-efficient analysis revealed that total phenols, flavonoids, and total reducing sugars appear to be particularly important, as they, directly and indirectly, affect *E. vittella* egg-laying choice. Multiple step-wise regression analyses of the number of eggs laid by *E. vittella* on different *Abelmoschus* species with host plant biochemical traits together explained the variability in the egg laying to the tune of 54% (R^2 = 0.54; $Y=26.71-0.30_{TP}+0.32_{TF}-1.39TS-4.41_{TRS}+8.29_{P}$; R^2=0.54; VIF=2.19). Further, combining the significant biochemical traits (TRS and P) with significant morphological traits (FL: fruit length, FW: fruit width, T: trichomes) based on *r/SE* explained the variability in the number of eggs laid by *E. vittella* to the tune of 78%; $Y=7.15+1.35_{FL}-3.64_{FW}+0.05_{T}-2.00_{TRS}+1.66_{P}$; R_2=0.78; VIF=4.56.

Keywords: Correlation, cultivated species, egg laying, okra, multiple linear regression, shoot and fruit borer, wild species.

INTRODUCTION

The survival and development of phytophagous lepidopteran insects primarily rely on their selection of appropriate oviposition sites on their host plants. The decisions made by gravid females in choosing suitable oviposition sites significantly influence the performance and survival of their offspring, ultimately impacting the species' reproductive success (García-Barros and Fartmann, 2009). The process of host plant selection for laying their eggs is influenced by a variety of factors, including morphological (such as the size of the plant or plant parts, shape, color, leaf hairs, cuticle thickness), (Keerthi *et al.,* 2023) and biochemical traits etc. (Carrasco *et al.*, 2015). Insects have evolved to detect and assess these traits that support their reproductive success, leading to positive preference-performance relationships (Beck, 1965; Kessler and Baldwin, 2002; Sharma, 2007; Dhillon and Sharma, 2004; War *et al.,* 2012; Da Silva *et al*., 2021; Ali *et al.,* 2019; Coapio *et al.,* 2018; Thakur *et al*., 2017). Okra shoot and fruit borer, *Earias vittella* (Fabricius) (Lepidoptera: Nolidae), is an economically important oligophagous pest that feeds on numerous host plants of Malvaceae. In okra, gravid female moths of *E. vittella* lay eggs singly on shoot tips, flower buds and tender fruits. The neonate larvae bore into delicate terminal shoots during the vegetative phase and fruit formation; they bore into flower buds and young fruits (Qasim *et al*., 2018). Damaged shoots wilt and dry out, and infested fruits have a distorted look and contain larval excrement, rendering them unfit for consumption, and crop losses often range from 3.5 to 90 percent (Hafeez *et al.*, 2019; Mandal *et al.*, 2006).

In the case of *E. vittella*, our earlier studies revealed that host plant morphological traits, namely number of branches (NB), stem diameter (SD), leaf length (LL), fruit length (FL), fruit width (FW) and trichomes density (T) significantly influenced the female moth egg laying choice. Step-wise linear regression equations showed that a combination of these morphological traits could explain the variability in the number of eggs laid by *E. vittella* to the tune of 79% (y=17.29-0.61NB-8.17SD+0.48LL+1.16FL-5.73FW+0.11T, R^2=0.79). Of these traits, fruit traits (fruit length, fruit width, and trichomes on fruit) were found to impact the egg-laying choice of moths exclusively. Further, consideration of only the fruit length (FL) alone was found to explain the maximum variability in egg laying choice of female moth *E. vittella* (R^2= 0.72) (Krishna Kumar *et al*.,

2023). However, in addition to the morphological traits, several studies revealed that biochemical traits such as phenols, flavonoids, sugars, etc. also found to influence the host plant preference of *E. vittella* during the egg laying, feeding vis-a-vis progeny fitness (Manju *et al.*, 2021; Kumar *et al.*, 2021; Sandhi *et al.*, 2017; Gautam *et al*., 2013; Koujalagi *et al*., 2009). The presence or absence of specific host plant biochemical traits can determine whether the particular host plant is preferred or avoided by insects (War *et al.*, 2012; Painter, 1951); understanding the potential host plant biochemical traits that are underlying the oviposition preference of *E. vittella* will serve as ready reckoners during host plant resistance breeding programs. Therefore, an attempt was made to explore the association of different host-plant biochemical traits with the oviposition preference of *E. vittella,* in wild and cultivated species of *Abelmoschus.*

MATERIALS AND METHODS

The present study was conducted at the Division of Crop Protection, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru, India (12°58'N, 77°35'E, 890 m above sea level) during 2021–2022. Seeds of selected wild species of *Abelmoschus*, *viz.*, *Abelmoschus tetraphyllus* (Roxb. ex Hornem.) Hochr., *Abelmoschus tuberculatus* Pal & Singh and *Abelmoschus angulosus* Wall. ex-Wight & Arn. (var. grandiflorus) along with the cultivated species (*Abelmoschus esculentus* L. (Moench) cv. Arka Anamika) were procured from the Division of Vegetable Crops, ICAR-IIHR, Bengaluru. The host plants were grown in polybags (6 x 8") containing a standard pot mixture (Red soil 40%; Coco peat 30%; Farm yard manure 30%) without any pesticide application (Agboyi *et al.*, 2019). Regular water sprays were given at frequent intervals to avoid insect pest infestation.

Insect culture maintenance

Okra fruits infested with *E. vittella* larvae were collected from the experimental fields of ICAR-IIHR. The larvae were reared by providing fresh immature okra fruits in plastic containers (13.63 × 8.25 × 4.88 cm) until pupation. The emerged adult moths were collected and released into net cages (1 × 1 × 1 m) for mating. The gravid females were separated and used for ovipositional preference studies.

Oviposition assays

Choice and no-choice oviposition assays with different host plants provide clues about the insect host plant's preference for egg-laying. The above-selected host plants were arranged randomly in a net cage and exposed to *E. vittella* (@ 2 moths/plant) for 48 hrs. In the no-choice assay, a single species of each of the six host plants was arranged randomly (N = 6) in net cages. The plants were exposed to gravid females of *E. vittella* (@ 2 moths/plant) for 48 hrs. Observations were recorded on the number of eggs laid on each host plant. Each assay was replicated six times at different times (Uzun *et al.,* 2015).

Biochemical traits

Observations on different biochemical traits were recorded, namely, total phenols (TP) (Singleton *et al*., 1999), total flavonoids (TF) (Zhishen *et al*., 1999), total and reducing sugar (TS& TRS) (Somogyi, 1952), total protein (P) (Lowry *et al.,* 1951), total free amino acids (FA) (Moore and Stein, 1954) and total antioxidants (AO) (Benzie and Strain, 1996) using double beam UV-visible spectrophotometer. For this investigation, 10-day-old okra fruits were utilized from a 55-day-old plant.

Morphological traits

Data on different morphological traits of host plants namely plant traits [plant height (PH, cm), number of branches/ plant (NB) and stem diameter (SD, cm)], leaf traits [number of leaves/ plant (NL), leaf length (LL, cm), petiole diameter (PD, cm)], fruit traits [number of fruits/ plant (NF), fruit length (FL, cm), fruit width (FW, cm), trichomes density on fruits (T, cm^2)] as per our earlier studies (Krishna Kumar *et al.,* 2023) was used.

Statistical analysis

Data on biochemical and morphological traits and the number of eggs laid were subjected to correlation analysis, and the correlation coefficient values were plotted in Corrplot using R 4.2.0. Path-coefficient analyses were also carried out between the plant traits and the number of eggs laid. To get further insights, a step-wise regression procedure (Ryan, 1997) was employed to select the most crucial plant traits (based on *r/SE*, a stringent criterion for identifying significant variables for regression analysis) influencing the variability in the egg-laying choice of *E. vittella*. This technique identified, stage by stage, trait(s) significantly related to egg-laying choice (y). Further, as a measure of the goodness-of-fit of the models developed, values pertaining to the Coefficient of Determination (R^2) (Agostid'no and Stephens, 1986) were calculated. The Variance Inflation Factor (VIF) was computed to test the multi-collinearity of variables.

Table 1. Descriptive statistics of biochemical traits of different *Abelmoschus* genotypes

Host plants	Total Phenols (mg GAE/100g)	Total Flavonoids (mg CE/100g)	Total sugar (g /100g)	Reducing Sugar (g/100g)	Protein (mg BSA/ 100g)	Free amino acids (mg/ 100g)	Antioxidant (mg AEAC/ 100 g)
A. angulus	111.50±10.28^{a} (89.3-147.8)	51.38±1.46^{a} (46.8-56.5)	11.46±0.55^{a} (9.9-13.1)	2.92±0.44^{a} (1.9-3.9)	3.56±0.24^{abc} (3.2-4.5)	3.02±0.26^{a} (2.1-3.7)	68.5±1.15^{c} (64.2-72.4)
A. tetraphyllus	78.52±4.77^{b} (71.2-88.2)	36.04±1.15^{b} (32.1-39.6)	12.31±1.26^{a} (11.8-13.2)	2.65±0.19^{ab} (2.4-3.4)	3.68±0.07^{ab} (3.5-3.8)	1.30±0.13^{d} (1.0-1.8)	39.33±0.73^{cd} (37.3-42.4)
A. tuberculatus	80.75±0.87^{e} (38.1-43.1)	19.63±0.61^{f} (17.9-22.1)	7.54±1.22^{bc} (7.1-8.2)	2.66±0.29^{ab} (2.0-3.4)	2.29±0.11^{g} (1.9-2.5)	2.78±0.20^{ab} (2.1-3.3)	34.03±0.91^{e} (31.8-38.2)
Arka anamika	40.52±2.86^{dc} (33.2-61.3)	24.85±0.84^{dc} (22.6-28.1)	6.13±0.31^{de} (5.3-6.9)	1.80±0.21^{c} (1.1-2.4)	3.31±0.06^{bcd} (3.2-3.5)	2.07±0.14^{c} (1.7-2.5)	46.39±3.03^{b} (40.1-59.6)
ACC 1685	40.11±2.32^{dc} (34.6-46.2)	20.31±1.18^{f} (17.5-25.9)	8.54±0.58^{b} (6.5-9.6)	2.06±0.30^{bc} (1.0-2.9)	2.81±0.20^{f} (2.4-3.4)	2.21±0.26^{bc} (1.6-2.9)	37.48±1.45^{cde} (32.2-41.8)
IIHR 356	56.64±4.89^{c} (61.2-69.3)	31.11±0.66^{c} (29.9-34.3)	5.60±0.31^{de} (4.9-6.7)	1.01±0.01^{d} (1.0-1.1)	3.90±0.05^{a} (3.7-4.0)	1.17±0.13^{d} (0.8-1.6)	65.16±1.13^{a} (60.8-69.5)
IIHR 358	39.83±2.36^{e} (34.1-48.1)	19.68±0.28^{f} (18.3-20.2)	5.58±1.51^{de} (4.2-7.1)	1.38±0.14^{cd} (1.0-1.9)	3.23±0.14^{cde} (2.9-3.7)	1.78±0.24^{cd} (1.1-2.6)	36.54±0.95^{cde} (32.2-38.6)
IIHR 379	55.29±1.26^{cd} (51.3-58.6)	25.66±1.79^{dc} (22.1-34.2)	5.19±2.09^{e} (4.9-5.4)	1.64±0.22^{cd} (1.2-2.5)	3.10±0.05^{def} (3.0-3.2)	2.08±0.32^{c} (1.0-3.0)	41.29±2.75^{c} (31.9-52.8)
IIHR 394	46.67±4.12^{dc} (39.6-61.9)	22.49±1.19^{ef} (20.1-28.1)	6.62±0.74^{cd} (4.1-8.3)	1.60±0.20^{cd} (1.0-2.2)	3.06±0.12^{def} (2.7-3.4)	2.78±0.27^{ab} (2.0-3.7)	38.49±1.84^{cde} (30.6-44.1)
IIHR 402	38.14±1.45^{e} (34.8-43.1)	18.76±1.36^{f} (14.8-24.6)	7.83±1.53^{bc} (6.4-9.6)	1.45±0.22^{cd} (1.0-2.1)	3.08±0.26^{ef} (2.5-3.7)	3.10±0.06^{a} (2.9-3.3)	34.86±1.44^{de} (30.8-39.8)
***F* (9,50)**	46.53	81.01	44.88	9.96	13.94	15.31	52.33
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
C D (0.05)	15.18	3.31	1.25	0.71	0.39	0.63	5.02

Figures in parentheses show the range of values; *P* = significanceat 5% level; CD (0.05) = critical difference at 5% level

RESULTS AND DISCUSSION

Among all the host plant biochemical traits studied, significantly higher amounts of total phenols (mg GAE/100g) were observed in the wild species *A. angulosus* var. *grandiflorus* (111.50±10.28mg GAE/100g) and the lowest was found in cultivated line namely IIHR 402 (38.14±1.45 mg GAE/100g)[$F_{(9, 50)}$ = 46.53; *P*<0.0001; CD = 15.18]. Similarly, the total flavonoid content was observed to be the highest in wild species *A. angluosus* var. *grandiflorus* (51.38±1.46mg CE/100g) and lowest in the cultivated line namely IIHR 402 (18.76±1.36 mg CE/100g) [$F_{(9,50)}$ = 81.01; *P*<0.0001; CD = 3.31]. Higher amounts of total sugars were observed in *A. tetraphyllus* (12.31±1.26g /100g) and the lowest were noticed in IIHR 379 (5.19±2.09 g /100g) [$F_{(9, 50)}$ = 44.88; *P*<0.0001; CD = 1.25]. Higher amounts of reducing sugar significantly were observed in *A. angulosus* var. *grandiflorus* (2.92±0.44g /100g) and lowest in IIHR 356 (1.01±0.01 g /100g) [$F_{(9, 50)}$ = 9.96; *P*<0.0001, CD = 0.71]. Observations on total protein content among the host plants revealed significantly higher amounts in *A. tetraphyllus* (3.68±0.07mg BSA /100g)and low protein in *A. tuberculatus* (2.29±0.11 mg BSA /100g) [$F_{(9, 50)}$ = 13.94; *P*<0.0001; CD = 0.39]. Significantly, the highest amounts of total free amino acids were observed in IIHR 402 (3.10±0.06 mg/ 100g) and the lowest in IIHR 356 (1.17±0.13 mg/ 100g) [$F_{(9, 50)}$ = 15.31; *P*<0.0001; C. D. = 0.63]. Significantly, the highest total antioxidant content was observed in *A. angluosus* (68.5±1.15 mg/100g) and the lowest in *A. tuberculatus* (34.03±0.91 mg/100g) [$F_{(9, 50)}$ = 52.33; *P* = <0.0001; CD = 5.02] (Table 1).

Variations in host plant traits, including nutritional

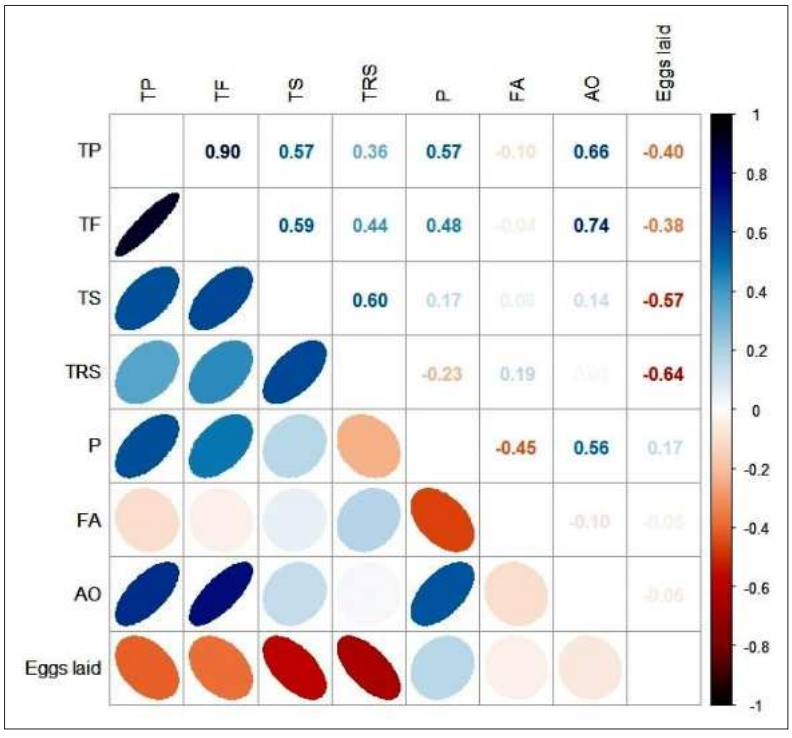

Fig. 1. **Correlation analysis of oviposition preference of *E. Vittella* and biochemical traits of *Abelmoschus* species. The correlation matrix shows that the lower left to high right ellipse shows a positive relationship; lower right to higher left shows a negative correlation. Thin ellipse size shows higher correlation coefficient value; the thicker one shows lower the value. Total phenols, TP; Total flavonoids, TF; Total sugar, TS; Total reducing sugar, TRS; Total protein, P; Total free amino acids, FA; Total antioxidant, AO**.

quality and defensive characteristics, significantly impact plant resistance against pests (Birke and Aluja, 2018). Ecological, physiological, and behavioral factors also influence insect oviposition preferences and offspring fitness (Balagawi *et al.,* 2013; Birke *et al.,* 2015). The relationship between female oviposition preference and offspring performance is strongly associated with their dietary habits and the absence of adverse effects from host plant biochemistry, crucial for maximizing offspring fitness (Hafsi *et al.,* 2016; Gripenberg *et al.,* 2010; Clark *et al.,* 2011). Thus, the initial phase of insect oviposition choice relies heavily on the diverse morphological and biochemical traits of host plants (Beck, 1965; War and Sharma, 2014).

Correlation analysis

Biochemical traits namely, total phenols (TP; r=-0.40; p<0.0001), total flavonoids (TF; r=-0.38; p<0.0001), total sugar (TS; r=-0.57; p<0.0001) and total reducing sugar (TRS; r=-0.64; p<0.0001) exhibited a significant negative correlation and a significant positive correlation was obtained from total protein (P; r=0.17; p=0.0138) with the number of eggs laid by *E. vittella*. Other traits such as total free amino acids (FA) and total antioxidants (AO) did not show any significant correlation (Figure 1).

Data analysis of biochemical traits indicates that higher levels of total phenols, total flavonoids, total sugars, and total reducing sugars in host plants negatively affect the oviposition behaviour of the okra shoot and fruit borer, *E. vittella*. In this study, wild species such as *A. tetraphyllus*, *A. angulosus* var. *grandiflorus*, and *A. tuberculatus* exhibited higher concentrations of phenols, flavonoids, total sugars, and reducing sugars compared to cultivated A. esculentus lines. Previous research on different Abelmoschus spp. also demonstrated that *A. tetraphyllus* and *A. angulosus* var. *grandiflorus* possess significant resistance to *E. vittella* due to their higher levels of total phenols, total sugars, and reducing sugars, while *A. tuberculatus* exhibited moderate field resistance with moderate levels of these compounds (Sandhi *et al.,* 2017; Doshi, 2004).

Most insects are negatively affected by phenolic compounds due to their toxic nature (Palial *et al.,* 2018; Dreyer and Campbell, 1987). Studies by Kumar *et al.* (2021), Halder *et al.* (2015), and Gautam *et al.* (2013) revealed that the presence of phenols in okra fruits has a detrimental effect on *E. vittella* infestation. Similarly, Sultani *et al.* (2011) reported that higher total sugar content reduces the ovipositional preference of

Table 2. Step-wise linear regression models to estimate *E. vittella* ovipositional preference using biochemical traits of *Abelmoschus* species

Variables	Model	R^2	VIF
TP	$Y= 37.26-0.20_{TP}$	0.16	-
TF	$Y= 37.53-0.44_{TF}$	0.14	-
TS	$Y= 46.47-2.71_{TS}$	0.33	-
TRS	$Y= 44.81-9.92_{TRS}$	0.41	-
P	$Y= 12.91+4.00_{P}$	0.03	-
TP+TF	$Y= 37.70-0.17_{TP}-0.09_{TF}$	0.16	1.19
TP+TS	$Y= 47.35-0.05_{TP}-2.42_{TS}$	0.34	1.51
TP+TRS	$Y=48.33-0.10_{TP}-8.81_{TRS}$	0.44	1.78
TP+P	$Y=3.33-0.38_{TP}+13.77_{P}$	0.40	1.68
TF+TS	$Y= 47.05-0.07_{TF}-2.55_{TS}$	0.33	1.50
TF+TRS	$Y= 47.05-0.14_{TF}-9.09_{TRS}$	0.42	1.72
TF+P	$Y=10.76-0.70_{TF}+10.59_{P}$	0.31	1.44
TS+TRS	$Y=50.29-1.42_{TF}-7.10_{P}$	0.46	1.86
TS+P	$Y=27.61-2.94_{TS}+6.47_{P}$	0.41	1.69
TRS+P	$Y=42.64-9.82_{TRS}+0.62_{P}$	0.41	1.68
TP+TF+TS	$Y=46.93-0.11_{TP}+0.15_{TF}-2.49_{TS}$	0.34	1.52
TP+TF+TRS	$Y=47.54-0.22_{TP}+0.32_{TF}-9.34_{TRS}$	0.45	1.82
TP+TF+P	$Y=3.02-0.40_{TP}+0.04_{TF}+13.81_{P}$	0.40	1.68
TP+TS+TRS	$Y=51.03-0.05_{TP}-1.18_{TS}-7.06_{TRS}$	0.47	1.88
TP+TS+P	$Y=16.26-0.24_{TP}-1.87_{TS}+11.68_{P}$	0.50	2.01
TP+TRS+P	$Y=25.48-0.23_{TP}-6.09_{TRS}+7.88_{P}$	0.45	1.97
TF+TS+TRS	$Y=50.29+0.001_{TF}-1.42_{TS}-7.11_{TRS}$	0.46	1.86
TF+TS+P	$Y=23.05-0.33_{TF}-2.23_{TS}+9.03_{P}$	0.45	1.81
TF+TRS+P	$Y=34.76-0.30_{TF}-7.52_{TRS}+4.21_{P}$	0.44	1.77
TS+TRS+P	$Y=39.51-1.77_{TS}-5.86_{TRS}+3.47_{P}$	0.48	1.93
TP+TF+TS+TRS	$Y=50.30-0.18_{TP}+0.36_{TF}-1.25_{TS}-7.56_{TRS}$	0.49	1.94
TP+TF+TS+P	$Y=15.32-0.32_{TP}+0.22_{TF}-1.96_{TS}+11.81_{P}$	0.51	2.04
TP+TF+TRS+P	$Y=25.46-0.33_{TP}+0.27_{TF}-6.63_{TRS}+7.65_{P}$	0.50	2.00
TP+TS+TRS+P	$Y=26.68-0.18_{TP}-1.33_{TS}-3.88_{TRS}+8.53_{P}$	0.53	2.13
TF+TS+TRS+P	$Y=35.11-0.18_{TF}-1.59_{TS}-4.89_{TRS}+5.33_{P}$	0.49	1.97
TP+TF+TS+TRS+P	$Y= 26.71-0.30_{TP}+0.32_{TF}-1.39_{TS}-4.41_{TRS}+8.29_{P}$	0.54	2.19

TP = Total phenols; TF = Total flavonoids; TS = Total sugar; TRS = Total reducing sugar; P = Total protein.

E. vittella. However, research by Sundararaj and David (1987) suggested that higher quantities of reducing sugars, particularly from immature fruit parts, may be favourable for *E. vittella*. Unlike the current study focusing on ovipositional preference, Sundararaj and David (1987) primarily examined larval food quality and reproductive biology. Furthermore, the present study indicates that higher total protein content in the host plant facilitates greater egg laying by female *E. vittella* moths, showing a significant positive correlation. Interestingly, cultivated *A. esculentus* lines exhibited higher protein content compared to wild species. Previous studies have also shown that higher total protein content from okra and cotton plants enhances fecundity in *E. vittella* (Basker *et al.,* 2014; Sundararaj and David, 1987).

The high levels of various biochemical parameters in wild species and their negative correlation with *E. vittella* egg laying suggest that a combination of these parameters rather than a single one influences ovipositional non-preference. Literature indicates that biochemical traits influence lepidopteran pests' egg-laying choices, serving as a significant factor in selecting suitable host plants for offspring fitness (Kogan and Ortman, 1978). In the case of *Abelmoschus* species, as observed in this study, biochemical traits such as phenols, flavonoids, and sugars play a crucial role in positively impacting ovipositional non-preference against the okra shoot and fruit borer, *E. vittella*.

Table 3. Step-wise linear regression models to estimate *E. vittella* ovipositional preference using a combination of host-plant (*Abelmoschus* species) morphological and biochemical traits

Variables	Model	R^2	VIF
FL+TRS	$Y= 4.90+1.20_{FL}-1.92_{TRS}$	0.73	3.70
FL+P	$Y= -3.76+1.33_{FL}+4.19_{P}$	0.75	4.08
FW+TRS	$Y= 32.06+7.30_{FW}-7.25_{TRS}$	0.50	1.99
FW+P	$Y= -7.11+12.93_{FW}+6.06_{P}$	0.40	1.67
T+TRS	$Y= 35.87+0.11_{T}-10.06_{TRS}$	0.51	2.05
T+P	$Y= 12.10+0.09_{T}+1.77_{P}$	0.10	1.11
FL+FW+TRS	$Y= 15.94+1.42_{FL}-3.83_{FW}-1.81_{TRS}$	0.74	3.89
FL+FW+P	$Y= -1.44+1.49_{FL}-2.79_{FW}+3.77_{P}$	0.76	4.19
FL+T+TRS	$Y= 12.46+1.09_{FL}+0.06_{T}-2.71_{TRS}$	0.76	4.19
FL+T+P	$Y= -3.68+1.30_{FL}+0.03_{T}+3.35_{P}$	0.76	4.24
FL+TRS+P	$Y= -0.81+1.28_{FL}-0.76_{TRS}+3.92_{P}$	0.76	4.10
FW+T+TRS	$Y= 27.18+5.82_{FW}+0.09_{T}-7.91_{TRS}$	0.57	2.31
FW+T+P	$Y= -6.51+12.30_{FW}+0.04_{T}+4.95_{P}$	0.41	1.71
FW+TRS+P	$Y= 19.44+8.28_{FW}-6.39_{TRS}+3.12_{P}$	0.51	2.05
T+TRS+P	$Y= 43.61+0.12_{T}-10.48_{TRS}-2.52_{P}$	0.52	2.09
FL+FW+T+TRS	$Y= 13.49+1.33_{FL}-4.18_{FW}+0.06_{T}-2.62_{TRS}$	0.78	4.49
FL+FW+T+P	$Y= -0.86+1.49_{FL}-3.38_{FW}+0.04_{T}+2.70_{P}$	0.77	4.42
FL+FW+TRS+P	$Y= 1.73+1.43_{FL}-2.82_{FW}-0.81_{TRS}+3.48_{P}$	0.76	4.22
FL+T+TRS+P	$Y= 3.22+1.17_{FL}+0.05_{T}-1.78_{TRS}+2.47_{P}$	0.77	4.34
FW+T+TRS+P	$Y= 26.99+5.84_{FW}+0.09_{T}-7.89_{TRS}+0.05_{P}$	0.57	2.31
FL+FW+T+TRS+P	$Y= 7.15+1.35_{FL}-3.64_{FW}+0.05_{T}-2.00_{TRS}+1.66_{P}$	0.78	4.56

FL = Fruit Length; FW = Fruit Width; T = Trichomes density; TRS = Total reducing sugar; P = Total protein.

Regression analysis

Based on *r/SE* (a stringent criterion for identifying significant variables for regression analysis), the biochemical traits *viz.,* total phenols (TP), total flavonoids (TF), total sugars (TS), total reducing sugar (TRS) and total protein (P) were further considered for multiple regression analysis. Step-wise regression analysis of all significant host-plant biochemical traits explained the variability in the number of eggs laid by *E. vittella* in the range of 16-54 per cent with lower VIF values (1.19-2.19, <10.0) suggesting that multicollinearity might not be a significant concern in these models. The regression equation that involved all the host plant biochemical traits explained a maximum of 54 per cent of the variability in the number of eggs laid by *E. vittella* ($Y = 26.71 - 0.30_{TP} + 0.32_{TF} - 1.39_{TS} - 4.41_{TRS} + 8.29_{P}$; $R^2 = 0.54$; VIF=2.19) (Table 2).

Multiple linear regression analysis conducted to further understand the relationship between the biochemical traits (TP, TF, TS, TRS & P) and the number of eggs laid by *E. vittella* could explain 54% of the variability in the moth egg laying choice. The study also considered the combined effect of both morphological and biochemical traits of the host plants in a step-wise regression model which revealed that together these traits could explain a substantial variability of 78% in the number of eggs laid by *E. vittella.* This suggests that combining both morphological (fruit length, fruit width, trichome density) and biochemical traits (total reducing sugars and total protein), could not improve the R^2 value significantly when compared to morphological traits (fruit length, fruit width, trichome density) alone was used (R^2 =76%; Krishna Kumar *et al.,* 2023). Therefore, as demonstrated by Krishna Kumar *et al.* (2023), host plant morphological traits, such as leaf length, stem diameter, number of branches, fruit characteristics (e.g., length, width, and trichomes density) might play a substantial role in shaping the ovipositional preference of *E. vittella* over biochemical traits that were explored in the present study.

The biochemical traits alongside previously generated morphological traits (the morphological trait data utilized in this study were sourced from the research conducted by Krishna Kumar *et al*., 2023) namely, fruit length (FL), fruit width (FW), trichomes (T), total reducing sugar (TRS) and total protein (P) were considered for multiple regression analysis. Step-wise regression analysis of all significant host-plant morphological and biochemical traits (based on *r*/SE) explained the variability in the number of eggs laid in the range of 41-78 per cent with acceptable VIF values (1.11-4.56, <10.0; indicating a lack of multi-collinearity). A total of 78 per cent of the variability in the number of eggs laid by *E. vittella* was explained by combining all the host plant morphological and biochemical traits ($Y = 7.15 + 1.35_{FL} - 3.64_{FW} + 0.05_{T} - 2.00_{TRS} + 1.66_{P}$; R^2=0.78; VIF=4.56; Table 3).

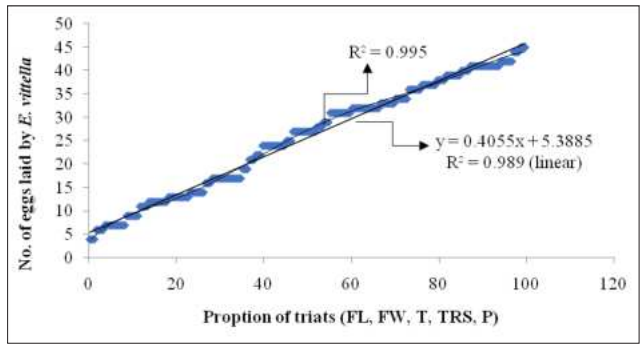

Fig. 2. Relationship between the proportion of host plant morphological and biochemical traits and number of eggs laid by *E. vittella.* Total phenols, TP; Total flavonoids, TF; Total sugar, TS; Total reducing sugar, TRS & Total protein, P.

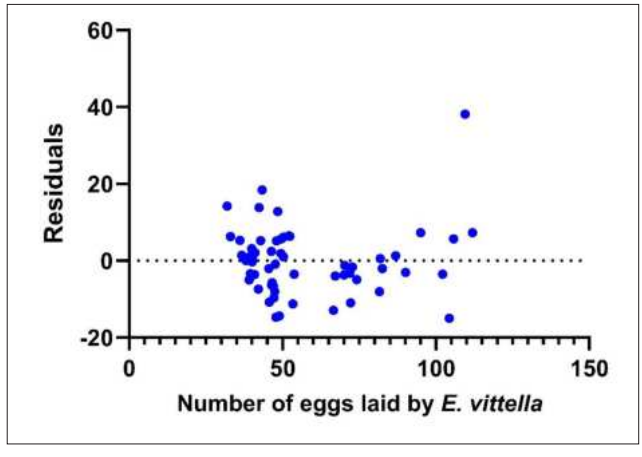

Fig. 3. Plot of the residuals against the egg laying of *E. vittella* with selected host plant (*Abelmoschus* species) morphological and biochemical traits as independent variables

The results of the polynomial models of different orders [(2), (3), (4), (5) and (6)] with all significant traits like fruit length, fruit width, trichomes, total reducing sugar and total protein increased the coefficient of determination to the maximum of 99% [R^2 = 0.9930, R^2 = 0.9937, R^2= 0.9943, R^2 =0.9948, for polynomial model orders (2), (3), (4) and (5) respectively] and the linear model explained to the tune of 98% variability in egg laying (Figure 2). Plotting the residuals observed and the estimated number of eggs laid by *E. vittella* using the host plant traits (FL, FW, T, TRS and P) showed a random dispersal of points across the x-axis (Figure 3).

Table 4. Direct and indirect effects of host plant (*Abelmoschus* species) biochemical traits on eggs laid by *E. vittella*

Pathways of association	Direct effects	Indirect effects
1. Total phenols		
a. Direct effect	0.86	
b. Indirect effect via		
Total flavonoids		0.86
Total sugar		0.58
Total reducing sugar		0.47
Total Protein		0.55
2. Total flavonoids		
a. Direct effect	-0.91	
b. Indirect effect via		
Total phenols		-0.91
Total sugar		-0.60
Total reducing sugar		-0.48
Total Protein		-0.58
3. Total sugar		
a. Direct effect	-0.04	
b. Indirect effect via		
Total phenols		-0.03
Total flavonoids		-0.03
Total reducing sugar		-0.03
Total Protein		-0.01
4. Total reducing sugar		
a. Direct effect	0.76	
b. Indirect effect via		
Total phenols		-0.42
Total flavonoids		-0.40
Total sugar		-0.63
Total Protein		0.16
5. Total Protein		
a. Direct effect	0.05	
b. Indirect effect via		
Total phenols		0.03
Total flavonoids		0.03
Total sugar		0.01
Total reducing sugar		-0.01

With data support from Krishna Kumar *et al.,* 2023

Table 5. Direct and indirect effects of host plant (*Abelmoschus* species) morphological and biochemical traits on eggs laid by *E. vittella*

Pathways of association	Direct effects	Indirect effects
1. Fruit length (cm)		
a. Direct effect	0.96	
b. Indirect effect via		
Fruit width (cm)		0.92
Trichomes density (cm^2)		0.24
Total reducing sugar		-1.13
Total Protein		-0.03
2. Fruit width (cm)		
a. Direct effect	-0.23	
b. Indirect effect via		
Fruit length (cm)		-0.18
Trichomes density (cm^2)		-0.04
Total reducing sugar		0.15
Total Protein		0.04
3. Trichomes density (cm^2)		
a. Direct effect	0.06	
b. Indirect effect via		
Fruit length (cm)		0.01
Fruit width (cm)		0.01
Total reducing sugar		0.002
Total Protein		0.03
4. Total reducing sugar		
a. Direct effect	0.20	
b. Indirect effect via		
Fruit length (cm)		-0.17
Fruit width (cm)		-0.13
Trichomes density (cm^2)		0.01
Total Protein		-0.04
5. Total Protein		
a. Direct effect	0.18	
b. Indirect effect via		
Fruit length (cm)		-0.004
Fruit width (cm)		-0.03
Trichomes density (cm^2)		0.08
Total reducing sugar		-0.04

With data support from Krishna Kumar *et al.,* 2023

Path co-efficient analysis

To reveal direct and indirect associations between biochemical traits and number of eggs laid by *E. vittella,* further analyses were carried out under path-coefficient analysis. The results showed that total phenols have a strong positive direct effect with a high magnitude (0.86). Total phenols also have positive indirect effects via total flavonoids (0.86), total sugar (0.58), total reducing sugar (0.47) and total protein (0.55). Total flavonoids have a strong negative direct effect (-0.91) and negative indirect effects via total phenols (-0.91), total sugar (-0.60), total reducing sugar (-0.48) and total protein (-0.58) with maximum magnitude. A weaker negative direct effect was observed with total sugar (-0.04) and its indirect effects also showed a negative effect of a very weak magnitude via total phenols (-0.03), total flavonoids (-0.03), total reducing sugar (-0.03) and total protein (-0.01). Total reducing sugar has a strong positive direct effect with high magnitude (0.76) and indirect effects via total phenols (-0.42), total flavonoids (-0.40), total sugar (-0.63) and positive indirect effects via total protein (0.16). Total protein has a weak positive direct effect with lesser magnitude (0.05) and total protein has very weak positive indirect effects via total phenols (0.03), total flavonoids (0.03), total sugar (0.01) and negative indirect effects via total reducing sugar (-0.01) (Table 4).

In the case of morphological and biochemical traits combined together, fruit length has a positive direct effect of 0.96 and significant positive indirect effects via fruit width (0.92) and relatively weaker indirect effects via trichomes density (0.24), total reducing sugar (-1.13) and total protein (-0.03). Fruit width has a negative direct effect with reasonable magnitude (-0.23) and negative indirect effects via fruit length (-0.18), trichomes density (-0.04), total reducing sugar (0.15) and total protein (0.04). Trichomes density has a positive direct effect with lesser magnitude (0.06) and has very weak indirect effects via fruit length (0.01), fruit width (0.01), total reducing sugar (0.002) and total protein (0.03). Total reducing sugar has a positive direct effect (0.20) and has negative indirect effects via fruit length (-0.17), fruit width (-0.13), trichomes density (0.01), and total protein (-0.04). Total protein has a positive direct effect (0.18); indirect negative effect via fruit length (-0.004), fruit width (-0.03), total reducing sugar (-0.04) and indirect positive effect via trichomes density (0.08) (Table 5).

In path-coefficient analysis, biochemical traits such as total phenols and total flavonoids showed strong positive and negative direct effects on *E. vittella* egg lying respectively. This suggests that they are closely related and may have a synergistic or antagonistic effect on *E. vittella*'s ovipositional preference. Total reducing sugars stood out with strong positive direct and negative indirect effects on *E. vittella* egg lying via total phenols, total flavonoids, and total sugars. These results revealed that biochemical traits such as total phenols and total flavonoids seem to be particularly important, as they have strong direct and indirect effects that might influence *E. vittella*'s ovipositional preference. When combining the morphological and biochemical traits of *Abelmoschus* species to understand their effects on the ovipositional preference of *E. vittella,* a strong direct effect by fruit length and total reducing sugars on egg laying was noticed indicating that these traits are highly influencing the egg laying choice of *E. vittella*. Other morphological traits like trichome density have weaker direct effects on *E. vittella* egg-laying choice. The variables namely fruit length and fruit width have reciprocal negative indirect effects on *E. vittella* egg laying. Additionally, both total reducing sugars and total protein were found to have positive and negative indirect effects on *E. vittella* egg laying. Thus, fruit length and total reducing sugars appear to be important traits, with strong direct and indirect effects on moth egg laying. This suggests that these variables may play a significant role in determining the attractiveness of host plants to *E. vittella*. In other words, the present study reveals that longer fruit length with higher total protein content is associated with increased oviposition, while greater fruit width with higher levels of total reducing sugars is associated with decreased oviposition. The present study also endorses previous findings which identified fruit length and total protein content as potential host-plant traits that positively influenced the egg-laying behaviour of *E. vittella* (Muthukumaran and Ganesan, 2017; Sundararaj and David, 1987). Conversely, studies also reported that fruit width and higher levels of total reducing sugars had an adverse effect on the number of eggs laid by *E. vittella* (Anitha and Karthika, 2018; Sultani *et al.*, 2011).

In conclusion, the present study clearly indicated that host plant morphological traits outweigh the host plant biochemical traits in the oviposition site selection process of *E. vittella*, in spite of the latter having a significant association with moth egg-laying choice. However, detailed studies including diverse germplasm might help to attain a more comprehensive understanding of the host plant traits that shape oviposition preference in okra fruit and shoot borer, *E. vittella*.

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REFERENCES

Agboyi, L. K., Mensah, S. A., Clottey, V. A., Beseh, P., Glikpo, R., Rwomushana, I., Day, R. and Kenis, M. 2019. Evidence of leaf consumption rate decrease in fall armyworm, Spodoptera frugiperda, larvae parasitized by Coccygidium luteum. *Insects*, **10**(11): 410.

D'Agostino, R. 2017. *Goodness-of-fit-techniques*. Routledge.

Ali, A., Rakha, M., Shaheen, F. A. and Srinivasan, R. 2019. Resistance of certain wild tomato (Solanum spp.) accessions to Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) based on choice and no-choice bioassays. *Florida Entomologist*, **102** (3): 544-548.

Karthika, N. 1970. Antixenosis resistance in okra accessions against shoot and fruit borer Earias vittella (Fab.). *Journal of Phytology*, **10**: 27-32.

Balagawi, S., Drew, R. A. and Clarke, A. R. 2013. Simultaneous tests of the preference-performance and phylogenetic conservatism hypotheses: is either theory useful? *Arthropod-Plant Interactions*, **7:** 299–313.

Baskar, K., Muthu, C. and Ignacimuthu, S. 2014. Effect of pectolinaringenin, a flavonoid from Clerodendrum phlomidis, on total protein, glutathione S-transferase and esterase activities of Earias vittella and Helicoverpa armigera. *Phytoparasitica*, **42**: 23-331. .

Beck, S. D. 1965. Resistance of plants to insects. *Annual review of entomology*, **10**(1): 207-232.

Benzie, I. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, **239** (1): 70–76.

Birke, A., Acosta, E. and Aluja, M. 2015. Limits to the host range of the highly polyphagous tephritid fruit fly *Anastrepha ludens* in its natural habitat. *Bulletin of Entomological Research*, **105**: 743–753.

Birke, A., Acosta, E. and Aluja, M., 2015. Limits to the host range of the highly polyphagous tephritid fruit fly Anastrepha ludens in its natural habitat. *Bulletin of Entomological Research,* **108**: 674–684.

Carrasco, D., Larsson, M.C. and Anderson, P., 2015. Insect host plant selection in complex environments. *Current Opinion in Insect Science*, **8:** 1-7.

Clark, K. E., Hartley, S. E. & Johnson, S.N. 2011. Does mother know best? The preference–performance hypothesis and parent–offspring conflict in aboveground–belowground herbivore life cycles. *Ecological Entomology*, **36**: 117–124.

Coapio, G. G., Cruz-López, L., Guerenstein, P., Malo, E. A. and Rojas, J. C. 2018. Oviposition preference and larval performance and behavior of Trichoplusia ni (Lepidoptera: Noctuidae) on host and nonhost plants. *Arthropod-plant interactions*, **12** (2): 267-276.

da Silva, P.V.D.C., Junior, C.B., Goncalves de Jesus, F., Hoffmann, L.V. and Pinto de Menezes, I.P., 2021. Antixenosis in cotton genotypes ('Gossypium hirsutum var. marie galante') to'Spodoptera frugiperda'(Lepidoptera: Noctuidae) mediated by trichome and gossypol densities. *Australian Journal of Crop Science*, **15** (12): 1435-1441.

Dhillon, M. K. and Sharma, P. D. 2004. Studies on biology and behaviour of Earias vittella (Lepidoptera: Noctuidae) for mechanisms of resistance in different cotton genotypes. *Crop Protection,* **23**: 235-241.

Doshi, K. M. 2004. Influence of bio-chemical factors on the incidence of shoot and fruit borer infestation in brinjal. *Capsicum and Egg Plant Newsletter*, **23**: 145-148.

Dreyer, D. L. and Campbell, B. C. 1987. Chemical basis of host plant resistance to aphids. *Plant Cell and Environment*, **10**: 353–361.

Feeny, P. 1995. Ecological opportunism and chemical constraints on the host associations of swallowtail butterflies. *Swallowtail butterflies: their ecology and evolutionary biology*, 9-16. .

García-Barros, E. and Fartmann, T. 2009. Butterfly oviposition: sites, behaviour and modes. In: Settele J, Shreeve TG, Konvicka M, van Dyck H (eds) Ecology of butterflies in Europe. Cambridge University Press, Cambridge, 29–42.

Gautam, H. K., Singh, N. N., Singh, C. and Rai, A. B. 2013. Morphological and biochemical characters in fruits against okra shoot and fruit borer (*Earias*

vittella F.). *Indian Journal of Entomology*, **75** (3): 189-193.

Gripenberg, S., Mayhew, P. J., Parnell, M. and Roslin, T. 2010. A meta□analysis of preference–performance relationships in phytophagous insects. *Ecology letters*, **13**(3), pp.383-393.

Hafeez, M., Jan, S., Nawaz, M., Ali, E., Ali, B., Qasim, M., Fernández-Grandon, G. M., Shahid, M. and Wang, M. 2019. Sub-lethal effects of lufenuron exposure on spotted bollworm *Earias vittella* (Fab): key biological traits and detoxification enzymes activity. *Environmental Science and Pollution Research*, **26** (14): 14300-14312.

Hafsi, A., Facon, B., Ravigné, V., Chiroleu, F., Quilici, S., Chermiti, B. and Duyck, P. F. 2016. Host plant range of a fruit fly community (Diptera: Tephritidae): does fruit composition influence larval performance? *BMC Ecology*, **16**: 40. DOI: https://doi.org/10.1186/s12898-016-0094-8.

Halder, J., Sanwal, S. K., Rai, A. K., Rai, A. B., Singh, B. and Singh, B. K. 2015. Role of physico-morphic and biochemical characters of different okra genotypes in relation to population of okra shoot and fruit borer, *Earias vittella* (Noctuidae: Lepidoptera). *Indian Journal of Agricultural Sciences*, **85**(2): 278-282.

Keerthi, M. C., Mahesha, H. S., Manjunatha, N., Gupta, A., Saini, R. P., Shivakumara, K.T., Bhargavi, H. A., Gupta, G. and Kulkarni, N. S., 2023. Biology and oviposition preference of fall armyworm, *Spodoptera frugiperda* (JE Smith)(Lepidoptera: Noctuidae) on fodder crops and its natural enemies from Central India. *International Journal of Pest Management*, **69**(3): 215-224.

Kessler, A. and Baldwin, I.T. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, **291**: 2141–2144.

Kogan, M. and Ortman, E. F. 1978. Antixenosis-A New Term Proposed to Define Painter's "Nonpreference" Modality of Resistance. *Bulletin of the Entomological Society of America*, **24** (2): 175–176.

Koujalagi, M., Gangappa, E., Chakravarthy, A. K., Pitchaimuthu, M., Kumar, N. R. and Thippaiah, M. 2009. Screening of okra hybrids and varieties for resistance to fruit borers. *Pest management of Horticultural Ecosystems*, **15** (2): 141-146.

Krishna Kumar, S., Pitchaimuthu, M., Jagadish, K. S. and Kamala Jayanti, P. D. 2023. Host Plant Traits Impact on the Egg Laying Choice of Female Fruit Borer Moth, *Earias vittella* (Fab.) in Okra. *Mysore Journal of Agricultural Sciences,* **57** (2): 156-168.

Kumar, R., Sing, P. P. and Ahmad, M. A. 2021. Morphological and biochemical responses associated with resistance to *Earias vittella* (Fabricious) (Lepidoptera: Noctuidae) in okra varieties. *Bangladesh Journal of Botany,* **50** (4): 1059-1066.

Lowry C. O., Oliverh., Rosebrough, N., Niraj., Farr, A. L. and Randall, R. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry,* **193** (1): 265–275.

Mandal, S. K., Sattar, A. and Gupta, S. C. 2006. Population dynamics of *Earias vittella* Fab. in okra as influenced by weather parameters in north Bihar. *Journal of Agrometeorology*, **8** (2): 260-265.

Manju, K. P., Lakshmi, K.V., Babu, B. S. and Anitha, K. 2021. Morphological and biochemical basis of resistance in okra to whitefly*, Bemisia tabaci* and okra yellow vein mosaic virus (OYVMV). *Journal of Entomology and Zoology Studies,* **9** (1): 1719-1728.

Moore, S. and Stein, W. H. 1954. A modified ninhydrin reagent for photometric determination of amino acids and related compounds. *Journal of Biological Chemistry,* **211**: 907–913.

Muthukumaran, N. and Ganesan, P. 2017. Antixenosis resistance *Earias vittella* in okra and their hybrid derivatives against shoot and fruit borer *Earias vittella* (Fab.). *Journal of Entomology and Zoological Studies*, **5** (4): 1884-1887.

Painter, R. H. 1951. Insect Resistance in Crop Plants. The Macmillen Company, New York, 520.

Palial, S., Kumar, S. and Sharma, S. 2018. Biochemical changes in the *Brassica-juncea-fruticulosa* introgression line after *Lipaphis erysimi* (Kaltenbach). *Phytoparasitica,* **46**: 499–509.

Panda, N. 1979. Principles of host-plant resistance to insect pests. Hindustan Publishing Company, New Delhi, India, 405.

Qasim, M., Husain, D., Islam, S.U., Ali, H., Islam, W., Hussain, M., Wang, F. and Wang, L. 2018.

Effectiveness of *Trichogramma chilonis* Ishii against spiny bollworm in Okra and susceptibility to insecticides. *Journal of Entomology and Zoology Studies,* **6**: 1576-1581.

Ryan, T. P. 1997, Modern Regression Methods. John Wiley and Sons, New Jersey, 515.

Sandhi, R.K., Sidhu, S.K., Sharma, A., Chawla, N. and Pathak, M.2017. Morphological and biochemical basis of resistance in okra to cotton jassid, *Amrasca biguttula biguttula* (Ishida). *Phytoparasitica*, **45** (3): 381–394. DOI: https://doi.org/10.1007/s12600-017-0589-7.

Sharma, H.C. 2007. Host plant resistance to insects: modern approaches and limitations. *Indian Journal of Plant protection*, **35** (2): 179-184.

Simmonds, M.S. 2001. Importance of flavonoids in insect–plant interactions: feeding and oviposition. *Phytochemistry,* **56** (3): 245-252.

Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means Folin-Ciocalteu reagent. *Methods in Enzymology,* **299**: 152–178.

Somogyi, M. 1952. Notes on sugar determination. *Journal of Biological Chemistry*, **195** (1): 19–23. DOI: https://doi.org/10.1016/S0021-9258(19)50870-5.

Stout, M. J. 2019. Plant-insect interactions, host-plant resistance, and integrated pest management. In (Ed.) Integrated management of insect pests. Burleigh Dodds Science Publishing, Cambridge. 191-224.

Sultani, M. S., Ram, S. and Dhankhar, S. K. 2011. Morphological and biochemical bases of resistance in selected okra genotypes against *Earias vittella* (Fabricius). *Journal of Insect Science* (Ludhiana), **24** (1): 33-40.

Sundararaj, R. and David, B.V. 1987. Influence of biochemical parameters of different hosts on the biology of *Earias vittella* (Fab.) (Noctuidae: Lepidoptera*). Proceedings: Animal Sciences,* **96**: 329-332.

Thakur, P., Rana, R. S. and Kumar, A. 2017. Biophysical characters of tomato varieties in relation to resistance against tomato fruit borer, *Helicoverpa armigera* (Hubner). *Journal of Entomology and Zoology Studies*, **5** (6): 108-112.

Uzun, F., Birgucu, A. K. and Karaca, I. 2015. Determination of oviposition preference of *Tuta absoluta* to tomato, pepper and eggplant. *Asian Journal of Agricultural and Food Sciences*, **3** (5): 569-578.

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S. and Sharma, H. C. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signal Behaviour*, **7**: 1306–1320.

Zhishen, J., Mengcheng, T. and Jianming, W. U. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, **64** (4): 555–559.

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Effect of weather parameters on the incidence of diamondback moth, *Plutella xylostella* (L.) in cabbage

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ABSTRACT: Studies were conducted to understand the effect of weather parameters on the incidence of diamondback moth, *Plutella xylostella* (L.) on cabbage at Horticulture Farm, Rajasthan College of Agriculture, Udaipur, India during r*abi,* 2019-20 and 2020-21. In both the seasons, a markedly high population of diamondback moth larvae was recorded (9.18 larvae/ plant) during the 2nd week of March, 2020 and 10.15 larvae/plant during the 3rd week of March, 2021, respectively. Similarly, the peak populations of diamondback moth pupae were noticed during the 4th week of February (6.45 pupae/ plant) and the 1st week of March (7.08 pupae/ plant). DBM larval and pupal populations had a significant positive correlation with maximum atmospheric temperature and sunshine hours while a significant negative correlation was observed with mean relative humidity during both years. The coefficient of multiple determination (R^2 =0.705) and (R^2 = 0.591) indicated that 70.50 and 59.10 per cent of variation in the larval populations during 2019-20 and 2020-21 respectively. Similarly, DBM pupal population directed a collective influence of 56.60 per cent (R^2=0.566) and 50.00 per cent (R^2 = 0.500) during 2019-20 and 2020-21, respectively.

Keywords: Diamondback moth, cabbage, seasonal incidence, weather parameters

INTRODUCTION

Cabbage (*Brassica oleracea* Var. *Capitata* L.) is a leafy vegetable grown for its edible enlarged terminal bud. It is cultivated widely in the tropical and temperate regions of the world. Many limiting factors have been attributed to low production; among them, the chief constraint is damage caused by the insect pest complex soon after germination till the harvesting. In India, a total of 37 insect pests have been reported to feed on cabbage (Lal, 1975). Among them, diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is recognised as the most prominent pest and considered as the most devastating insect pest of cruciferous crops worldwide. Diamondback moth, hereafter "DBM", is a dominant pest in more than 100 countries across the globe. DBM considered to be one of the most devastative pests of cabbage and other Brassica crops worldwide (Talekar and Shelton, 1993; Shelton and Badenes-perez, 2006). It influences cruciferous plants, peculiarly *Brassica* crops in particular cabbage, cauliflower, broccoli, brussels sprout, kale and turnip (Talekar, 1992; Alam, 1992). DBM manifests a marked preference for cabbage and cauliflower as these crops equip olfactory and gustatory stimuli for successful selection and colonization with fleshy and succulent leaves. It destructs the crop by feeding on the foliage and infests by multitudes of larvae which hinders the growth of the plant leading to a notable reduction in yield. The yield loss is evaluated to vary from 31 to 100 per cent (Abraham and Padmanabhan, 1968) and 52 per cent to 100 per cent (Anuradha, 1997; Cardleron and Hare*,* 1986).

The modifying cropping pattern, monoculture, intensive cultivation of high yielding varieties, negligence of crop rotation, non-adoption of summer ploughing besides negation of other cultural practices and injudicious use of insecticides have aggravated this pest problem in cruciferous vegetables. Commercial consideration of cabbage crop has compelled the growers to go for frequent and injudicious use of insecticides for better marketable yield. As a result, DBM has developed resistance to most commonly used insecticides (Atumurirava *et al.,* 2011; Zhou *et al.,* 2011) and was reported as the first species to develop resistance to some toxins of *Bacillus thuringiensis* (Tabashnik and Cushing, 1987; Talekar and Shelton, 1993). To reduce yield losses caused by DBM, farmers routinely follow chemical control, due to the lack of reliable alternatives and the availability of relatively cheaper insecticides (Talekar and Shelton, 1993). Furthermore, it has led to several problems *viz.,* multiple resistance to commonly used insecticides (Ribeiro *et al.,* 2014), pesticide resurgence (Nemato *et al.*, 1984), residue problems, in-efficiency of natural enemies and environmental pollution, etc. In current estimation, the area, production and productivity of cabbage in Rajasthan have declined rapidly from 1.29 lakh ha, 19.66 lakh metric tonnes and 15.27 metric

tonnes per ha, respectively in the year 2016-17 to 1.20 lakh ha, 11.69 lakh metric tonnes and 9.74 metric tonnes per ha, respectively in the year 2017-18 and numerous farmers abandoned the cultivation of cabbage in the state (Anonymous, 2018). Weather conditions play important role in managing DBM, therefore, the weather parameters are studied in the present study to understand their influence on DBM larval and pupal populations.

MATERIALS AND METHODS

In order to study the weather parameters and their multiple corrections on DBM larval and pupal populations, the experiment was laid out at the Horticulture Farm, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur. Udaipur region comes under the agro-climatic zone, "Sub-humid Southern Plain and Aravalli Hills of Rajasthan", which is situated at an altitude of 582.17 meters above mean sea level, at 24˚35' N latitude and 74˚42' E longitude. The region is characterized by a sub-tropical climate with typical winters and summers. The average annual rainfall ranges from 592.5 mm to 620 mm. The maximum rainfall is received from mid-June to September with scanty showers during the winter season.

Table 1. Seasonal incidence of diamondback moth on cabbage during *Rabi,* 2019-20

SMW	Atmospheric Temperature (°C)			Relative Humidity (%)			Sunshine (Hrs.)	Mean / plant		
	Max.	Min.	Mean	Morning	Evening	Mean		Larvae	Pupae	Infestation (%)
48	26.30	13.30	19.80	88.90	58.00	73.45	3.80	0.62	0.00	1.67
49	24.00	8.10	16.05	86.10	31.40	58.75	5.60	0.88	0.00	2.50
50	24.30	7.50	15.90	88.00	37.70	62.85	6.50	1.13	0.00	5.83
51	23.50	6.60	15.05	82.40	40.10	61.25	7.20	2.05	0.00	8.33
52	21.50	4.70	13.10	82.60	39.40	61.00	5.80	2.62	0.17	12.50
1	21.20	6.80	14.00	86.70	49.30	68.00	5.10	3.52	0.27	20.83
2	22.10	5.20	13.65	81.70	40.40	61.05	7.10	4.17	0.43	25.00
3	21.00	6.40	13.70	86.40	42.00	64.20	5.60	4.65	0.73	27.50
4	24.70	7.90	16.30	82.70	31.30	57.00	8.60	5.48	1.53	30.83
5	22.30	4.50	13.40	85.00	36.30	60.65	8.60	6.27	2.07	42.50
6	23.00	4.40	13.70	80.30	34.40	57.35	8.60	6.72	2.67	38.33
7	28.50	7.70	18.10	77.40	21.90	49.65	8.60	7.30	4.23	39.17
8	28.10	9.00	18.55	76.00	29.00	52.50	7.90	8.20	6.45	45.83
9	30.20	10.90	20.55	73.40	26.60	50.00	7.60	8.65	2.03	48.33
10	26.60	10.20	18.40	71.90	34.40	53.15	9.00	9.18	1.03	54.17
Coefficient of correlation (r) between population and atm. temp.							Maximum Temp.	0.50	0.55*	-
							Minimum Temp.	0.05	0.04	
							Mean Temp.	0.31	0.34	
Coefficient of correlation (r) between population and relative humidity							Morning RH	-0.85*	-0.57*	
							Evening RH	-0.62*	-0.62*	
							Mean	-0.77*	-0.65*	
Coefficient of correlation (r) between population and sunshine								0.78*	0.57*	

SMW = Standard Meteorological Week; *Significant at 5% level

Table 2. Seasonal incidence of diamondback moth on cabbage during *Rabi,* 2020-21

SMW	Atmospheric Temperature (°C)			Relative Humidity (%)			Sunshine (Hrs)	Mean / plant		
	Max.	Min.	Mean	Morning	Evening	Mean		Larvae	Pupae	Infestation (%)
49	30.40	10.20	20.30	76.10	27.30	51.70	8.80	0.65	0.00	0.83
50	24.50	12.10	18.30	85.40	52.10	68.75	4.10	0.87	0.00	3.33
51	23.30	4.30	13.80	80.90	26.70	53.80	7.90	1.62	0.00	6.67
52	22.50	3.80	13.15	81.90	27.80	54.85	8.30	2.32	0.00	9.17
1	24.00	8.70	16.35	85.70	46.70	66.20	3.40	3.07	0.12	14.17
2	22.80	9.70	16.25	90.60	51.40	71.00	4.30	4.28	0.20	15.83
3	27.30	7.50	17.40	87.90	32.90	60.40	8.30	4.83	0.47	17.50
4	24.50	4.10	14.30	83.10	28.10	55.60	8.80	5.53	1.02	19.17
5	26.10	4.40	15.25	76.10	23.10	49.60	8.60	6.20	1.40	21.67
6	26.60	5.90	16.25	75.60	22.30	48.95	8.70	7.12	2.13	32.50
7	29.00	8.00	18.50	74.40	23.10	48.75	8.30	8.20	1.98	36.67
8	29.60	8.90	19.25	65.40	18.30	41.85	9.30	8.68	4.08	39.17
9	32.30	11.40	21.85	64.90	23.10	44.00	9.60	9.28	7.08	49.17
10	33.10	13.10	23.10	54.40	26.50	40.45	9.50	9.53	3.37	52.50
11	33.30	14.70	24.00	57.40	27.10	42.25	8.50	10.15	1.50	58.33
Coefficient of correlation (r) between population and atm. temp.							Maximum Temp.	0.70*	0.66*	-
							Minimum Temp.	0.35	0.32	
							Mean Temp.	0.58*	0.54*	
Coefficient of correlation (r) between population and relative humidity							Morning RH	-0.76*	-0.68*	
							Evening RH	-0.54*	-0.52*	
							Mean	-0.72*	-0.67*	
Coefficient of correlation (r) between population and sunshine								0.53*	0.54*	

SMW = Standard Meteorological Week; *Significant at 5% level

Seedlings of cabbage variety, Golden acre recommended for the zone was transplanted on prepared field, with uniformly sized plots of 4.5 m x 4.5 m laid out in RBD during the last week of October, 2019-20 and first week of November, 2020-21. Intercultural operations were performed as per the package of practices recommended by the Directorate of Research, MPUA&T, Udaipur, Rajasthan. The population of DBM on cabbage was recorded at weekly intervals for the appearance of DBM larva and pupae in the course of each sampling. The prevalence of DBM infestation was recorded in terms of the number of larvae and pupae per plant on 10 randomly selected plants. The extent of damages by DBM larvae was recorded by sampling randomly selected 20 plants per plot from six plots. Weekly meteorological data of abiotic factors *viz*., atmospheric temperature (maximum and minimum), relative humidity (morning and evening) and sunshine (hrs) were obtained from Agromet observatory, Rajasthan College of Agriculture, Udaipur.

The abiotic factors *viz*., atmospheric temperature (maximum and minimum), relative humidity (morning and evening) and sunshine (hrs) were recorded during the crop season and their simple correlation with the

population of insect pests and natural enemies were evaluated by the Karl Pearson formula of correlation coefficient (Fowler *et al*., 1998). The calculated t-value obtained was compared with tabulated t-value 5 per cent level of significance.

RESULTS AND DISCUSSION

The data on seasonal incidence of DBM on cabbage during *Rabi,* 2019-20 has been presented in Table 1. Multiple correlation analysis presented in Table 3. The DBM larvae started appearing from the 1st week of December, 2019 (48th SMW) and reached its peak of 9.18 larvae/ plant during the 2nd week of March, 2020 (10th SMW). The larval populations endured till the harvest of crop. During the peak incidence, the mean temperature, mean relative humidity and sunshine hrs were 18.40 □, 53.15 % and 9.00 hrs, respectively. The extent of damage or per cent plant infestation by the larvae of DBM were ranged from 1.67-54.17 per cent. The larval incidence had a significant positive correlation (r = 0.50) with maximum atmospheric temperature and sunshine hours (r = 0.78). Although, a significant negative correlation (r = - 0.77) was observed with mean relative humidity. Meagre total rainfall of 3.00 mm was documented during the *Rabi,* 2019-20 and hence no significant relations were observed between rainfall and insect populations. The percentage plant infestation by DBM larvae was 1.67 - 54.17 during *Rabi,* 2019-20. Similarly, the DBM pupal population appeared from the 4th week of December, 2019 (52nd SMW) and reached its crest of 6.45 pupae/ plant during the 4th week of February, 2020 (8th SMW) and prevailed till the harvest of crop. At the time of peak, pupae per plant, the mean temperature, mean relative humidity and sunshine were 18.55 □, 52.50% and 7.90 hrs, respectively. The pupal population had a significant positive correlation with maximum atmospheric temperature (r = 0.55) and sunshine hours (r = 0.57) whereas significant negative correlation (r = -0.65) was observed with mean relative humidity.

The multiple linear regression analysis directed that atmospheric parameter (mean atmospheric temperature, mean relative humidity and total sunshine) obligated a combined encouragement on DBM larval population. The coefficient of multiple determinations (R^2) was 0.705 indicating that 70.50 per cent of the variation in the larval populations was explained by the explanatory variables. In multiple linear regression equation, the regression coefficient b_1= 0.150 depicts that X_2 (mean relative humidity) and X_3 (total sunshine) constant, with one degree increase in X_1 (mean temperature) commanded on the average to about 0.150 per cent increase in DBM larval populations. In a similar way, b_2= -0.139 means that holding X_1 and X_3, constant and 1 per cent increase in X_2 (mean relative humidity) led from average to about 0.139 per cent decrease in DBM larvae. Likewise, b_3=-0.865 means that holding X_1 and X_2 constant and 1 hr increase in X_3 (sunshine) led average to about 0.865 per cent increase in DBM larvae. Similarly, the multiple linear regression analysis for pupal population directed a collective influence of 56.60 per cent (R^2=0.566) on DBM pupal population. The coefficient of multiple determinations (R^2) was 0.566 indicating that 56.60 per cent of variation in the larval populations was explained by the explanatory variables. The regression coefficient b_1= 0.222 held X_2 (mean relative humidity) and X_3 (total sunshine) constant, with one degree increase in X_1 (mean temperature) directed on the average to about 0.222 per cent increase in DBM pupal population. likewise, b_2= -0.082 which held X_1 and X_3, constant and 1 per cent increase in X_2 (mean relative humidity) led from average to about 0.082 per cent decrease in DBM pupae and b_3=0.520 which held X_1 and X_2 constant and 1 hr increase in X_3 (sunshine) led from average to about 0.520 per cent increase in DBM pupae.

The data on seasonal incidence of DBM on cabbage during *Rabi,* 2020-21 is presented in Table 2. Multiple correlation analysis presented in Table 3. The incidence of DBM larvae initiated in the second week of December (49th SMW, 2020) with count of 0.65 larvae/plant. Afterward, the infestation elevated and reached its peak (10.15 larvae/plant) during the third week of March (11th SMW, 2021), while the prevailing mean atmospheric temperature, mean relative humidity and total sunshine were 24.00 °C, 42.25% and 8.50 hrs, respectively. The extent of damage or per cent plant infestation by the larvae of DBM were ranged from 0.83-58.33 per cent. The DBM larval population exhibited positively significant correlation with mean temperature (r= 0.58) and sunshine (r= 0.53), while negatively non-significant with mean relative humidity (r= -0.72). Meagre total rainfall of 12.60 mm was recorded during the *Rabi,* 2020-21, hence no significant relations were observed between rainfall and insect populations. The percentage plant infestation by DBM larvae was 0.83 - 58.33 during *Rabi,* 2020-21. Likewise, the DBM pupal population noticed from the 1st week of January (1st SMW, 2021) and touched its crest of 7.08 pupae/ plant during the 1st week of March (9th SMW, 2021). During peak pupae per plant, the mean temperature, mean relative humidity and sunshine were 21.85 □, 44.00% and 9.60 hrs, respectively. The pupal population had a significant positive correlation with mean atmospheric temperature

(r = 0.54) and sunshine hours (r = 0.54). Although, a significant negative correlation (r = -0.67) was observed with mean relative humidity.

The multiple linear regression analysis revealed that atmospheric parameters (mean atmospheric temperature, mean relative humidity and total sunshine) obliged a collective reassurance on DBM larval population. The coefficient of multiple determination (R^2) was 0.591 indicating that 59.10 per cent of variation in the DBM larval populations was explained by the explanatory variables. In multiple linear regression equation, the regression coefficient b_1= 0.205 means that held X_2 (mean relative humidity) and X_3 (total sunshine) constant, with one degree increase in X_1 (mean temperature) directed on average 0.205 per cent increase in DBM larval population. In the same way, b_2= -0.283 means that holds X_1 and X_3, constant and 1 per cent increase in X_2 (mean relative humidity) led from average to about 0.283 per cent decrease in larval populations. Equally, b_3=-0.447 holds X_1 and X_2 constant and 1 hr increase in X_3 (sunshine) led from average to about 0.447 per cent decrease in number of larvae. The multiple linear regression analysis for pupal population had a collective influence on DBM pupal population. The coefficient of multiple determinations (R^2) was 0.500 indicating that 50.00 per cent of variation in the DBM pupal populations was explained by the explanatory variables. The regression coefficient b_1= 0.166 means that held X_2 (mean relative humidity) and X_3 (total sunshine) constant, with one degree increase in X_1 (mean temperature) focused on the average to about 0.166 per cent increase in DBM pupal population. Similarly, b_2= -0.095 means that held X_1 and X_3, constant and 1 per cent increase in X_2 (mean relative humidity) led from average to about 0.095 per cent decrease in DBM pupae and b_3= 0.088 means that held X_1 and X_2 constant and 1 hr increase in X_3 (sunshine) led from average to about 0.088 per cent increase in DBM pupal populations.

Similarly, Mahendran *et al.* (2017) observed that initial larval and pupal populations of *P. xylostella* were seen during the 52nd meteorological week, with a mean density of 1.27 to 1.30 larvae and pupae/ 10 plants and peak populations during 9th SMW with 102.55 to 111.15 larvae and pupae/10 plants. They witnessed that larval and pupal populations were positively significant with sunshine hours as well as maximum and minimum temperature during the first year of the experiment while positive non-significant during the second year, respectively while relative humidity was negatively significant during the first year and negatively non-significant during the second year.

In the present investigation, DBM larval incidence had a significant positive correlation with maximum atmospheric temperature and sunshine hrs whereas, mean relative humidity had a significant negative correlation during both the seasons. Meagre total rainfall of 3.00 mm and 12.60 mm were recorded during the

Table 3. Multiple linear regression for key weather parameters on diamondback moth on cabbage during *Rabi,* 2019-20 and 2020-21

Season	Insect	Regression equation	Regression coefficients			Multiple correlation coefficient (R)	Coefficient of multiple determination (R^2)
			b_1	b_2	b_3		
Rabi, 2019-20	Diamondback moth larvae	$Y = 4.447+(0.150)X_1+(-0.139)X_2+(0.865)X_3$	0.150*	-0.139*	0.865*	0.839	0.705 (70.50%)
Rabi, 2019-20	Diamondback moth pupae	$Y = -0.688+(0.222)X_1+(-0.082)X_2+(0.520)X_3$	0.222*	-0.082*	0.520*	0.752	0.566 (56.60%)
Rabi, 2020-21	Diamondback moth larvae	$Y = 20.399+(0.205)X_1+(-0.283)X_2+(-0.447)X_3$	0.205*	-0.283	-0.447*	0.769	0.591 (59.10%)
Rabi, 2020-21	Diamondback moth pupae	$Y = 2.927+(0.166)X_1+(-0.095)X_2+(0.088)X_3$	0.166*	-0.095*	0.088*	0.707	0.500 (50.00%)

*Significant at 5 per cent level of significance; Observations = 11; Y = Dependent variable; insect populations;

X_1 = Mean Atm. Temperature (°C); X_2 = Mean Relative Humidity (%); X_3 = Mean Sunshine (hour) (mm)

Partial regression coefficient $b_{1:}\ b_{yx1.x2x3,}\ b_{2:}\ b_{yx2.x1x3,}\ b_{1:}\ b_{yx3.x1x2}$

Rabi, 2019-20 and 2020-21 seasons, respectively, hence no significant relations were noticed between rainfall and insect populations. The results obtained from the present experiment revealed that the incidence of DBM was significantly affected by temperature and sunshine positively whereas relative humidity negatively Similarly, Venkateswarlu *et al.* (2011) observed DBM larval populations appearance during the 1st week of February and peak incidence (7.9 larvae /plant) during the 1st week of March. They also revealed that maximum temperature among different abiotic factors had a significant positive correlation while mean relative humidity had a significant negative correlation with the population of *P. xylostella.* Patra *et al.* (2013) found that the maximum population of the *P. xylostella* was documented on 1st March and 23rd February of about 13.60 and 14.33 larvae per plant during a couple of seasons, respectively on cabbage crop and the maximum temperature had a significant effect in elevating the population of *P. xylostella*. Similarly, Rahimgul and Sasya (2016), conducted research on the cabbage cultivar Golden acre during *Rabi,* 2015-2016 at Allahabad and reported that DBM larvae commenced from the 2nd week of February with an average 0.25 larvae/plant and reached a peak level of 3.40 larvae/ plant at 4th week of March. Jat *et al.* (2017) also reported the incidence of DBM in the 4th week of January and peak during the 4th week of February (13.10 larvae/five plants). They found that DBM population exhibited a positive significant correlation with temperature and a negative non-significant correlation with relative humidity and rainfall. Similarly, Jat *et al.* (2017) evidenced that infestation of DBM was started during the 2nd week of December during *Rabi,* 2012-13 and 2013-14 and reached its crest with a mean population of 5.40 and 5.20 larvae/plant in the 5th and 6th SMW during both the years, respectively. They found that DBM population exhibited a positive correlation with mean temperature and a negative correlation with mean relative humidity.

During the study period, the larval incidence of DBM on cabbage started from the 1st week of December, 2019 and the second week of December during the *Rabi,* 2019-20 and 2020-21, respectively. During both seasons, a noticeably high population of DBM larvae was recorded as 9.18 larvae/ plant during the 2nd week of March, 2020 and 10.15 larvae/plant during the 3rd week of March, 2021, in the respective crop season. The DBM pupal population was heeded from the 4th week of December and the 1st week of January during both years, respectively. The peak populations were noticed during the 4th week of February (6.45 pupae/ plant) and the 1st week of March (7.08 pupae/ plant). DBM larval and pupal populations had a significant positive correlation with maximum atmospheric temperature and sunshine hrs while a significant negative correlation was observed with mean relative humidity during both years. The coefficient of multiple determination (R^2 =0.705) and (R^2 = 0.591) indicated that 70.50 and 59.10 per cent of variation in the larval populations during 2019-20 and 2020-21 respectively. Similarly, DBM pupal population directed a collective influence of 56.60 per cent (R^2=0.566) and 50.00 per cent (R^2 = 0.500) during 2019-20 and 2020-21, respectively. The information is helpful for developing efficient pest management strategies against DBM populations in Southern Rajasthan and similar agro-ecological regions.

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REFERENCES

Abraham, E. V. and Padmanabhan, M. D. 1968. Bionomics and control of the diamondback moth, *Plutella xylostella* Linn. *Indian Journal of Agricultural Science,* **38**: 513-519.

Alam, M. M. 1992. Diamondback moth and its natural enemies in Jamaica and some other Caribbean Islands. Diamondback moth and other crucifer Pests: Proceedings of the Second International Workshop, Tainan, Taiwan, AVRDC, Taiwan, pp 233-243.

Glance, H. S. A. A. 2018. Horticulture Statistics Division Department of Agriculture. *Cooperation & Farmers' Welfare Ministry of Agriculture and Farmers' Welfare Government of India*, p.9.

Anuradha, M. 1997. Possibility on integration of bio-agent for the management of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera:Yponumeutidae). Ph.D. Thesis, University of Agricultural Sciences, Bangalore.

Atumurirava, F., Furlong, M., Srinivasan, R., Shelton, A. and Collinsm, H. 2011. Diamondback moth resistance to commonly used insecticides in Fiji. Paper presented at the Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests.

Cardleron, J. J. and Hare, C. J. 1986. Control of diamondback moth in South East Asia by profenofos. In Diamondback moth management, Proceedings of the First International Workshop (Eds. N. S. Talekar and T. D. Griggs) AVRDC, Taiwan, pp. 347- 357.

Fowler, J., Cohen, L. and Jarvis, P. 1998. Practical Statistics for Field Biology, (2nd Edition), John Wiley and Sons, Chichester, West Sussex, England.

Jat, G. C., Lekha, Jat, S. K. and Yadav, P. C. 2017. Effect of Weather Parameters on the Incidence of Major Insect Pests of Cabbage. *International Journal of Agricultural Science,* **9**(16): 4133-4135.

Jat, M. K., Rana, B. S. and Jain, A. K. 2017. Seasonal incidence of major insect pests of cabbage with their natural enemies. *Indian Journal of Entomology,* **79**(3): 257-260.

Lal, O. P. 1975. A Compendium of insect pest of vegetables in India. *Bulletin of Entomological Research,* **16**: 31-56.

Mahendran, B., Sharma, R. K. and Sinha, S. R. 2017. Population dynamics of natural enemies of *Plutella xylostella* and *Pieris brassicae* on cauliflower. *Indian Journal of Entomology,* **79**(4): 411-416.

Nemato, H., Kiritani, K. and Ono, H. 1984. Enhancement of the intrinsic rate of natural increase induced by treatment of the Diamondback moth, *Plutella xylostella* L. with sublethal concentration of methomyl. *Japanese Journal of Applied Entomology and Zoology,* **28**(3): 150-155.

Patra, S., Dhote, V. W., Alam, S. K. F., Das, B. C., Chatterjee, M. L. and Samanta, A. 2013. Population dynamics of major insect pests and their natural enemies on cabbage under new alluvial zone of West Bengal. *Journal of Plant Protection Sciences,* **5**(1): 42-49.

Rahimgul, S. and Sasya, T. 2016. Efficacy of chemical insecticides and botanicals in the management of diamondback moth (*Plutella xylostella*) in cabbage (*Brassica oleracea var. capitata* L.). *International Journal of Multidisciplinary Research and Development,* **3**(6): 101-104.

Ribeiro, L. M. S., Wanderley-teixeira, V., Ferreira, H. N., Teixeira, A. A. C. and Siqueira, H. A. A. 2014. Fitness costs associated with field-evolved resistance to chlorantraniliprole in *Plutella xylostella* (Lepidoptera: Plutellidae), *Bulletin of Entomological Research,* **104** (1): 88-96.

Shelton, A. M. and Badenes-perez, F. R. 2006. Concepts and applications of trap cropping in pest management. *Annual Review of Entomology*, **51:** 285-308.

Tabashnik, B. E. and Cushing, N. L. 1987. Leaf residue Vs tropical bioassays for assessing insecticide resistance in the diamondback moth. *Plutella xylostella* (L.) *Plant Protection Bulletin,* **35**: 11-14.

Talekar, N. S. 1992. Management of diamondback moth and other crucifer pests. *Proceedings of the Second International Workshop*, Shanhua, Taiwan, *AVRDC, Taiwan,* 10-14, December 1990, p. 603.

Talekar, N. S. and Shelton, A. M. 1993. Biology, ecology, and management of diamondback moth. *Annual Review of Entomology*, **38**: 275–301.

Venkateswarlu, V., Sharma, R. K., Chander, S. and Singh, S. D. 2011. Population dynamics of major insect pests and their natural enemies in cabbage. *Annals of Plant Protection Sciences*, **19**(2): 272-77.

Zhou, L., Huang, J. and Xu, H. 2011. Monitoring resistance of field populations of diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) to five insecticides in South China: A ten-year case study. *Crop Protection*, **30**(3): 272-278.

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Preference of brinjal hadda beetle, *Henosepilachna vigintioctopunctata* (Fabricius) (Coccinellidae: Coleoptera) to different solanaceous weed plant hosts

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ABSTRACT: Surveys were conducted to record the incidence of hadda beetle *Henosepilachna vigintioctopunctata* on Solanaceous weed hosts during the brinjal off-season during 2021 and 2022. The maximum mean population of hadda beetle was recorded on *Physalis angulata* (20.59) followed by *Solanum trilobatum* (6.88), *Datura metal* (6.26) and *Solanum nigrum* (6.06). The preference and biology of hadda beetle observed on the solanaceous weed hosts at the Department of Entomology, Faculty of Agriculture, Annamalai University, Tamil Nadu, India. Feeding preference towards the solanaceous hosts was *P.angulata >S. nigrum>S. trilobatum>D. metal*. The oviposition preference was *P.angulata >D. metal>S. nigrum>S. trilobatum*.The shotest total larval period was on *P.angulata*(12.55 days) followed by *S. nigrum* (13.30 days), *S. trilobatum* (14.30 days) and *D. metal* (15.75 days). The maximum female longevity observed on *P. angulata* (54.35 days) followed by *S. nigrum* (52.30 days), *D. metal* (37.20 days) and *S. trilobatum* (36.20 days). Similarly, the longevity male was maximum on *P. angulata* (56.55 days) followed by *S. nigrum* (53.60 days), *S. trilobatum* (40.40 days) and *D. metal* (35.25 days).

Keywords: Hadda beetle, off-season, weed hosts, host preference, brinjal

INTRODUCTION

Brinjal, *Solanum melongena* Linnaeus is one of the most important solanaceous vegetable crops majorly cultivated in tropical and sub-tropical countries of the world (Sarker *et al*., 2006). India is one of the major Brinjal producers globally after China with the production of 12.61 million metric tons in the fiscal year 2023 (Statista, 2023). Brinjal is a highly nutritious vegetable renowned for its rich phosphorus, iron, and B complex vitamins, eggplant serves as a vital component of diets with health benefits (Gurubuz *et al.,* 2018).

The Brinjal cultivation in India is affected by many insect pests such as fruit and shoot borer, leafhoppers, mites, whiteflies, aphids, thrips, spotted/ hadda beetles, leaf roller, stem borer, and blister beetle. Among them, the Hadda beetle, *Henosepilachna vigintioctopunctata*(Fabricius) (Coccinellidae: Coleoptera) is one of the most destructive defoliating insect pests. Both the immature and adult stages of the hadda beetles feed on the chlorophyll content of the leaves resulting in skeletonization and drying in later stages. It affects the plant growth and leading to heavy economic yield loss (Ali *et al*., 2017; Sharma *et al*., 2017).

Hadda beetle distribution is reported in many tropical and sub-tropical countries. In India apart from Brinjal, the Hadda beetle is reported as the key pest of many cultivated plants and weed plants of Solanaceae and Cucurbitaceae such as potato, tomato, *S. bonariense*, *Datura stramonium* L., *D. metel* L., *D. innoxia* Mill*, S.nigrum* L., *S. torvum* L., *S. trilobatum*, *Physalis* sp., *Withania somnifera* L., *Momordica charantia* L., *Benincasa cerifera* Savi, *Cucumissativus* L., *Luffa cylindrica* Roem., *Coccinia grandis* (Mohansundaram and Uthamaswamy, 1973; Islam *et al*., 2011; Manikandan *et al.*, 2019a).

The chance of survivability of the Hadda beetle is higher due to its wider host range. The beetles move to their alternate host when unfavorable conditions such as pesticide applications, and unavailability of crops occur in the fields. Though the farmer follows weed-free practices in their fields many alternate host plants are available to the pests in unmanaged ecosystems. For effective pest management practices availability of alternate hosts during the off-season and their interaction with pests should be known. Keeping in this view, the present experiment was conducted to know the potential off-season host plants of the hadda beetles in the ecosystem and their impact on the biology of Hadda beetles.

MATERIALS AND METHODS

2.1. Survey for the alternate host of *H. vigintioctopunctata*

A field survey was conducted on roadside landscapes for the alternate hosts of *Henospilachna*

vigintioctipunctata in the major egg plant-growing villages of Kollidam block, Mayiladuthurai district, Tamil Nadu, India. The ten villages were selected for survey *viz.,* Puthur, Serugudi, Pudhupattinam, Madhanam, Achalpuram, Thandavankulam, Arasur, Mahendrapalli and Thirumullaivasal. Five spots in the roadside landscapes for each village were pre-located and the weekly surveys were conducted during May-June in 2021 and 2022. The data were recorded based on damage symptoms and the presence of insect stages on different weed hosts. Weekly data from all ten villages were averaged and expressed as the mean for May and June 2021 and 2022.

2.2. Preference of *H. vigintioctopunctata* towards solanaceous hosts

2.2.1. Laboratory rearing of *H. vigintioctopunctata*

To ensure accuracy in preference among solanaceous weed hosts, beetles were not multiplied on selected weed hosts. Brinjal was used as the host for insect rearing. Adults of *H. vigintioctopunctata*were collected from the unsprayed brinjal fields in Annamalai University, Chidambaram, Tamil Nadu, India. Released into potted brinjal plant (Variety: Annamalai brinjal) in the cage. Once the egg laying was completed adults were removed and the plant was kept undisturbed in laboratory conditions at 27±2˚C, 85% RH. Immediately after hatching, the grubs were transferred on to fresh and healthy host plant leaves by using a soft brush. The cut end of each leaf petiole was wrapped with wet cotton to prevent water loss from the leaves and kept plastic trays covered with a fine cotton cloth. Leaves were replenished once in two days. The grubs were reared until adult emergence and the above process was repeated throughout the study period.

2.2.2. Feeding preference test by the free and no-choice method:

Laboratory-maintained third-in star grubs were used for the experiment under laboratory conditions of 28±2^{0}C and 90 per cent relative humidity. Fresh and undamaged leaves of five solanaceous weed hosts were collected from the pot culture. Leaf discs were made in the size of 3.7cm^2. For the free-choice method, 20 cm diameter plastic Petri plates were used for the experiment. The filter paper was placed inside the Petri plates and the leaf discs of selected hosts were placed equidistantly. For the no-choice test, 9cm diameter Petri plates were used with single leaf discs of selected host plants kept in the individual Petri plates. The pre-starved (for four hours) test insects were released in the Petri plates and kept in undisturbed condition. The above setup was replicated thrice under a completely randomized design. Leaf area fed by insect was calculated using graph sheets48 hours after grub release.

2.2.3. Oviposition preference test by the free and no-choice method:

For the free-choice test, one plant for each host was placed at an equal distance within the oviposition cage, and five pairs of freshly emerged adults were released. For the No-choice test, each test host plants were kept individually in the cage and a pair of freshly emerged adults was released. The number of eggs laid on the plant was counted at 10 days after the release of adults. Three replications were and maintained data collected and pooled together.

2.3. Effect of solanaceous weed hosts on developmental stages of *H. vigintioctopunctata*

The newly hatched larvae from the laboratory insect culture were released on the fresh leaves of selected host plants kept in 30 cm diameter individual plastic trays and continuously provided with the same host plants. The egg masses collected from the culture reared on selected host plants were kept on 20 cm diameter individual plastic trays for each host plant. Once larvae started to emerge from eggs, fresh foliage of selected host plants was regularly provided until pupation. All the treatments were replicated three times with twenty number of larvae per replication, and each developmental stage was recorded daily. Based on the observations incubation period, in star *viz*., larval period, pre-pupal period, pupal period and total developmental stage period were calculated.

2.4. Effect of solanaceous weeds hosts on oviposition, fecundity and longevity of *H. vigintioctopunctata*

The newly hatched adults reared from selected hosts were collected and kept in plastic trays allowing for matting. The mating pairs were collected and released into transparent plastic jars provided with twigs of selected host plants. All the treatments were replicated three times with twenty pairs of adults per replication. Based on the observations, pre-oviposition period, oviposition period, post-oviposition period, fecundity, and adult longevity (male and female) were calculated.

2.5. Statistical analysis

The data obtained from the field survey and field evaluation were analysed statistically using randomized block design (RBD) and all the laboratory experiment data were analysed using completely randomized block design (CRD) as per the methods described by Panse and Sukhatme (1978).

RESULTS AND DISCUSSION

The survey on the incidence of hadda beetle on solanaceous weed hosts was conducted during May and June in of 2021 and 2022. These months were reported as times of reduced brinjal cultivation or off-season by Manikandan *et al*. (2019a). The survey was conducted on the four solanaceous weed hosts located in the undisturbed ecosystems particularly along the sides of the canals and roadside landscapes at the survey locations. While farmer removes the weeds acting as alternate hosts from fields, weed hosts in undisturbed ecosystems such as roadsides and canal bunds and may still be available to pests. Hence, the hosts located in the roadsides and canal bunds were selected as survey spots. Earlier findings revealed the pest incidence in alternate host located in undisturbed ecosystem like roadside landscape. Manikandan and Rengalakshmi (2023) reported the incidence of leaf-twisting weevils on Indian butter trees on roadsides. Additionally, Manikandan *et al*. (2022a) reported the heavy incidence of Red pumpkin beetles on calotrop is on the roadside when the cucurbit plants were not available or not accessible.

3.1. Incidence of *H. vigintioctopunctata* on solanaceous weed hosts

Many phytophagous insect pests feed on multiple hosts, but most of them feed on the host plants from the same families or related families. Surveying the pest incidence on alternate hosts from the same family of economic crops can effectively highlight the importance of these alternate hosts in survey locations. Saravanraman *et al.* (2016) surveyed alternate hosts for the sesame webworm, *Antigastra catalaunalis* in the Pedalaceae family in Cuddalore district, Tamil Nadu. Meanwhile, Manikandan *et al.* (2019a) surveyed the solanaceous weed hosts for the incidence of hadda beetle, *Henosepilachna vigintioctopunctta* in Cuddalore district, Tamil Nadu.

The result of the survey conducted for the incidence of hadda beetle on solanaceous weed hosts such as *D. metal* (Picture-1), *S. trilobatum* (Picture-2), *S. nigrum* (Picture-3) and *P. angulata* (Picture-4) during off-season showed that the maximum mean population during May 2021 was recorded on *P.angulata* (19.44) followed by *S. trilobatum* (6.81), *S. nigrum* (5.62) and *D. metal* (2.66). During June 2021 the maximum incidence recorded on *P.angulata* (16.50) and the incidence of hadda beetle were statistically non-significant on *S. trilobatum* (6.78), *D. metal* (6.21) and *S. nigrum* (5.92). A similar trend was found in the results of May and June 2022 (Table 1). These findings were supported by Manikandan *et al.* (2019a), who also recorded the maximum incidence of hadda beetles on *P. angulata* among the solanaceous plants, as well as by Isaianbu and Manikandan (2020).

Table 1. Incidence of *H. vigintioctumpunctata* on different solanaceous weeds hosts

Host plant	Mean number of insects/ plant				Mean
	May, 2021	June, 2021	May, 2022	June, 2022	
S.nigrum	5.62 (2.57)^{a}	5.92 (2.63)^{a}	6.21 (2.68)^{a}	6.50 (2.74)^{a}	6.06
P.angulata	19.44 (4.52)	16. 50 (4.17)^{b}	22.12 (4.81)	20.20 (4.59)^{b}	20.59
S.trilobatum	6.81 (2.79)^{b}	6.78 (2.79)^{a}	7.21 (2.86)^{b}	6.72 (2.77)^{a}	6.88
D.metal	6.10 (2.66)^{a}	6.21 (2.68)^{a}	6.32 (2.70)^{a}	6.40 (2.72)^{a}	6.26
SE(d)	0.057	0.077	0.062	0.105	
C.D.	0.117	0.159	0.128	0.217	

Each value is the mean of ten replications Values in the parentheses are square root transformed Value with different alphabets differs significantly

Fig. 1. Hadda beetle, *H. vigintioctopunctata* infestation on *D. metal*

Fig. 2. Hadda beetle, *H. vigintioctopunctata* infestation on *S. trilobatum*

Fig. 3. Hadda beetle, *H. vigintioctopunctata* infestation on *S. nigrum*

Fig. 4. Hadda beetle, *H. vigintioctopunctata* infestation on *P. angulata*

3.2. Feeding preference of *H. vigintioctipunctata* on solanaceous weed hosts

Data of leaf area consumption of *H. vigintioctipunctata*in free choice test on the solanaceous weed hosts at 48 hours after release (HAR) of larvae showed that the maximum leaf area consumption recorded on *P.angulata* (6.33 cm^2) followed by *S. nigrum* (4.21 cm^2), *S. trilobatum* (3.44 cm^2) and *D. metal* (1.11 cm^2). In the no-choice test, leaf area consumption at 48 HAR data revealed that the highest leaf area consumption was on *P.angulata* (2.65 cm^2) followed by *S. nigrum* (1.13 cm^2), *S. trilobatum* (0.38 cm^2), *D. metal* (0.31 cm^2) (Figure-1).

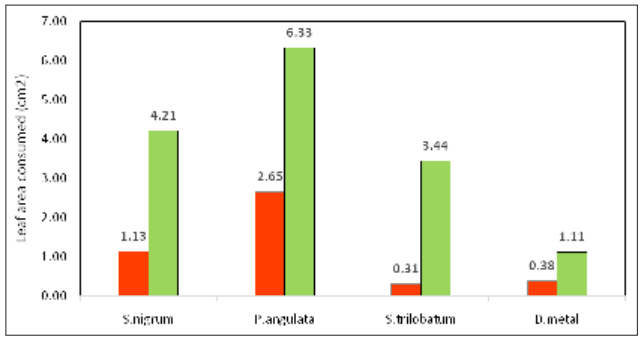

Fig. 1. Feeding preference of *H. vigintioctopunctata* to different Solanaceous hosts

Although some insect pests feed on the host plants from the same family the preference level varies from host to host. Imura and Ninomiya (1998) also recorded the feeding of hadda beetle by image processing method on different hosts and reported the variability in the leaf consumption. Manikandan *et al.* (2022b) reported the variation in the preference of pumpkin beetle, *Aulocophora* spp. towards the host plants belonging to the cucurbitaceous family under *in-vitro* conditions. Several earlier reports indicated that hadda beetle infestation varies among various solanaceous plants including weed hosts (Rajagopal and Trivedi, 1989; Katakura *et al.,* 1988).

Manikandan *et al.* (2019a) evaluated the variation in the feeding preference of hadda beetles towards different host plants and reported the highest leaf area consumption recorded on *P.angulata* (0.89 cm^2) followed by *S. nigrum* (0.44 cm^2), *D. metal* (0.21 cm^2), *S. trilobatum* (0.16 cm^2) and (0.13 cm^2) and *S. xanthocarpum* (0.07cm^2).

The variation in the feeding preference or incidence of pests is not only reported between the species but also reported within the species of host plants. Germplasm or cultivars of several crop species such as groundnut, sesame, black gram, tomato, bitter gourd, banana, mango etc., showed the difference in pest infestation resulting

from biophysical, biochemical factors of the germplasms or cultivars (Saravanaraman *et al.,* 2019; Manikandan *et al*., 2019b, Arunbabu *et al.,* 2020; Manikandan and Selvanarayanan, 2020; Manikandan *et al.,* 2020a; Manikandan *et al.,* 2020b; Manikandan *et al.,* 2022c).

3.3. Oviposition preference of *H. vigintiopunctata*on solanaceous weed hosts

Data on the number of eggs numbers laid at 10 days after release (DAR) data showed that the maximum number of eggs recorded on *P.angulata* (97.67) followed by *D. metal* (77.33), *S. nigrum* (65.33) and *S. trilobatum* (57.67). Conversely, in the no choice test resulted in the number of eggs laid varied among the host, with the highest count on *P. angulata* (57.67) followed by *D. metal* (40.33), *S. nigrum* (29.67) and *S. trilobatum* (20.33) (Figure-2).The finding of Nagia *et al,*(1992) supports the result that a maximum number of hadda beetle eggs laid on *Physalisminima* Linn. Manikandan *et al.* (2019a) evaluated the oviposition preference of hadda beetles towards solanaceous weed hosts and reported

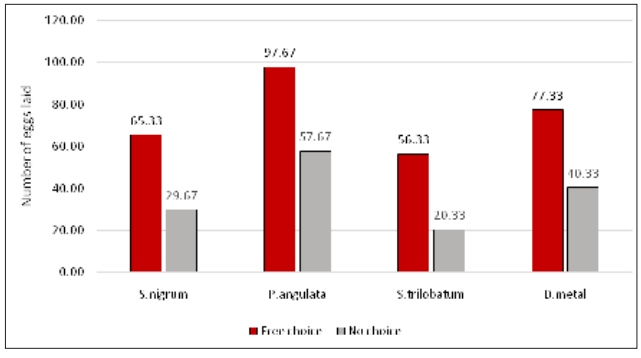

Fig. 2. Oviposition preference of *H. vigintioctopunctata* to different Solanaceous hosts

the maximum number of eggs counted on *P.angulata* followed by *D. metal*, *S. nigrum*, *S. trilobatum* and *S. xanthocarpum*similar trends observed by Isaianbu and Manikandan (2020) under semi-field or net cage condition.

3.4. Effect of solanaceous host plants on the development of *H. vigintiopunctata*

The effect of solanaceous host plants on the biology of the developmental stage was observed and the results showed that the shortest incubation period observed on *P.angulata* which was statistically significant compared to other hosts such as *D. metal*, *S. nigrum* and *S. trilobatum.*

The shortest first instar duration observed in the larvae fed on*P.angulata* (2.15 days) which was on par with *S. nigrum* (2.40days) followed by *S. trilobatum* (2.80days) and *D. metal* (3.20days). The shortest and longest second in star observation on the host plants *viz., P. angulata* and *D. metal*. There is no significant difference in the influence of host plants on the third instar duration whereasthe fourth instar duration fed on *P. angulata* and *S. nigrum* was non-significant and the longest duration recorded on *D. metal* (4.80 days). The total larval period was shortest (12.55 days) in *P.angulata* followed by *S. nigrum* (13.30 days), *S. trilobatum* (14.30 days) and *D. metal* (15.75 days).

There was no significant difference observed during the prep-pupal period among the insects fed on different solanaceous hosts. The pupal period was shortest on*P. angulata* (3.35 days) which was statistically on par with *S. nigrum* (3.45 days) and the longest pupal period was observed on the insects fed on *D. metal* (4.20 days).

Table 2. Effect of solanaceous weed hosts on development stages of *H. vigintioctopunctata*.

Host plant	Incubation period (days)		Larval period (days)								Total larval period (days)		Pre-pupal period (days)		Pupal period (days)		Total duration (days)	
			First in star		Second in star		Third in star		Fourth in star									
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
S.nigrum	4.65 (2.38)^{b}	0.023	2.40 (1.84)^{a}	0.035	3.10 (2.02)^{ab}	0.016	3.70 (2.16)	0.041	4.10 (2.25)^{a}	0.032	13.30 (3.78)^{b}	0.042	2.15 (1.77)	0.031	3.45 (2.11)^{a}	0.031	18.90 (4.46)^{b}	0.045
P.angulata	4.20 (2.28)^{a}	0.030	2.15 (1.77)^{a}	0.022	2.90 (1.97)^{a}	0.026	3.65 (2.15)	0.026	3.85 (2.20)^{a}	0.019	12.55 (3.68)^{a}	0.021	1.90 (1.70)	0.022	3.35 (2.08)^{a}	0.026	17.80 (4.33)^{a}	0.026
S.trilobatum	4.75 (2.40)^{b}	0.021	2.80 (1.95)^{b}	0.031	3.30 (2.07)^{b}	0.025	3.80 (2.19)	0.022	4.40 (2.32)^{b}	0.024	14.30 (3.91)^{c}	0.025	2.15 (1.77)	0.022	3.85 (2.20)^{b}	0.019	20.30 (4.61)^{c}	0.027
D.metal	4.85 (2.42)^{b}	0.017	3.20 (2.05)^{c}	0.022	3.65 (2.15)^{c}	0.030	4.10 (2.25)	0.027	4.80 (2.41)^{c}	0.020	15.75 (4.09)^{d}	0.030	2.25 (1.79)	0.038	4.20 (2.28)^{c}	0.026	22.20 (4.81)^{d}	0.037
SE(d)	0.03		0.04		0.04		0.04		0.03		0.04		0.04		0.04		0.05	
C.D.	0.07		0.08		0.07		N/A		0.07		0.09		N/A		0.07		0.10	

Each value is the mean of three replications @ twenty insects per replication Values in the parentheses are square root transformed Value with different alphabets differs significantly

The significant influence of host plants on the hadda beetles' developmental stages was observed during the experiments. The total duration of developmental stages was shortest in the insects fed on *P.angulata* (17.80 days) followed by *S. nigrum* (18.90 days), *S. trilobatum* (20.30 days) and the longest duration observed on *D. metal* (22.20 days).

In over all observation ofthe larval period on the different hosts, results were supported by Saravanan and Vipin (2017) who also reported the influence of host plants on larval development. They evaluated the different solanaceous host plants on the developmental stages of hadda beetles and their results agreed with our findings, showing that the hadda beetles fed with *D. metal* resulted in the longest larval stages, pupal duration and total development period. The results strongly indicated the shortest developmental stages of hadda beetles on*P. angulata*, Similarly,Sivasankari *et al.* (2021) also reported the shortest developmental stages of hadda beetles fed on *P. minima.*

3.5. Effect of solanaceous host plants on oviposition, fecundity and longevity of the *H. vigintiopunctata*

The effect of solanaceous host plants on the oviposition, fecundity and longevity of the *H. Vigintiopunctata* was recorded and the results revealed that host plants had a significant influence on the test insect. The shortest pre-oviposition observed in the hadda beetles fed on *P.angulata* (8.35 days) followed by *S. nigrum* (9.20 days), *S. trilobatum* (11.45days) and *D. metal* (12.95days).

The shortest oviposition period observed on the hadda beetles fed on host plants *viz., D. metal* (17.60 days) followed by *S. trilobatum* (23.10 days), *S. nigrum* (36.90 days) and *P. angulata* (39.40 days). A similar trend was observed on post-oviposition periods of hadda beetles fed on the solanaceous hosts.The maximum number of eggs laid by hadda beetles fed on *P.angulata*(480.75/female) followed by *S. nigrum* (435.8/female), *S. trilobatum* (224.3/female) and *D. metal* (120.75/female).

Influence of solanaceous hosts observed on the longevity of female and male beetles, the maximum female longevity oberved on *P. angulata* (54.35 days) followed by *S. nigrum* (52.30 days), *D. metal* (37.20 days) and *S. trilobatum* (36.20 days). Similarly, the longevity male was maximum on *P. angulata* (56.55 days) followed by *S. nigrum* (53.60 days), *S. trilobatum* (40.40 days) and *D. metal* (35.25 days). The findings of Saravanan and Vipin (2017) supported the results by reporting the shortest oviposition period and post-oviposition period, less fecundity and minimum longevity observed on hadda beetles fed on *D. metal.*The results strongly expressed the shortest developmental stages of *P. angulata*, similarly, Sivasankari *et al.* (2021) also reported the shortest developmental stages of hadda beetles fed on *P. minima.*

The alternate host plants of crop pests may indirectly affect crop cultivation by acting as reservoirs for pests, thereby protecting them from unfavorable environments. On the other hand, they may serve as pest refugia, which are susceptible to insecticides but also support beneficial insects such as natural enemies of crop pests

Table 3. Effect of solanaceous weeds hosts on oviposition, fecundity and longevity of adults of *H. vigintioctopunctata*

Host plant	Pre-oviposition period (Days)		Oviposition period (Days)		Post-oviposition period(Days)		Fecundity (Number of eggs/ Female)		Longevity (days)			
									Female		Male	
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
S.nigrum	9.20 (3.19)^{b}	0.014	36.9 (6.15)^{c}	0.041	7.55 (2.92)^{c}	0.026	435.8 (20.89)^{c}	0.15	52.3 (7.30)^{b}	0.023	53.6 (7.39)^{c}	0.027
P.angulata	8.35 (3.06)^{a}	0.021	39.4 (6.35)^{d}	0.035	8.75 (3.12)^{d}	0.020	480.75 (21.92)^{d}	0.26	54.35 (7.44)^{c}	0.029	56.55 (7.58)^{d}	0.042
S. trilobatum	11.45 (3.53)^{c}	0.021	23.1 (4.90)^{b}	0.061	5.85 (2.61)^{b}	0.035	224.3 (15.00)^{b}	0.20	36.2 (6.10)^{a}	0.029	40.4 (6.43)^{b}	0.067
D. metal	12.95 (3.73)^{d}	0.018	17.6 (4.30)^{a}	0.079	4.75 (2.40)^{a}	0.021	120.75 (11.00)^{a}	0.19	37.2 (6.18)^{a}	0.037	35.25 (6.02)^{a}	0.042
SE(d)	0.03		0.08		0.04		0.26		0.04		0.07	
C.D.	0.05		0.16		0.07		0.52		0.08		0.13	

Each value is the mean of three replications @ twenty insects per replication Values in the parentheses are square root transformed Value with different alphabets differs significantly

and pollinators by providing nectar and pollen (Selvam *et al.,* 2019; Ayyamperumal *et al.,* 2020; Manikandan *et al.,* 2023). The preference and survival potential of crop pests on alternate host plants may vary from species to species of both the pest and the host plants. Based on the nature of the relationship between the pest and alternate host, farmers can decide whether to remove the alternate host or allow it near the field. Some alternate hosts may have negligible support for pests, while others may be more attractive economically than the crop itself. Therefore, they can be used as attractant plants in pest management programs.

REFERENCES

Ali, S. S., Ahmed, S. S., Rizwana, H., Bhatti, F., Khoso, A., Mengal, M. I. and Shahwani, S. A. 2017. Efficacy of different bio-pesticides against major sucking pests on brinjal under field conditions. *Journal of Basic and Applied Sciences*, **13**: 133-138.

Arunbabu, J., Manikandan, P. and Selvanarayanan, V. 2020. Screening of green gram, *Vigna radiata* (Linn.)accessions against aphids, *Aphis craccivora* (Genn.) (Aphididae: Hemiptera) in two coastal districts of Tamil Nadu. *Pestology,* **44**: 20-25.

Ayyamperumal, M., Manikandan, P. and Suguna, K. 2020. Conservation biological control (CBC) – Tool for natural pest Control. In Organic Farming for Sustainable Agriculture Ed. Muthukumar, A. and Suthinraj, T., *JPS Scientific Publications,* India. pp: 198 – 211.

Gurubuz, N., Uluişik, S., Frary, A., Frary, A. and Doganlar, S. 2018. Health benefits and bioactive compounds of eggplant. *Food Chemistry*, **268**: 602–610.

Imura, O. and Ninomiya, S. 1998. Quantitative measurement of leaf area consumption by *Epilachna vigintioctopunctata* (Fabricious) (Coleoptera: Coccinellidae) using image processing. *Applied Entomology and Zoology*, **33**:491-495.

Isaianbu, A. and Manikandan, P. 2020.Trap cropping efficacy of an alternate host *Physalis angulata* against brinjal hadda beetle *Epilachna vigintioctopunctata* (Coccinellidae: Coleoptera). *Journal of Entomology and Zoology Studies*, **8**: 712-715.

Islam, K., Islam, M. S. and Ferdousi, Z. 2011. Control of *Epilachna vigintioctopuntata* Fab. (Coleoptera: Coccinellidae) using some indigenous plant extracts. *Journal of Life Earth Science,* **6**: 75-80.

Katakura, H., Abbas, I., Nakamura, K. and Saraji, H. 1988. Records of Epilachnie crop pests (Coleoptera, Coccinellidae) in Sumatra Barat, Sumatra, Indonesia. *Kontyu*, **56**: 281-287.

Manikandan, P. and Rengalakshmi, R. 2023. The first record of leaf-twisting weevil *Apoderus tranquebaricus* Fab (Curculionidae: Coleoptera) on the Indian butter tree, *Madhuca longifolia. Ecofarming,* **3**: 318-320..

Manikandan. P., Saravanaraman, M., Selvanarayanan, V. and Suguna, K. 2019a. Incidence and Host Preference of Brinjal Hadda Beetle *Epilachna vigintioctopunctata* (Fabricius)(Coccinellidae: Coleoptera) on Different Solanaceous Weed Hosts. *International Journal of Advances in Agricultural Science and Technology*, **6**:1-13.

Manikandan, P., Saravanaraman, M., Suguna, K. and Selvanarayanan, V. 2022b. Preference of pumpkin beetles, *Aulacophora* spp. (Coleoptera: Chrysomelidae) to different cucurbitaceous hosts. *Pest Management in Horticultural Ecosystems*, **28**(2): 58-63.

Manikandan, P. and Selvanarayanan, V. 2020a. Reaction of groundnut germplasm against leaf caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). *Plant Archives*, **20**:3003-3006.

Manikandan, P., Saravanaraman, M. and Selvam, K. 2022a. The exploration of the damage potential of red pumpkin beetle *Aulocophora* sp. on *Calotrpis* sp. *Ecofarming*, **02**:184-187.

Manikandan, P., Saravanaraman, M., Suguna, K. and Selvanarayanan, V. 2020b. Incidence and preference of mango hopper, *Idioscopus clypealis* Leth (Hemiptera: Cicadellidae) on different mango varieties. *Pestology,***44**: 49-53.

Manikandan, P., Saravanaraman, M., Suguna, K. and Selvam, K. 2019b. Host Plant Resistance- A Fundamental Approach to Pest Management. In Recent Trends in Insect Pest Management (Volume-1). Ed. Raju, S.V.S. and Sharma, K.R.AkiNik Publication, New Delhi pp. 85-121. ISBN-978-93-5335-754-2

Manikandan, P., Saravanaraman, M., Saravanan, M. and Selvanarayanan, V. 2022. Inducing insect resistance in sesame by innovative genetic manipulation using mutagens. In Genetic methods and tools for managing crop pests. Ed. Chakravarthy, A. K. Springer, Singapore. pp.623-640.

Manikandan, P., Malarvannan, S., Ranjith, R., Willcox, B. K., Rengalakshmi, R., and Deepa, S. 2023. Weed flora in field margins: An essential off-season resource for insect pollinators in Moringa (Moringa oleifera Lam.). Paper presented at the Global Development Conference 2023-Biodiversity and Sustainable Development, Universidad San Francisco de Quito (USFQ), Quito, Ecuador, October 31 – November 01. Global Development Network.

Manikandan, P., Saravanaraman, M., Selvam, K. and Suguna, K. 2021. Screening of bitter gourd, *Momordica charantia* accessions against cucurbit fruit fly, *Bactrocera cucurbitae* (Tephritidae: Diptera). *International Journal of Entomology Research,***45**: 907-912.

Manikandan, P., Selvanarayanan, V. and Sabesan, T. 2020. Genetic variability of leaf caterpillar resistance traitsamong groundnut (*Arachis hypogea*L.) germplasm. *International Journal of Entomology Research,* **5**: 82-85.

Mohansundaram, M. and Uthamaswamy, S. 1973. A note on the *Epilachna* spp. commonly found in Tamil Nadu with observation on the host range and distribution. *Science Culture*, **39**: 305-306.

Nagia, D. K., Kumar, S., Sharma, P., Meena, R. P and Saini, M. L. 1992. Mass multiplication of *Henosepilachna vigintioctopunctata*(Fabricius) (Coleoptera : Coccinellidae) on *Physalis minima* L. *Plant Protection Bulletin*. Faridabad. **44**: 24-25.

Panse, V.C. and Sukhatme, P.V. 1978. Statistical methods for Agricultural workers. III Rev. Ed. ICAR, New Delhi.

Rajagopal, D. and Trivedi, P. 1989. Status, bioecology and management of epilachna beetle, *Epilachna vigintioctopunctata*(Fab.) (Coleoptera : Coccinellidae) on potato in India : a review. *Tropical Pest Management*, **35**: 410-413.

Saravanan, L. and Vipin, C. 2017. Population growth potential of *Epilachna vigintioctumpunctata* (F.) (Coleoptera: Coccinellidae) on some solanaceous, medicinal plants. *International Journal of Pest Management,* **63**: 82-91.

Saravanaraman, M., Selvanarayanan, V. and Saravanan, K. 2016. Sesame webworm, *Antigastra catalaunalis* duponchel (Crambidae: Lepidoptera) survives on a new alternate host in Southern India. *International Journal of Entomology Research*,**1**: 46-48.

Saravanaraman, M., Manikandan, P., Suguna, K. and Selvanarayanan, V. 2019. Varietal preference of Pseudostem Weevil *Odoiporus longicollis* (Oliv.) (Coleoptera: Curculionidae) towardsdifferent banana varieties. *Journal of Insect Science,***32**: 81-84.

Sarker, R. H., Yesmin, S. and Hoque, M. I. 2006. Multiple shoot formation in eggplant (*Solanum melongena* L.). *Plant Tissue Culture Biotechnology,* **16**: 53-61.

Selvam, K., Shiva, N. and Manikandan, P. 2019. Ecological Engineering: A Sustainable Tool for Insect Pest Management. In Recent Trends in Insect Pest Management (Volume-2). Ed. Raju, S.V.S. and Sharma, K.R. AkiNik Publication, New Delhi. pp.21-53. ISBN-978-93-5335-992-8.

Sharma, J. H. and Tayde, A. R. 2017. Population dynamics of brinjal fruit and shoot borer, *Leucinodes orbonalis* Guen. and hadda beetle, *Epilachna vigintioctopunctata* Fab. on brinjal at Allahabad agro climatic region. *International Journal of Current Microbiology and Applied Sciences*, **6**:2055-2060.

Sivasankari, S., Kathirvelu, C. and Kannan, R. 202. Impact of host plants on the biology of hadda beetle, *Henosepilachna vigintioctopunctata* fab. In laboratory condition. *International Journal of Entomology Research,* **6**:293-295.

Statista, 2023. Statista.com/Production volume of brinjal in India from the financial year 2015 to 2022, with an estimate for 2023. Accessed on 16.02.2024. https://www.statista.com/statistics/1038975/india-production-of-eggplant

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Behavioural ecology of mango leaf webber, *Orthaga exvinacea* Hampson

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ABSTRACT: Behavioural studies of mango leaf webber, *Orthaga exvinacea* were carried out both in the laboratory and field conditions, majorly focusing on neaonate larva, mating, oviposition and intra specific competition. The feeding behaviour of *O. exvinacea* was found to be gregarious *i.e.,* a group of larvae coalesce together and scrape the chlorophyll content of young leaves. However, the later instars start feeding voraciously by making webs joining the adjacent leaves along with the shoots. The female moths laid eggs individually or in groups (8-14 eggs/cluster) on the lower surface of leaves near the midrib, while eggs were occasionally laid on the upper surface of the leaves during the early hours of photophase. Under field conditions, a series of observations revealed that the eggs were commonly laid in well-established older webs. During scotophase, several behavioural transitions in both male and female moths were observed while studying the mating behavior under laboratory conditions. Larval migration among the brood members of *O. exvinacea* was also noticed during the study.

Keywords: *Orthaga exvinacea*, mango, behavioural ecology, mating behavior, photophase, scotophase, brood members

INTRODUCTION

Mango leaf webber, *Orthaga exvinacea* Hampson (Pyralidae: Lepidoptera) is one of the important leaf-feeding insect pests in mango (*Mangifera indica* L.) responsible for low productivity (Reddy *et al*., 2013; Jayanthi *et al*., 2020). Though it was considered a minor pest earlier, in the recent past it has gained importance in all mango-growing locations and it has become a major pest in intensively farmed areas on both young and old plants a like (Srivastava and Verghese, 1983; Srivastava, 1997). *O. exvinacea* becomes a serious pest in extensively cropped areas of Uttar Pradesh, Uttaranchal, and Andhra Pradesh (Singh *et al*., 2006). *O. exvinacea* is responsible to cause ~35 percent yield loss under favourable environmental conditions (Tandon and Srivastava, 1982). The infestation of leaf webber begins in June and lasts until December. The caterpillars start webbing the leaves and feed on the entire leaf, leaving the midribs and veins. The heavily infested trees have a burnt appearance from distance, and significant levels of leaf webber infestation results in partial to complete failure of flower emergence (Verghese, 1998; Kavitha *et al*., 2005). Ninety percent of the defoliated/ skeletonised branches dried and did not fruit in the next season due to webber infestation (Singh, 1988). *O. exvinacea* is responsible to cause ~35 percent yield loss under favourable environmental conditions (Tandon and Srivastava, 1982).

MATERIAL AND METHODS

Maintenance of test insects and host plants

Mango webber, *Orthaga exvinacea*

The cultures of target insect pest, *O. exvinacea* were established in the laboratory for continuous supply throughout the study period. The webs consisting of larvae, *O. exvinacea* were collected from the experimental fields of IIHR to establish initial culture. For this, mango plants were thoroughly searched for any webber infestation and the active webs with live larvae were identified. The active webs were carefully cut using secateurs and gently placed into a polythene cover (40 cm length x 30 cm width) and tied with rubber bungs to avoid any escape of larvae. The polythene covers containing webs were brought to the laboratory and the webs were transferred to insect cages (54 x 50 x 45 cm) carefully. These insect cages were placed at ambient conditions (27 ± 1°C, 75±2% RH and 14L: 10D) and provided with fresh host plant (*Mangifera indica* L.) leaves on a daily basis until pupation. The pupae were collected when they formed and sexing was done by examining the location of genital slit in relation to anal slit (Khosravi and Sendi, 2010) under stereo microscope (Leica M205A). After sexing, the male and female pupae were placed in separate cages (30 x 30 x 30 cm) until adult emergence. The freshly emerged moths were provided with sugar /honey solution as food. These virgin insects

were used for further studies on mating behaviour. To establish mated population, the freshly emerged male and female moths were released in to a cage (30 x 30 x 30 cm) and allowed to mate for 24 h.

Host plants

The host plants of target insect pest *i.e*., mango (*M. indica*) were maintained continuously under glass house conditions for experimental use. Two to three years old mango grafts (cv. Banganpalli) were obtained from the Nursery Unit of ICAR-IIHR, Bengaluru and were planted in the pots (27 cm height x 30 cm diam.) containing red soil and farm yard manure (in 1:1 ratio). All the standard agronomic practices were followed without any insecticidal sprays. The field collected larvae, *O. exvinacea* (2-3 larvae/plant) were released on mango potted plants to create the webber infestation and these infested mango plants were used for all further studies.

Oviposition behaviour

To observe the oviposition behaviour of *O. exvinacea,* individual healthy mango plants were kept in a net cage (1 x 1 x 1 m) under glass house conditions at Division of Plant Protection, ICAR-IIHR and exposed to gravid female moths. A total of five one day old gravid female moths (n = 5) from the above established insect culture was released in to each net cage. The host plants were exposed to gravid female moths continuously for 24h and observations were made on egg laying behaviour and egg hatching time with the help of magnified hand lens.

Behaviour of neonate larvae

The behaviour of neonate larvae was observed in the early photophase with the help of magnified lens and torch light on mango webber infested plants. The neonate larvae emerged from the eggs (laid by moths in the above experiment) were observed closely on potted mango plants under glass house conditions. Observations were made on feeding behaviour, time taken for webbing, larval migration to form separate webs, behaviour while pupation and identification of pupation site.

Mating behaviour

Mating behaviour of *O. exvinacea* was studied by releasing freshly emerged male and female moths in to a square glass mating chamber (30 x 30 x 30 x 30 cm). The mating chamber containing moths was placed in darkness and observations were made with the help of torch light covered with red colour cellophane tape to avoid any photo disturbance to moths. To understand the mating behaviour pattern, the observations were recorded during the early scotophase (6.00 PM) to early photophase (6.00 AM), when moths were active. Observations were recorded for every 10 min interval. A total of 30 mating episodes were observed. The different behavioural events (resting period (R), swift antennal movements (SAM), walking with antennal swift movement (WK), flight and fluttering of the wings (FL), mate tracking (MT), mate approaching (MA) and mating (M) were noticed in both male and female moths. All the behavioural observations were compiled across the episodes and mating behavioural ethogram was developed. Behavioural transitions were also calculated and analysed by first order markov model.

Intraspecific competition

Intraspecific larval competition within the web between the brood members of *O. exvinacea* was studied in the experimental orchards of Division of Fruit Crops, IIHR, Bengaluru. A total of 25 webs were tagged randomly on webber infested mango plants (cv. Arka Udaya). Each individual web was thoroughly examined initially for the presence of different life stages of *O. exvinacea* namely egg, larval instars and pupa with the help of magnified hand lens.

RESULTS AND DISCUSSION

Egg laying behaviour/ Oviposition

The egg-laying behaviour was studied under glasshouse conditions. Freshly emerged male and female *O. exvinacea* moths were collected from the above-established culture and allowed for mating in insect cages (30×30×30 cm) for 24 hr. Potted healthy mango plants were placed in a net cage (1 x 1 x 1 m) and post-mating, the female moths (n = 5) were released onto the above-potted mango plants to study the gravid female moth egg-laying behaviour. The female moths of *O. exvinacea* laid eggs singly as well as in groups (8-14 eggs/cluster) on the lower surface of leaves near the midrib and occasionally eggs were also laid on the upper surface of the leaves during the early hours (4.00 to 6.00 AM) of photophase. The freshly laid eggs of *O. exvinacea* were oval, flat, creamish yellow in colour when laid singly. However, when laid in groups no proper shape was observed. Under field conditions, a series of

observations revealed that the eggs were often laid into the well-established older webs. The eggs hatched within 3-4 days of egg laying in the early hours of photophase (4.30 to 7.30 AM).

Rao *et al.* (2020) reported that the egg-laying and egg-hatching time of the mango red banded caterpillar, *Deonalis sublimbalis* Snellen was observed in the early hours of photophase in the mango orchard. Similar results were found by Kavitha *et al*. (2005) in *O. exvinacea*. Patel *et al.* (2007) reported that webber moths of *O. euadrusalis* are preferred to lay eggs on the lower surface of the mango leaves singly as well as in clusters near the midrib, sometimes the eggs were also laid on the upper surface of leaves as well as on tender mango twigs. Beria *et al.* (2008) also reported the same in *O. euadrusalis*. Further, we observed that *O. exvinacea* moths preferred to lay eggs on infested mango plants than on healthy plants under field conditions. Under field conditions, a series of observations revealed that the moths laid eggs in already-established older webs. Wee (2016) observed that the diamondback moth, *P. xylostella* laid a greater number of eggs on larvae-infested cabbage plants than on intact uninfested cabbage plants. Similar reports were made by Ntiri *et al*. (2018) and Sokame *et al*. (2019) on stem borer, *Chilo partellus* Swinhoe on maize. Jayanthi *et al*. (2020) reported in detail about the oviposition behaviour of *O. exvinacea* in conspecific webs and observed the presence of eggs, multiple larval stages, and pupae that differ by many days within the same web that indicated the sequential oviposition behaviour of multiple gravid moths rather than oviposition by a single moth. Thus, during oviposition, *O. exvinacea* moths laid eggs near or in conspecific webs, demonstrating their social facilitation habit, indicating the existence of conspecifics can be an essential habitat selection cue for *O. exvinacea*.

Behaviour of neonate larvae

Under glasshouse conditions, detailed observations were made on the neonate larval behaviour on potted mango plants. The newly hatched larvae passed through seven instars by moulting six times. The neonates were often found to form a group on the under surface of the mango plant leaves and slowly initiated the scrapping of chlorophyll content. Larval feeding behaviour varied among the different larval instars; the larvae were found to be gregarious in the early instars as a group of larvae coalesced together and started scraping the chlorophyll content of the leaves and leaf webbing was found to be absent during this period. However, the middle and late larval instars (from third to fifth instars) initiated the leaf webbing and fed on the leaves along the edges leaving only the midrib and veins. During the sixth instar, larval feeding was found to be reduced comparatively and complete feeding cessation was noticed during the seventh instar and the grown-up larvae entered into the prepupal stage. The prepupa stayed concealed in old larval galleries which are made from frass and dried pellets of excreta and started spinning the cocoon with the help of silken threads secreted by their silk glands along with dried pellets of excreta and later moulted to form a strong pupal case. The site of pupation was observed in the web itself in both the glass-house as well as field conditions.

The time taken for the complete construction of the web ranged from 20-25 days almost coinciding with first-generation phenological stages from egg hatching to pupal formation. The mid-instar caterpillars (III to IV instars) were actively involved in making the web as they started secreting the silken threads in higher amounts as compared to early instar larvae (I and II instars). During the developmental period of larva, migration was also observed in the early photophase (5.00-7.30 am) as few larvae were moved to nearby leaves (especially II and III instars) and started feeding voraciously to form new web. The same behaviour was observed under the field conditions.

Butani (1979) reported that *O. exvinacea* caterpillars scrape the leaf surface in the early stages and subsequently web the tender shoots and leaves, with multiple caterpillars being found in a single web. Kannan and Rao (2006) observed that *O. exvinacea* caterpillars loosely web numerous shoot leaves together and feed by defoliating from within. Haseeb *et al*. (2000) also reported that the larvae of *O. euadrusalis* start scraping the leaves after hatching and forming webs by feeding voraciously in later stages. Patel *et al.* (2007) reported that caterpillars scrape the leaf surface for chlorophyll initially and web the leaves by feeding on edges to the midrib, leaving a network of veins behind. The site of pupation was observed in the web itself in both the glasshouse and also in the field conditions. Mallikarjuna (2019) reported similar results as they studied the detailed biology of *O. exvinacea* in both laboratory and field conditions. Similar behaviour was reported by many researchers (Sajitha and Gokuldas, 2015; Gundappa *et al*., 2016; Kasar *et al*., 2017; Kerketta *et al*., 2021).

Mating behavioural profiles

During the scotophase period, the moths were found to be very active and exhibited a series of behavioural events compared to photophase. The detailed mating behaviour inventory revealed major behaviours *viz.,* resting period (R), swift antennal movements(SAM), walking with antennal swift movement (WK), flight and fluttering of the wings (FL), mate tracking (MT), mate approaching (MA) and mating (M) in both male and female moths. Among the series of events, most of the time both sexes exhibited swift antennal movement (SAM) (326.33±26.28 min) followed by a resting period (300.67±22.68 min) and mating (68.67±7.45 min). Further, both sexes walked along together showing SAM (53.34±8.90min) and resorted to flight mode with often fluttering of the wings and SAM (38.67±9.18min). Both sexes approached each other (12.00±1.80 min) and tracked each other (8.67±2.35). However, the peak time of resting was recorded during the period from 6.00 PM to 8.30 PM and 4.30 AM to 6.00 AM. Both sexes walked with SAM majorly between 9.30 PM to 10.30 PM and 2.00 AM to 2.30 AM. Intermittent flight and wing fluttering with SAM were often noticed between 11.00 PM to 12.30 PM. Both male and female moths tracked each other during the late scotophase *i.e.,* from 1.00 AM to 2.00 AM and approached each other between 2.00 AM to 3.00 AM. The mating period was observed between 3.00 AM to 4.30 AM (Fig.1). Interestingly, throughout the scotophase (8.00 PM to 5.00 AM) moths continuously exhibited swift antennal movements which were totally absent during the photophase. Further, a compilation of transition behavioural frequencies (Table 1a) and transition behavioural rates (Table 1b) (as per Markov first order) revealed that more behavioural transitions were noticed between the resting period and SAM in both sexes (αR,SAM= 0.57; αSAM,R= 0.48). Similar transitions were also observed among the behaviours namely mate approaching and SAM in both the sexes (αMA,SAM= 0.31; αSAM,MA= 0.12), followed by SAM (in both sexes) to walking with SAM (in both sexes, αSAM, WK= 0.13; αWK,SAM= 0.37), followed by mate tracking and SAM (αMT,SAM= 0.08; αSAM,MT= 0.06), flight and wing fluttering and SAM (in both sexes, αFL,SAM= 0.45; αSAM,FL=0.10).

Further, moths often walked followed by and resting period (in both sexes, αWK,R= 0.28; αR,WK=0.24). Moreover, transitions were also observed between the flight and fluttering of the wings and resting period (αFL,R= 0.32; αR,FL = 0.18), mate approaching and walking with antennal swift movement (αMA,WK= 0.00; αWK,MA =0.05), walking with antennal swift movement followed by flight and fluttering of the wings (αWK,FL= 0.13; αFL,WK = 0.15), mate approaching and mate tracking (αMA,MT= 0.00; αMT,MA = 0.50), mate tracking and flight and fluttering of the wings (αMT,FL= 0.00; αFL,MT= 0.07).

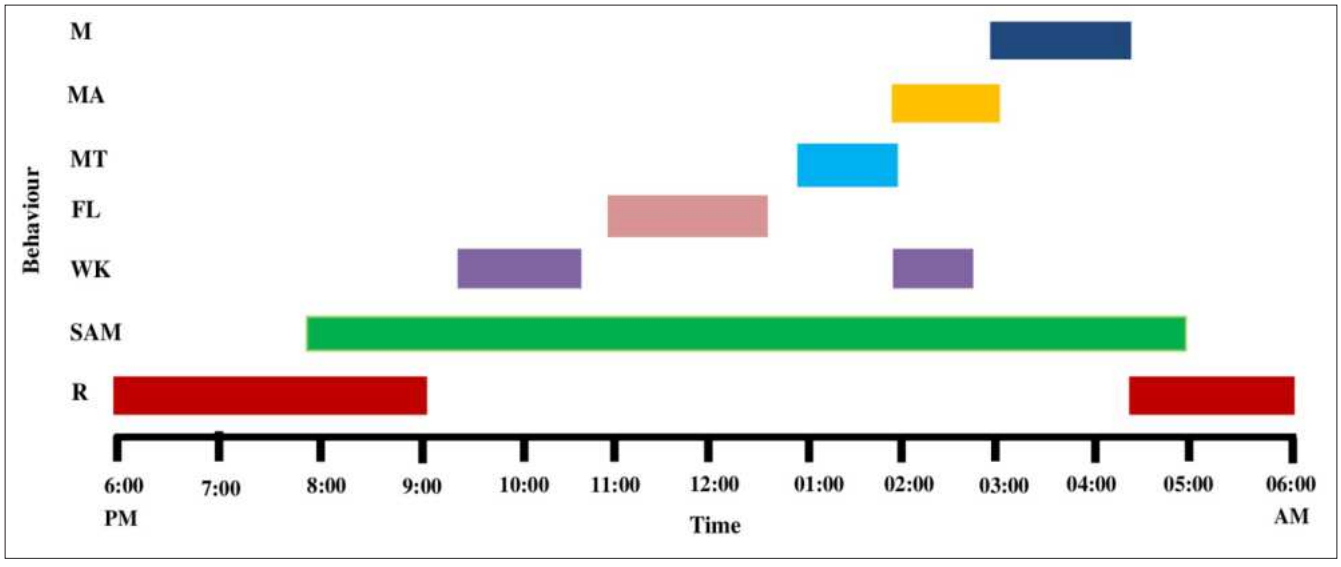

Fig.1. Behavioural dynamics of the adult mango leaf webber, *O. exvinacea* moths during scotophase

(R = Resting, SAM = Swift antennal movement, WK = Walking with antennal swift movement, FL = Flight and fluttering of the wings, MT = Mate tracking, MA = Mate approaching and M = Mating).

However zero transition was also found between resting period and mate approaching (αR,MA,= 0.00; αMA,R= 0.00), mating approach and flight and fluttering of the wings (αMA,FL= 0.00;αFL,MA= 0.00), mating and walking with antennal swift movement (αM,WK= 0.00; αWK,M= 0.00), mating and flight and fluttering of the wings (αM,FL= 0.00; αFL,M= 0.00) and mating and mate tracking (αM,MT= 0.00; αMT,M= 0.00) (Fig. 2).

Similarly, the calculated behavioural transition rates (%) also revealed that the major behaviour transitions were MA to M (68.57%) followed by M to R (58.82%), R to SAM (57.33%), MT to MA (50.00%), SAM to R (48.48%), FL to SAM (45.00%), M to SAM (41.18%), WK to SAM (36.84%), FL to R (32.50%), MA to SAM (31.43%), WK to R (28.95%), R to WK (24.00%), MT to WK (22.22%), MT to R (19.45%), R to FL (18.67%)

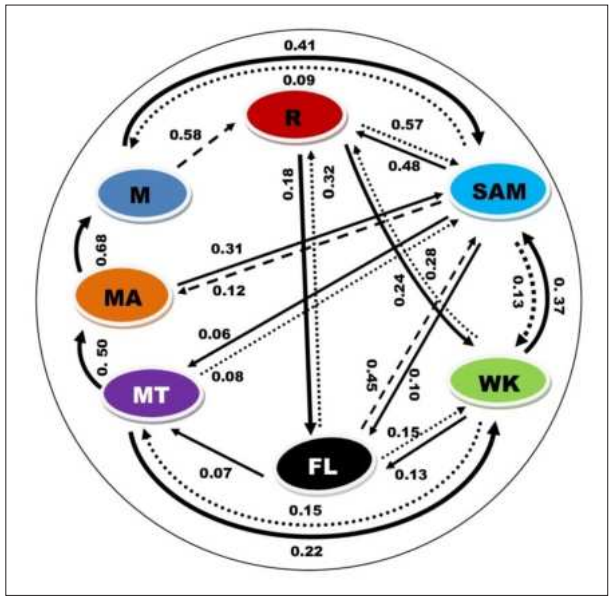

Fig.2. Markov state space diagram of the mating behaviour transitions in mango leaf webber, *O. exvinacea.*

(R = Resting, SAM = Swift antennal movement, WK = Walking with antennal swift movement, FL = Flight and fluttering of the wings, MT = Mate tracking, MA = Mate approaching and M = Mating).

Table1. Transition matrix of the behaviour of *O. exvinacea* based on first order markov model

a. Behaviour transition frequencies

	R	SAM	WK	FL	MT	MA	M	Total
R	-	43	18	14	0	0	0	75
SAM	32	-	9	7	4	8	6	66
WK	11	14	-	5	6	2	0	38
FL	13	18	6	-	3	0	0	40
MT	7	3	8	0	-	18	0	36
MA	0	11	0	0	0	-	24	35
M	30	21	0	0	0	0	-	51
Total	93	110	41	26	13	28	30	349

b. Behaviour transition rates

	R	SAM	WK	FL	MT	MA	M
R	-	α R,SAM =0.57	α R,WK =0.24	α R,FL =0.18	α R,MT =0.00	α R,MA =0.00	α R,M =0.00
SAM	α SAM,R =0.48	-	α SAM,WK =0.13	α SAM,FL =0.10	α SAM,MT =0.06	α SAM,MA =0.12	α SAM,M =0.09
WK	α WK,R =0.28	α WK,SAM =0.37	-	α WK,FL =0.13	α WK,MT =0.15	α WK,MA =0.05	α WK,M =0.00
FL	α FL,R =0.32	α FL,SAM =0.45	α FL,WK =0.15	-	α FL,MT =0.07	α FL,MA =0.00	α FL,M =0.00
MT	α MT,R =0.19	α MT,SAM =0.08	α MT,WK =0.22	α MT,FL =0.00	-	α MT,MA =0.50	α MT,M =0.00
MA	α MA,R =0.00	α MA,SAM =0.31	α MA,WK =0.00	α MA,FL =0.00	α MA,MT =0.00	-	α MA,M =0.68
M	α M,R =0.58	α M,SAM =0.41	α M,WK =0.00	α M,FL =0.00	α M,MT =0.00	α M,MA =0.00	

c. Per cent of behaviour transitions

	R	SAM	WK	FL	MT	MA	M	Total
R	-	57.33	24.00	18.67	0.00	0.00	0.00	100.00
SAM	48.48	-	13.63	10.60	6.06	12.12	9.10	100.00
WK	28.95	36.84	-	13.16	15.79	5.26	0.00	100.00
FL	32.50	45.00	15.00	-	7.50	0	0	100.00
MT	19.45	8.33	22.22	0	-	50.00	0	100.00
MA	0	31.43	0	0	0	-	68.57	100.00
M	58.82	41.18	0	0	0	0	-	100.00
Total	188.2	220.11	74.85	42.43	29.35	67.38	77.67	

WK to MT (15.79%), FL to WK (15.00%), SAM to WK (13.63%), WK to FL (13.16%), SAM to MA (12.12%), SAM to FL (10.60%), SAM to M (9.10%), MT to SAM (8.33%), FL to MT (7.50%), SAM to MT (6.06%) and WK to MA (5.26%) (Table 1c).

A similar experiment was carried out by Rao *et al.* (2020) in mango red banded caterpillar, *D. sublimbalis* under laboratory conditions. The results revealed that major behavioural transitions were observed between 6.00 PM to 6.00 AM in *D. sublimbalis* as swift antennal movement (SAM) (493.45±37.32 min) followed by resting period (179.5±20.48 min), walking along with SAM (77.93±12.17 min), resorted to flight with often fluttering of the wings and SAM (51.72±10.78 min), approaching each other (10.68±3.26 min) and tracked each other (5.17±2.30). Kelley (2016) observed the

mating behaviour of odd beetle, *Thylodrias contractus* Motschulsky in order to investigate the biosynthesis of sex pheromones. Agee and Webb (1969) recorded the mating behaviour of bollworm, *Helicoverpa zea* Boddie under laboratory conditions. Haynes and Birch, 1984 reported that the mating behaviour of the artichoke plume moth, *Platyptilia carduidactyla* Riley has shown a series of behavioural events such as wing fanning, flying, flying upwind, landing, wing fluttering while walking, approaching the female, rapidly fluttering the wings towards the female and making a copulatory attempt.

Intraspecific competition

In the present study, larval competition and migration in *O. exvinacea* were investigated under field conditions. A total of 25 webs were selected randomly in the mango orchard to observe larval competition and migration in *O. exvinacea.* During the observations each web was observed slowly without disturbing much with the help of a magnified hand lens. Interestingly, we found different instars (first to seventh instar) in a single web. A series of different behavioural dynamics were observed throughout the observation period. The larvae moved out from the web and at the same time, some larvae were found entering into the webs from nearby webs. This observation clearly indicates that there is competition between the larval populations within the web. This competition may be due to a shortage of food or space. We have also observed the migration behaviour of larvae to nearby webs. There is no relevant research available, particularly on *O. exvinacea,* regarding intraspecific competition among brood members. Rao *et al*. (2020) reported that few larvae go to nearby fruits in the early morning hours as part of their migration behaviour which is observed in mango red banded caterpillar, *D. sublimbalis.*

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REFERENCES

Agee, H. R. and Webb, J. C. 1969. Effects of ultrasound on capture of *Heliothis zea* and *Ostrinia nubilalis* moths in traps equipped with ultraviolet lamps. *Ann. Entomol. Soc. Am*, **62**(6):1248-1252.

Beria, N. N., Acharya, M. F. and Kapadia, M. N. 2008. Biology of mango leaf webber, *Orthaga exvinacea. Annal Plant Prot Sci*, **16**:218-219.

Butani, D. K. 1979. Insects and fruits. Periodical Expert Book Agency, Delhi.

Gundappa. S. P., Rajkumar. B., Verma. S. and Misra A. K. 2016. Incidence of shoot gall psylla, *Apsylla cistellata* Buckton on mango in India. *In World mango conference* at Islamia University of Bahawalpur, Pakistan.

Haseeb, M., Abbas, S. R., Srivastava, R. P. 2000. Missing links in the biology of mango leaf webber, *Orthaga euadrusalis* Walker (Lepidoptera: Pyralidae). *Insect Environ,* **6**(2): 25-29.

Haynes, K. F. and Birch, M. C. 1984. Mate-locating and courtship behaviors of the artichoke plume moth, *Platyptilia carduidactyla* (Lepidoptera: Pterophoridae). *Environ. Entomol*, **13**(2):399-408.

Jayanthi, P. D. K., Kempraj, V. and Ravindra, M. A. 2020. Lepidopteran webber, *Orthaga exvinacea* oviposits amidst conspecific colonies: a social facilitation gone unnoticed. *Curre. Sci*, **119**(5):823.

Kannan, M. and Rao, N. V. 2006. Influence of age and stage of the host plant on insect pests of mango (*Mangifera indica* L.). *Int. J. Agric. Sci,* **2**:351-353.

Kasar, N., Marak, J. C., Das, U. K. and Jha, S. 2017. Incidence and distribution pattern of leaf webber (*Orthaga exvinacea* Hamp.) on mango. *J. Entomol. Zool*, **5**(2): 1196-1199.

Kavitha, K., Vijaya Lakshmi, K. and Anitha, V. 2005. Mango leaf webber, *Orthaga euadrusalis* walker (Pyrallidaea: Lepidoptera) in Andhara Pradesh, *Insect Env*, **11**(1): 39-40.

Kelley, P. J. 2016. Investigation into the sex pheromone of the adult female odd beetle, *Thylodrias contractus* Motschulsky, 1839.

Kerketta, A., Sharma, S., Tomar, D. R., Katlam, D. B., Bisen, D. R. and Chaure, D. N. 2021. Studies on mango leaf webber, *Orthaga* spp. with special

reference to seasonal incidence. *The Pharma Innovation Journal*, **10**(6):521-529.

Mallikarjuna, C. J. 2019. Seasonal incidence, biology and management of mango leaf webber, *Orthaga exvinacea* Hampson (Pyralidae: Lepidoptera). *M. Sc. (Hort.) Thesis,* University of Horticultural Sciences, Bagalkot, India.

Ntiri, E. S., Calatayud, P. A., Musyoka, B., Van den Berg, J. and Le Ru, B. P. 2018. Influence of feeding-damaged plants on the oviposition responses within a community of female moths. *Phytoparasitica,* **46**:607-615.

Patel, D. B. Korat, D. M. and Borad, P. K. 2007. Bionomics and behaviour of Mango leaf webber, *Orthaga euadrusalis* Walker, *Karnataka J. Agric. Sci,* **20**(3): 644-647.

Rao, G. K., Sujatha, A., Suneetha, P. and Jayanthi, P. D. 2020. Behavioural insights of red banded mango caterpillar *Deanolis sublimbalis* snellen.

Reddy, D. S. 2013. Relative incidence of leaf webber, *Orthaga exvinacea* Hamp. on varieties and hybrids of mango (*Mangifera indica* L.). *Pest Manage. Horti. Ecosyst*, **19**(2): 234-236.

Sajitha, N. and Gokuldas, M. 2015. Biochemical analysis of silk proteins of the mango leaf webber, *Orthaga exvinacea* Hampson (Lepidoptera: Pyralidae). *J. Entomol. Zool,* **3**(5): 285-290.

Singh, G. 1988. Biology of two defoliator pests of mango under North Indian Conditions. *Acta Hortic,* **231:** 625-628.

Singh, R., Lakhanpal, S. C. and Karkara, B. K. 2006. Incidence of mango leaf webber, *Orthaga euadrusalis* (Hampson) in high density plantation of mango at Dhaulakhan in Himachal Pradesh. *Insect Environ*, **11:** 178-179.

Sokame, B. M., Ntiri, E. S., Ahuya, P., Torto, B., Le Ru, B. P., Kilalo, D. C. and Calatayud, P. A. 2019. Caterpillar-induced plant volatiles attract conspecific and heterospecific adults for oviposition within a community of lepidopteran stemborers on maize plant. *Chemoecology*, **29**(3): 89-101.

Srivastava, R. P. and Verghese, A. 1983. The mango leaf webber and its control. *Indian J. Horti*, **28**(2): 21-22.

Srivastava, R. P. 1997. Mango insect pest management. International Book Distributing Co, Lucknow, India, pp. 272.

Tandon, P. L. and Srivastsva, R. P. 1982 Notes on new pest of mango in India. *Sci. Cult,* **48**(2): 78-80.

Verghese, A. 1998. Management of mango leaf webber. A vital package for panicle emergence, *Insect Environ*, **4:** 17-19.

Wee, S. L. 2016. Effects of conspecific herbivory and mating status on host searching and oviposition behavior of *Plutella xylostella* (Lepidoptera: Plutellidae) in relation to its host, *Brassica oleracea* (Brassicales: Brassicaceae). *Fla. Entomol*, **99**(spl):159-165.

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Frugivorous birds and mammalian pests of cultivated fig, *Ficus carica* L. in Punjab, India

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ABSTRACT: The present paper discusses the frugivorous bird and mammalian pest species on cultivated fig, *Ficus carica* L. in Punjab, India. This study was conducted for 10 years from 2014 to 2024. A total of 11 frugivorous pests including 9 bird species namely; Indian Grey Hornbill *Ocyceros birostris* (Scopoli), Asian Koel *Eudynamys scolopacea* (Linnaeus), House Crow *Corvus splendens* (Vieillot), Jungle Babbler *Turdoides striata* (Dumont), Red-vented Bulbul *Pycnonotus cafer* (Linnaeus), Common Myna *Acridotheres tristis* (Linnaeus), Brown-headed Barbet *Megalaima zeylanica* (Gmelin), Coppersmith Barbet *Megalaima haemacephalus* (Statius Muller), Rose-ringed Parakeet *Psittacula krameri* (Scopoli) falling under 5 orders and 8 families and two mammals (Indian flying fox, *Pteropus giganteus* (Brunnich) and Northern palm squirrel, *Funambulus pennantii* (Wroughton) were recorded to act as pests of fruits of *F. carica* at the three locations of the Punjab state, India. Passeriformes was found to be the dominating amongst all with birds of four families damaging the fruits. Amongst all, Rose-ringed parakeet and Northern palm squirrel were observed causing significant damage to the fruits throughout the fruiting season. Rose-ringed parakeet was observed causing damage at all the three locations surveyed. All the recorded species are of least concern status as per IUCN. These pests caused 18.3-29.4 per cent damage on fig fruits at different locations in Punjab.

Keywords: *Ficus carica* L, avian, depredatory, frugivore, pests, punjab

INTRODUCTION

Ficus carica Linnaeus, commonly known as 'Fig', is one of the earliest cultivated fruit trees in the world. It is a deciduous, perennial tree belonging to the family Moraceae. The fig tree is one of the unique *Ficus* species widely spread in the tropical and subtropical countries which has edible fruits with high commercial value. The fig is juicy and sweet when ripe, gummy with latex before ripening. Because of the high content of beneficial compounds in fresh or dried fig fruits, their consumption should be encouraged as a potential healthy alternative for sweets (Robert and Maja, 2016). Fig consists of numerous varieties with significant genetic diversity and outstanding pharmacological activities that are of remarkable commercial importance. Black Fig 1 and Brown Turkey are the important varieties being grown in Indian Punjab (Anonymous, 2024).

Like other fruit crops, figs are also attacked by many pests. Worldwide, 100 species of insects and other arthropods have been reported to attack fig trees (Singh *et al*., 2022). Atwal and Dhaliwal (2009) reported 50 insect species feeding on fig trees in India. Previously, Singh and Kaur (2017a) recorded 14 insect and mite pests and 1 pollinator from Punjab to be associated with fig trees. Recently, Singh *et al*. (2022) have check listed 48 species of insect-pests and 4 of mite-pests infesting *F. carica* worldwide. Though, lots of data on invertebrate pests of *F. carica* are available but rarely any information are available on its vertebrate pests.

In Africa, Ostrich *(Struthio camelus)* has been recorded eating the introduced *Ficus carica* (Cramp, 1977). In Australia, Rooke (1983) found Silver eye (*Zosterops lateralis*) preferring figs as alternatives to grapes. In the Canary Islands, *Corvus corax* regurgitated pellets of up to 980 *F. carica* seeds (Nogales *et al*., 1999). Tracey *et al*. (2007) also found many species of birds like European blackbirds (*Turdus merula*), mynas (*Acridotheres tristis*), noisy friarbird (*Philemon corniculatus*), rainbow lorikeet (*Trichoglossus haematodus*), red wattlebird (*Anthochaera carunculata*) and scaly-breasted lorikeet (*Trichoglossus chlorolepidotus*) causing damage to figs in Australia. Singh *et al*. (2022) also observed Garden Warbler, (*Sylvia borin* (Boddaert)), ground and tree squirrels to be pests of fig.

Ficus is the most important plant genus for tropical frugivores (Corner, 1988; Berg, 1989) and is being described as 'keystone resources' in tropical forests for potentially sustaining frugivores through lean periods of low fruit availability (Korine *et al*., 2000). Shanahan *et al*. (2001) check listed a small number of reptiles and fish, 1274 bird and mammal species in 523 genera and 92 families known to eat fruits of various *Ficus* species. In terms of the number of species and genera of fig-eaters and the number of fig species eaten, they identified the avian families interacting most with *Ficus* to be Columbidae, Psittacidae, Pycnonotidae, Bucerotidae, Sturnidae and Lybiidae. Among mammals, the major fig-eating families they check listed were Pteropodidae, Cercopithecidae, Sciuridae, Phyllostomidae and Cebidae. O'Brien *et al*. (1998) credited the high calcium levels in figs as one of the main reasons of its dietary consumption by tropical frugivores.

Birds can incur harm to the yields as well as loss to the agronomists in every phase of yields directly from planting until harvesting (Dhindsa *et al.,* 1993; Dhindsa and Saini, 1994; Manakadan & Pittie, 2001; Malhi, 2008; Kale *et al.,* 2014; Grimmett *et al.,* 2014; Kler and Kumar, 2015a). Globally, avian pests cause severe damage to crops in many agricultural systems (Linz *et al*., 2011; 2015). Australia, for example, loses a$290 million annually to crop damage by 60+ bird species (Tracey *et al*., 2007). The United States experiences US$189 million in fruit crop loss (Anderson *et al*., 2013) and US$47 billion in commercial grain crop loss due to birds (Pimentel *et al*., 2005). Severe productivity loss to birds also occurs in regions of Asia (Gupta *et al*., 1998; Kale *et al*., 2014), Europe (Pinowski and Zając, 1990; Hake *et al*., 2010), and Africa (de Mey and Demont 2013). Birds like Jungle Myna (*Acridotheres fuscus* (Wagler)), House Crow (*Corvus splendens* Vieillot), Common Myna (*Acridotheres tristis* (Linnaeus)), White cheeked Bulbul (*Pycnonotus leucogenys* (Gray) and Brahminy Starling (*Sturnia pagodarum* (Gmelin)) harm the fruit plants particularly of grapes greatly in Himachal Pradesh, India (Patyal and Rana, 2006). As a result, many crop producers prioritize reducing bird populations in their agricultural fields to protect the crops (Avery and Werner, 2017).

Managing bird damage in crops is generally depends on a few cultural (trap cropping, altered sowing time), physical (visual scarring and audio devices), chemical methods (repellent, pesticides) and botanical repellents (Dhindsa and Saini, 1994; Anonymous, 2002).–The objective of the present study was to determine the diversity of frugivorous bird and mammal pests of cultivated Fig trees in Punjab.

MATERIALS AND METHODS

Study area

To study the abundance, diversity and the pest activities of frugivorous bird and mammal species in relation to fig trees, three districts of the Punjab state, namely Ludhiana, Bathinda and SBS Nagar were selected. In Ludhiana, the survey area was College Orchard of the Punjab Agricultural University (PAU), Ludhiana. The University Campus is situated on outskirts of city towards the west at latitude of 30˚ 56' N, and longitude of 75˚ 52' E and 247 m above sea level on the Ferozepur Road. Punjab Agricultural University site comprises of agronomic grounds, orchard plantations, official campus and housing areas. In Bathinda, the observations were taken from trees planted at Krishi Vigyan Kendra and Regional Research Station of the University (Latitude 30°18’ N, Longitude 74°94’E) at Dabwali Road. At these stations, Black Fig variety of fig has been planted. In district SBS Nagar, PAU Zonal Research Station for Kandi area at Ballowal Saunkhri was selected as study area. This station is located in the Shivalik foothills of Punjab, (latitude 31° 6’ 5”N and longitude 76° 27’ 26” E) at height of 355 m above sea level. At this research Farm, trees of Fig (Variety Brown Turkey; Age >15 years) which bears medium to large sized fruits, are grown. The research work was carried out at Ludhiana from 2014 to 2024 during May-June while at the other locations; the studies were conducted during 2021- 2024.

Identification of the birds and mammals was done with the help of key given by Ali (2002) and Grimmett *et al.* (2014). The nomenclature given by Manakadan and Pittie (2001) was followed. Point count method by Javed and Kaul (2002) was followed.

The fauna recorded during the surveys were presented with their taxonomic position, place of occurrence, IUCN status, food and habitat details. The locations were visited weekly from 8.00 am to 10.00 am in the morning and from 4.00 pm to 6.00 pm in evening during May-June to observe the fruit damage. For this, the method of fruit damage assessment suggested by Patyal and Rana (2006) was followed.

Instruments used

Digital Camera (Nikon P 500), Nikon Binocular (8X50) for observing birds.

RESULTS AND DISCUSSION

A total of 11 frugivorous pests including 9 bird species and 2 mammals were recorded as pests of fruits of cultivated fig, *F. carica* at the three locations of the Punjab state, India (Table 1). The bird species namely Indian Grey Hornbill (*Ocyceros birostris* (Scopoli)), Asian Koel (*Eudynamys scolopacea* (Linnaeus)), House Crow (*Corvus splendens* Vieillot), Jungle Babbler (*Turdoides striatus* (Dumont)) Red-vented Bulbul (Pycnonotus cafer (Linnaeus)), Common Myna (*Acridotheres tristis* (Linnaeus)), Brown-headed Barbet (*Megalaima zeylanica* (Gmelin)), Coppersmith Barbet (*Megalaima haemacephala* (Statius Muller)), Rose-ringed Parakeet **(***Psittacula krameri* (Scopoli)**)** were observed and fall under 5 orders and 8 families. Amongst mammalian fauna, there was Indian Flying Fox (*Pteropus giganteus*) and a rodent (Northern palm squirrel, *Funambulus pennantii*) recorded to be frugivorous on the fig trees (Plate 1). Amongst all, Rose-ringed parakeet and Northern Palm Squirrel were observed causing significant damage to the fruits throughout the season. The rest were found to damage the fruits occasionally. All the recorded species are of least concern as per IUCN (2020). Rose-ringed parakeet was observed as a frugivorous species on *F. carica* at all the three locations surveyed (Table 1). Jungle Babbler, Brown-headed Barbet and Red-vented Bulbul were only found causing damage at Ludhiana. Coppersmith Barbet, Northern Palm Squirrel and Indian Flying Fox were recorded only from district Bathinda. Asian Koel, Common myna and Indian grey hornbill were recorded from both Ludhiana and Bathinda locations of the survey. House crow as a fruit eating species of fig

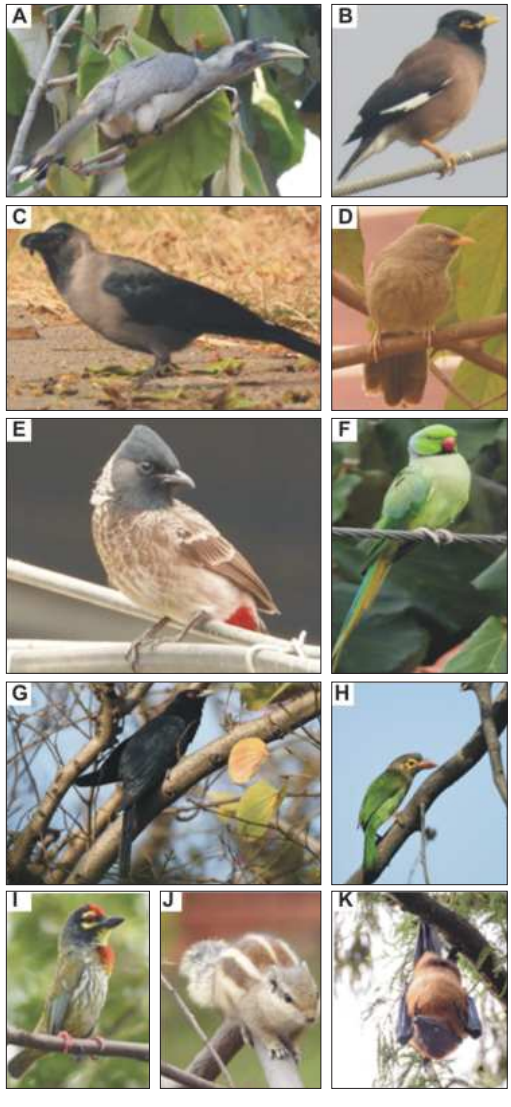

Plate.1. Frugivorous vertebrates of *F. carica*, a. Indian Grey Hornbill, b. Common Myna, c. House Crow, d. Jungle Babbler, e. Red-vented Bulbul, f. Rose-ringed Parakeet, g. Asian Koel, h. Brown-headed Barbet, i. Coppersmith barbet, j. Northern palm squirrel (five-striped palm squirrel) and k. Indian flying fox

was recorded from Ludhiana and SBS Nagar districts. Both the sexes of Asian Koel were found feeding on fig fruits. These pests were found causing 18.3-29.4 per cent damage on fig fruits at different locations in Punjab (Fig 1). Shanahan *et al*. (2001) also check listed the avian families like Psittacidae, Pycnonotidae, Sturnidae and Sciuridae causing significant damage to *Ficus* fruits as observed in the present studies. In the Canary Islands, *Corvus corax* was found feeding on *F. carica* seeds (Nogales *et al*., 1999). Tracey *et al*. (2007) also found mynas (*Acridotheres tristis*), causing damage to figs in Australia. Singh *et al*. (2022) also observed ground and tree squirrels to be pests of fig.

1. **Common Myna, *Acridotheres tristis* (Linnaeus, 1766) (Sturnidae)** It is also known as *Lalri/Gutar/ Shark* in local language. It is a familiar bird in areas adjoining human habitations. Common Myna population along with Rock Pigeon has increased in urban areas in recent years because of supplemental feeding sites (Ali and Ripley 1983; Dhindsa *et al.,* 1993; Dhindsa and Saini, 1994; Ali, 2002).

Size: Its body size is 23 cm.

Identifying features: The body is dark brown, with lustrous black head; yellow legs, bill and naked patch below and behind eye. A large white patch on the wings is visible in flight and under tail coverts are also white. Sexes are alike. Young ones are duller, less dark brown than adults, with the ashy brown head rather than black (Kler and Kumar, 2015b; Kaur and Kumar, 2018).

Resident status: It is resident and having wide distribution.

Habitat: Found almost everywhere except in very dense forests. Stays in family parties of 5 or 6 birds except in the breeding season joining into flocks sometimes of many hundred, roosting communally in large trees, reed-beds and fields. Railway stations warehouses and other large sheds are preferred roosts in urban areas (Kler and Kumar, 2015a; Kaur and Kumar, 2018).

Breeding: The breeding season ranges from April-August with two-three successive broods being raised. Nest is usually a messy collection of twigs, roots, and rubbish stuffed in holes in trees, earth banks, walls of houses or between the ceiling and roof. Same site often

Table 1. Depredatory birds and mammalian pests recorded on cultivated fig, *Ficus carica* L. in Punjab during 2014 to 2024

Scientific Name	Common Name	Recorded from	Status	Food	IUCN status	Habitat
Class Aves						
Order Coraciiformes						
Family Bucerotidae						
Ocyceros birostris (Scopoli, 1786)	Indian Grey Hornbill	B, L	R	F,I	LC	AB
Order Cuculiformes						
Family Cuculidae						
Eudynamys scolopacea (Linnaeus, 1758)	Asian Koel	B, L	R	F, I	LC	AB
Order Passeriformes						
Family Corvidae						
Corvus splendens Vieillot,1817	House Crow	S, L	R	O	LC	AB
Family Leiothrichidae						
Turdoides striatus (Dumont, 1823)	Jungle Babbler	L	R	F, I	LC	AB

Family Pycnonotidae						
Pycnonotus cafer (Linnaeus,1766)	Red-vented Bulbul	L	R	I,P,F	LC	AB
Family Sturnidae						
Acridotheres tristis (Linnaeus,1766)	Common Myna	B, L	R	I,F	LC	AB
Order Piciformes						
Family Megalaimidae						
Megalaima zeylanica (Gmelin, 1788)	Brown-headed Barbet	L	R	F,P	LC	AB
Megalaima haemacephala (Statius Müller, 1776)	Coppersmith Barbet	B	R	F, I	LC	AB
Order Psittaciformes						
Family Psittacidae						
Psittacula krameri (Scopoli,1769)	Rose-ringed Parakeet	B, S, L	R	F,P,G	LC	AB
Class Mammalia						
Order Chiroptera						
Pteropus giganteus (Brünnich, 1782)	Indian flying fox	B	R	F	LC	A
Order Rodentia						
Family Sciuridae						
Funambulus pennantii Wroughton, 1905	Northern palm squirrel or five-striped palm squirrel	B	R	F,G,P	LC	AB

Habitat: Type A-Agricultural Habitat; Type B-Residential area; **Status**: R–Resident; **Food Habit**: I-Insectivorous; G-Granivorous; F-Fruits/berries; P-Plants/aquatic vegetation/nectar; O-Omnivorous; **IUCN Status**: LC-Least Concern; **Recorded from:** B-Bathinda, S- SBS Nagar and L-Ludhiana.

used year after year. Lays 4 to 6 turquoise blue eggs (Ali and Ripley, 1983; Dhindsa and Saini, 1994; Grimmett *et al.,* 2014).

Feeding habits: It is an omnivorous bird and can feed on fruit, grain, insects and everything else that can be eaten like kitchen waste, small animals such as baby mice, frogs, lizards and crabs,

and flower-nectar. Often seen following grazing cattle or the plough for insects and invertebrates. Also observed in large number at waste/garbage disposal sites (Dhindsa and Saini, 1994; Manakadan and Pittie, 2001; Malhi, 2008; Kale *et al.,* 2014).

Damaging status: Causes some damage to orchard fruit and cereal crops, but is also beneficial as a destroyer of serious agricultural pests (Kale *et al.,* 2014).

2. **House Crow, *Corvus splendens* Vieillot, 1817 (Corvidae)** House Crow commonly known as *Kan* in vernacular language is an opportunist birds and can feed on variety of food available. Because of wide-spread distribution, it has been noted causing damage on maize and other cereals at sowing stages. Large numbers of House crows were observed at waste/garbage dumps and at animal flaying centres in villages and small towns (Ali, 2002; Dhindsa and Saini, 1994; Kaur and Kumar, 2018a).

Size: Its body size is approximately 43 cm.

Identifying features: It has glossy black body with greyish nape, neck (all round), upper breast and upper back. Bill, legs, forehead, crown and throat are contrasting glossy black. Sexes are alike.

Resident status: It is resident bird, very adaptable and it associates with human habitation and cultivation.

Habitat: Inseparable from human habitations; abundant to pest proportions in many urban and metropolitan areas. Its habitat has wide range; commonly found in rural/urban areas, cultivation and forest edges (Ali and Ripley, 1983; Dhindsa *et al.,* 1993; Dhindsa and Saini, 1994; Kler and Kumar, 2015a).

Breeding: Its breeding season ranges from April-August. Nest is usually an untidy platform of sticks and twigs intermixed with iron wire, threads, etc. placed in the fork of a branch, with a cuplike depression in the centre lined with coir, and other fibers. Lays 4-5, pale blue green eggs, speckled and streaked with brown, in one clutch (Kler and Kumar, 2015; Kaur and Kumar, 2018).

Feeding habits: It is bold, cunning and omnivorous scavenger. Gregarious behaviour noted during feeding and roosting times. Commonly seen at rubbish dumps. Food includes grain, fruits, flower-nectar, eggs and young or sickly birds, lizards, small rodents, land crabs, kitchen scraps, and garbage (Dhindsa and Saini, 1994; Kale *et al.,* 2014; Grimmett *et al.,* 2014; Kler and Kumar, 2015b).

Damaging status: Crow causes damages to crops by pulling out freshly sown seeds of cereals, pulses, oilseeds and feeding on matured maize cobs and horticultural crops (Dhindsa *et al.,* 1993; Malhi, 2008; Kale *et al.,* 2014; Kler and Kumar, 2015a).

3. **Indian Grey Hornbill, *Ocyceros birostris* (Scopoli, 1786) (Bucerotidae)** It is also known as *Dhan Chidi* in vernacular language. It is a common hornbill, mostly arboreal and seen in pairs (Ali and Ripley, 1983).

Size: Its body size is 61 cm.

Identifying features: It is a medium sized clumsy brownish grey bird having long tail with white tip and dark sub terminal band. Heavy curved bill is dual toned - black and yellow, with a peculiar pointed protuberance or casque. In females, casque is smaller in size. Juveniles are like adult but have no casque (Dhindsa *et al.,* 1993; Kler and Kumar, 2015a).

Resident status: It is resident and having wide distribution.

Habitat: Largely arboreal, but will occasionally descend to the ground. Usually found in open woodlands, plantations, gardens, and parks in cities.

Breeding: The breeding season ranges from March-June. It is a cavity nester and builds nest in a hole of tree, wall, ceiling etc. Nest is usually a natural hollow in an old tree-trunk, sometimes enlarged to suit. Eggs, normally 2 or 3, rarely 4, dull glossless white (Dhindsa *et al.,* 1993; Kler and Kumar, 2015a).

Feeding habits: The Indian grey Hornbill largely feed on fruits, especially wild figs (*Ficus* spp.), berries and flower petals; also insects and lizards, mice and other small animals.

Damaging status: It is both beneficial as well as harmful in nature as it feeds on insects as well as fruit and berries.

4. **Red-vented Bulbul, *Pycnonotus cafer* (Linnaeus, 1766) (Pycnonotidae)** It is also known as *Bulbul or Guldum* locally. It has been commonly observed throughout Punjab. It is included in the list of the world's 100 worst invasive alien species as it has established in many countries where it has been introduced (Dhindsa and Saini, 1994; Ali, 2002; Kaur and Kumar, 2018a).

Size: Its body size is approximately 20 cm.

Identifying features: It has earth-brown colour with a short crest which gives squarish appearance to the head. It has black throat and scale-like markings on back and breast. Rump is white and vent is red. Blackish tail has white tip, evident in flight. Sexes are alike.

Resident status: It is a resident species.

Habitat: It lives in pairs or in small loose flocks according to season usually keeping itself to lower or middle level of trees and bushes. Dry scrub, open forest, cultivated lands, gardens and roadside avenues are the preferred sites.

Breeding: It breeds in the months of February-October. Nest is cup shaped generally made of rootlets, placed in shrubs, hedges, tree, or sometimes inside buildings. It lays 2 - 4 eggs of pinkish white colour with purplish brown markings (Dhindsa *et al.,* 1993; Kler and Kumar 2015a).

Feeding habits: Fruits and berries, flower nectar, insects, grains and even kitchen waste constitutes its food. It is an efficient pollinating and seed-disseminating agent.

Damaging status: Solitary bird or pair has been noted to cause damage to ripening fruits especially in kitchen gardens. Damage level may be moderate to high on individual or solitary fruit plant (Dhindsa *et al.,* 1993; Dhindsa and Saini, 1994; Kale *et al.,* 2014).

5. **Rose-ringed Parakeet, *Psittacula krameri* (Scopoli, 1769) (Psittaculidae)** Rose-ringed Parakeet is also commonly known as *Tota.* It is one of the most destructive depredatory bird species in cultivated areas of Punjab. There has been observed a shift in its preferred trees for nesting from traditional to agro forestry trees in recent years. Large flocks are often observed at grain store facilities. Orchard owners use variety of methods, both traditional and mechanical like cracker fire gun/acetylene gas powered guns to scare them away from fruiting trees. Netting has been found to be the most successful and efficient method to reduce parakeet damage. The birds clamber about among the twigs and gnaw into the half-ripe fruits, one after another, wasting far more than they actually eat (Ali and Ripley, 1983; Dhindsa *et al.,* 1993; Ali, 2002; Kler and Kumar, 2015b; Kaur and Kumar, 2018).

Size: Its body size is approximately 42 cm.

Identifying features: A vibrant bright green parakeet with a short, deeply hooked red coloured bill. Males have a rose-pink and black collar which is absent in females but they have an indistinct emerald-green ring around the neck. Juveniles are like female. Male acquires pink-and-black collar in the third year (Dhindsa *et al.,* 1993; Kler and Kumar, 2015a).

Resident status: It is resident bird, very adaptable and it associates with human habitation and cultivation.

Habitat: Its habitat also included grain storage facilities, markets, open forests, gardens and vicinity of habitation.

Breeding: Its breeding season ranges from February-April. Nest is usually an unlined hollow in a tree-trunk, usually some small natural hole cut and enlarged to size. It prefers readymade nest-hole of barbet or woodpecker. Holes in rock scarps and walls of ruined buildings are commonly occupied, many pairs often nesting close to each other in a loose colony. Lays 3-5 pure white roundish oval eggs (Dhindsa *et al.,* 1993; Kler and Kumar 2015).

Feeding habits: The foraging behaviour of parakeet is gregarious in nature. Feeds and roosts in large flocks. Fruits, cereal, grain, and wild as well as cultivated seeds; flower-petals and nectar form its main diet (Dhindsa *et al.,* 1993; Kale *et al.,* 2014; Kler and Kumar 2015a).

Damaging status: It is observed to cause serious damages to cereals, pulses, oilseeds, fruits and vegetables in standing crops, orchards and gardens (Dhindsa *et al.,* 1993; Kale *et al.,* 2014; Kler and Kumar 2015).

6. **Asian Koel, *Eudynamys scolopaceus* (Linnaeus, 1758) (Cuculidae)** It is the most well known song bird of the region. Usually arboreal and confined to inner canopy of trees and seldom showing itself (Dhindsa and Saini, 1994; Ali, 2002).

Size: Its body size is approximately 43 cm.

Identifying features: Males are glossy black with yellowish green bill and crimson eyes noticed by distinctive shrieking calls: Females are dark brown above with tailfeathers and wing-quills barred with white. Chin, throat and fore neck has white spots, barred on rest of underparts. Juveniles more or less like adult, sex for sex, but female far darker and more sooty above with blackish head, throat and breast; thus closer in the character of its plumage, especially upperparts, to male rather than to adult female as usually seen in birds. This probably serves as survival tactic amidst the black nestlings of its normal fosterers, the House and Jungle crows. Bill is black and not green as in the adult (Ali, 2002).

Resident status: It is resident, nomadic and local bird.

Habitat: It inhabits lightly wooded areas like gardens, orchards, and groves of trees in and around human habitation.

Breeding: Brood-parasitic almost exclusively on House and Jungle crows cunningly laying in their nests. Its breeding season ranges from March to August. Eggs very similar in appearance to crows' but smaller and greenish in ground colour, profusely blotched and speckled with reddish brown. (Malhi, 2008; Grimmett *et al.,* 2014). In the present study, male and female koels were observed to sit together on fig trees while eating the fruits.

Feeding habits: Largely feeds on fruits, berries, nuts, hairy caterpillars, bugs and various insects, terrestrial snails, eggs of small birds and flower nectar (Malhi, 2008; Kale *et al.,* 2014; Grimmett *et al.,* 2014).

Damaging status: Adults being largely frugivorous, cause some damage to fruits in orchards and gardens (Dhindsa *et al.,* 1993; Kale *et al.,* 2014; Grimmett *et al.,* 2014).

7. **Brown-headed Barbet**, ***Megalaima zeylanica*** **(Gmelin, 1788) (Megalaimidae)** In vernacular language, it is called *Bada Basanta,* usually an arboreal bird (Ali, 2002).

Size: Its body size is approximately 27 cm.

Identifying features: it has a chubby, heavy-billed grass-green feathers with head, neck, upper back and breast brown, having conspicuous orange bare patch around eyes, sexes alike (Ali, 2002).

Resident status: It is resident bird.

Habitat: It is commonly found in places where fruiting trees are available especially various species of wild fig, whether in gardens, orchards or groves of trees (Malhi, 2008; Grimmett *et al.,* 2014; Kler and Kumar, 2015a).

Breeding: Breeding season ranges from January to June. Eggs are glossless white (Dhindsa and Saini, 1994; Ali, 2002).

Feeding habits: Feeds on fruits, berries and sometime winged termites (Malhi, 2008; Kale *et al.,* 2014).

Damaging status: Being largely frugivorous, cause some damage to fruits in orchards and gardens specifically wild and cultivated species of fig (Dhindsa and Saini 1994; Dhindsa *et al.,* 1993; Malhi, 2008; Kale *et al.,* 2014).

8. **Coppersmith Barbet,** ***Megalaima haemacephala*** **(Statius Müller, 1776) (Megalaimidae)**, known as *Basanta* in vernacular, it is a commonly heard but hard to see, entirely arboreal bird (Dhindsa *et al.,* 1993; Ali, 2002; Grimmett *et al.,* 2014; Kler and Kumar, 2015b).

Size: Its body size is approximately 17 cm.

Identifying features: A small green barbet with yellow throat, crimson breast and forehead, and green-streaked yellowish underparts. Tail is short, truncated and distinctly triangular in-flight silhouette. Sexes are almost alike, the female being little duller. Juveniles lack the red colour and are duller (Malhi, 2008; Kale *et al.,* 2014; Grimmett *et al.,* 2014; Kler and Kumar, 2015a).

Resident status: Resident; common and very widely distributed

Habitat: Inhabits lightly wooded countryside, roadside avenues and groves of tree near villages and cultivation, and in urban gardens and compounds

Breeding: Breeding season ranges from November to June. Nest is generally a shaft excavated in a dead or decaying softwood branch ending in a slightly widened chamber. Lays 2-4 white, longish ovals eggs on bare wood at bottom of shaft (Dhindsa *et al.,* 1993; Kler and Kumar, 2015a).

Feeding habits: Banyan, peepal and other wild figs are preferred food. Also eats drupes and berries. Occasionally eats moths and flying termites captured in air.

Damaging status: It has been observed to cause minor damages to fruits (Dhindsa *et al.,* 1993; Malhi, 2008; Kale *et al.,* 2014).

9. **Jungle Babbler,** ***Turdoides striatus*** **(Dumont, 1823) (Leiothrichidae) c**ommonly called *Jungli Serhri* in vernacular. Very gregarious bird and noisy seen all through the year in parties of six to twelve, and thus also called 'Seven Sisters' (Ali and Ripley, 1983; Kaur and Kumar, 2018).

Size: Its body size is approximately 25 cm.

Identifying features: Earthy brown colour, untidy appearance with creamy white eyes, yellowish bill and legs. Head and nape are a little greyer. Rump and tail-coverts buff; tail rufous brown, belly is creamy buff. Sexes are alike (Dhindsa and Saini, 1994; Ali 2002).

Resident status: It is resident and fairly common bird.

Habitat: Inhabits open forest, urban gardens and cultivated areas around human habitation.

Breeding: Breeds from March to September. Nest are generally a loosely put together cup of twigs, roots, grass, placed in bushes and trees in gardens, orchards, hedges etc. Lays 4-6 eggs deep turquoise blue in colour (Ali, 2002; Grimmett *et al.,* 2014).

Feeding habits: Mainly feeds on insects like grasshoppers, ants, beetles, cockroaches, caterpillars, etc., and spiders. Also eats grains, seeds, figs, Lantana and other berries (Ali 2002; Dhindsa *et al.,* 1993; Dhindsa and Saini, 1994; Malhi, 2008; Kale *et al.,* 2014; Grimmett *et al.,* 2014).

Damaging status: It is observed to cause minor damage to crops.

10. **Indian flying fox,** ***Pteropus giganteus*** **(Brunnich, 1782) (Pteropodidea) c**ommonly called *Chamgidar* in vernacular. It is native to Indian sub-continent and one of the large bat species (Ramakrishna *et al.,* 2017).

Size: Body length 16-22 cm, wingspan ranges from 1.2-1.5 meter.

Identifying features: It has lightly streaked black back with grey, a pale, yellow-brown mantle, a brown head, and dark, brownish underparts. It has large eyes, simple ear (Francis and Priscilla, 2008; Ramakrishna *et al.,* 2017; Prasad, 2020).

Resident status: It is resident bat and widely distributed throughout Indian subcontinent.

Habitat: It roosts in large colonies on open tall tree branches, especially near human residences, agricultural land in urban areas or in abandoned buildings or ruins and prefers to be in close proximity to bodies of water (Francis and Priscilla, 2008;. Ramakrishna *et al.,* 2017).

Breeding: Breeding season ranges from July to October and give birth to 1-2 pups and reproductive maturity occurs at 18-24 months.

Feeding habits: It is frugivorous in nature and feeds on fruits and berries (Francis and Priscilla, 2008; Ramakrishna *et al.,* 2017).

Damaging status: It can be a pest as it poaches ripe fruits in orchards (Ramakrishna *et al.,* 2017; Prasad, 2020) and was also observed to be causing damage at different locations in Punjab in the present studies.

11. **Northern Palm Squirrel, *Funambulus pennantii* Wroughton, 1905 (Sciuridae)** Commonly called *Gilehri or Galad* in vernacular, it is the most common mammal in urban areas with a shrill, bird like call often accompanied by tail jerks and cause damage to several fruit and cereal crops (Kenward, 2008; Chakravarthy, 2012).

Size: Head and body length 13-16 cm, Tail length 14-16 cm

Identifying features: It has five pale stripes on its greyish brown or olive brown body. The tail does not have a mid-ventral line and resembles a grey bottlebrush. The top coat color ranges from grayish brown to almost black, while the head is usually grayish to reddish brown.

Resident status: It is resident squirrel and widely distributed.

Habitat: A semi-arboreal squirrel found in grasslands, scrublands, plantations, urban gardens, rural and forested areas. Northern palm squirrels are gregarious and up to 10 may inhabit a tree.

Breeding: Breeding occurs several times a year, usually with different partners each time. Able to reproduce throughout most, if not all, of the year. Females have 2 to 3 litters yearly. Litter sizes range from 1 to 5 (Kenward, 2008; Chakravarthy, 2012). Commonly nest in the branches of trees, holes in the tree trunk or in man-made structures such eaves of houses, attic spaces, electricity boxes, etc. Squirrel nests are made of grasses, threads, wool, cotton, jute fibers and other fibrous materials

Feeding habits: Northern palm squirrels are herbivorous and omnivorous. They typically feed on a wide variety of foods including seeds, nuts, buds, young bark, leaves, insects, flowers, and grubs. They have also been known to eat baby birds. They feed both in trees and on the ground and store food for later use (Kenward, 2008; Chakravarthy, 2012).

Damaging status: It can be a pest commonly eating buds and seeds of food producing plants

Habitat: Type A-Agricultural Habitat; Type B-Residential area; **Status**: R–Resident; **Food Habit**: I-Insectivorous; G-Granivorous; F-Fruits/berries; P-Plants/aquatic vegetation/nectar; O-Omnivorous; **IUCN Status**: LC-Least Concern; **Recorded from:** B-Bathinda, S- SBS Nagar and L-Ludhiana.

FRUIT DAMAGE

Data on fruit damage by various depredatory and mammalian pests of *F. carica* at different locations in the present study during 2021-2024 are presented in Figure 1. During 2021, 20.0 to 28.3% damage in *F. carica* fruits was observed across the locations studied, being maximum and minimum at Ludhiana and Bathinda, respectively. The fruit damage varied from 18.3 to 26.5% during 2022. It remained maximum at Ludhiana and minimum at Bathinda locations. During 2023, fruit damage observed between 19 to 27.9 % which increased up to 29.4 % during 2024 at Ludhiana (Fig 1).

The extent of the damage caused to different crops varied between study plots and also within plants and trees. Slack and Reilly (1994) reported that the damage to the citrus trees was greatest in the top canopy, almost four times greater than damage to the middle and lower branches of the" trees. Similar pattern of damage was recorded in peach and almonds (Dhindsa and Saini 1994). Prasad and Verghese (1985) recorded a similar pattern of damage in guava, with most of the damage occurring in the upper central core of the tree. Subramanya (1994) stated that the parakeet damage was positively correlated with the height of the plant, and was greater on varieties

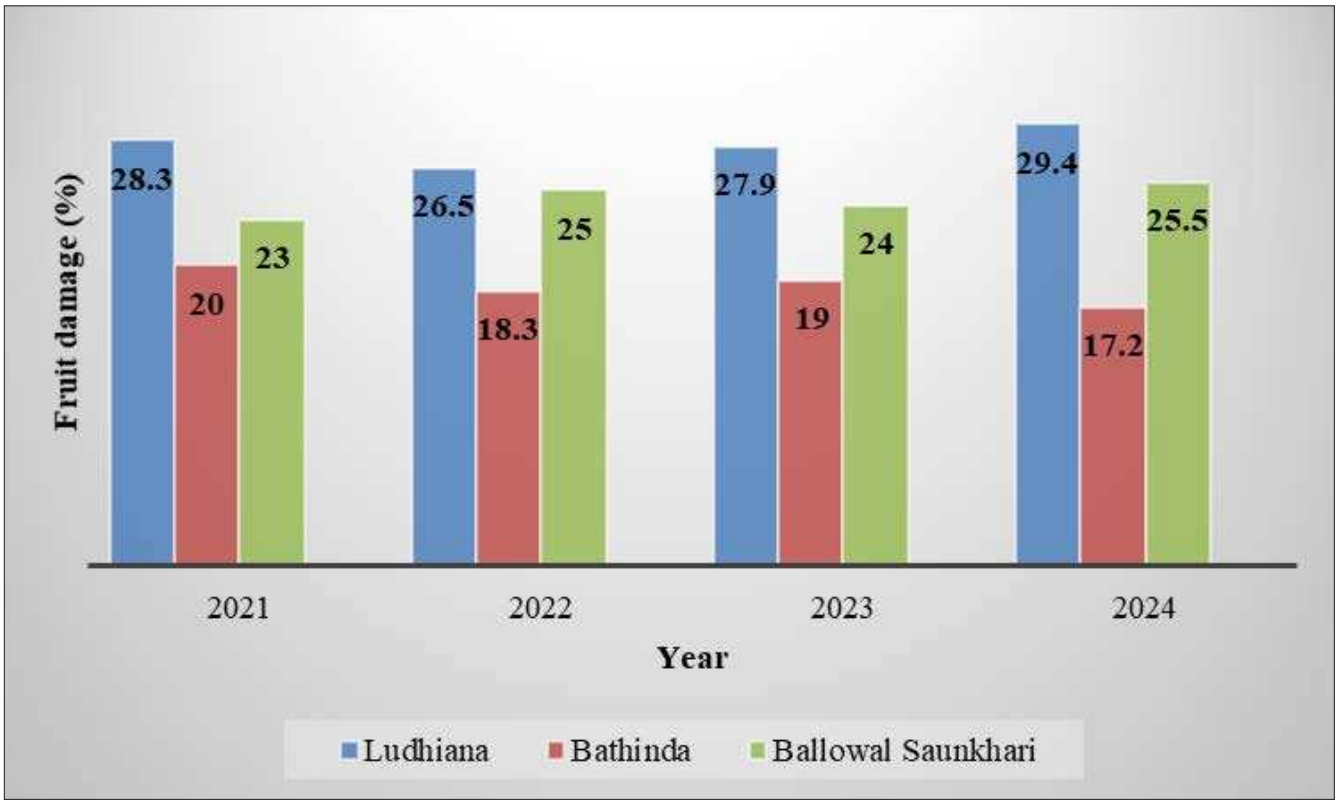

Fig.1. Fruit damage by various depredatory and mammalian pests of *F. carica* at different locations during 2021-2024

with the erect heads. This was thought to be due to the better visibility, and hence the predators can be avoided. The edges of the fields have more damage (Kler 2015), especially where the perching and refuging sites are available near to the crops, and away from the human disturbances (Saini *et al.,* 1992). Damage to oilseed crops *viz.* brassica, sunflower, and canola, were also reported apart from a variety of orchards trees (Khan and Aziz 1993; Ali and Ripley 1983; Dhindsa and Saini 1994; Kler and Kumar 2015). A loss of 25-100% to mangoes, guava, brassica, and sunflower by parakeet was reported by Prasad and Verghese (1985) in India. Saini *et al.* (1992) analyzed the gut contents of the Rose-ringed parakeets for one year which was consisted of cereals (45%), tree orchards (38%) and oilseeds (16%).

Depletion of indigenous tree cover and invasion of exotic tree species directly affects the distribution of avian and mammalian fauna. Old and indigenous trees account to be an important substrate for nesting in the form of dense canopies and cavities. Indigenous trees must be promoted over exotic ones because the services provided by them are already part of local ecology.

Among feeding habits, omnivorous species were in higher proportion as compared to other diets. Invasion of few migratory birds and mammals was also observed in indigenous trees during the fruiting periods. The study suggests that different communities make use of indigenous trees in different ways. So, plantation of these trees must be popularized over exotic trees.

Out of the eleven recorded species, Rose-ringed parakeet and northern palm squirrel were found to cause significant damage to the fruits of *F. carica* (Plate 2). The rest of the species were found to cause the damage occasionally. Though, the recorded species are protected under The Indian Wild Life (Protection) Act, 1972 suitable eco-friendly methods are needed to be worked out for their management. Using effective integrated pest management approaches, there may be opportunities to simultaneously reduce disservices, enhance services and conserve biodiversity. We can achieve the objective of sustaining current and future human well-being within ecological limits. Hence, there is a need for interdisciplinary research in the development of eco-friendly depredatory bird and animal management techniques as well as enhancing beneficial avian population in the agro-ecosystem. The positive relationships between people, birds, and sustainable agriculture may be a key starting point to develop a shared conservation vision for the future.

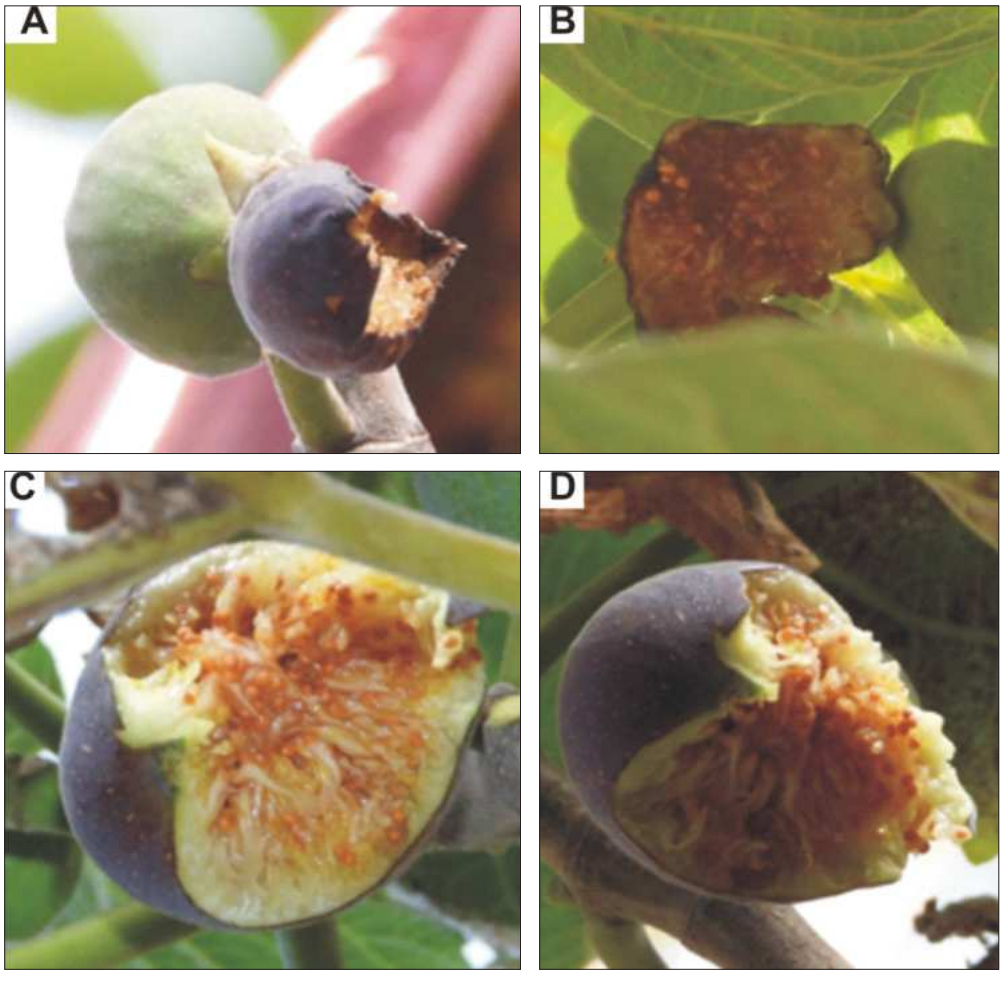

Plate.2. Fruits of *F. carica* damaged by bird and mammal pests

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REFERENCES

Ali, S., and Ripley, S. D. 1983. *Handbook of the birds of India and Pakistan*, Oxford University Press, New York. pp. 332.

Ali, S. 2002. *The book of Indian birds*, Bombay Natural History Society. Oxford University Press, Bombay. pp. 402.

Anderson, A., Lindell, C. A., Moxcey, K. M., Siemer, W. F., Linz, G. M., Curtis, P. D., Carroll, J. E., Burrows, C. L., Boulanger, J. R., Steensma, K. M. M. and Shwiff, S. A. 2013. Bird damage to select fruit crops: The cost of damage and the benefits of control in five states. *Crop Protection*, **52:** 103-109.

Anonymous. 2002. *Research accomplishment of agricultural ornithology (ICAR)*. Technical Bulletin-II, Project Coordinator, AINP on Agricultural Ornithology, ANGR Agricultural University, Hyderabad. pp. 37.

Anonymous. 2024. *Package of Practices for Cultivation of Fruits*, Punjab Agricultural University, Ludhiana. pp. 200.

Atwal, A. S. and Dhaliwal, G. S. 2009. *Agricultural Pests of South Asia and their Management*, Kalyani publishers, Ludhiana. pp. 616.

Avery, M. L. and Werner, S. J. 2017. Frightening devices. In Linz, G.M., Dolbeer, R.A., Avery, M.L. (Eds.), *Ecology and management of blackbirds (Icteridae) in North America* (pp. 159-174). CRC Press, Taylor & Francis, Boca Raton, FL.

Berg, C. C. 1989. Classification and distribution of Ficus. *Experientia*, **45:** 605-611.

Chakravarthy, A. K. and Thyagaraj, N. E. 2012. The palm squirrel in coconut plantations: ecosystem services by therophily. *Mammalia* **76**(2): 193-199. DOI:10.1515/mammalian-2011-0073.

Corner, E. J. H. 1988. Wayside Trees of Malaya, Vol. 2. 3rd edition. United Selangor Press, Kuala Lumpur, Malaysia. pp. 698-699.

Cramp, S. 1977. *Handbook of the Birds of Europe, the Middle East and North Africa, (The Birds of the Western Palearctic)*. Vol. I. Ostrich to Ducks. Oxford University Press, Oxford, UK.

De Mey, Y. and Demont, M. 2013. Bird damage to rice in Africa: evidence and control. In M. C. S. Wopereis, D. E. Johnson, N. Ahmadi, E. Tollens, & A. Jalloh (Eds.), *Realizing Africa's rice promise* (pp. 241-249). CABI. https://doi.org/10.1079/9781845938123.0241

Dhindsa, M. S. and Saini, H. K. 1994. Agricultural ornithology: an Indian perspective. *Journal of bioscience*, **19**(4): 391-402.

Enos, J. K., Ward, M. P. and Hauber, M. E. 2021. A review of the scientific evidence on the impact of biologically salient frightening devices to protect crops from avian pests. *Crop Protection*, **148**: 105734.

Faanes, C. A. 1987. Breeding birds and vegetation structure in western North Dakota wooded draws. *Prairie Naturalist*, **19**(4): 209-220.

Grimmett, R., Inskipp, T. and Inskipp, C. 2014. *Birds of the Indian Subcontinent*, Christopher Helm, London. pp 1-556.

Gupta, M. K., Rajan, B. and Baruha, R. 1998. Parakeet damage to sugarcane. *Indian Journal of Sugarcane Technology*, **46:** 953–967.

Hake, M., Månsson, J. and Wiberg, A. 2010. A working model for preventing crop damage caused by increasing goose populations in Sweden. *Ornis Svecica*, **20**(3): 225-33.

IUCN. 2020. https://www.iucn.org/resources/conservation-tools/iucn-red-list-threatened-species. website visited on 08 Jan 2020.

Javed, S. and Kaul, R. 2002. *Field Methods for Bird Surveys*. Bombay Natural History Society; Department of Wildlife Sciences, Aligarh Muslim University, Aligarh and World Pheasant Association, South Asia Regional Office (SARO), New Delhi, India.

Kale, M. A., Dudhe, N., Kasambe, R. and Bhattacharya, P. 2014. Crop depredation by birds in Deccan Plateau, India. *International Journal of Biodiversity*, (947683), 8. https://doi.org/10.1155/2014/947683.

Kaur, N. and Kumar, M. 2018. Avian diversity in relation to indigenous trees. *Journal of Entomology Zoology Studies*, **6**(3): 1739-1745.

Kenward, R. E. 2008. The causes of damage by red and grey squirrels. *Mammal Review*, **13**(2-4): 159-166.

Kahn, N. A., Samiuilah and Aziz, O. 1993. Response of mustard to seed treatment with pyridioxine and basal and foliar application of nitrogen and phosphorus. *Journal of plant nutrition*, **16**(9): 1651-1659.

Kler, T. K. and Manoj, K. 2015a. Avian fauna in agricultural habitats of Punjab State. *Agricultural Research Journal*, **52**(3): 83-90.

Kler, T. K. and Kumar, M. 2015b. Prevalence of bird species in relation to food habits and habitat. *Journal of Agricultural Research*, **52**(1): 50-53.

Korine, C., Kalko, E. K. and Herre, E. A. 2000. Fruit characteristics and factors affecting fruit removal in a Panamanian community of strangler figs. *Oecologia*, **123**: 560-568.

Linz, G. M., Homan, H. J., Werner, S. J., Hagy, H. M. and Bleier, W. J. 2011. Assessment of bird-management strategies to protect sunflowers. *BioScience*, **61**(12): 960-970.

Linz, G. M., Bucher, E. H., Canavelli, S. B., Rodriguez, E. and Avery, M. L. 2015. Limitations of population suppression for protecting crops from bird depredation: A review. *Crop Protection*, **76**: 46-52.

Malhi, C. S. 2008. Status of avifauna in agro-ecosystem of Punjab- some additions to the checklist of Birds. *Environment and Ecology Research,* 26 (2A), 899-906.

Manakadan, R. and Pittie, A. 2001. Standardised common and scientific names of the birds of the Indian subcontinent. *Buceros*, **6**(1): 1-37.

Mathew, D. N., Narendran, T. C. and Zacharias, V. J. 1983. A comparative account of the food habits of some species of birds affecting agriculture. *Journal of the Bombay Natural History Society*, 75(Suppl.): 1178-1197.

Nogales, M., HernÁndez, E. C. and Valdés, F. 1999. Seed dispersal by common ravens Corvus corax among island habitats (Canarian Archipelago). *Ecoscience*, **6**(1): 56-61.

O'Brien, T. G., Kinnaird, M. F., Dierenfeld, E. S., Conklin-Brittain, N. L., Wrangham, R. W. and Silver, S. C. 1998. What's so special about figs?. *Nature*, **392**(6677), 668-668.

Patyal, S. K. and Rana, R. S. 2006. "Bird damage to Kinnow fruits in Himachal Pradesh and evaluation of management techniques against them," *Pest Management and Economic Zoology,* **14**: 157-161.

Pimentel, D., Zuniga, R. and Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological economics*, **52**(3): 273-288.

Pinowski, J. and Zając, R. 1990. Damages to crops caused by birds in Central Europe. *In Proceedings of the General Meetings of the Working Group on Granivorous Birds* (pp. 333-347).

Prasad, S. 2020. Report of Indian Flying Fox, *Pteropus giganteus* (Brunnich) (Chiroptera, Pteropodidae) in Dumraon, Bihar. *International Archive of Applied Sciences and Technology*, **11**(1): 42-45. DOI:.10.15515/iaast.0976-4828.11.1.4245.

Prasad, V. G. and Verghese, A. 1985. Birds as pests of horticultural crops. *Bulletin of Entomological Research*, **26**(1): 94-96.

Rajpar, M. N. and Zakaria, M. 2011. Bird species abundance and their correlationship with microclimate and habitat variables at natural wetland reserve, Peninsular Malaysia. *International Journal of Zoology*, Article ID 758573, 17 pages. DOI:10.1155/2011/ 758573.

Ramakrishna, S., Sharief, R., Ateeth, B., Khan, A. and Jayashankar, M. 2017. Indian flying fox, *Pteropus giganteus* (Brunnich, 1782) in the backyard of Bengaluru. *The Multidisciplinary Journal Carmelight*, **10**(2): 81-88.

Veberic, R. and Mikulic-Petkovsek, M. (2016). Phytochemical composition of common fig (*Ficus carica* L.) cultivars. In *Nutritional composition of fruit cultivars* (pp. 235-255). Academic Press.

Rooke, I. J. 1983. *Research into the Biology of the Silvereye Leading to Methods for Minimizing Grape Damage in Vineyards of South-west Australia*. Technical Series No. 2. Agricultural Protection Board of Western Australia, Perth.

Saini, H. K., Dhindsa, M. S. and Toor, H. S. 1992. Food of the rose ringed parakeet *Psittacula krameri:* a quantitative study. *Journal of the Bombay Natural History Society*, **91:** 96-102.

Shanahan, M., So, S., Compton, S. G. and Corlett, R. (2001). Fig-eating by vertebrate frugivores: a global review. *Biological reviews*, **76**(4): 529-572.

Singh, S. and Kaur, G. 2017. Biodiversity of insect and mite pests infesting fig in the Indian Punjab. *Acta Hortic.* 1173: 257-262. DOI: 10.17660/ ActaHortic.2017.1173.44.

Singh, S. and Kaur, G. 2017. Evaluation of fig germplasm against insect and mite pests in North-West India. *Acta Hortic.* 1173: 245-250. DOI: 10.17660/ ActaHortic.2017.1173.42.

Singh, S., Li, Z., Zhang, Y., Grieshop, M. J., Giliomee, J., Cocuzza, G. M. and Sandhu, R. K. 2022. Arthropod pests of fig and their management. In *The Fig: Botany, Production and Uses* (pp. 332-366). GB: CABI.

Slack, J. and Reilly, T. 1994. The economics of orchard netting. In *Proceedings of the Bird and Bat Control for Horticulture and Aquaculture Seminar* (Vol. 18, pp. 42-54).

Subramanya, S. 1994. Non-random foraging in certain bird pests of field crops. *Journal of biosciences*, **19**(4): 369-380.

Tracey, J., Bomford, M., Hart, Q., Saunders, G. and Sinclair, R. 2007. Managing Bird Damage to Fruit and Other Horticultural Crops. Bureau of Rural Sciences, Canberra, Australia.

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Biology and morphometrics of *Thrips parvispinus* (Karny) (Thysanoptera: Thripidae) on chilli, *Capsicum annuum* L.

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ABSTRACT: The Southeast Asian thrips, *Thrips parvispinus* (Thysanoptera: Thripidae), has recently invaded India, posing a significant threat to the country's chilli industry. Laboratory studies were conducted at the Department of Agricultural Entomology, College of Agriculture, Raichur, to understand the growth and development of this invasive pest on chilli. The study was conducted at controlled conditions of 25 ± 1 °C and a relative humidity of 65 ± 5%. The results revealed that the mean egg incubation period was 5.74 ± 0.34 days, with egg dimensions measuring 0.23 ± 0.01 mm in length and 0.13 ± 0.01 mm in breadth. The first and second larval instars lasted 2.16 ± 0.24 days and 3.76 ± 0.42 days, respectively, with a total larval duration of 5.92 ± 0.66 days. The pre-pupal stage lasted 1.83 ± 0.23 days, while the pupal stage lasted 4.17 ± 0.50 days. Adult males had an average lifespan of 5.55 ± 0.46 days, whereas adult females lived for 10.18 ± 0.47 days. The pre-oviposition, oviposition, and post-oviposition periods were 2.51 ± 0.24 days, 3.58 ± 0.25 days, and 4.09 ± 0.37 days, respectively. The mean fecundity was 15.53 ± 3.46 eggs per female. This study provides the first documented report on the biological parameters of *T. parvispinus* in the invaded regions.

Keywords: Biology, *Capsicum annuum,* Chilli thrips, Invasive pest, Fecundity, Morphometrics.

INTRODUCTION

Thrips (Thysanoptera: Thripidae) are among the most significant pests affecting a wide variety of crops. Within the insect order Thysanoptera, *Thrips* is one of the largest genera, containing several agricultural important species. In India alone, 44 species have been documented with a new geographical distribution records (Rachana and Varatharajan, 2017). Among the members of Thripidae, the *Thrips parvispinus* also known as South East Asian thrips, tobacco thrips, western thrips, and Taiwanese thrips was recently reported from India, where it devastated over 0.4 million hectares of chilli crop (Timmanna *et al*. 2022). This species is predicted to continue spreading and establishing itself in India, due to the large-scale cultivation of its major hosts, *viz*., papaya and chilli, coupled with the favourable meteorological conditions. These factors contribute to significant population increases and yield losses of 10 to 30 percent compared to other countries. The districts of Warangal, Khammam, and Guntur in Andhra Pradesh have experienced the highest levels of infestation, with thrips numbers ranging from 10 to 20 per flower (Janyala, 2021; Directorate of Plant Protection Quarantine and Storage of India, 2021).

In India, the first recorded occurrence of *T. parvispinus* on papaya (*Carica papaya*) was documented in Karnataka in 2015 (Tyagi *et al*., 2015). Originally native to Southeast Asian countries, *T. parvispinus* has been reported in Australia, Thailand, North America, Europe, Malaysia, and Africa, establishing itself as a global and polyphagous pest (Waterhouse, 1993; Zhang *et al*., 2011; Lim, 1989). Following its invasion, *T. parvispinus* has been found on a variety of host plants, including paprika, watermelon, mums, dahlia, cotton, mango, bitter gourd, marigold, and tamarind (Nagaraju *et al*., 2021; Rachana *et al*., 2022; Roselin *et al*., 2021). Reports of severe infestations on chilli plants (*Capsicum annuum*) have emerged from Gujarat (Lodaya *et al*., 2022), Telangana, Andhra Pradesh (Timmanna *et al*., 2022; Veeranna *et al*., 2022), and Karnataka (Basavaraj *et al*., 2022). Despite its widespread documentation, a detailed understanding of the biology of *T. parvispinus* in the newly invaded regions remains lacking. Therefore, it is hypothesized that studying the biological parameters of *T. parvispinus* will provide essential information for effective management.

MATERIALS AND METHODS

The biology of *T. parvispinus* was studied in the insectary of the Department of Agricultural Entomology, College of Agriculture, Raichur, University of Agricultural Sciences, Raichur (16°15' N latitude and 77°20 E' longitudes at 398.37 m above mean sea level)

during 2022-23. The controlled room temperature of 25±1 °C and relative humidity (65±5 %) were maintained during the study period.

Maintenance of pure culture of thrips

The cultivation of chilli plants involved the use of earthen pots, which were protected from pests by being covered with nylon net cages (0.15 × 0.15 mm) (Fig 1). A single pair of male and female adults of *Thrips parvispinus* were obtained from the thrips infected chilli field and introduced to these enclosed plants (Fig 1). The adults for biological studies were collected from stock culture and reared in a cylindrical tubes made out of overhead projector (OHP) sheets. The cylindrical OHP sheets of 21 cm length and 6 cm diameter were open at both ends; one end was pressed gently into the soil, and the other end was covered with a plastic plate with mesh top (Fig 2). Fresh chilli seedling was put inside the cylindrical tube. A pair of adult thrips were released for oviposition in such OHP cylindrical tubes.

Fig.1. Maintenance of *T. parvispinus* pure culture for biology study

Pre-ovipositional, ovipositional and post ovipositional period

The cylindrical OHP sheet tubes were opened daily and the leaves were taken after following the procedure of chlorophyll bleaching and staining method and observed for oviposition under stereo zoom binocular microscope (Nikon SMZ745) and continued till the first egg laid and duration was recorded as pre-oviposition period. The procedure, which was adopted for the pre-ovipositional period, was continued further, provided with fresh seedlings, and observed the oviposition until the last egg deposition (Fig 2). The duration between the first and the last egg laid was recorded as an ovipositional period.

Fig. 2. Experimental set up for study of preoviposition, oviposition, post oviposition and fecundity studies of *T. parvispinus*

Fecundity count using chlorophyll bleaching and staining method

The mated females (n= 15) were collected from pure culture and allowed to lay eggs on chilli leaf. The oviposited leaves were collected daily, and each female was given fresh leaves until they died. The leaves were immersed into a beaker containing 100 ml of Dimethyl sulfoxide (DMSO). This beaker with leaflets and DMSO were kept in the water bath at 60- 70□C for 30 minutes. After removing chlorophyll, the leaves were kept on a petri plate (Fig 3). Later, the bleached leaflets were stained with acetocarmine 1% solution for 1 to 2 hours (Fig 3). The stained eggs were observed, counted and measured under the stereo zoom trinocular microscope (Nikon SMZ745) and its measuring software. The total number of eggs deposited by each female was documented.

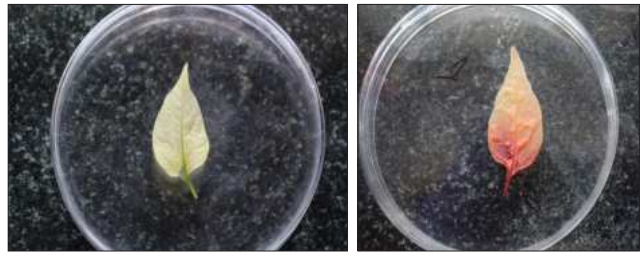

Fig.3. Chlorophyll bleaching and staining of chilli leaves

The incubation period

To determine the incubation period, the oviposited leaves, which were used in earlier fecundity studies, were transferred from the rearing tubes to a sterile, moist cotton wad in a petri plate at a temperature of 25±1 °C and a relative humidity of 65±5 % per cent in the laboratory. The hatching of newly laid eggs on chilli leaves placed inside the rearing tubes was monitored,

and fifteen observations were made, including the time between egg laying and the emergence of the first instar larva.

Larval instars

To study the larval instars, a fine-toothed, moistened camel hairbrush was used to transport newly hatched larvae onto a leaf arena on a wet cotton pad in petri dishes. Every two or three days, fresh leaves were brought into the arena; *i.e.* three leaves were used in this experiment, and vaseline was used all over the leaf corners. This way, the larval instars did not escape from the leaf arena (Fig 4). Examining the shed skin with a binocular microscope (Nikon SMZ745) and recording the number of larval instars and the days needed to complete each larval instar.

Fig.4. Experimental set up for larval rearing of *T. parvispinus*

Prepupa and Pupa

The dull second instars larvae of *T. parvispinus* were shifted with a camel hairbrush to another Petri plate containing one mm thick of fine black soil for easy pupation (Fig 5). The visual observations *viz*., length of the wing pad up to the end or half to the abdomen and antennae position, were considered to differentiate the pre-pupa and pupa.

Fig.5. Experimental set up for pre-pupa and pupal stages of *T. parvispinus*

Adult longevity

The duration of adult longevity in thrips is observed from the time they emerge until they die in an OHP sheet cylindrical tube.

Morpho-metric measurements and statistical analysis

All morphological stages, including the egg, larva, pre-pupa, pupa, and adult, were observed, and the length (mm) and breadth (mm) measurements were taken using a trinocular stereo zoom microscope (Nikon SMZ25) and the imaging programme NIS-Elements D version 5.02.03 (64 bit). The mean durations of different biological parameters were worked out using Excel 2013. Similarly, the mean and standard deviation of morphometric values of various stages were worked out.

RESULTS AND DISCUSSION

Egg

A female thrips lays eggs irregularly on the leaf. The eggs are bean-shaped and shiny white, with a pair of tiny brownish-red eyes visible through the chorion at the anterior end just before hatching (Fig. 6). The incubation period ranges from 5.00 to 6.00 days, averaging 5.74 ± 0.34 days (Fig. 7 and Table 1). On average, the eggs measure 0.23 ± 0.01 mm in length and 0.13 ± 0.01 mm in breadth (Table 2).

Larval instars

Upon hatching, the larva emerged headfirst, wriggling in all directions and leaving the eggshell inside the leaf tissue. Chilli thrips have two larval instars, first and second, with distinct characteristics. The newly hatched first instar larva was translucent white, gradually turning light yellow with seven-segmented antennae and red eyes (Fig. 6). This stage lasted between 2.00 and 3.00 days, averaging 2.16 ± 0.24 days (Fig. 7 and Table 1). The first instar larva had an average length of 0.35 ± 0.02 mm and a width of 0.11 ± 0.01 mm (Table 2). The second instar larva was dark yellowish with red eyes, distinct antennal segments with pale brownish setae, and a stout, elongated body (Fig. 6). This stage lasted an average of 3.76 ± 0.42 days, ranging from 3.00 to 5.00 days (Fig. 7 and Table 1). The second instar larva had an average length of 1.06 ± 0.02 mm and a width of 0.22 ± 0.01 mm (Table 2).

Pre-pupa and Pupa

The pre-pupa was dark yellow with red eyes and two pairs of small, transparent white wing pads that extended

almost one-third of the way down the abdominal segment, with antennae pointing forward (Fig. 6). The pre-pupa was markedly different from the second instar, being dull and immobile. In contrast, the second instar was very active and fast-moving. The pre-pupal phase lasted between 1 and 2 days, with an average duration of 1.83 ± 0.23 days (Fig. 7 and Table 1). The average length and width of the pre-pupa were 0.93 ± 0.01 mm and 0.19 ± 0.01 mm, respectively (Table 2). The pupa was dark yellow with dark red eyes, with antennae positioned backward over the head towards the pro-thorax and longer wings extending to the sixth abdominal segment (Fig. 6). The pupal stage lasted 3 to 5 days, averaging 4.17 ± 0.50 days (Fig. 7 and Table 1). The average length and width of the pupa were 0.86 ± 0.01 mm and 0.20 ± 0.01 mm, respectively (Table 2).

Adults

Female: The females of *T. parvispinus* are brownish-black with yellow legs and fringed wings that are brown at the base and black at the ends. The thorax is brown, and the abdomen is black. They have seven-segmented antennae. Females are larger than males and can be identified by their dark brown colour and the well-developed saw-like ovipositor (Fig. 6). The female lifespan ranges from 8 to 11 days, with an average duration of 10.18 ± 0.47 days (Fig. 7 and Table 1). The total life cycle of females was in the range of 25.00 – 29.00 days with an average of 27.84 ± 0.89 days. The average length and width of females are 1.29 ± 0.09 mm and 0.32 ± 0.01 mm, respectively (Table 2).

Male: The males were smaller than the females, with their whole body being yellow (Fig. 6). Male longevity ranged from 4.00 to 6.00 days, with an average duration of 5.55 ± 0.46 days (Fig. 7 and Table 1). The total life cycle of males was in the range of 22.00- 24.00 days with an average of 23.21 ± 0.56 days. The average length and width were 0.88 ± 0.03 mm and 0.17 ± 0.01 mm, respectively (Table 2).

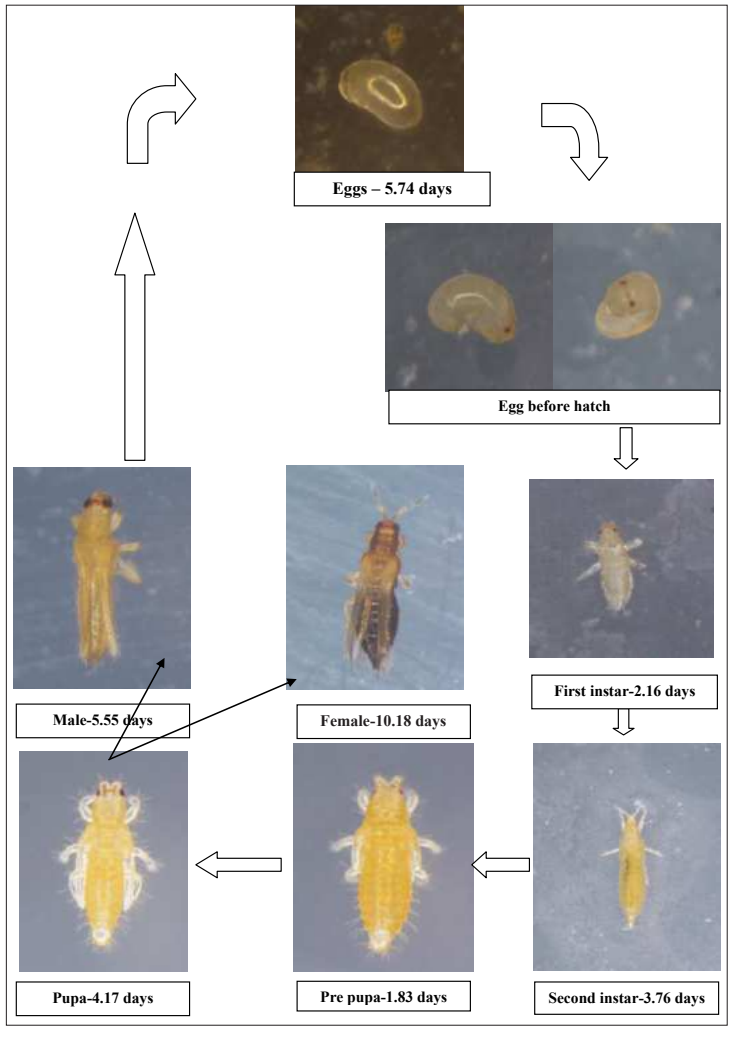

Fig.6. Life cycle of *T. parvispinus* on chilli

Table 1. Biology of *T. parvispinus* on chilli under laboratory conditions

Stage of development	Range (days)	Mean ± SD
Incubation period	5 - 6	5.74 ± 0.34
I instar	2 - 3	2.16 ± 0.24
II instar	3- 5	3.76 ± 0.42
Pre –pupa	1-2	1.83 ± 0.23
Pupa	3-5	4.17 ± 0.50
Pre-ovipositional period	2-3	2.51 ± 0.24
Ovipositional period	3-4	3.58 ± 0.25
Post ovipositional period	3-5	4.09 ± 0.37
Adult longevity		
Female	8-11	10.18 ± 0.47
Male	4 - 6	5.55 ± 0.46
Adult life cycle		
Total female life cycle	25 - 29	27.84 ± 0.89
Total male life cycle	22 - 24	23.21 ± 0.56
Total Average life cycle	24 - 27	25.53 ± 0.70
Number per female		
Fecundity	12 - 22	15.53 ± 3.46

Note: n=15, SD-Standard deviation

Reproductive parameters

The pre-ovipositional period ranged from 2.00 to 3.00 days, averaging 2.51 ± 0.24 days (Fig. 7 and Table 1). The ovipositional period ranged from 3.00- 4.00 days with an average of 3.58 ± 0.25 days (Fig. 7 and Table 1). The post-ovipositional phase lasted 3–5 days, averaging 4.09 ± 0.37 days (Fig. 7 and Table 1). The fecundity ranged from 12.00 to 22.00 eggs, averaging 15.53 ± 3.46 eggs per female (Table 1).

Table 2. Morphometric studies of different life stages of thrips, *T. parvispinus*

Life stage	Length (mm)		Breadth (mm)	
	Range	Mean ± SD	Range	Mean ± SD
Egg	0.21- 0.23	0.23 ± 0.01	0.12 - 0.15	0.13 ± 0.01
I Instar	0.29 – 0.37	0.35 ± 0.02	0.10 – 0.13	0.11 ± 0.01
II Instar	1.02 – 1.08	1.06 ± 0.02	0.21 – 0.24	0.22 ± 0.01
Pre-pupa	0.91 - 0.96	0.93 ± 0.01	0.18 - 0.19	0.19 ± 0.01
Pupa	0.82 - 0.87	0.86 ± 0.01	0.18 – 0.21	0.20 ± 0.01
Adult (male) Adult	0.82 - 0.93	0.88 ± 0.03	0.14 – 0.18	0.17 ± 0.01
(female)	0.95 - 1.34	1.29 ± 0.09	0.31 – 0.33	0.32 ± 0.01

Note: n=15

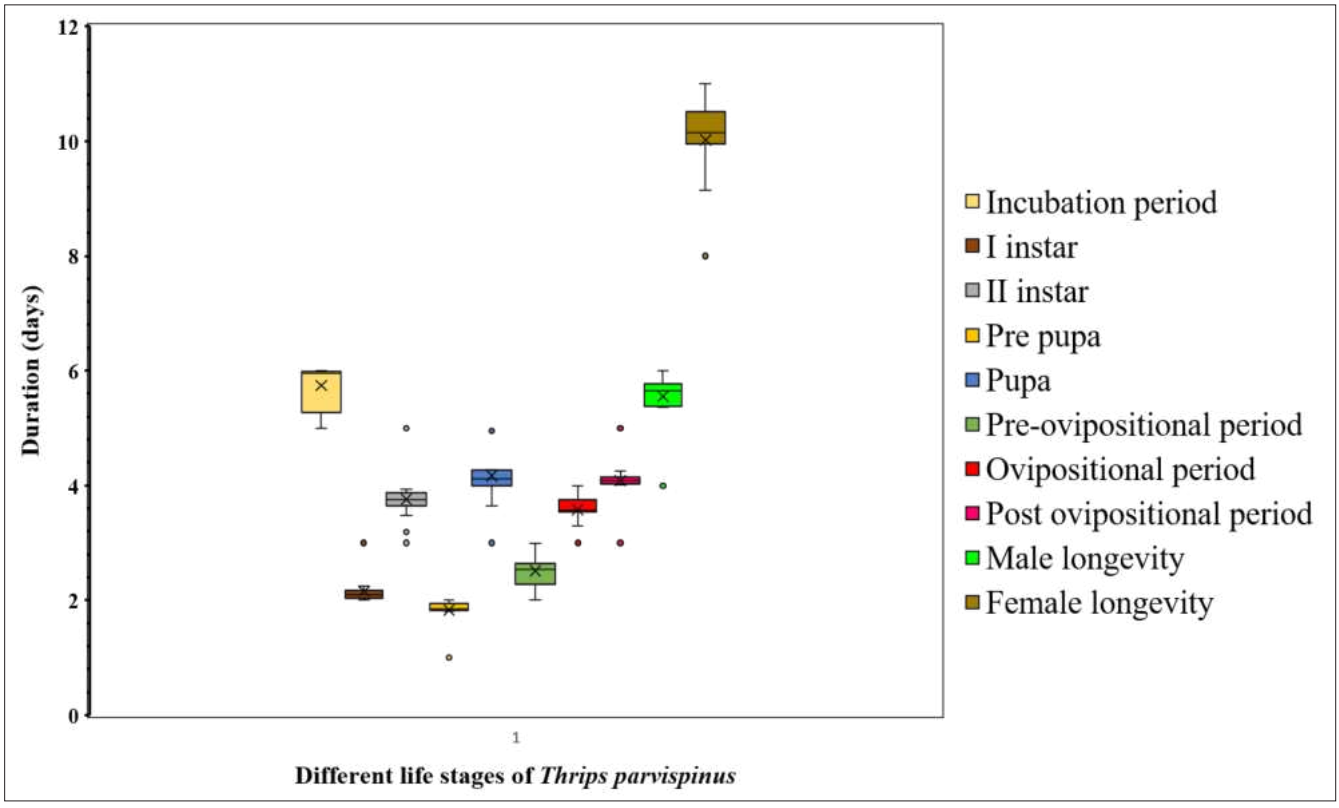

Fig.7. Box plot of the life cycle stages of *T. parvispinus*

The current findings align with Hutasoit *et al*. (2017), who reported average durations for egg, first instar larva, second instar larva, prepupa, and pupa as 4.79, 1.36, 3.54, 1.08, and 1.96 days, respectively. They also noted mean values for the pre-oviposition period, life cycle, adult lifespan, and fecundity as 1.11 days, 13.68 days, 8.55 days (female), 6.00 days (male), and 15.33 eggs, respectively. These figures are similar to our findings, which reported averages of 5.74, 2.16, 3.76, 1.83, and 4.17 days for the same developmental stages, with mean values of 2.51 days for the pre-oviposition period, 25.53 days for the life cycle, 10.18 days (female) and 5.55 days (male) for adult lifespan, and 15.53 eggs for fecundity. These variations can be attributed to environmental changes, as suggested by Hutasoit *et al*. (2019), who highlighted that temperature and host plants influence pest development duration.

Our results partially agree with Murai *et al*. (2010), who found mean generation times of 37.6, 24.8, and 18.8 days and mean fecundity of 50, 69, and 56 eggs at 20, 25, and 30°C, respectively. The mean generation time at 25°C (23.35 days) aligns with our findings, but fecundity differs due to varying climatic conditions, such as the photoperiod used in their study. Ahmed *et al*. (2023) reported an egg period of 4-5 days, first and second larval stages of 2-3 days each, prepupa and pupa stages of 2-3 days each, adult female lifespan of nine days, male lifespan of six days, and a total lifecycle of 13-14 days with a fecundity of up to 15 eggs per female. These findings partially conflict with ours, likely due to differences in host plants used in the studies.

Given the limited information on the biology of *T. parvispinus*, our study draws on data from studies on *Thrips orientalis* and *Thrips tabaci* for reference. The comparable life cycle duration of *T. parvispinus* and *T. orientalis*, despite significant differences in fecundity, underscores the need for further research to understand the role of host plants and local climatic conditions in pest development. Devi and Roy (2019) observed similar developmental stages: incubation period (2-6 days), first instar (2-3 days), second instar larva (4-6 days), prepupa (2-3 days), pupal duration (3-5 days), pre-ovipositional phase (1-3 days), post-ovipositional period (2-3 days), average life cycle (22-28 days), and fecundity (18-42 eggs per female). However, differences in the post-ovipositional period and fecundity may be due to the nutritional status of the host and laboratory conditions.

Johari *et al*. (2014) examined the body size variations of *T. parvispinus* in the lowland and highland areas

of Jambi Province, Indonesia. They found that in the lowland area, long-sized thrips measured 1.42 ± 0.065 mm, medium-sized thrips were 1.32 ± 1.15 mm, and short-sized thrips were 0.122 ± 0.051 mm. The thorax width measurements were 0.30 ± 0.017 mm for long-sized thrips, 0.28 ± 0.021 mm for medium-sized thrips, and 0.29 ± 0.027 mm for short-sized thrips. Our findings somewhat correspond to the medium-length adult thrips from the lowland areas (100-500 m above sea level), which is similar to the mean sea level range in Raichur.

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REFERENCES

Ahmed, M. Z., Revynthi, A. M., McKenzie, C. L. and Osborne L. S. 2023. *Thrips parvispinus* (Karny), an emerging invasive regulated pest in the United States. [https://mrec.ifas.ufl.edu/lsolab/thrips/thripsparvispinus/].[accessed on 28 April 2023].

Basavaraj, K., Sreenivas, A. G., Prasad, P. B. and Rachana, R. R. 2022. First report of invasive thrips, *Thrips parvispinus* (Karny) (Thysanoptera: Thripidae) infesting chilli, *Capsicum annuum* L. in Kalaburagi, Karnataka, India. *Journal of Experimental Zoology India*, **25**: 191- 194.

Devi, M. S. and Roy, K. 2019. Biology of *Thrips tabaci* Lindemann on onion cultivar under laboratory conditions. *Annals of Biology*, **35**(2): 299-302.

Directorate of Plant Protection Quarantine and Storage of India. 2021. Joint survey report on chilli flower thrips conducted in Telangana state. Faridabad, India: Directorate of Plant Protection Quarantine and Storage.

Hutasoit, R. T., Hermanu, T. and Rully, A. 2019. The abundance and diversity of thrips (Thysanoptera: Thripidae) on chili (*Capsicum annum* L.) and cayenne (*Capsicum frutescens* L.) in Bogor. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, **19**(1): 33-41.

Hutasoit, R. T., Triwidodo, H. and Anwar, R. 2017. Biology and demographic statistics of *Thrips parvispinus* Karny (Thysanoptera: Thripidae) in chili pepper (*Capsicum annuum* Linnaeus). (Biologi dan statistik demografi *Thrips parvispinus* Karny (Thysanoptera: Thripidae) pada tanaman cabai (*Capsicum annuum* Linnaeus).) *Jurnal Entomologi Indonesia*, **14**(3): 107-116.

Janyala, S. 2021. Insect hits 9L acres of chilli crop in south India; experts point to use of pesticide. The New Indian Express, 12 December 2021. Available at https://indianexpress.com/article/ india/insect-hits-chilli-crop-south-india-7668378/.

Johari, A., Herlinda, S., Pujiastuti, Y., Irsan, C. and Sartiami, D. 2014. Morphological and genetic variation of *Thrips parvispinus* (Thysanoptera: Thripidae) in chili plantation (*Capsicum annuum* L.) in the lowland and highland of Jambi Province, Indonesia. *American Journal of Bioscience*, **2**: 17-21.

Lim, W. H. 1989. Bunchy and malformed top of papaya cv. Eksotika caused by *Thrips parvispinus* and *Cladosporium oxysporum. Mardi Research Journal*, **17**: 200- 207.

Lodaya, J. P., Suthar, M., Patel, H. C., Sisodiya, D. B., Acharya, R. R., Raval, A. T., Trivedi, N. P. and Mohapatra, A. R. 2022. Status of invasive species of thrips, *Thrips parvispinus* (Karny) infesting chilli grown in middle Gujarat. *Omega*, **5**(3): 6.

Murai, T., Watanabe, H., Wataru, T., Adati, T. and Okajima, S. 2010. Damage to vegetable crops by *Thrips parvispinus* Karny (Thysanoptera: Thripidae) and preliminary studies on biology and control. IXth International Symposium on Thysanoptera and Tospoviruses, 31 August- 4 September 2009*,* [ed. by Persley, D., Wilson, C., Thomas, J., Sharman, M., Tree, D.]. 166 pp.

Nagaraju, D. K., Uppar, V., Ranjith, M., Sriharsha, R. G., Verma, O. M. and Ravi, P. 2021. Occurrence of *Thrips parvispinus* (Karny) (Thripidae: Thysanoptera) in major chilli (*Capsicum annum*) growing areas of Karnataka. *Insect Environment*, **24**: 523-532.

Rachana, R. R. and Varatharajan, R. 2017. Checklist of Terebrantian Thrips (Insecta: Thysanoptera) recorded from India. *Journal of Threatened Taxa*, **9**: 9748-9755.

Rachana, R. R., Roselin, P., Amutha, M., Sireesha, K. and Narasa reddy, G. 2022. Invasive pest, *Thrips parvispinus* (Karny) (Thysanoptera: Thripidae) a looming threat to Indian agriculture. *Current Science*, **122**: 211-213.

Roselin, P., Sharma, K. and Rachana, R. R. 2021. Diversity of floral thrips from Western Ghats of Karnataka. *Indian Journal of Entomology*, **83**: 407-410.

Timmanna, H., Baradevanal, G., Surpur, S., Raghavendra, D., Doddachowdappa, S., Shashank, P. R., Mallaiah, K. K. and Bedar, J. 2022. Diagnosis and potential invasion risk of *Thrips parvispinus* under current and future climate change scenarios. *Peer J*, **6**: 38-68.

Tyagi, K., Kumar, V., Singha, D. and Chakraborty, R. 2015. Morphological and DNA barcoding evidence for invasive pest thrips, *Thrips parvispinus* (Thripidae: Thysanoptera), newly recorded from India. *Journal of Insect Science*, **15**: 1-4.

Veeranna, D., Reddy, R. U., Moguloju, M. and Padmaja, G. 2022. Report on heavy infestation and damage by invasive thrips species, *Thrips parvispinus* (Karny) on chilli in Telangana state of India. *Journal of Pharma Innovation*, **11**: 3845-3848.

Waterhouse, D. F. 1993. The major arthropod pests and weeds of agriculture in Southeast Asia: distribution, importance and origin. Monograph No. 1. Australian Centre for International Agricultural Research. Canberra.

Zhang, H., Xie, Y. and Li, Z. 2011. Identification key to species of thrips genus from China (Thysanoptera, Thripidae), with seven new records. *Zootaxa*, **2810**: 37-46.

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Occurrence and seasonal abundance of *Thrips tabaci* Lindeman on onion in the north transition zone of Karnataka, India

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ABSTRACT: Studies were conducted in four consecutive seasons from *kharif,* 2022 to *rabi,* 2023, on onion crop to understand the thrips, *Thrips tabaci* Linderman incidence. The identity of the species was confirmed through morphological characteristics as well as sequence amplified product of *Cytochrome c oxidase subunit I (COI)* gene (GenBank No. PP838743). The phylogenetic analysis revealed that the *T. tabaci* population is closely related to the sequence of MZ882441 and MT991561 from China and New Delhi, India, respectively. Incidence in the *Kharif* season has experienced minimal thrips infestation compared to the *Rabi* season, where severe infestation was noted. The peak thrips population occurred in the 6th Standard Meteorological Week (SMW) of 2022 and 2023, highlighting the role of weather in thrips population dynamics. Correlation analysis indicated that maximum temperature exhibited a significant positive correlation, while relative humidity showed a significant negative correlation. Additionally, rainfall was found to have a cleansing effect on thrips populations, resulting in lower incidences during the *Kharif* season. However, during the *Rabi* season, the coincidence of higher temperatures with low relative humidity contributed to the proliferation of thrips, making them a significant threat to bulb onion production. This study provides a comprehensive information on the occurrence, species confirmation, nature of damage, seasonal incidence and associated weather parameters with *T. tabaci* in onion crops.

Keywords: correlation, *Cytochrome c oxidase subunit I*, incidence, population dynamics, thrips.

INTRODUCTION

Onion, *Allium cepa* L. (Amaryllidaceae), is one of the most widely cultivated and consumed vegetables worldwide. India is the second largest producer of onion in the world, next only to China, and the crop occupies an area of approximately 1.94 million hectares, with a production of 26.64 million MT in the year 2021. Karnataka is ranked the highest onion producer, next to Maharashtra in India. However, the onion market in India is often volatile due to various factors such as weather conditions, pest infestations and fluctuations in demand and supply. Among the biotic factors, onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) is the consensus important pest. The thrips, *T. tabaci* is one of the most economically important insect pests of onion and causes significant yield loss worldwide (Gill *et al.,* 2015). Larvae and adults mainly cause economic damage, and reducing the photosynthetic processes produces smaller bulbs (Boateng *et al.,* 2014). However, the pest status of onion thrips can be attributed to various factors, *i.e.,* polyphagous nature, high reproductive rate, short generation time, high survival of cryptic (non-feeding pre-pupa and pupa) instars, and ability to reproduce without mating. The damage due to the thrips in onion induces more excellent ethylene production when the saliva from the thrips comes into contact with damaged tissues, which causes the ripening and senescence of leaves. Extensive feeding by thrips in onion not only results in plant stunting and reduced bulb yield, but it also predisposes the plants to various fungal and bacterial pathogens, leading to further decreases in bulb yield and could allow pathogens to infect the plant, causing quality reductions in storage.

Climate change has influenced the growth and development of insect pests (Bergant et al., 2005). Although these pests existed earlier with minimal damage, global warming now favors their proliferation, exacerbating the problem. However, there is hope in the form of integrated pest management (IPM) programs. Accurate identification of pest species is a fundamental step in these programs. Thrips, being minute insects, require reliable identification. This study addresses species confirmation, incidence and the potential influence of weather parameters on thrips abundance in the North Transition Zone of Karnataka, India.

MATERIALS AND METHODS

Study site

The present investigation was undertaken at the Main Agricultural Research Station (MARS), University of Agricultural Sciences (UAS), Dharwad (15 0 49'N; 74 0 96'E) for four consecutive seasons from 2022-2023 in field condition. The popular and ruling onion varieties, *Bheema super* and *Bheema Shakti* were taken during the *Kharif* and *Rabi* seasons, respectively. The seedlings were raised as per the practice package developed by UAS Dharwad. The 6-8 weeks onion seedlings were transplanted to the main field with a spacing of 15 × 10 cm and an area of 10 × 10 m. The crop was raised as per the package of practices except for insecticide spray.

Morphological identification

In a fixed plot, observation was taken from pest initiation until the end of the pest activity during the study period. The adult and immature stages of *T. tabaci* insects were collected from the infected plants using a fine camel hair brush and kept in plastic vials containing 70% ethanol. The collected specimens were identified at the species level using the taxonomic key by Amutha and Rachana (2023). Voucher specimens were deposited in the National Insect Museum at the ICAR- National Bureau of Agricultural Insect Resources, Bengaluru, India.

Molecular confirmation

Genomic DNA extraction

Genomic DNA was extracted from the individual sample of thrips using a DNA extraction kit (Qiagen DNeasy, Hilden, Germany) following the manufacturer's protocols with slight modification (Swapnarani *et al*., 2023; Shivakumara*et al*., 2024).

Polymerase chain reaction (PCR) and COI gene sequencing

PCR amplification was performed by using a 658bp region near the 5′ terminus of the COI gene by using a standard protocol (Hebert *et al.,* 2003). The COI gene primers used for amplification were: forward primer (LCO 1490 5′-GGTCAACAAATCATAAAGATATTGG-3′) and reverse primer (HCO 2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer *et al.,* 1994). Polymerase Chain Reaction (BioRad C1000™) was carried out by using 200 µL volume PCR tubes (Tarsons, Kolkata, India). An aliquot of 25 µL contained 12.5 µL of 2 × reSource™ Taq Mix (resource Taq DNA Polymerase, 6 mM $MgCl_2$, 2 mMdNTPs) (Source Bioscience, UK), 1 µL of each 10 µM primer, 8.5 µL of molecular biology grade water (Sigma Aldrich) and 2 µL of template DNA. PCR was performed with initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94 °C for 0.30 min, primer annealing at 48 °C for 0.30 min and extension at 72 °C for 0.30 min. Then, a final extension was performed at 72 °C for 5 min before storing the reaction at 4°C. The PCR products were separated on 1.5% agarose gel electrophoresis (Sambrook and Russell, 2001). PCR products were sequenced by Eurofins Genomics India Pvt Ltd, Bengaluru, India. The homology, insertions, deletions, stop codons and frameshifts were checked by using NCBI-BLAST and ORF finder. The samples were bi- directionally sequenced and checked for homology, insertions and deletions, stop codons and frameshifts by using the Basic Local Alignment Search Tool (BLASTn, http://www.ncbi.nlm.nih.gov), with the sequence of similar or related genera retrieved from National Center for Biotechnology Information (NCBI). The partial sequence of study isolate was deposited in Gen Bank, NCBI database and an accession number was obtained.

Phylogenetic analysis

The phylogenetic analysis was carried out using MEGA-11 (Tamura *et al.,* 2021) to explain the relationship between the study isolate (PP838743) and other thrips populations from different world geographical regions. Similar to our target sequence, the sequences were downloaded from NCBI and GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/). All nucleotide sequences under study were aligned using the Clustal-W tool of MEGA-11. The Neighbor-Joining method inferred the evolutionary history (Saitou and Nei, 1987). The optimal tree is shown. The evolutionary distance was computed using the Maximum Composite Likelihood method (Tamura *et al.,* 2004) and is the unit of the number of base substitutions per site. This analysis involved 27 nucleotide sequences of *T. tabaci*. All ambiguities were removed for each sequence pair (pairwise deletion option). There were a total of 677 positions in the final dataset. The evolutionary history was exhibited based Kimura-two-parameter model with 1000 bootstrap replications (Tamura *et al.,* 2021).

Nature of damage and severity

The thrips population was counted on ten randomly

selected plants at weekly intervals from 15 days after transplanting till harvest. Both adult and immature stages of thrips were counted by opening the neck region of the plant, and the mean thrips per plant were worked out. Thrips damage rating was calculated at 45, 60 and 90 DAT by adopting the following damage scale adopted by Njau *et al.* (2017). Where 0= Damage, 1= 0.1-20% leaf area damaged, 2= 20.1- 40% leaf area damaged, 3=40.1-60% leaf area damaged, 4=60.1-80% leaf area damaged, 5=80.1-100% leaf area damaged.

Correlation of weather parameters with thrips incidence

The effect of different weather parameters i.e. maximum temperature (X_1), minimumtemperature (X_2), morning relative humidity (X_3), evening relative humidity (X_4), and rainfall(X_5) on the incidence of the thrips species on onion crop was worked out to know therelationship of pest incidence with weather parameters. All the statistical analysis was performed using Microsoft Excel 2010 software.

RESULTS AND DISCUSSION

Taxonomic investigations led to the identification of the thrips species as *Thrips tabaci* Lindeman (Thysanoptera: Terebrantia: Thripidae).

Diagnosis

Female macroptera (Fig. 1): Abdominal pleurotergites with closely spaced rows of regular, fine microtrichia; lateral margins of tergites with microtrichia on sculpture lines; tergite IX with one pair of campaniform sensilla, anterior pair absent; antennal segment V not sharply paler than IV.

Fig. 1. *Thrips tabaci*

All the search analysis results revealed that the analyzed species belongs to *T. tabaci.* Alignment of the *T. tabaci* mtCOI sequences was found to have no deletions or insertions and no stop codons, consistent with the amplified DNA arising from functional genes. The sequence generated by the study (PP838743) showed 100% identity with *T. tabaci* (MN036460), which was submitted from China on Onion. Further, the sequence and the specimen details were submitted to the BOLD database and DNA barcodes were generated.

The phylogenetic tree was constructed from *T. tabaci* MtCOI data from this study. The present phylogenetic analysis consisted of 27 MtCOI sequences including one sequence generated from this study, 26 sequences were downloaded from the NCBI Genbank database which includes the sequences across the different geographical regions of the world, showcasing the global reach of our study. One sequence of common blossom thrips, *Frankliniella schultzei* Trybom (Thysanoptera: Thripidae) COl gene with NCBI accession number KF144133 served as an out-group. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei parameter model (Tamura *et al.,* 2013). The tree generated by combining the NCBI Genbank sequences showed one major clade (Fig. 2). All the *T. tabaci* populations were clustered in one clade and had a high degree of geographical representation from different regions, emphasizing the breadth of our study. The study isolate was closely associated with MZ882441 and MT991561 the sequence originated from Yunnan, China and New Delhi, India respectively.

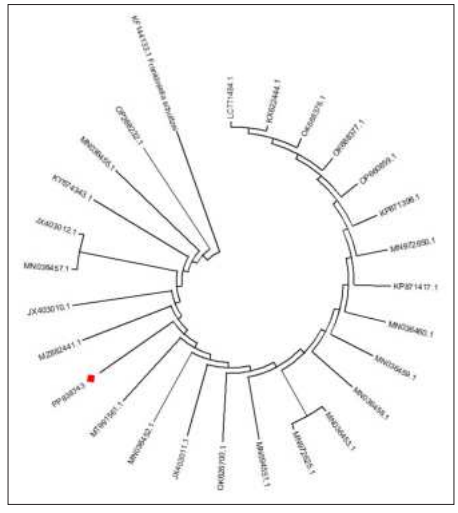

Fig. 2. The phylogenetic relationships of the study isolate from the Onion population using 658 bp mitochondrial cytochrome oxidase I (CO I) sequence of *Thrips tabaci* using the Maximum Likelihood method based on the Tamura-Nei model. The isolate from the current study is marked with a diamond. The tree was formed using the MEGA-11 program.

Both larvae and adults have very distinctive feeding behavior by punching through the leaf surface and then extracting sap from plant and it release substances that helps to pre-digest the leaf tissue and consume mesophyll cells normally. Ultimately leads to loss of chlorophyll and reduced photosynthetic efficiency. The results were by Boating *et al.* (2014). Further, damage appears as silvery patches or streaks on the leaves (Fig 3). Theincidence started from the 31st SMW *i.e.,* August 1st week and reached a peak (10.5 thrips/plant) at the 38th SMW *i.e.,* 3rd week of September in *Kharif* 2022 (Fig 4).

Fig. 3. Damage caused by *Thrips tabaci* on onion

In *Kharif* 2023, thrips infestation began in the 33rd Standard Meteorological Week (SMW), which is the third week of August and peaked in the 38th SMW, which is the last week of September, with a significant infestation of 22.60 thrips per plant (Fig. 4). Unlike in 2022, there was no monsoon rain during the *Kharif* season of 2023. This absence of rain likely contributed to the continuous presence of thrips throughout the season.

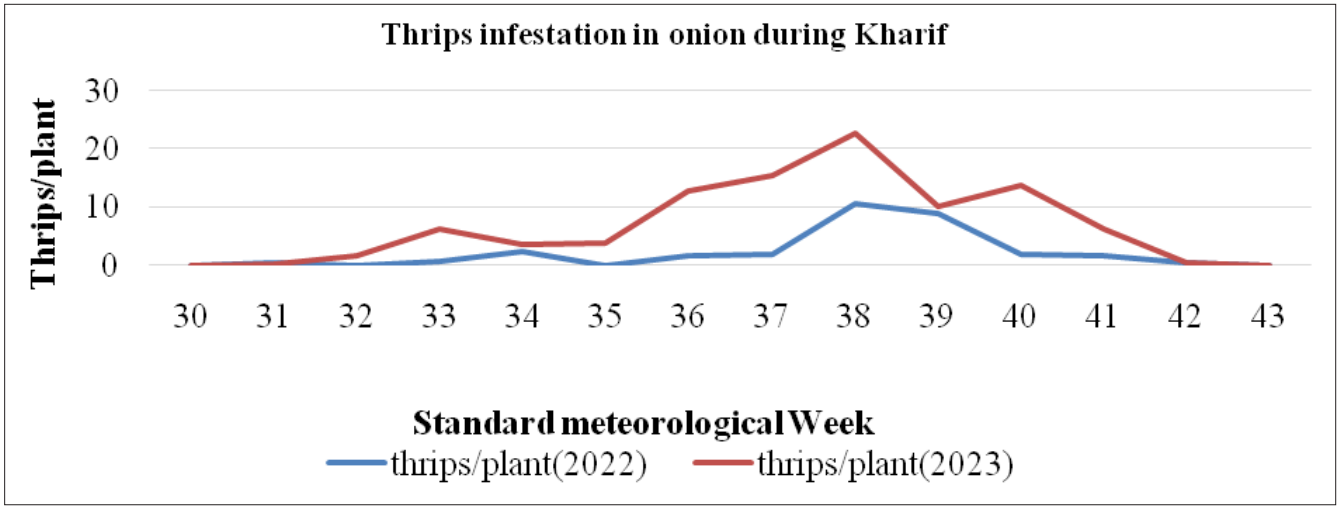

Fig. 4. Population dynamics of thrips in onion during *kharif* 2022 and *kharif* 2023

During this study period, the infestation was much less compared to other season because monsoon rain might have washed out the thrips. Both rainfall and the total number of rainydays showed a negative significant effect on thrips infestation. This suggests that increased rainfall and rainy days correlate with decreased thrips infestation during *Kharif* (Table 1).

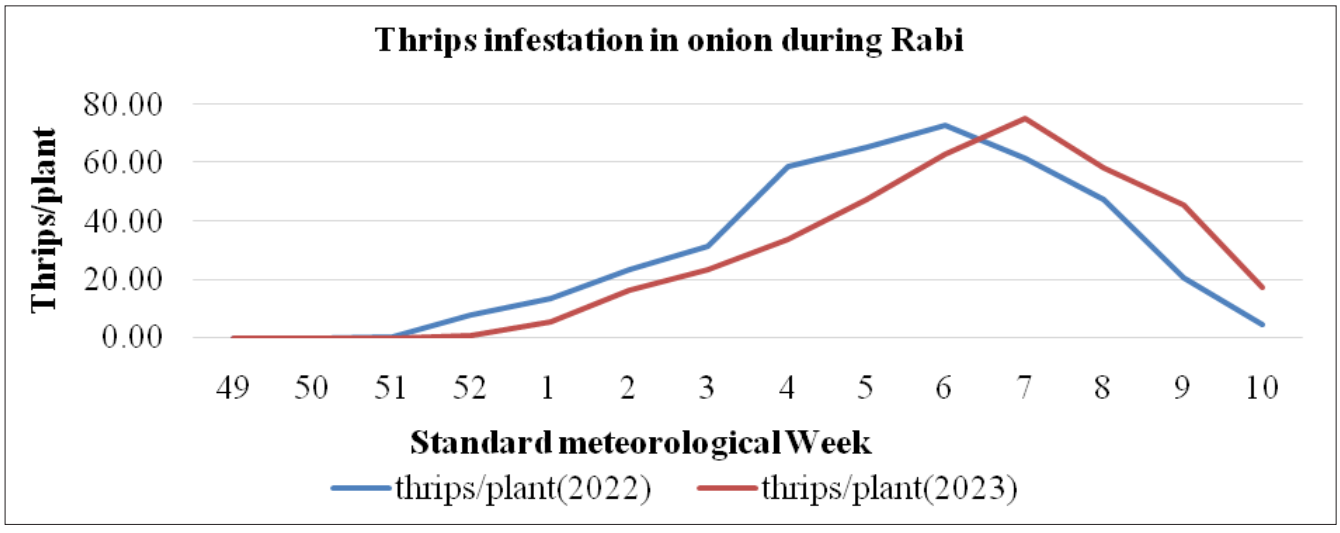

Fig. 5. Population dynamics of thrips in onion during *rabi* 2022 and r*abi* 2023

In *rabi* 2022 and 2023, thrips incidence began in the 51st Standard Meteorological Week (SMW), which corresponds to late December and peaked in the 6th and 7th SMW of the respective years (Fig. 5). The peak populations recorded were 72.60 thrips per plant in 2022 and 75.10 thrips per plant in 2023. Maximum temperature and morning and afternoon relative humidity were significant factors influencing thrips incidence (Table 1), with higher temperatures and lower humidity associated with increased thrips populations. These results align with those given by Akashe *et al.* (2016) where they observed that *Thrips palmi* Karny incidence was positively correlated with maximum temperature while negatively correlated with Morning RH, afternoon RH and rainfall in sunflowers. Linear regression analysis predicted that maximum temperature was the most important weather factor which influenced thrips to the tune of 92.1 % (Fig. 6) while rainfall, morning and afternoon relative humidity had only 18.5 %, 38.2% and 30.2% influence on incidence during *Rabi,* respectively. However, rainfall and rainy days did not significantly affect thrips populations during these *Rabi* seasons (Table 1). Since the thrips population is more during the *rabi* seasons (2022 and 2023), leaf feeding damage also increased, and the damage percentage increased as the crop age progressed. At 90 DAT, the leaf damage scale was three, corresponding to 45.2 and 48.8 percent leaf damage during *Rabi* 2022 and *Rabi* 2023, respectively (Table 3).

Table 1. Correlation matrix of weather parameters with the incidence of thrips in onion from 2022-2023

	Correlation coefficient (r)					
Season	**Max. Temperature**	**Min. Temperature**	**Morning RH**	**Afternoon RH**	**Rainfall (mm)**	**Rainy days**
Kharif 2022	0.389^{NS}	-0.051^{NS}	-0.044^{NS}	-0.155^{NS}	-0.535*	-0.573*
Rabi 2022	0.633*	-0.441^{NS}	-0.647*	-0.617*	-0.375^{NS}	--
Kharif 2023	0.157^{NS}	-0.349^{NS}	-0.259^{NS}	-0.231^{NS}	-0.023^{NS}	-0.092^{NS}
Rabi 2023	0.663**	-0.024^{NS}	-0.553*	-0.609*	-0.223^{NS}	-0.223^{NS}

NS: Non significant; *Significant at 0.05 level; ** significant at 0.01 level; -- : no rainy days; RH: Relative humidity (%)

Table 2. Multiple regression model for thrips incidence

Thrips incidence	Regression equation	R^2
Kharif 2022	Y=(0.677)T.Max + (-2.287) T.Min + (0.829) Morning RH + (-0.351) Afternoon RH + (-0.079) rainfall + (0.243) rainy days + (-16.678)	0.592
Rabi 2022	Y=(7.330) T.Max + (1.410) T.Min + (-1.218) Morning RH + (-0.132) Afternoon RH + (-1.005) rainfall + (-135.263)	0.518
Kharif 2023	Y=(-1.863)T.Max + (-7.618) T.Min + (-0.64) Morning RH + (-0.220) Afternoon RH + (-0.224) rainfall + (3.245)rainy days + 289.13	0.538
Rabi 2023	Y=(11.326)T.Max + (-5.696) T.Min + (-1.574) Morning RH + (0.932) Afternoon RH + (-0.183) rainfall + (-169.23)	0.623

T. Max: Temperature Maximum, T. Min: Temperature Minimum, RH: Relative Humidity

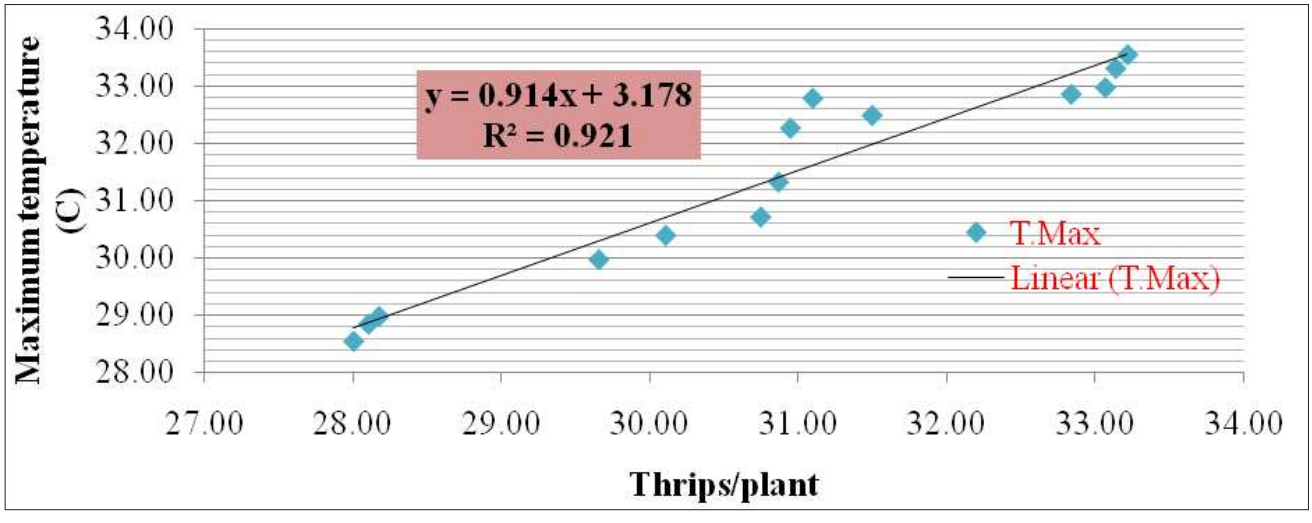

Fig. 6. Effect of Maximum Temperature on incidence of thrips during Rabi (pooled data of 2022and 2023)

This study reveals the influence of weather parameters on the incidence of thrips. In the *Kharif* season, the population was very low, and the same observation was made by Dharmatti and Beeraganni (2013). Likewise, Liu (2005) also recorded a significant difference in thrips population between onion growing seasons. Higher temperature coupled with lesser relative humidity supports the buildup of the thrips population in matured crops, revealing that hot and dry climate promotes higher incidence in onion. These results were on par with observations made by Choudhary (2016) on the incidence of thrips in cowpeas. The peak population of *T. tabaci* occurred within the maximum temperature range of 30-33oC as recorded by Karuppaiah *et al.* (2018) in garlic, and the minimum temperature ranged from 14-19 °C. This suggests that certain temperature thresholds are conducive to thrips proliferation. In this study, rainfall, rainy days, and minimum temperature showed a negative, non-significant effect on thrips incidence in *Rabi*, consistent with findings by Karar *et al.* (2014). Lower relative humidity, both in the morning and afternoon, facilitated thrips establishment in onion crops. This finding is supported by the results of a study by Kumar *et al.* (2015). Foliar feeding damage by the thrips was recorded by giving a leaf damage score. Though the incidence is very low during this season, the maximum damage score was 1, corresponding to 4.27 percent leaf damage at 90 DAT (Table 3). Since thrips were washed out by rain, there was no continuous feeding irritation on foliage. Therefore, the greening appearance was more. Leaf feeding damage score was one throughout the season (Table 3). On the other hand, multiple regression studies revealed that weather factors influenced 59.2, 51.8, 53.8 and 62.3 percent on the incidence of *T. tabaci* on onion in *Kharif* 2022, *Rabi,* 2022, *Kharif,* 2023 and *Rabi,* 2023, respectively (Table 2).

Table 3. Thrips damage rating on onion leaves from *Kharif* 2022-*Rabi* 2023

Cropping Season	45DAT		60DAT		90DAT	
	Percent leaf damage	Damage score	Percent leaf damage	Damage score	Percent leaf damage	Damage score
Kharif 2022	0	0	3.67	1	4.27	1
Rabi 2022	6.0	1	17.3	1	45.2	3
Kharif 2023	0	0	4.5	1	12.0	1
Rabi 2023	6.17	1	16.9	1	48.8	3

DAT: Days after transplanting

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REFERENCES

Akashe, V. B., Jadhav, J. D., Bavadekar, V. R., Pawar, P. B. and Amrutsagar, V. M. 2016. Forewarning model for sunflower thrips (*Thrips palmi* Karny) in western Maharashtra scarcity zone. *Journal of Agrometeorology*, **18**(1): 68-70.

Amutha, M. and Rachana, R. R. 2023. Species diversity of thrips on cotton. *Indian Journal of Entomology,* **85**(1): 78–82.

Bergant, K., Trdan, S., Znidarcic, D., Crepinsek, Z. and Kajfez-Bogataj, L. 2005. Impact of climate change on developmental dynamics of *Thrips tabaci* (Thysanoptera: Thripidae): Can it be quantified? *Environmental Entomology*, **34**(4): 755-766.

Boateng, C. O., Schwartz, H. F., Havey, M. J. and Otto, K. 2014. Evaluation of onion germplasm for resistance to Iris yellow spot (Iris yellow spot virus) and onion thrips, *Thrips tabaci*. *Southwestern Entomologist*, **39**(2): 237-260.

Choudhary, J. S. 2016. Influence of weather parameters on population dynamics of thrips and mites on summer season cowpea in Eastern Plateau and Hill region of India. *Journal of Agrometeorology*, **18**(2): 296-299.

Dharmatti, P. R. and Beeraganni, K. M. 2013. Seasonal abundance of onion thrips, *Thrips tabaci* Lindeman. *International Journal of Plant Protection*, **6**(2): 428-431.

Folmer, R. H. A., Nilges, M., Folkers, P. J. M., Konings, R. N. H. and Hilbers, C.W. 1994. A model of the complex between single-stranded DNA and the single-stranded DNA binding protein encoded by gene V of filamentous bacteriophage M13. *Journal of Molecular Biology*, **240**(4): 341-357.

Gill, H. K., Garg, H., Gill, A. K., Gillett-Kaufman, J. L. and Nault, B. A. 2015. Onion thrips (Thysanoptera: Thripidae) biology, ecology and management in onion production systems. *Journal of Integrated Pest Management*, **6**(1): 6.

Haider Karar, G. A., Hameed, A., Ahmad, G. and Ali, A. 2014. Losses in onion (*Allium cepa*) due to onion thrips (*Thrips tabaci*) (Thysanoptera: Thripidae) and effect of weather factors on population dynamics of thips. *World Applied Sciences Journal,* **32**(11): 2250-2258.

Hebert, P. D., Cywinska, A., Ball, S. L. and DeWaard, J. R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **270**(1512): 313-321.

Karuppaiah, V., Soumia, P. S., Gawande, S. J., Mahajan, V. and Singh, M. 2018. Influence of dibbling time and weather factors on seasonal dynamics of thrips (*Thrips tabaci* Lindeman) on garlic in Maharashtra. *Journal of Agrometeorology*, **20** (4): 311-314.

Kumar, J., Singh, P. P., Satyanarayana, P. and Rani, D. D. 2015. Effect of Abiotic Factors on the Population Dynamics of Thrips, (*Thrips tabaci* Lind.) on Onion Crop. *Bioscience Trends*, **8**(6): 1515-1518.

Njau, G. M., Nyomora, A. M., Dinssa F. F., Chang, J. C., Malini, P., Subramanian, S. and Srinivasan, R. 2017. Evaluation of onion (*Allium cepa*) germplasm entries for resistance to onion thrips, *Thrips tabaci* (Lindeman) in Tanzania. *International Journal of Tropical Insect Science*, **37**(2): 98-113.

Raut, A. M., Pal, S., Wahengbam, J. and Banu, A. N. 2020. Population dynamics of onion thrips (*Thrips tabaci*lindeman, Thysanoptera; Thripidae) and varietal response of onion cultivars against onion thrips. *Journal of Entomological Research*, **44**(4): 547-554.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, **4**(4): 406-425.

Sambrook, J. and Russell, D.W. 2001. Detection of DNA in agarose gels. *Molecular Cloning, A Laboratory Manual, (3rd Ed.) Cold Spring Harbor Laboratory Press, New York*, 5-14.

Shivakumara, K. T., Chinapolaiah, A., Keerthi, M. C., Ramya, R. S. and Gotyal, B. S. 2024. Identification and characterization of novel resistant genotypes of *Gymnema sylvestre* (Retz.) R. Br. ex Sm. against invasive mealybug species, *Phenacoccus solenopsis* Tinsley and *Paracoccus marginatus* Williams and Granara de Willink for sustainable pest management. *Journal of Applied Research on Medicinal and Aromatic Plants*, **39**: 100534.

Swapnarani, K., Pal, S. and Shivakumara, K. T. 2023. Biology and integrative taxonomy of leaf folder, *Helcystogramma hibisci* (Stainton, 1859): a pest of musk mallow, *Abelmoschus moschatus* (L.) Medik. *Animal Biology*, 1(aop), 1-14.

Tamura, K., Nei, M. and Kumar, S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, **101**(30): 11030-11035.

Tamura, K., Stecher, G. and Kumar, S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, **38**(7): 3022-3027.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, **30**(12): 2725-2729.

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Biology and morphometry of common Mormon butterfly, *Papilio polytes* L. (Papilionidae: Lepidoptera) on acid lime

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ABSTRACT: The common Mormon, *Papilio polytes* L. is one of the important butterfly pests on citrus crops. The biology of *P. polytes* was studied under laboratory conditions at the Department of Agricultural Entomology, College of Agriculture, Vijayapura, Karnataka, India. The average fecundity was 21.70 eggs per female butterfly. The incubation, total larval and pupal period were observed to be of 3.83, 14.88 and 13.36 days, respectively. The total lifecycle of male and female butterfly was completed in 39.42 and 41.47 days, respectively. Pre-oviposition, oviposition and post-oviposition period were 1.18, 5.96 and 1.20 days, respectively.

Keywords: Acid lime, *Papilio polytes*, biology, morphometry, incubation period, larval period

INTRODUCTION

The common Mormon, *P. polytes* L. is one of the serious butterfly pests whose larval forms cause economic damage to crops by devouring large quantity of foliage during their development on citrus crops (Singh, 1993; Dileepkumar *et al*., 2022). The caterpillars are voracious feeders of young seedlings and cause death of the seedling within no time (Resham *et al*., 1986). The larvae of this butterfly are of regular occurrence in nurseries, young plantation and on newflush of grown up trees. Severe infestation of pest results in complete defoliation of the tree and decreased photosynthetic activity leading to reduction in vigour, plant growth and finally fruit yield (Butani and Jotwani, 1975). The pest is also known to feed on other hosts belong to family rutaceae (Corbet and Pendlebury, 1992; Haribal, 1992; Gunathigalraj *et al*., 1998). In recent times, increase in area under cultivation of acid lime in northern parts of Karnataka attracted many insect pests to cause severe damage to crop (Dileepkumar *et al*., 2022). Among them, *P. polytes* is one of the butterfly pests which have been limiting production under both nursery and field conditions along with *P. demoleus*. The morphological differentiation of immature stages of both the species of butterfly needs greater attention for correct identity of the pest. The literature on biology and morphometry of *P. polytes* is very meagre in India. So, the present study was undertaken to know developmental behaviour of pest on acid lime (*Citrus aurantifolia* Swingle) under laboratory conditions. This information may form valuable basis for correct identity and initiation of suitable control measures against this pest.

MATERIALS AND METHODS

The field culture of *P. polytes* was collected from the acid lime orchards near College of Agriculture, Vijayapura, Karnataka. The collected larvae were reared on acid lime leaves placed in petri plates and fresh leaves were renewed twice in a day till the end of larval stage in the laboratory (27±2ºC, 65-70%RH). The pupae were collected and placed in an oviposition cage for adult emergence. The adult butterflies on emergence were provided with acid lime seedlings as substrate for the oviposition. The cotton soaked in 10% sugar solution was provided as food source. Ten pairs of adults were maintained separately in ovipositional cages to record the fecundity. Freshly laid eggs were collected every day and larval rearing was continued till pupation. The observations on life stages *viz*., egg, larva, pupa and adults were recorded daily. The parameters like pre-oviposition period, oviposition period, post-oviposition period, incubation period, larval period (instars-wise), pre-pupal period, pupal period, adult longevity (Male and Female) and total length of life cycle in days were observed and mean duration for completion of each stage was computed. The morphometric observations on diameter, length and width of various life stages *viz*., egg, larva, pupa and adults was measure with the help of stereo binocular microscope which was arranged with a camera at the apex of the eye piece. Later the mean values were computed with statistical analysis.

RESULTS AND DISCUSSION

Egg: The female *P. polytes* deposited egg singly on dorsal and ventral surface of leaves, young twigs and sometimes on stem portion of the plant. Freshly laid

Table 1. Biology of common Mormon butterfly, *Papilio polytes* L. reared on acid lime under laboratory conditions

Life stage parameter	Duration (days) Mean± SD
Fecundity	21.70±1.45
Incubation period	3.83± 0.24
Larval period	
I instar	2.39±0.25
II instar	2.21±0.25
III instar	2.56±0.36
IV instar	3.29±0.26
V instar	4.43±0.20
Total larval period	14.88±0.63
Pre-pupal period	1.00±0.00
Pupal period	13.36±0.59
Adult longevity	
Male	6.35±0.33
Female	8.39±0.35
Total life cycle	
Male	39.42±0.41
Female	41.47±0.39
Pre-oviposition	1.18±0.26
Oviposition	5.96±0.43
Post-oviposition	1.20±0.25

SD- Standard Deviation

eggs were small, spherical, greenish yellow and smooth in appearance. One day after, eggs were turn into pale yellow to cream colour with black taint on the surface of the egg and gave finely roughened appearance on surface (Fig. 1). Eggs become brownish black at one day before hatching. The fecundity was varied from 18 to 23 number of eggs per female and average fecundity was 21.70 ± 1.45 eggs per female. The average incubation period was 3.83 ± 0.24 days (Table 1).The diameter of egg was ranged from 1.13 to 1.35 mm with an average of 1.28 ± 0.06 mm (Table 2).The present observations on morphological descriptions of eggs of *P. polytes* were supported by Khan *et al.* (2019) and Islam *et al.* (2017). The similar incubation period were recorded in earlier reports (Suwarno *et al.,* 2007; Jaafar *et al.,* 2014; Islam *et al.,* 2017).

Larva: The larvae of *P. polytes* were moulted four times and passed through five larval instars during the completion of larval stage. First instar larva was yellowish dark brown dorsally and dark brown laterally. Hypognathous head with mandibles and two conspicuous primary setae. White bands encircles first and last abdominal segment of the body (Fig. 2). The terminal abdominal segment bears whitish brown caudal horns. Larvae with small and large setae covering lateral sides of the body. The average length and width of first instar larva was 7.74 ± 0.13 and 1.22 ± 0.06 mm, respectively (Table 2). The second instar larva was looking like late first instar larva except in size and length of the body. The anterior parts of body heighten and widened than posterior part of the body. Larva with more extricates white marking on middle and posterior parts of the body (Fig. 3). As the age of larva was increased, white patches become more widened dorsally and laterally of the body. The average length and width of second instar larva was 8.72 ± 0.12 and 1.68 ± 0.04 mm, respectively. Third instar was dark brown with distinguishing white

furrow on lateral sides of thoracic and abdominal segments. Three pairs of white-bluish spots were present on dorsum of second to fourth abdominal segments (Fig. 4). Caudal horns were thicker, conspicuous, snow-white in colour and present on last abdominal segment. A pair of white spiky projection was visible on dorsum of fifth abdominal segment.The average length and width of third instar larva was 13.34 ± 0.28 and 2.42 ± 0.08 mm, respectively. Fourth instar larva more or less similar to third instar larva but has greasy appearance with greenish coloration of body. The lateral white streak completely enclosed the first and second abdominal segments (Fig. 5). Metathoracic segment bears four conspicuous white bluish spots dorsally. The average length and width of fourth instar larva was 31.19 ± 0.94 and 4.80 ± 0.09 mm, respectively. Unlike to early instars, fifth instar larva was drastically different in physical appearance. Newly moulted fifth instar larva was light green, later the colour was gradually changed to pure green. Dorsally there were two transverse sinus marking on the body, one is connected two eye spots present laterally on meta-thoracic segment and another one occurs between dorsum of meta-thorax and first abdominal segment. A pastel purplish bluish slit was existing in sinus marking.

Fig 1. Egg

Fig 2. First instar

Fig 3. Second instar

Fig 4. Third instar

Fig. 5. Fourth instar

Fig 6. Fifth instar

Fig. 7. Pre-pupa

Fig.8. Green pupa

Fig. 9. Brown Pupa

Female adult

Male adult

Table 2. Morphometry of common Mormon butterfly, *Papilio polytes* reared on acid lime under laboratory conditions

Particulars	Width (mm)		Length (mm)	
	Mean± SD	Range	Mean± SD	Range
Egg (Diameter)	1.28±0.06	1.13-1.35	-	-
I instar	1.22±0.06	1.10-1.45	7.74±0.13	7.42-7.91
II instar	1.68±0.04	1.59-1.73	8.72±0.12	8.46-8.85
III instar	2.42±0.08	2.29-2.55	13.34±0.28	12.99-14.00
IV instar	4.80±0.09	4.71-5.12	31.19±0.94	29.14-31.90
V instar	8.02±0.07	7.93-8.13	36.48±0.42	35.79-37.15
Pupae	7.97±0.13	7.57-8.15	30.58±0.64	29.39-31.70
Male adult	57.66±0.78	55.97-58.92	29.60±0.18	29.15-29.87
Female adult	65.62±0.86	64.05-66.65	30.69±0.37	30.15-31.50

SD- Standard Deviation

Two pairs of bluish spots were present on dorsal side of third and fourth abdominal segments. Three pairs of oblique black to brownish bars were present on either side of the abdominal segments; first pair of oblique bar was arising from the base of lateral side of third abdominal segment and ended on dorsum of fourth abdominal segment. Second pair of bar was present on lateral sides of six abdominal segment and short third bar occurred an each sides of last abdominal segment(Fig. 6). The three pairs of thoracic and five pairs of abdominal pseudo legs were more conspicuous than earlier instars. Thoracic legs were whitish and spongy at the base and bears terminal hook like structure. Abdominal pseudo-legs were whitish, stumpy and spongy in appearance. Fleshy, pink, forked structure osmeterium was present on prothoracic region of larva, normally hidden but can be exerted outside when caterpillar was disturbed by external stimuli. The average length and width of fifth instar larva was 36.48 ± 0.42 and 8.02 ± 0.07 mm, respectively. The total larval period was 14.88 ± 0.63 days (Table 1). Similar morphological observations were recorded by Islam *et al.* (2017) and Khan *et al.* (2019) on different larval instars of *P. polytes*.

Pupa: Mature fifth instar larva was stopped feeding, becomes inactive, body shortened gradually in length and attached to substrate with frontal and posterior parts of the body by bending upward mid dorsally (Pre-pupa) (Fig. 7). The average pre-pupal period was 1.00 ± 0.00 days. The pupa was formed one day after pre-pupal stage. The pupa of *P. polytes* is a chrysalis and was attached to substrate posteriorly with cremaster at the anal end and held in a position with help of silken griddles run dorsally on middle of the body. Two pupal forms were found, one being green morph which was predominantly green with two yellowish diamond patches on ventral side of abdominal segment. The brown morph was grayish to dark shades of brown (Fig. 8, 9). Pupae were having characteristic pair of cephalic horns, dorsal thoracic hum and a pair of caudal horn. Pupa turned black with appearance of black wings just before eclosion. The average pupal period was 13.36 ± 0.59 days (Table 1).The average length and width of pupa was 30.58 ± 0.64 and 7.97 ± 0.13 mm, respectively(Table 2).The pupal period of 9 to 15 days reported by Gaikwad and Bhawane (2013), Minh *et al.* (2015) and Islam *et al.* (2017) which corroborates the present study.

Adults: Adults of *P. polytes* were large, black colored with wide wing spread. The head, thorax and abdomen jet black colored with blackish scales all over the body. Head with hooked club shaped antenna and long curved proboscis. Thorax consists of three pair dusky black legs and two pairs of wing which are attractively colored on both the sides. Both female and male sexes were tailed giving the name swallowtails. Female was black with attractive red and white colored spots over the wings. Fore wing with pale streaks between the longitudinal veins. Hind wing with four elongated white spots and paler series of narrow red spots on outer margins of wing

(Fig. 10). The male was black, forewing with series of white spots decreasing in size towards the apex and hind wing with discal band of elongated white spots which were well separated from each other (Fig. 11). Similar morphological characters were observed in the earlier findings (Islam *et al.,* 2017; Suwarno *et al.,* 2007). The longevity of adult male and female butterfly was 6.35 ± 0.33 and8.39 ± 0.35 days, respectively. The male sex of *P. polytes* was completed life cycle on an average of 39.42 ± 0.41 days, whereas life cycle of female butterfly was completed in41.47 ± 0.39 days (Table 1).The average length and width of male adult butterfly was 29.60 ± 0.18 and 57.66 ± 0.78 mm, respectively whereas average length and width of female adult butterfly was 30.69 ± 0.37 and 65.62 ± 0.86 mm, respectively (Table 2). In accordance to present study Islam *et al.* (2017) recorded total developmental period of 32 to 64 days for *P. polytes* and opined that life cycle duration varies according to weather conditions. The pre-oviposition, oviposition and post-oviposition period was 1.18 ± 0.26, 5.96 ± 0.43 and 1.20 ± 0.25 days respectively.

The common Mormon, *P. polytes* is occurring in serious proportions and causing significant damage to acid lime plants in northern parts of Karnataka. There is an ambiguity in morphological differentiation of immature stages of *P. polytes* with other closely related species of butterflies. The information generated during present study would be helpful for correct identity of pest by differentiating from other closely related species to initiate suitable control measures.

REFERENCES

Butani, D. K. and Jotwani, M. G. 1975. Trends in the control of insect pests of fruit crops in India. *Pesticides,* **9**: 39-149.

Corbet, A. S. and Pendlebury, H. M. 1992. The butterflies of the Malay Peninsula. Fourth Edition, Malayan Nature Society, Kuala Lumpur.

Dileepkumar, N. T., Biradar, A. P., Mallapur, C. P., Kulkarni, S. and Venugopal, C. K. 2022. Survey on insect pests of acid lime, *Citrus aurantifolia* in northern Karnataka, India. *Journal of Farm Sciences*, **35**: 456-459.

Gaikwad, S. M. and Bhawane, G. P. 2013. Life history including behavior, parasitism and population density of *Papilio polytespolytes* (L). *International Symposium on Entomology*, September 04, 2013, Orlando, Florida, USA.

Gunathilagaraj, K., Perumal, T. N. A., Jayaram, K. and Kumar, M. G. 1998. Some south Indian butterflies. Nilgiri wildlife and environment assocaition, Tamil Nadu.152.

Haribal, M.1992. The butterflies of Sikkim Himalaya and their natural history, Sikkim nature conservation foundation, Gangtok. 217.

Islam, M. S., Islam, A. T. M. F., Rahman, M. M. and Yamanaka, A. 2017. Biology and morphometrics of the common mormon butterfly, *Papilio polytes* Linnaeus (Lepidoptera: Papilionidae) rearing in laboratory condition. *University Journal Zoology. Rajasthahi University,* **36**: 49-56.

Jaafar, I., Cheng, S. and Hurzaid, A. 2014. Development of eggs and larvae of the common swallowtail butterfly, *Papilio Polytes* (L.) (Lepidoptera: Papilionidae) in Malaysia. *Malayan Nature Journal,* **65**: 47-53.

Khan, M. M. H., Molla, M. N. and Rahman, M. A. 2019. Development, host preference and leaf consumption of the lime swallow tail butterfly, *Papilio polytes Romulus* Cramer (Papilionidae: Lepidoptera) on citrus. *Journal of Asiatic Society Bangladesh,* **45**: 111-125.

Minh, H. G., Giang, H. T. T. and Trang, H. T. Q. 2015. Studies on some aspects of the biology and ecology of citrus butterfly *Papilio demoleus* (Papilionidae: Lepidoptera) on citrus in Vietnam. *Journal of Tropical Asian Entomology,* **4**: 20-27.

Resham, B.T., Fanindra, P. N. and Butani, D. K.1986. Insect pests of citrus in Nepal and their control. *Pestology,* **10**: 24-27.

Singh, S. P.1993. Species composition and diapause in citrus butterflies. *Journal of Insect Science,* **6**: 48-52.

Suwarno., Salmah, M. R. C., Hassan, A. A. and Norani, A. 2007. Effect of different host plants on the life cycle of *Papilio polytes* Cramer (Lepidoptera: Papilionidae) (Common mormon butterfly). *Journal of Bioscience,* **18**: 35-44.

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Studies on seasonal incidence of leaf webber, *Spoladea recurvalis* F. and *Spodoptera litura* F. on amaranthus (*Amaranthus*sp. L)

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ABSTRACT: Seasonal incidence of leaf webber, *Spoladea recurvalis* F. and *Spodoptera litura* F. on amaranthus (*Amaranthus*sp. L) was studied during 2017-18 in Tamil Nadu, India. The incidence of leaf webber was recorded throughout the season, the infestation was observed from the first week of observation (1st SMW) but a considerable larval population was recorded from the 05th SMW to the 10th SMW with the range of 1.22 to 2.31 larvae/ plant. The infestation of leaf caterpillars was observed from the 1st SMW and the incidence increased from the 5th SMW (0.15 larvae/plant) and reached a peak during the 10th SMW (1.14 larvae/plant). The fluctuation in the incidence of leaf caterpillars occurred throughout the survey. The correlation between the leaf webber incidence and weather parameters revealed that leaf webber incidence had a strong negative correlation with rainfall (-0.445**) and minimum temperature (-0.286*) and had a non-significant negative correlation with minimum temperature (-0.238^{NS}) whereas leaf webber non-significantly positively correlated with relative humidity (-0.175^{NS}). The leaf caterpillar had a significant negative correlation with maximum temperature (-0.346**), minimum temperature (-0.487**), and rainfall (-0.303*) whereas significantly positively correlated with relative humidity (0.348**).

Keywords: Amaranthus, defoliators, leafwebber, leaf caterpillar, incidence

INTRODUCTION

Amaranthus which is native to tropical America, has been widely distributed throughout the tropics and is considered a popular leafy vegetable. The Amaranthus plant's leaves are used as vegetables and its seeds are used as cereal. The amaranthus leaves are rich in calcium, phosphorus, folic acid, potassium, iron and vitamins A, B and C. Insect pests are the major limiting factor in amaranthus cultivation, insect pests *viz*., Amaranthus stem weevil- *Hypolixus truncatulus* (F.), Amaranthus leaf webber, *Spoladia recurvalis* (Fab.), Amaranthus leaf webber, *Eretmocera impactella*, *Psara basalis* F., Podfly-*Gitona distigma*, Leaf cater pillar, *Eupterote mollifera* Wlk., leaf worms (*Spodoptera* spp.), leaf rollers (*Sylepta derogota*), leaf miners (*Liriomyza* spp.), spider mites (*Tetranychus* spp.), stem boring weevils (*Hypolixus haereus*), bugs (*Asparia armigera*) were reported to cause damage to amaranthus (Okunlola *et al.,* 2008).

Among the above pests, amaranthus leaf webber and leaf caterpillar are important on the amaranthus cultivated for its leaf vegetable. It affects the market quality and consumer preference by defoliating the leaves and also the presence of larval stages on the leaves. To avoid those pests farmers spray pesticides, which may cause severe health hazards to the consumers because of the short harvesting time. Pest and pesticide usage can be reduced by many alternate methods of pest management available such as botanicals, Insect growth regulators, biocontrol, host plant resistance, and ecological engineering etc., (Arivudainambi *et al*., 2010; Manikandan and Kannan 2019 and 2020; Selvam *et al.*, 2019; Manikandan *et al.,* 2019; Ayyamperumal *et al.,* 2020).

Understanding pest population dynamics and their relationship with weather parameters is very important to make decisions on the correct method and correct time of pest management to avoid economic losses. In this view, the experiment was conducted to understand the population dynamics of major defoliators in amaranthus leaf cultivation.

MATERIALS AND METHODS

Studies were conducted in a farmer's field in the Vallampadugai village, Kumaratchi block of the Cuddalore of Tamil Nadu during 2017 and 2018. The field area was one acre and the local cultivar of amaranthus was cultivated. There was no pesticide sprayed throughout the survey period. Observations were recorded during the morning hours (8.30 - 9.30 am). Twenty five random plants per location representing North, South, East and West of the amaranthus field were selected and examined for pest incidence. The incidence of leaf webber, *S. recurvalis* was recorded by counting the number of larvae present both on the leaves and within the webbed

Table 1. Seasonal incidence of major defoliators of amaranthus concerning weather parameters in "*Rabi* 2017and 2018"

SMW	Temperature (°c)		Relative Humidity (%)	Rainfall (mm)	No.of larva/ Plant	
	Maximum	Minimum			*S. recurvalis*	*S. litura*
			***Rabi* 2017**			
01	30.2	20.1	85	0.0	0.47	0.11
02	30.1	19.1	87	0.0	0.81	0.1
03	29.2	20.7	85	54.2	0.2	0.0
04	28.7	22	91	73.6	0	0.0
05	29.2	20.6	88	0.0	1.22	0.15
06	29.9	18.3	87	0.0	1.46	0.18
07	30	21.2	85	0.0	2.31	0.71
08	31.7	18.7	87	0.0	1.76	0.92
09	30.1	21.8	86	0.0	2.14	1.11
10	33.2	24.4	88	0.0	1.65	1.14
11	33.2	23.8	87	13	0.17	0.39
12	33.9	22.9	87	0.0	0.72	0.21
13	33.6	22.1	86	0.0	0.79	0.2
14	35.7	25.2	84	0.0	0.46	0.08
15	35.8	28.6	84	0.0	0.62	0.0
16	38.3	26.4	80	0.0	0.23	0.0
17	39.2	26.9	79	0.0	0.12	0.0
			***Rabi* 2018**			
01	29.3	20.2	89	0.0	1.43	0.09
02	27.8	21.4	92	155.3	0.0	0.0
03	28.3	20.5	88	0.0	1.61	0.12
04	28.4	19.9	91	0.0	1.56	0.45
05	28.4	18.5	91	0.0	0.96	0.61
06	29.4	18.5	90	0.0	1.72	1.1
07	30.1	21.4	92	0.0	1.63	1.16
08	29.5	20.3	90	0.0	2.13	1.01
09	30.9	17.8	91	0.0	1.78	0.97
10	31.4	19	87	0.0	1.59	1.1
11	31.8	24.4	87	23.5	0.23	0.0
12	33.8	23.3	89	0.0	2.12	0.09
13	34.3	24.9	82	0.0	3.09	0.17
14	34.3	24.3	86	0.0	3.13	0.21

15	33.8	25.4	86	4.2	3.22	0.37
16	35.2	25.3	84	0.0	2.65	0.76
17	36	25.5	85	0.0	1.03	1

SMW- Standard Meteorological Week

Table 2. Seasonal incidence of major defoliators of amaranthus concerning weather parameters in "*Kharif* 2017 and 2018"

SMW	Temperature (°c)		Relative Humidity (%)	Rainfall (mm)	No.of larva/ Plant	
	Maximum	Minimum			*S. recurvalis*	*S. litura*
			***Kharif* 2017**			
27	37.6	26.4	78	0.0	0.34	0.07
28	36.7	25.3	85	1	0.94	0
29	34.3	25.2	84	0.0	1.15	0.19
30	35.8	26.1	79	3	1.24	0.26
31	37.4	25.9	81	13	0.19	0.16
32	35.7	25.6	81	0.0	1.02	0.25
33	35.1	24.8	84	50	0.0	0.0
34	32.6	24.3	89	17	0.1	0.0
35	33.2	25.4	84	7	0.67	0.0
36	33.6	26.1	83	4	0.89	0.0
37	34.1	24.9	82	6	0.62	0.0
38	31.8	25.5	82	40	0	0.0
			***Kharif* 2018**			
27	35.4	24.8	84	34.8	0.0	0.0
28	35.1	26.4	83	0.0	0.31	0.05
29	36.1	26.2	80	4	0.27	0.09
30	36.7	25.9	82	15.8	0.12	0
31	36.3	25	84	20.4	0.06	0
32	35.2	25.1	95	28.4	0.04	0
33	35.4	25.6	81	10.2	0.53	0.02
34	35.1	25.3	85	12.4	0.41	0.04
35	33.8	24.2	89	132.6	0.0	0.0
36	35	25	84	0.0	1.23	0.09
37	35.2	25.7	86	0.4	2.41	0.16
38	34	25.2	86	18.8	0.14	0

SMW- Standard Meteorological Week

leaves. To assess the leaf caterpillar, *S. litura* the number of larvae present on the leaves of the plant was recorded. All the observed data was pooled together, averaged and expressed as the number of larvae per plant. The incidence of the above pests was plotted in Randomized Block Design (RBD). The weather information was collected from the Meteorological Observation Unit, Annamalai University, Annamalainagar. The relationship between the pests and weather factors was estimated by Pearson correlation coefficient, and regression using IBM SPSS (IBM SPSS, 2022).

RESULTS AND DISCUSSION

The level of pest incidence may not be similar throughout the year. It varies from season to season in many crops (Manikandan *et al.,* 2021; Selvam *et al.,*2022). The prevailing weather factors of the season would be the major reason behind the difference in the seasonal incidence of the pest. One to a few or more weather parameters may affect the pest population by directly affecting its life cycle or by changing the constituents in the crop plants. This research investigated the seasonal incidence of amaranthus leaf webber *Spoladea recurvalis* and *Spodoptera litura* on amaranthus and the role of weather parameters on their incidence.

The result of the observations recorded during the *rabi* 2017 showed that the incidence of leaf webber occurred throughout the season,from the first week of observation (1stSMW) but a considerable larval population was recorded from the 05th SMW to the 10th SMW with the range of 1.22 to 2.31larvae/ plant. A sudden reduction in larval incidence was observed in the 2nd, 3rd and 11th SMW which received rainfall of 54.2, 73.6 and 13.00 mm respectively.A similar trend was observed in the incidence of leaf webber during *rabi* 2018 with the larval population ranging from 0.00 to 3.22 larvae/ plant and considerable larval incidence recorded throughout the season(Table 1). During *Kharif* 2017 leaf webber incidence was observed almost throughout the season, but considerable incidence was observed on three observation days. Incidence ranges between 0.00 to 1.24 larvae per plant. A similar trend was observed in *Kharif* 2018 with the population ranging between 0.00 to 2.41 larvae per plant (Table 2). In overall observation incidence of *S. recurvalis* occurs in both seasons. These findings are supported by Othim *et al.* (2018a) who also reported the incidence of amaranthus leaf webber in both seasons. Findings made conformity with the findings of Aderolu *et al.* (2013) that the presence of *S. recurvalis* in both the rabi and Kharif season in Nigeria.

The correlation between the leaf webber incidence and weather parameters during the *Rabi* 2017 and 2018 revealed that the relative humidity (0.341*) had a significant strong positive correlation, and maximum temperature (0.837**) whereas the rainfall (-0.595**) had a significant negative correlation. The non-significant negative correlation of the larval incidence was recorded with maximum temperature (-0.014^{NS}) and minimum temperature (-0.062^{NS}). All the weather parameters during *Kharif* 2017 and 2018 had a non-significant relationship with leaf webber incidence. Though the weather parameters had positive and negative correlation occurrence of leaf webber was noted throughout the year which might be due to the adaptivity of *S. recurvalis* towards different climate conditions (Aderolu *et al.,* 2013).

The infestation of leaf caterpillars was observed from the 1stand the incidence increased from the 5th SMW (0.15 larvae/plant) and reached a peak during the 10th SMW (1.14 larvae/plant). The fluctuation in the incidence of leaf caterpillars, though the infestation was observed on twelve observation days a considerable level (above 1 larvae/ plant) of incidence was observed only on two observation days. Similarly, *Rabi* 2018 data also showed the population fluctuation of leaf caterpillars, with a considerable level of incidence observed only on the 06th, 7th, 8th, 10th and 17th SMW 1.1, 1.16, 1.01, 1.1 and 1.0 larval/ plant respectively. The data on leaf caterpillar incidence during *Kharif* 2017 and 2018 revealed the lower incidence of leaf caterpillars on amaranthus. Compared to *rabi* the incidence was very low. The larval population ranges between 0.0 to 0.26 and 0.0 to 0.09 per plant during *Kharif* 2017 and 2018 respectively. The result is supported by the findings of Maninkandan and Selvanarayanan (2020) who also reported a lower incidence of *S. litura* during the *Kharif* season.

The overall observation of the incidence of *S. recurvalis* and *S. litura* revealed that the amaranthus leaf webber, *S. recurvalis* incidence was more and notably destructive on amaranthus compared to leaf caterpillar *S. litura*. Othim *et al.* (2018b) studied the defoliators of amaranthus and reported that leaf webber, *S. recurvalis* was the major damage-causing pest than the *S. litura*.

The correlation between the leaf caterpillar incidence and weather parameters during the *Rabi* 2017 and 2018 revealed that the relative humidity (0.197^{NS}) had a non-significant positive correlation whereas the rainfall (-0.363*) had a significant negative correlation. The non-significant negative correlation of the larval incidence was recorded with maximum temperature (-0.150^{NS})

Table 3. Correlation of major defoliators of amaranthus concerning weather parameters

Weather parameters	Coefficient of correlation (r) for pest population with parameters in the season					
	Rabi		*Kharif*		*Over all seasons*	
	S. recurvalis	*S. litura*	*S. recurvalis*	*S. litura*	*S. recurvalis*	*S. litura*
Maximum Temperature	-0.014^{NS}	-0.150^{NS}	0.060^{NS}	0.321^{NS}	-0.238^{NS}	-0.346**
Minimum Temperature	-0.062^{NS}	-0.304^{NS}	0.252^{NS}	0.392^{NS}	-0.286*	-0.487**
Relative Humidity	0.341*	0.197^{NS}	-0.239^{NS}	-0.344^{NS}	0.175^{NS}	0.348**
Rainfall	-0.595**	-0.363*	-0.118^{NS}	0.043^{NS}	-0.445**	-0.303*

* -Significant at 0.05%, ** -Significant at 0.01% level of probability, ^{NS}- Non Significant

Table 4. Stepwise linear regression of major defoliators of amaranthus and weather parameters

Pest	Regression model	Regression coefficient (R^2)
	Rabi	
S. recurvalis (Y1)	-2.01-0.08x_1+0.080+0.05 x_3 -0.02x_4	0.235
S. litura(Y2)	-6.69+0.06x_1-0.05 x_2- 0.07x_3 -0.01x_4	0.308
	Kharif	
S. recurvalis (Y1)	0.789-0.03x_1+0.02 x_2+0.01x_3 -0.01x_4	0.236
S. litura(Y2)	-0.10+0.01x_1+0.01 x_2-0.004x_3 -0.001x_4	0.235
	Over the seasons	
S. recurvalis (Y1)	0.284 -0.08x_1+0.01 x_2+0.04x_3 -0.02x_4	0.317
S. litura(Y2)	-1.04+0.02x_1-0.06 x_2+0.03x_3 -0.004x_4	0.358

X_1= Maximum Temperature, X_2= Minimum Temperature, X_3= Relative Humidity, X_4= Rainfall

and minimum temperature (-0.304^{NS}). All the weather parameters during *Kharif* 2017 and 2018 had a non-significant relationship with leaf caterpillar incidence.

The relationship between the weather factors and major defoliators over the seasons revealed that leaf webber incidence had a strong negative correlation with rainfall (-0.445**) and minimum temperature (-0.286*) and had a non-significant negative correlation with minimum temperature (-0.238^{NS}) whereas leaf webber non-significantly positively correlated with relative humidity (-0.175^{NS}). The leaf caterpillar had a significant negative correlation with maximum temperature(-0.346**), minimum temperature(-0.487**), and rainfall (-0.303*) whereas significantly positively correlated with relative humidity (0.348**). Aswal and Bisht (2017) also reported a negative relationship between rainfall and *S. recurvalis* and a positive relationship with relative humidity. Manikandan *et al.* (2021) studied the incidence of some lepidopteran pests of mango and revealed that leaf webber, shoot borer and leaf caterpillars had a negative correlation with rainfall, and maximum and minimum temperature whereas pests had a positive relationship with relative humidity.

Regression analysis on the influence of weather parameters *viz.,* maximum temperature, minimum temperature, relative humidity and rainfall during *Rabi* on the leaf webber and leaf caterpillar *viz.,* 23.5 per cent (R2=0.235), 30.8 per cent (R2=0.308). Altogether the influence of weather parameters on leaf webber and leaf caterpillar during *Kharif* extent to 23.6 per cent (R2=0.236) and 23.5 per cent (R2=0.235). The regression equation developed for the major defoliators of amaranthus leaf webber and leaf caterpillar with relevant to the weather parameters for the *Rabi* and

Kharif seasons (Table 4). These results revealed the less per cent influence of weather parameters on the incidence of *S. recurvalis* and *S. litura.* These findings were supported by Othim *et al.* (2018a and 2018b) and Aderolu *et al.* (2013) who reported the less influence of climatic factors on the incidence of *S. recurvalis* and *S. litura*on amaranthus.

REFERENCES

Aderolu, I. A., Omooloye, A. A. and Okelana, F. A. 2013. Occurrence, abundance and control of the major insect pests associated with amaranths in Ibadan, Nigeria. *Entomology Ornithol Herpetol*, **2** (112): 2161-0983.

Arivudainambi, S., Selvamuthukumaran, T. and Baskaran, P. 2010. Efficacy of herbal extracts in management of amaranth leaf caterpillar. *International Journal of Vegetable Science*, **16** (2): 167-173.

Aswal, J. S. and Bisht, B. S. 2017. Population dynamics of *Hymenia recurvalis* (Fab.) (Amaranth leaf webber) in Garhwal Hills of Uttarakhand. *Indian Journal of Hill Farming,* **30**: 242-250.

AVRDC. 2003. Progress Report. Asian vegetable Research and Development centre, Shanhua, Taiwan, 80-90.

Manikandan, P. and Kannan, K. 2020. Evaluation of Novaluron 10 EC against Amaranthus leaf caterpillar *Spoladea recurvalis* (Fab.) for their feeding, growth and development. *Pestology,* **44**: 29-33.

Manikandan, P. and Kannan, R. 2019. Efficacy of an insect growth regulator on Amaranthus leaf caterpillar *Spoladea recurvalis* (Fab.) (Lepidoptera: Crambidae). *Pest Management Horticultural Ecosystem*, **24**: 241-246.

Manikandan, P. and Selvanarayanan, V. 2020. Reaction of groundnut germplasm against leaf caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). *Plantarchives*, **20:** 3003-3006.

Manikandan, P., Saravanaraman, M., Suguna, K. and Selvam, K. 2019. Host Plant Resistance: A Fundamental Approach to Pest Management. In: Recent Trends in Insect Pest Management, Raju, S.V.S. and Sharma, K. R. (Eds.). *AkiNik Publication*, New Delhi, 85-121.

Manikandan, P., Suguna, K. and Saravanaraman, M. 2021. Population dynamics of defoliating insect pests of mango in the coastal agroecosystem of Tamil Nadu. *Pest Management in Horticultural Ecosystem*, **27**: 196-200.

Okunlola, A. I., Ofuya, T. I. and Aladesanwa, R. D. 2008. Efficacy of plant extracts on major insect pests of selected leaf vegetables in south western Nigeria. *Nigeria Agriculture Journal*, **3**:181-184.

Othim, S. T. O., Kahuthia-Gathu, R., Akutse, K. S., Foba, C. N. and Fiaboe, K. K. M. 2018a. Seasonal occurrence of amaranth Lepidopteran defoliators and effect of attractants and amaranth lines in their management. *Journal of Applied Entomology,***142:** 447-456.

Othim, S. T. O., Srinivasan, R., Kahuthia-Gathu, R., Dubois, T., Dinssa, F. F., Ekesi, S. and Fiaboe, K. K. M. 2018. Screening for resistance against major lepidopteran and stem weevil pests of amaranth in Tanzania. *Euphytica*, **214** (10):182.

Selvam, K., Shiva Narendran and Manikandan, P. 2019. Ecological Engineering: A Sustainable Tool for Insect Pest Management. In: Recent Trends in Insect Pest Management, Raju, S.V.S. and Sharma, K.R. (Eds.). *AkiNik Publication*, New Delhi, 21-53.

Selvam, K., Shiva, N., Manikandan, P., Archunan, K. and Saravanaraman, M. 2022. Studies on seasonal incidence and diversity of major pests in black gram under rainfed conditions. *Journal of Entomology Research,* **46**: 300-305.

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Assessment of various pollen substitutes for sustaining brood development in *Apis cerana himalaya* Fabricius during the dearth period in Manipur conditions

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ABSTRACT: Five pollen substitutes were evaluated during 2022 for their effect on brood development of *Apis cerana himalaya* at Central Agricultural University, Imphal, Manipur, India. Six flours *viz*., green gram flour, soybean flour, wheat flour, rice bean flour, black gram flour and maize flour were chosen as main substrates of pollen substitutes. Five different treatments were prepared by mixing the flours with yeast and either honey solution or sugar solution. These diets were fed to *A. cerana himalaya* colonies in the form of patties on top bars during dearth period (June to August). Brood area development was observed the most in the colonies given T_3 (Rice bean flour (30%) + Yeast (30%) + Honey (40%)) with 777.66 cm^2 of brood area. The colonies given with T_5 (Maize flour (50%) + Yeast (20%) +Sugar (30%)) showed 649.13 cm^2 brood area followed by T_4(Black gram flour (50%) +Yeast (20%) +Honey (30%)) with 553.60 cm^2, T_2(Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)) showed 427.40 cm^2 and T_1 (Green gram flour (50%) + Yeast (20%) + Honey (30%)) showed 384.13 cm^2 of brood area. T_6 was control *i.e*., the colonies were only given with sugar solution which showed the least brood area as 333.93 cm^2. All the diet was found to be better as compared to control.

Keywords: *Apis cerana himalaya*, pollen substitute, brood, rice bean flour, maize flour, green gram flour, black gram flour, soybean flour, wheat flour

INTRODUCTION

Apiculture involves the controlled management of diverse honey bee species within artificial hives. Honey bees valuable not only for producing honey, beeswax, pollen, and propolis, but also for their exceptional ability to effect cross pollination in several crops of economic importance. Apiculture being closely associated with agriculture, plays a vital role in sustaining ecosystems. In beekeeping colony maintenance during dearth pesriods when adequate floral resources are not available is challenging. Scarce food resources exacerbate the decline in colony vigour. The only nutrition on which the honey bees depend are pollen and nectar from the flowers of different plants. Nectar is a carbohydrate source, while pollen supplies the bees with the proteins, lipids, vitamins, and minerals needed to rear larvae (DeGroot, 1953; Manning, 2001). The abundant availability of food source can be seen in honey flow season, whereas during dearth period there is a scarcity of food source which effect the strength of the colony.

To avoid this situation efforts should be made to formulate highly nutritional and palatable pollen substitutes for the colonies to overcome unfavourable conditions so as to strengthen the beekeeping. The necessity to formulate artificial diets for the honey bees has been undertaken by many researchers (De-Grandi *et al*. 2008; Saffari *et al*. 2010a, b; Sihag and Gupta, 2011; Morias *et al*. 2013; Gemeda, 2014; Kumar and Agrawal, 2014; Pande *et al*. 2015; Shehata, 2016; Abd el-Wahab *et al*. 2016).

Apis cerana himalaya, a subspecies of the Asian honeybee, is found in the northeastern region of India and plays a crucial role in the ecosystem. This subspecies exhibits unique adaptations to the local climate and is well-suited for beekeeping in the region (Thakur *et al*. 2012). Limited studies have been conducted on *Apis cerana himalaya* in comparison to other honeybee species. To fully understand its ecology, behaviour, and potential for sustainable development in the northeastern hill region of India, more research is necessary.

The current research work was to compare the efficacies of five different diets along with control for the brood development of *A. cerana himalya* colonies.

MATERIALS AND METHODS

Studies were conducted with the colonies of *A. cerana himalaya* during June to September,2022, considered a dearth period in Manipur. The Apiary maintained by Department of Entomology, College of Agriculture, Central Agricultural University, Imphal, Manipur. The experiment consisted of five treatments (diets), each with three replications, along with a control. Each diet

(Fig.3) weighing about 10 g was prepared freshly and provided to bee colonies at three days interval. The diets were given on the top bar of hive for consumption. The initial observation was conducted 14 days after the first feeding. Subsequently, at each 14-day interval, a new comb was selected for measuring various parameters. A measuring frame, consisting of a wire grid with squares of 1 cm (Fig.2), was used for these measurements. The parameters recorded were: Sealed brood, Unsealed brood, Eggs laid area, Total Brood area and Pollen store.The collected data were tabulated and subjected to statistical analysis (ANOVA) following Completely Randomized Design (CRD).

Fig.1. Top bar feeding

Fig. 2. Measuring frame

Fig. 3. Pollen Substitutes (Diets)

RESULTS AND DISCUSSION

The results revealed that feeding pollen substitutes to the bee colonies increased the brood area much faster compared to the control colonies which was only given with sugar solution (2:1).

Effect of diet formulation on sealed brood area

The effect of various diet formulations on sealed brood area is shown in table 1 Maximum sealed brood area (336.60 cm^2) was recorded in the colonies fed with T_3 (Green gram flour (50%) + Yeast (20%) + Honey (30%)), followed by 223.30 cm^2 when colonies with fed with T_5 (Maize flour (50%) + Yeast (20%) +Sugar (30%)). Other treatments i.e., T_1, T_2, and T_4 also shows increase in the sealed brood area as 127.33 cm^2, 135.00 cm^2 and 203.33 cm^2, respectively. The least development (116.73 cm^2) was seen in the colonies taken as control which was only given with sugar solution of ratio 2:1. The above findings on sealed brood area can be compared with the work of Kumari and Kumar (2020) who reported that the colonies treated with pollen substitute containing Defatted Soy flour and Parched Gram shows 1938.3 cm^2 sealed brood in *Apis mellifera*.

Effect of diet formulation on Unsealed brood area

The data given in table 2 on effect of feeding diet formulations on Unsealed brood revealed that maximum of unsealed brood was 219.25 cm^2 when the colonies were fed with T_3 followed by 207.75 cm^2 when the colonies were given with T_5. The colonies showed increase in unsealed brood area from first feeding to the last feed. The colonies also showed increase in unsealed brood area when given with T_1, T_2 and T_4, respectively. The data can be compared with the finding of Kumar and Agrawal (2014), who reported a maximum unsealed area of 65% when given with diet containing soy flour. Pandey (2008) also studied the effect of various diets on unsealed broods and reported maximum area under unsealed brood was found to be 21.67% in treatment containing Mandua + honey + Yeast extract + Multivitamins + Pollen pellets.

Effect of diet formulation on egg area

The colonies fed with T_3 showed more area (221.26 cm^2) where eggs are laid followed by the colonies fed with T_5 showed 214.53 cm^2. The mean observation recorded for T_1, T_2 and T_4 were 133.33 cm^2, 140.53 cm^2 and 148.53 cm^2, respectively. Colonies provided with only sugar solution showed less result. However, all the colonies which were given with pollen substitute showed good result as compared to the control colonies. The data can be compared with the finding of Kumar and Agrawal (2014), who reported maximum egg laying as 76% when given with a diet containing soy flour.

Effect of diet formulation on total brood area

Total brood was calculated by adding sealed brood, unsealed brood and egg area of all the colonies. Maximum brood area was recorded as 777.66 cm^2 when the colonies were given with $_{T3}$ containing Rice bean flour whereas least brood area (333.93 cm^2) was recorded in control colonies. All other treatments showed significant increase in brood area as compared to

the control colonies. When the colonies were treatment with T_5 it showed 649.13 cm^2 brood area. T_2 and T_1 showed 427.40 cm^2 and 384.13 cm^2 of brood area. The present study is comparable with the findings of Kumar *et al.* (2013), who reported that diet 3 (defatted soy four, brewer's yeast and soy protein hydrolysate powder) proved to be most effective with 2155.3 cm^2 brood area, 5.8 total bee covered frames and 11509 bee population. A similar study was again conducted by Kumar *et al.* (2013), who reported that the maximum (peak) amount of sealed brood area was observed in the colonies given diet 3 (723.4 cm2 per colony).

Table 1. Effect of diet formulation on sealed brood area (cm^2)

Diet	Treatment	DATE					Mean
		14 June	**28 June**	**12 July**	**26 July**	**9 Sep**	
T_1	Green gram flour (50%) + Yeast (20%) + Honey (30%)	103.33	112.00	134.33	141.33	148.33	127.33
T_2	Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)	108.33	122.33	140.00	152.33	163.33	135.00
T_3	Rice bean flour (30%) + Yeast (30%) + Honey (40%)	311.00	327.00	345.00	346.66	352.66	336.60
T_4	Black gram flour (50%) +Yeast (20%) +Honey (30%)	128.00	184.33	223.33	236.00	244.33	203.33
T_5	Maize flour (50%) + Yeast (20%) +Sugar (30%)	185.00	208.00	229.00	242.33	251.33	223.3
T_6	Sugar solution (2:1)	46.66	109.33	131.00	137.66	146.00	116.73
	SE(m)	2.47	2.12	2.45	2.17	2.50	0.79
	$CD_{(0.05)}$	7.70	6.61	7.62	6.75	7.79	2.47

Table 2. Effect of diet formulation on Unsealed brood area(cm^2)

Diet	Treatment	Day after treatment					Mean
		14 June	**28 June**	**12 July**	**26 July**	**9 sep**	
T_1	Green gram flour (50%) + Yeast (20%) + Honey (30%)	75.33	111.33	121.66	143.66	164	119.62
T_2	Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)	117.33	132.00	152.00	164.66	187	150.83
T_3	Rice bean flour (30%) + Yeast (30%) + Honey (40%)	187.33	192.00	208.00	245.33	266.33	219.25
T_4	Black gram flour (50%) +Yeast (20%) +Honey (30%)	160.33	182.00	195.00	225.33	246	203.73
T_5	Maize flour (50%) + Yeast (20%) +Sugar (30%)	179.00	187.33	202.00	231.33	257.33	207.75
T_6	Sugar solution (2:1)	47.33	96.66	119.33	129.33	136	109.60
	SE(m)	1.55	1.73	1.92	2.05	2.43	2.01
	$CD_{(0.05)}$	4.85	5.39	5.99	6.40	7.58	6.28

Table 3. Effect of diet formulation on Egg-laid area(cm^2)

Diet	Treatment	Day after treatment					Mean
		14 June	**28 June**	**12 July**	**26 July**	**9 Sep**	
T_1	Green gram flour (50%) + Yeast (20%) + Honey (30%)	110.00	134.00	136.00	138.00	148.66	133.33
T_2	Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)	112.33	136.66	146.66	152.00	155.00	140.53
T_3	Rice bean flour (30%) + Yeast (30%) + Honey (40%)	199.66	210.33	214.00	233.66	248.66	221.26
T_4	Black gram flour (50%) +Yeast (20%) +Honey (30%)	113.33	144.00	149.33	166.33	169.66	148.53
T_5	Maize flour (50%) + Yeast (20%) +Sugar (30%)	197.66	200.00	203.66	226.66	244.66	214.53
T_6	Sugar solution (2:1)	98.00	110.00	11.66	118.66	125.66	112.8
	SE(m)	4.23	3.07	3.26	2.96	2.92	3.14
	$CD_{(0.05)}$	13.20	9.56	10.18	9.24	9.10	9.81

Table 4. Effect of diet formulation on Total Brood area(cm^2)

Diet	Treatment	Day after Treatment					Mean
		14 June	**28 June**	**12 July**	**26 July**	**9 Sep**	
T_1	Green gram flour (50%) + Yeast (20%) + Honey (30%)	288.66	356.00	392.00	423.00	461.00	384.13
T_2	Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)	338.00	386.00	438.66	469.00	505.33	427.40
T_3	Rice bean flour (30%) + Yeast (30%) + Honey (40%)	698.66	729.33	767.00	825.66	867.66	777.66
T_4	Black gram flour (50%) +Yeast (20%) +Honey (30%)	402.33	510.33	567.66	627.66	660.00	553.6
T_5	Maize flour (50%) + Yeast (20%) +Sugar (30%)	562.00	595.33	634.66	700.33	753.33	649.13
T_6	Sugar solution (2:1)	192.00	322.33	362.00	385.66	407.66	333.93
	SE(m)	5.44	5.26	3.44	6.42	7.57	4.77
	$CD_{(0.05)}$	16.95	16.40	10.71	20.00	23.60	14.86

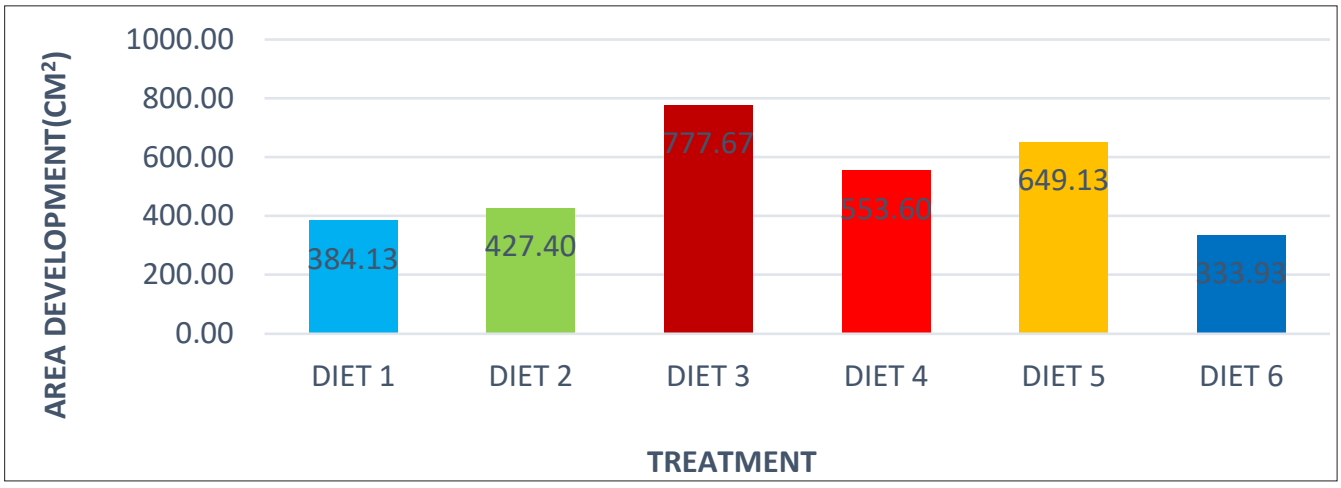

Fig. 4. Diagrammatic representation of brood area (cm²)

Table 5. Effect of diet formulation on Pollen store area (cm²)

DIET	TREATMENT	DATE					MEAN
		14 JUN	**28 JUN**	**12 JUL**	**26 JUL**	**9 SEP**	
T_1	Green gram flour (50%) + Yeast (20%) + Honey (30%)	19.50	22.00	25.00	29.00	33.00	25.70
T_2	Soybean flour (30%) + Wheat flour (30%) + Yeast (10%) + Sugar (30%)	20.66	23.00	26.00	31.00	34.00	26.66
T_3	Rice bean flour (30%) + Yeast (30%) + Honey (40%)	26.33	27.00	31.66	37.00	40.00	32.40
T_4	Black gram flour (50%) +Yeast (20%) +Honey (30%)	22.00	24.00	28.67	31.33	36.00	28.73
T_5	Maize flour (50%) + Yeast (20%) +Sugar (30%)	23.66	24.66	29.67	34.00	38.00	30.00
T_6	Sugar solution (2:1)	17.66	20.00	24.00	28.00	29.00	23.73
	SE(m)	0.69	0.64	0.75	0.64	0.58	0.68
	$CD_{(0.05)}$	2.22	1.97	2.30	1.97	1.78	2.08

Effect of diet formulation on pollen store area

Pollen is the main source of protein to honey bees, which get scarce during dearth period. The area under pollen store was measured for each colony to analysis the best treatment showing pollen store. Data collected in Table 6 and fig 6 revealed that though the pollen store area was less compared to other above parameters maximum area was seen in T_3 i.e., 32.40 cm² followed by 30.00 cm² of area under T_5. Among the other treatments T_4 showed 28.75 cm², T_2 showed 26.66 cm² and T_1 showed 25.70 cm². Only 23.73 cm² area of honey store was measured from colonies taken as control. The present data, which shows that all the pollen substitutes had pollen storage when compared with the control, is in accordance with the work of Vijayakumari et al. (2021), who reported that the colonies fed with T_4 – Roasted Bengal gram powder (30%) + Skimmed milk powder (25%) + Honey (34%) + Brewer's yeast (10%) + multivitamin (1%) had the greatest pollen storage (68.62 cm²), followed by T_1 – Bee pollen (65%) + Honey (34%) + multivitamin (1%) of about (61.62 cm²). The other pollen substitute had a smaller pollen storage than T_4 and T_1. The control colonies were observed to have the least amount of

pollen storage area compared to the other treatments (44.62cm^2).

Based on findings, it can be concluded that Diet containing Rice bean flour shows best result to increase the brood rearing activity during the dearth period. Our research shows that providing sufficient nutrition can sustain bee colonies and encourage brood rearing, even during unfavorable conditions when rearing rates are at their lowest.

REFERENCES

Abd el-Wahab, T. E., Ghania, A. M. M. and Zidan, E. W. 2016. Assessment of a new pollen supplement diet for honey bee colonies and their effect on some biological activities. *International Journal of Agricultural Technology*, **12**: 55-62.

De-Grandi, H., Waardell, G. and Ahumanda, S. F., Rinderer, T. E., Danka, R. and Pettis, J. 2008. Comparison of pollen substitute diets for honey bees: Consumption rates by colonies and effects on brood and adult populations. *Journal of Apicultural Research*, **47**: 265-270.

De Groot, A. P. 1953. Protein and amino acid requirements of the honeybee (*Apis mellifica* L.). *Physiologia Comparata et d'Ecogia*, **3**: 195-285.

Gemeda, T. K. 2014. Testing of effect of dearth period supplementary feeding of honeybee (*Apis mellifera*) on brood development and honey production. *International Journal of Advance Research*, **2**: 319-324.

Kumar, R. and Agrawal, O. P. 2014. Comparative performance of honey bee colonies fed with artificial diets in Gwalior and Panchkula region. *Journal of Entomology and Zoology Studies*, ***2***(4): 104-107.

Kumar, R., Mishra, R. C. and Agrawal, O. P. 2013. Effect of feeding artificial diets to honey bees during dearth period under Panchkula (Haryana) conditions. *Journal of Entomological Research*, **37**(1): 41-46.

Kumar, R., Rajpoot, G. S., Mishra, R. C. and Agrawal, O. P. 2013. Effect of feeding various diet formulations to honey bee colonies during dearth period under Gwalior (India) conditions. *Munis Entomology and Zoology*, **8**(1): 267-272.

Kumari, I. and Kumar, R. 2020. Pollen Substitute Diet for *Apis mellifera*: Consumption and Effects on Colony Parameters in Sub-Tropical Himalaya. *Indian Journal of Agricultural Research*, **54**(2):147-153.

Manning, R. 2001. Fatty acids in pollen: a review of their importance to honey bees. *Bee World*, **82**: 60-70.

Morais, M. M., Turcatto, A. P., Francoy, M. T., Goncalves, L. S., Cappelari, F. A. and Jong, D. D. 2013. Evaluation of inexpensive pollen substitute diets through quantification of haemolymph proteins. *Journal of Apicultural Research*, **52**: 119-121.

Pande, R., Karnatak, A. K. and Pande, N. 2015. Development of nectar supplements for dearth period management of honey bees (*Apis mellifera* L.) colonies in foothills of Shivalik range of Himalayan. *The Bioscan* **10**: 1599-1603.

Pandey, A. 2008. Studies on the evaluation of various diets as pollen substitutes for *Apis mellifera* L. and foraging behaviour of *Apis* spp. on different flowering plants. M.Sc. (Agriculture) Thesis, Submitted to G.B. Pant University of Agriculture & Technology, (Pantnagar, Uttarakhand) India.

Saffari, A., Kevan, P. J. and Atkinson, J. 2010a. Consumption of three dry pollen substitutes in commercial apiaries. *Journal of Apicultural Science*, **54**: 13-20.

Saffari, A., Kevan, P. J. and Atkinson, J. 2010b. Palatability and consumption of patty: formulated pollen and pollen substitutes and their effects on honey bee colony performance. *Journal of Apicultural Science*, **54**: 63-71.

Shehata, I. A. A. 2016. Evaluation of Carniolan and Italian honey bee colonies fed on artificial diets in dearth and flowering periods under Nasr city conditions. *International Journal of Environment*, **5**: 19-25.

Sihag, R. C. and Gupta, M. 2011. Development of an artificial pollen Substitute/supplement diet to help tide the colonies of honey bees (*Apis mellifera* L.) over the dearth season. *Journal of Apicultural Research*, **55**: 15-29.

Thakur, N. A., Firake, D. M., Behere, G. T., Firake, P. D. and Saikia, K. 2012. Biodiversity of agriculturally important insects in north eastern Himalaya: an overview. *Indian Journal of Hill Farming*, **25**(2), 37-40.

Vijayakumari, N., Saravanan, P. A., Chitra, N. and Rajendran, L. 2021. Effect of pollen substitutes on influencing the colony growth of Asiatic hive bee, *Apis cerana indica* F. (Hymenoptera: Apidae). *Ecology, Environment and Conservation*, **27**: 415-420.

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Biology of major lepidopteran pests of *Jasminum sambac* in Kerala

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ABSTRACT: The jasmine bud borer, *Hendecasis duplifascialis* and jasmine leaf webworm*, Nausinoe geometralis,* were identified as the major lepidopteran pest of *Jasminum sambac* in Kerala*.* Detailed studies on their biology revealed that *H. duplifascialis* has an egg period of 3.35 ± 0.11 days, a larval period of 11.15 ± 0.08 days, and a pupal period of 5.25 ± 0.10 days. The female moths of this species exhibit a longevity of 5.8 ± 0.10 days, while the male moths live for 3.9 ± 0.15 days. The egg, larval, and pupal period of *N. geometralis* was 3.35 + 0.11, 10.65 + 0.20, and 6.65 ± 0.11 days, respectively. The adults recorded longevity of 6.7 ± 0.15 and 4.8 ± 0.13 days, respectively, for female and male moths.

Keywords: *Hendecasis duplifascialis, nausinoe geometralis,* bud borer, leaf webworm, jasmine.

INTRODUCTION

Jasmine, known as the queen of fragrance, is extensively cultivated in India. The genus *Jasminum* comprises over 200 species, many of which are synonyms, with only ninety-two being genuinely recognized (Menninger, 1970; Abdulkhader and Kumar, 1995). The commercially significant *Jasminum* species in India is *Jasminum sambac* (L.) cultivated over 12,250 hectares, yielding 65,230 tonnes loose and 1,700 tonnes cut flowers (Pirithiraj, 2020). Jasmine cultivation is affected by approximately 50 distinctive insect species of eight insect orders (Harini *et al.,* 2018). These crops face threats from insects, mites, diseases, and nematodes. Major pests include the jasmine budworm (*Hendecasis duplifascialis* Hampson), galleryworm (*Elasmopalpus jasminophagus* Hampson), leaf webworm (*Nausinea geometralis* Guenee), leaf roller (*Glyphodes unionalis* Hubner), blossom midge (*Contarinia maculipennis* Felt), and red spider mite (*Tetranychus urticae* Koch) (David, 1958).

Despite Kerala's favorable climate and soil conditions, the potential for cultivating jasmine commercially remains largely untapped. In Kerala, *J. sambac* is primarily grown as an ornamental plant in homesteads (KAU, 2016). However, recognizing the substantial potential of jasmine as a commercial crop, entrepreneurs and self-help groups are gradually beginning to explore its commercial cultivation (Swathy, 2022). As an emerging crop in commercial agriculture, it is crucial to have basic knowledge of jasmine pests and their biology to implement effective control measures under the prevailing climatic conditions of Kerala. However, the number of studies focusing on the pests and diseases of jasmine from Kerala is lower. In this context, the current study focuses on identifying major lepidopteran pests of *J. sambac* in Kerala and their biology.

MATERIALS AND METHODS

The samples of lepidopteran pests of jasmine were collected from three locations in districts*, viz.,* Thiruvananthapuram, Kollam, and Alappuzha of Kerala, for documentation, and their percentage incidence was calculated from 20 randomly selected jasmine plants from each di.

Biology of major lepidopteran pests

The study on the biology of major lepidopteran pests identified during documentation was conducted at the Department of Agricultural Entomology, Vellayani.

The initial culture of larvae of jasmine budworm, *Hendecasis duplifascialis* and jasmine leaf webworm, *Nausinoe geometralis* (Guenee) was collected from the field. The larva was kept in rearing bottles with young tender leaves and buds. Emerged adults were transferred to separate rearing containers @ 1: 1 (male: female).

Two pairs of adults were released into each rearing bottle with tender shoots of jasmine and shoots bearing buds for *N. geometralis* and *H. duplifascialis*, respectively. The base of the *shoot* was secured with moist cotton to prevent drying. The container was closed using muslin cloth and undisturbed for mating and oviposition. A cotton ball dipped in diluted honey (5%) was provided as food (Gajera *et al*., 2012). The moths were observed at every 2 to 3h. Forty-eight hours after mating, the leaves and buds were observed for the eggs of the leaf webworm and the budworm, respectively.

The eggs were collected using a moist brush and kept for larval emergence. The egg, larval, pupal period, and adult longevity were recorded during the experiment. Observations were taken from twenty pairs.

RESULTS AND DISCUSSION

Percentage incidence

The lepidopteran pests, jasmine budworm, *Hendecasis duplifascialis* Hampson), jasmine leaf webworm, *Nausinoe geometralis* (Guenee), *Nausinoe perspectata* (Fabricius), shoot webworm, *Margaronia unionalis* Hubner), jasmine gallery worm, *Elasmopalpus jasminophagus* (Hampson) were documented from Thiruvananthapuram, Kollam, and Alappuzha districts of Kerala from twenty different locations in each district. From the Table.1 it is evident that the major lepidopteran pests infesting jasmine in Kerala are jasmine budworm and leaf webworm. Even though the infestation of shoot webworm and jasmine gallery worm was documented, their percentage of incidence was scarce.

Table 1. Percentage incidence of major lepidopteran pests in *Jasminum sambac* from Thiruvananthapuram, Kollam, and Alappuzha districts of Kerala.

Scientific name	*Percentage incidence			Average
	Thiruvananthapuram	Kollam	Alappuzha	
H. duplifascialis	90	100	90	93.33
N. geometralis	60	10	45	38.33
M. unionalis	5	5	0	3.33
E. jasminophagus	10	0	0	3.33

Table 2. Biology of jasmine borers

Parameters	*Hendecasis duplifascialis*			*Nausinoe geometralis*		
	No. of days		Mean ± SE	No. of days		Mean± SE
	Minimum	Maximum		Minimum	Maximum	
Egg period	3	4	3.35 ± 0.11	3	4	3.35 ± 0.11
Larval period	11	12	11.15 ± 0.08	9	12	10.65 ± 0.20
Pupal period	5	6	5.25 ± 0.10	6	7	6.65 ± 0.11
Adult longevity						
Male	3	4	3.9 ± 0.10	4	5	4.8 ± 0.13
Female	5	6	5.8 ± 0.13	6	7	6.7 ± 0.15
Total lifecycle						
Male	23	24	23.7 ± 0.11	24	26	25.2 ± 0.09
Female	24	27	25.56 ± 0.20	26	29	27.6 ± 0.09

Biology of major lepidopteran pests

The major lepidopteran pests identified during the initial documentation were later studied to get better insights into the biology of the pest. This was conducted at the laboratory of the College of Agriculture, Vellayani. The result of the study is represented as the duration of different stages of the budworm and leaf webworm in Table 2.

Jasmine bud borer, Hendecasis duplifascialis Hubner *(Lepidoptera: Crambidae)*

The life cycle of *H. duplifascialis* is depicted in Fig. 1.The egg of *H. duplifascialis* was round, creamy white, and eggs laid on flower stems and closed buds. The egg period was 3.35± 0.11 days, whereas the larval period extended to 11.15±0.08 days with five instars. The first two instars were creamy white with a dark brown

head and prothoracic shield. The third instar resembled the initial instars but differed in their size. The fourth and fifth instar larvae were yellowish green and green, respectively.

In the pre-pupal stage, the larva stopped feeding and crawled to the lower part of the container for pupation. The pupa was dark brown and oblong, with a pupal period of 5.25 ±0.10 days. The adults were small, creamy white with well-developed scaly proboscis and wavy wing and abdomen markings. The longevity of female and male moths was 5.8 ± 0.13 and 3.9 ± 0.15 days, respectively. The total lifecycle (egg to death of adult) was longer for females, with 25.56+ 0.20 days, whereas the males had 23.7± 0.20 days.

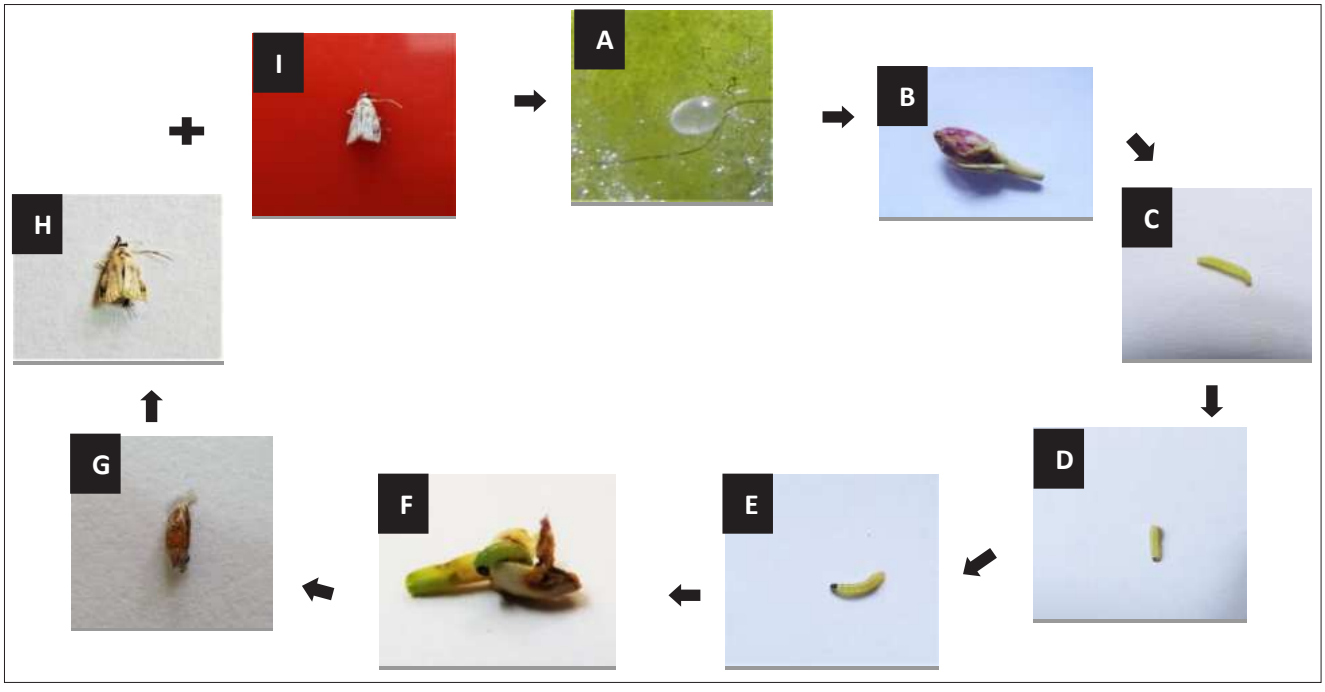

Fig.1. Life cycle of jasmine budworm *Hendecasis duplifascialis:* A: Egg; B: 1st insatr; C: 2nd instar; D: 3rd instar; E: 4th instar; F: 5th instar; G: Pupal stage; H: Female moth; I: Male moth

Similar findings were observed in the studies conducted by Chaitanya and Kumar (2018) on the biology of budworm *H. duplifascialis*. They found that the incubation period, total larval, and pupal period were 3.40 ± 0.23, 11.60 ± 1.30, and 5.80 ± 0.19 days, respectively. The longevity of male and female moths was 3.10 ± 0.23 and 4.10 ± 0.31 days, respectively. Atwal and Dhaliwal (2002) studied the biology of jasmine budworm. They reported that their egg, larval, and pupal periods lasted for 3 to 5 days, 11 to 17 days, and 6 to 8 days, respectively, with an adult longevity of 2 to 3 days. Thus, the total lifecycle was recorded as 20 to 23 days. Muthukrishnan *et al.* (2005) described the larvae of budworms as green with prominent dark brown or black colored heads, and adults were small moths with white and black wavy margins on the hind wings, similar to our findings.

Jasmine Leaf Webworm Nausinoe geometralis (Gunee) *(Lepidoptera: Crambidae)*

The life cycle of *N. geometralis* is depicted in the Fig. 2. The egg was laid singly or in small groups on the under surface of the leaf lamina. The freshly laid egg was small, oblong, and translucent, which turned to greenish yellow in later stages. The egg period lasted for 3.35 ± 0.11 days. The neonate larva was yellowish-white with a reddish-yellow head. The head was less broad than the body. They were found in groups on the lower surface of webbed leaves. The second and third instar had small hairs on their yellowish-green body with black dots on the lateral sides. The brown head capsule and body hairs were more prominent and visible in the third instar larva. The fourth instar larva was green with dark brown or black head capsule. The black dots on the prothorax were more prominent, with two triangular markings, whereas the black spots on the dorsal lateral line faded towards the abdomen. The fully grown fifth instar larva had four rows of black spots on the thoracic region. The larval period was 10.65 ± 0.20 days with five instars.

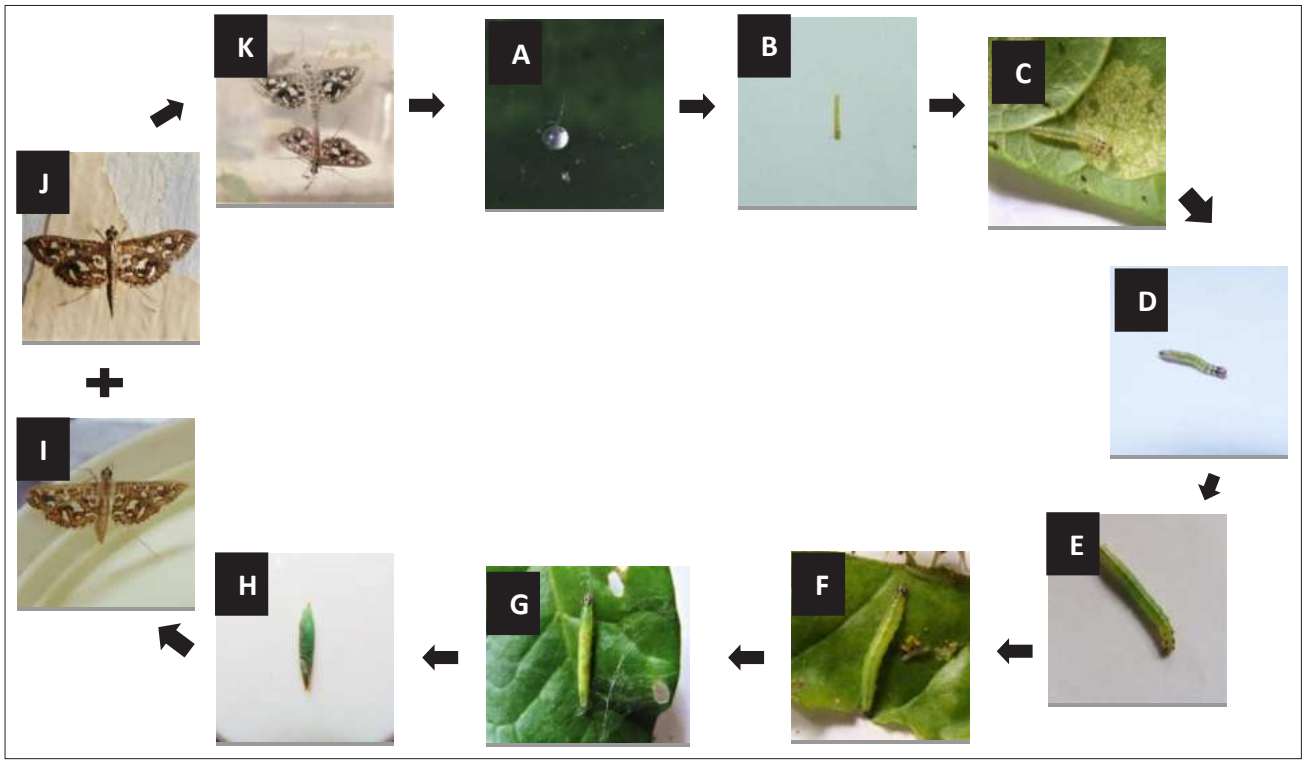

Fig.2. Life cycle of jasmine leaf webworm *Nausinoegeometralis*: A: Egg; B: 1st instar; C: 2nd instar; D: 3rd instar; E: 4th instar; F: 5th instar; G: prepupal stage; H: Pupal stage; I: Female moth; J: Male moth; K: Mating.

In the pupal stage, the larva stopped feeding. In later stages, the size of the larva was gradually reduced and sluggish, and their color changed to a yellowish-green color. The larva pupated within the web on a silken thread. The pupa was green, spindle-shaped, with tapering ends. However, the color gradually turned brown in later stages. The pupal period was 6.65 ± 0.11 days.

The newly emerged adult moth was medium-sized with a pale brown body tapered towards the anal region. The wings were brown with irregular white spots and small wavy margins. The abdomen was brown with white markings. The female moth was slightly larger than the male moth. The longevity of female and male moths was 6.7 ± 0.15, 4.8 ±0.13 days, respectively. The total life cycle (egg to death of adult) was longer for females, with 27.6 ± 0.09 days, whereas the males had 25.2± 0.09 days.

Fewer studies regarding the biology of leaf webworms than jasmine budworms. Gajera *et al.* (2012) reported the average larval period and pupal period of *N. geometralis* as 9.98 ± 0.84 days and 7.84 ± 0.37 days, respectively, concurrent with our findings. The total lifecycle was reported to be complete in 26.62 + 2.57 days and 28.90 + 2.11 days for males and females, respectively, similar to the current findings.

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REFERENCES

Abdulkhader, J. B. and Kumar, N. 1995. Genetic resources of jasmine. In Advances in Horticulture, Ornamental plants Eds. Chadha, K. L. and Bhattacharjee, S. K., Malhotra Publishing House, New Delhi, 121- 132.

Atwal, A. S. and Dhaliwal, G. S. 2002. Agricultural pests of South Asia and their management. Kalyani Publishers, Ludhiana.

Chaitanya, T. K. and Kumar, K. 2018. Biological study of jasmine bud worm, *Hendecasis duplifascialis* Hampsn. (Pyraustidae: Lepidoptera) from India. *International Journal of Current Microbiology and Applied Sciences,* **7**: 2884-2888.

David, S. K. 1958. Insects and mites affecting jasmine in Madras state. *Madras Agricultural Journal,* **45**:146-150.

Gajera, D. K., Patel, K. A., Patel, R. S., Toke, N. R. and Patil, K. S. 2012. Biology of leaf webworm, *Nausinoe geometralis* (Guenee) on jasmine under laboratory conditions. *International Journal of Plant Protection,* **5**: 120-123.

Harini, K., Elanchezhyan, K., Murugesan, N., Allwin, L. and Prabhu, T. 2018. Seasonal incidence and management of budworm, *Hendecasis duplifascialis* (Hampson) in *Jasminum sambac* L. *International Journal of Advances in Agricultural Science and Technology,* **5**: 42-51.

KAU (Kerala Agricultural University). 2016. Package of practices recommendations: Crops (15th Ed.). Kerala Agricultural University, Thrissur.

Menninger, E. A. 1970. Flowering vines of the world: An encyclopedia of climbing plants. Heart side Press Inc. New York.

Muthukrishnan, N., Ganapathy, N., Nalini, P. and Ragendran, R. 2005. Pest management in horticultural crops. New Madura publishers, Madurai.

Pirithiraj, U., Soundararajan, R. P., Justin, C. G. L. and Lakshmanan, V. 2020. *Journal of Entomology and Zoology Studies,* **8**: 680-684.

Swathy, I. M. 2022. Documentation and management of pests of jasmine, *Jasminum* spp, M. Sc. Thesis, Department of Agricultural Entomology, College of Agriculture, Vellayani.

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Levels of insecticide resistance in *Phthorimaea absoluta* (Meyrick) populations collected from South India

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ABSTRACT: *Phthorimaea absoluta* (Meyrick) has become a serious threat to global tomato production. Management of an invasive pest mainly relies on insecticides because of immediate effect. In this study, susceptibility of second instar larvae of *P. absoluta* collected from different locations of south India to seven insecticides belonging to different chemical groups was evaluated to determine the lethal concentration (LC) values. Leaf dip bioassay method revealed that the LC_{50} values in *P. absoluta* varied to different tested insecticides among different populations collected from south India. The median lethal concentrations of all the insecticides against *P. absoluta* ranged from 1.4 to 106.0 ppm. Among tested insecticides, chlorantraniliprole showed more toxicity to second instar larvae of *P. absoluta* which recorded lowest LC_{50} values, followed by cyantraniliprole, spinosad, indoxacarb, flubendiamide and the least susceptibility of *P. absoluta* larvae were recorded to neonicotinoids (acetamiprid and imidacloprid). Regarding resistance ratio, maximum of 7.8-fold resistance to acetamiprid was recorded when compared to remaining six insecticides.

Keywords: *Phthorimaea absoluta*, median lethal concentration, chlorantraniliprole, cyantraniliprole, spinosad, indoxacarb, flubendiamide, acetamiprid, imidacloprid

INTRODUCTION

Insect pest invasions have been rapidly increasing worldwide and with increased movement of people and goods from one country to another, there are high chances for increased numbers of invasive species conquering many regions (Pimentel *et al.,* 2001). These invasive agricultural pest species are widely recognized as a major threat to agro-ecosystems and agricultural production. One such invasive species that have been wreaking havoc to tomato crop worldwide is tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) which is also commonly called as tomato borer or tomato pinworm (Campos *et al.,* 2014a). *T. absoluta* native to South America and was first described in Peru in 1917 as *Phthorimaea absoluta* (Meyrick, 1917) (Desneux *et al.,* 2010). This has other synonyms: *Scrobipalpuloides absoluta* (Povolny, 1987), *Scrobipalpula absoluta* (Povolny, 1964) and (EPPO 2005). *Tuta absoluta* again was renamed as *Phthorimaea absoluta* based on cladistic analysis of morphology (Chang and Metz, 2021)

Phthorimaea absoluta has become a serious threat to global tomato production. In India, incidence of this pest was first recorded at Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bengaluru (Sridhar *et al.,* 2014) Maharashtra (Shashank *et al.,* 2015) and Malnad region of Karnataka (Kalleshwaraswamy *et. al.,* 2015). Its invasion to India has led to major economic impact on tomato production as its infestation starts from nursery stage of the crop itself and when the infestation pressure is high, besides attacking vegetative stage of the crop, infestation is observed both on developing and mature fruits. Due to its distinctive feeding behavior (larvae mine in the leaf mesophyll forming irregular, transparent mines) makes it a challenging insect pest to manage (Kandil *et al.,* 2020).

While managing invasive pests, insecticides are often the first choice. Though, insecticides are one of the important components in IPM program, indiscriminate and continuous use of same insecticides could lead to development of resistance in insect pests and may also become harmful to the natural enemies of insect pest. Hence, there is a need to generate information on resistance levels of an invasive pest, *P. absoluta* over a broad geographical range of population, which provides information regarding natural variation in identifying those insecticides where resistance is more likely to develop (Kumar *et al.,* 2020).and also will be helpful in developing Insecticide Resistance Management (IRM) strategies. Therefore, the present study was carried out to know the level of resistance to commonly used insecticides *viz.,* chlorantraniliprole, cyantraniliprole, flubendiamide, spinosad, indoxacarb, acetamiprid and

imidacloprid which were tested against field collected population of *P. absoluta* from Karnataka, Tamil Nadu, Maharashtra and Andhra Pradesh.

MATERIALS AND METHODS

Insecticides

Commercial formulations of regularly used insecticides for the management of tomato leafminer belonging to different chemical groups with various modes of action as defined by the Insecticide Resistance Action Committee (IRAC) were selected and purchased from local market for bioassay studies *viz.,* chlorantraniliprole (coragen 18.5 SC, DuPont India Ltd. Hyderabad), cyantraniliprole (benevia 10.26 OD, DuPont India Ltd. Hyderabad), flubendiamide (fame 39.35 SC, Bayer crop science Mumbai), indoxacarb (kingdox 14.5 SC, Gharda chemicals Ltd. Mumbai), spinosad (tracer 45 SC, Dow Agro Science India Ltd. Mumbai), acetamiprid (pride 20 SP, Syngenta India Ltd. Mumbai) and imidacloprid (confidor 17.8 SL, Bayer crop science Mumbai) were used in bioassay studies.

Maintenance of insect culture and host plant in the laboratory

Insect population

Population for this study were collected from ten districts representing four states of South India *viz.,* Karnataka (Davanagere, Chamarajnagar, Chikkamagaluru and Raichur), Maharashtra (Kolhapur and Solapur), Tamil Nadu (Coimbatore and Madurai) and Andhra Pradesh (Chittoor and Kurnool). From each sampling site, tomato leaves infested with different instars of *P. absoluta* larvae were collected into large plastic bags (45 cm X 30 cm). Approximately 100-200 larvae were collected from each site. The samples were transferred into a plastic box and covered with muslin cloth to avoid stress on insects. The plastic boxes containing the infested leaves with larvae were opened in insect-proof rearing cages containing fresh tomato seedlings, so that larvae could resume normal development. The rearing cages were maintained at 25 ± 2.5 °C, 65% RH and 16:8 h light: dark photoperiod. The second instar larvae of F_1 generation were used for insecticidal bioassay and the experiment was carried out at Toxicology Laboratory, Department of Agricultural Entomology, College of Agriculture, Shivamogga.

Rearing of *P. absoluta* in laboratory

The field collected *P. absoluta* populations were maintained and reared on healthy tomato leaves separately in boxes with 25 ± 2.5 °C room temperature and 65 ± 5% relative humidity, fully mined leaves were replaced with fresh tomato leaves to the larvae until pupation. The pupae were collected from the tray and placed them in insect breeding boxes and the box was kept in adult emergence cage. Newly emerged adults were provided with 10 per cent honey solution with cotton swab. Thirty-day old tomato seedlings grown in small pots were kept in the adult emergence cage for oviposition and number of eggs laid were observed visually. If an adequate number of eggs were observed (*i.e.,* more than 150–200 eggs/plant), then the plant was carefully removed and new plants were placed in the oviposition area to allow continuation of the oviposition. The seedlings with eggs were kept in separate cages and observed for hatching. The hatched larvae maintained by providing fresh seedlings and the culture was maintained continuously in laboratory and insect proof net house without any insecticide application. Field populations from different locations were collected from unsprayed fields and reared separately for one generation and F_1 populations were used for toxicity studies. Second instar larvae were used for laboratory experiments.

Host Plant material

Tomato plants (JKTH 811- hybrid widely cultivated in university jurisdiction) were maintained in pest free insect-proof cages under laboratory conditions and in polyhouse. No insecticides were used during the plant development phase.

Bioassay method

Laboratory bioassay of insecticides on *P. absoluta* was carried out as per the IRAC test method No. 022. Median lethal concentrations (LC_{50} and LC_{90} values) for these insecticides were estimated through leaf dip bioassay method. Accurate dilutions of the different insecticides from the identified commercial product were prepared. Prior to bioassays, bracketing was done for every insecticide to fix concentrations causing approximately 10 to 90 per cent mortality of *P. absoluta* larvae. Initially stock solutions of maximum concentration were prepared and serially diluted to obtain desired concentrations. Bioassay for every insecticide was conducted at five concentrations in geometric progression and the experiment was conducted under laboratory conditions. Each insecticide concentration was replicated thrice. The use of a wetter/spreader (non-ionic adjuvant) is highly recommended in order to obtain optimal leaf coverage and hence in all cases, the selected surfactant triton X 100 agent was used in the study.

Tender young tomato leaves of uniform size were collected from 30 days seedlings grown in the polyhouse were used for the bioassay. Leaflets were individually dipped in each insecticide concentration for three to five seconds with gentle agitation, ensuring that the entire surface is treated equally. Then the treated leaflets were dried with the leaf axial surface facing upwards at room temperature for 15 min on moist tissue paper cut to fit the insect breeding box. The control leaves were dipped in the distilled water without insecticide. Second-instar *P. absoluta* larvae were carefully removed from the galleries in infested tomato leaves maintained in the cage and starved for a period of four hour. It was also ensured that larvae never been under starvation stress during the study. Twenty second-instar larvae were released for each concentration by using a fine soft brush on the treated leaf in the box as previously described and the box lid was immediately fitted to prevent larvae from escaping. Three boxes were used served as replication for each treatment, resulting in a total of 45 to 60 larvae per concentration. All treatments were placed under laboratory conditions (26 ± 2 ◦ C, 50-60% RH, 16:8 h L:D). A moist cotton plug was attached at the cut end of the leaf and the leaf was then placed in a transparent insect breeding box (9 cm). The same procedure was followed for all the doses and for all the insecticides, starting with the "untreated" control then followed by the more diluted dose and advancing progressively to the higher concentrations. The mortality was assessed after 24 h of exposure at different intervals. The data on final values of mortality (LC_{50}) were represented at 72 hrs. Larvae were recorded as moribund if no coordinated movement or deficient response to external stimulus was observed and were considered dead if they failed to right-back themselves when turned upside down with a brush. Mortality was estimated from the total number of dead and moribund insects. If insect vitality could not be clearly determined (live or moribund), the larvae were carefully extracted from the leaf to observe the responses when undistracted by the leaf epidermis.

Data analysis

The mortality data were corrected by the Abbott's formula (Abbott 1925) and subjected to Probit analysis using the statistical program SPSS version 16.0 software (IBM SPSS, Armonk, New York, USA) to obtain the LC_{50}, fiducial limits (95%), slopes and Chi-square values.

Relative potency

The LC_{50} of the susceptible population among the field collcted population was used for the calculation of relative potency for each insecticide. In the absence of a characterised susceptible reference strain, results are compared with those of the most susceptible population *i.e.,* the population which has a least value of LC_{50} assumed as susceptible population for each insecticide. Relative potency ratio was thus estimated to know the potency of the active ingredients used in the study by the formula LC_{50} of the least susceptible population divided by the LC_{50} of the most susceptible population (Deshmukh *et al.,* 2020).

RESULTS AND DISCUSSION

Bioassay of insecticides on *P. absoluta* collected from different locations of South India. Susceptibility to different insecticides having different mode of action among field collected tomato leaf miner populations during 2017-19 from ten locations from different districts of Karnataka, Maharashtra, Tamil Nadu and Andhra Pradesh was studied by following leaf dip bioassay method. Per cent mortality of *P. absoluta* larvae was checked from 24 h up to 72 h after treatment. The insecticides had a substantial difference in larval mortality at 72 h after treatment. Hence, final values are represented for 72 h after treatment. The ranges of LC_{50} and LC_{99} values calculated for *P. absoluta* populations from various locations are presented in Table 1. The results indicated that populations of *P. absoluta* among ten locations had variable responses to tested insecticides in this experiment.

The slopes of the dose response curves of the tested populations were high and varied among *P. absoluta* populations, but they were not extremely broad. In all cases, the slopes for insecticides were more than two, presumably indicating greater homogeneity/uniformity among *P. absoluta* populations collected from different locations. In response to diamide exposure, the tested populations showed highest slopes. For chlorantraniliprole the slopes ranged from 1.9 to 2.6 with an average of 2.2 (SE = 0.21). The slope values for cyantraniliprole among different population ranged from 1.7 to 2.6. High slopes of the response line to flubendiamide were observed, ranging from 2.2 to 3.6 and resulting in an average of 2.6 (SE = 0.2).

The calculated LC_{50} value of *P. absoluta* for chlorantraniliprole from different locations ranged from 1.4 to 4.7 ppm at 72 h after treatment. Resistance ratio (RR) varied from 1.1-fold to 3.3-fold, which might be considered within the natural variability range or developing low resistance (Table 1). For cyantraniliprole the LC_{50} ranged from 2.7 ppm to 9.2 ppm and resistance ratio (RR) varied from 1.5-fold to 3.4-fold showing a fairly homogeneous response among the populations. Madurai

Table 1. Relative toxicity of insecticides against field population of *Phthorimaea absoluta* from different locations by leaf dip bioassay

Population	n	LC_{50} (ppm)	Fiducial limits (95%)	LC_{95} (ppm)	Fiducial limits (95%)	Slope ± SE	Chi-square	RR ratio	Recommended field dose (ppm)
Chlorantraniliprole									
Davanagere	360	1.4	1.0-1.9	6.9	4.3-17.3	2.4 ± 0.2	3.0	-	55
Chikkamagaluru	360	1.6	1.3-1.9	8.8	6.3-14.5	2.2 ± 0.2	1.6	1.1	55
Madurai	270	1.7	1.4-2.1	11.0	7.5-19.6	2.0 ± 0.2	2.2	1.2	55
Chittoor	360	1.8	1.5-2.3	13.4	8.8-25.9	1.9 ± 0.2	2.9	1.2	55
Coimbatore	270	2.3	1.9-2.8	14.5	10.0-25.7	2.0 ± 0.2	2.2	1.6	55
Solapur	360	2.4	1.9-2.9	16.6	11.0-30.8	1.9 ± 0.2	2.5	1.7	55
Chamarajanagar	360	2.5	2.1-3.0	10.7	7.5-18.8	2.6 ± 0.3	2.5	1.7	55
Raichur	270	2.6	1.8-3.9	15.3	8.3-65.0	2.1 ± 0.2	4.3	1.8	55
Kurnool	270	3.2	2.7-3.9	17.5	12.5-28.5	2.2 ± 0.2	1.5	2.2	55
Kolhapur	360	4.7	4.0-5.6	21.2	15.8-32.3	2.5 ± 0.2	2.0	3.3	55
Cyantraniliprole									
Madurai	270	2.7	2.3-3.3	16.3	11.5-27.4	2.1 ± 0.2	2.9	-	184
Kurnool	270	4.1	2.7-6.0	19.6	11.0-85.6	2.4 ± 0.2	5.5	1.5	184
Coimbatore	270	4.2	3.0-5.9	21.7	12.8-66.1	2.3 ±0.2	3.8	1.5	184
Raichur	360	5.4	3.2-9.0	30.3	15.3-198.2	2.2 ±0 .2	6.9	2.0	184
Solapur	360	5.5	4.6-6.7	32.6	22.9-54.7	2.1 ± 0.2	2.9	2.0	184
Chikkamagaluru	270	6.2	4.4-9.2	46.6	24.4-181.9	1.8 ± 0.2	3.2	2.2	184
Chamarajanagar	360	6.3	5.3-7.5	28.2	20.9-43,5	2.5 ± 0.2	1.9	2.3	184
Davanagere	360	6.6	3.5-10.5	27.9	15.4-215.5	2.6 ± 0.3	7.7	2.4	184
Kolhapur	270	8.2	6.8-9.9	49.8	34.8-84.1	2.1 ± 0.2	2.7	3.0	184
Chittoor	270	9.2	5.6-16.2	85.9	36.6-834.8	1.7 ± 0.2	4.9	3.4	184
Flubendiamide									
Madurai	270	2.3	1.7-3.0	6.6	4.6-12.7	3.6 ± 0.3	4.1	-	100
Solapur	270	2.6	1.8-3.6	10.9	6.8-28.0	2.6 ± 0.2	4.3	1.1	100
Coimbatore	270	2.7	1.9-3.8	9.5	6.1-23.8	3.0 ±0.2	5.4	1.1	100
Kurnool	270	2.9	1.7-4.9	15.4	7.7-110.0	2.2 ± 0.2	7.5	1.2	100
Davanagere	360	5.1	4.3-6.0	21.6	16.5-31.6	2.6 ±0.2	2.9	2.2	100
Chittoor	270	8.1	6.0-10.9	29.0	19.2-63.5	2.9 ±0 .2	4.2	3.5	100
Chamarajanagar	360	9.0	6.5-12.4	40.2	25.0-102.8	2.5 ±0.2	3.7	3.9	100
Chikkamagaluru	360	11.0	6.5-18.4	50.0	26.6-309.4	2.5 ±0.2	8.3	4.7	100
Raichur	360	11.3	9.6-13.3	44.1	33.8-64.9	2.7 ±0.3	2.4	4.9	100
Kolhapur	270	16.2	6.7-35.7	86.6	38.2-4087.8	2.2 ± 0.2	13.1	7.0	100
Indoxacarb									
Solapur	270	2.8	1.9-4.1	20.0	10.5-78.0	1.9 ± 0.2	3.5	-	145
Coimbatore	270	3.8	3.0-4.8	40.9	24.8-89.7	1.6 ±0.1	2.7	1.3	145
Kurnool	270	4.6	2.6-8.4	25.0	12.1-236.6	2.2 ± 0.2	9.3	1.6	145
Chittoor	270	5.2	3.5-7.8	54.6	26.0-269.9	1.6 ±0.1	3.0	1.8	145
Raichur	360	5.4	3.2-9.0	54.3	24.2-417.4	1.6 ±0.1	4.9	1.9	145
Chikkamagaluru	360	6.1	4.1-9.6	56.0	26.1-319.2	1.1 ±0.1	3.7	2.1	145
Davanagere	360	6.5	3.8-11.3	75.7	30.9-847.6	1.5 ±0.1	4.4	2.3	145
Chamarajanagar	270	6.6	3.9-11.7	103.5	38.2-1481.6	1.3 ± 0.1	3.9	2.3	145
Madurai	270	7.7	6.2-9.8	82.3	49.8-183.6	1.6 ± 0.1	2.8	2.7	145
Kolhapur	270	12.2	8.5-18.7	109.3	53.0-524.1	1.7 ± 0.2	3.3	4.3	145

Spinosad									
Madurai	270	2.1	1.2-3.4	13.4	6.9-75.8	2.0 ± 0.2	6.0	1	144
Kurnool	270	2.6	2.2-3.1	14.5	10.4-23.5	2.2 ± 0.2	1.3	1.2	144
Solapur	270	3.1	1.9-5.3	17.3	8.5-128.7	2.0 ± 0.2	6.6	1.4	144
Chittoor	270	3.9	2.7-5.4	18.2	11.0-55.5	2.4 ± 0.2	4.3	1.8	144
Coimbatore	270	4.7	3.9-5.6	27.0	19.2-44.3	2.1 ± 0.2	1.4	2.2	144
Davanagere	360	5.1	4.4-6.0	21.4	16.4-31.2	2.6 ± 0.2	2.7	2.4	144
Chikkamagaluru	360	5.4	4.5-6.4	28.2	20.4-44.9	2.2 ± 0.2	1.2	2.5	144
Kolhapur	270	6.6	5.5-7.9	36.9	26.4-60.1	2.2 ± 0.2	1.5	3.1	144
Chamarajanagar	360	8.2	6.6-10.2	68.4	44.0-135.1	1.7 ± 0.2	1.0	3.9	144
Raichur	270	9.3	6.7-12.6	51.6	31.6-129.1	2.2 ± 0.2	3.1	4.4	144
Acetamaprid									
Solapur	270	2.9	2.0-4.2	14.2	8.2-43.6	2.3 ± 0.2	4.6	1.0	200
Kurnool	360	6.1	4.1-9.6	56.0	26.1-319.2	1.7 ± 0.1	3.7	2.1	200
Kolhapur	270	7.0	5.8-8.6	45.6	30.6-83.8	2.0 ± 0.2	1.0	2.4	200
Chittoor	270	13.7	11.3-16.8	89.3	59.9-163.	2.0 ± 0.2	1.2	4.7	200
Coimbatore	270	14.2	11.7-17.4	86.8	58.6-158.7	2.0 ± 0.2	1.4	4.8	200
Chamarajanagar	270	14.3	11.9-17.4	91.6	62.8-161.0	2.0 ± 0.2	1.8	4.9	200
Raichur	360	16.6	10.-27.3	135.2	6.0-979.0	1.8 ± 0.2	4.3	5.7	200
Madurai	270	17.2	14.2-21.0	111.7	74.9-204.3	2.0 ± 0.2	1.3	5.9	200
Chikkamagaluru	360	17.4	14.5-21.2	105.8	72.1-188.1	2.1 ± 0.2	1.5	6.0	200
Davanagere	270	22.9	13.9-49.7	121.3	53.8-2518.9	2.7 ± 0.2	7.7	7.8	200
Imidacloprid									
Kurnool	270	26.4	22.0-31.7	149.1	106.5-243.9	2.1 ± 0.2	1.6	-	60
Coimbatore	270	53.0	44.2-63.5	297.6	212.6-486.0	2.1 ± 0.2	1.5	2.0	60
Solapur	360	60.4	49.4-74.5	453.4	296.4-867.8	1.8 ± 0.2	2.8	2.2	60
Raichur	360	62.9	52.9-74.2	294.9	222.9-437.7	2.4 ±0 .2	2.7	2.3	60
Chikkamagaluru	360	66.2	55.2-79.3	372.2	265.9-608.1	2.1 ± 0.2	1.5	2.5	60
Davanagere	360	71.4	60.2-84.7	347.8	255.4-543.0	2.3 ± 0 .2	1.2	2.7	60
Madurai	270	76.8	64.4-91.3	403.1	292.6-643.1	2.3 ± 0.2	1.8	2.9	60
Chamarajanagar	360	81.2	67.1-98.3	515.4	356.6-889.2	2.0 ± 0.2	0.2	3.0	60
Chittoor	270	92.7	77.3-111.1	521.4	372.4-852.0	2.1 ± 0.2	1.5	3.5	60
Kolhapur	270	106.0	88.4-127.0	595.2	425.2-972.1	2.1 ±0 .2	1.5	4.0	60

*n = sample size

*LC_{50} - Lethal concentration which kills 50% of exposed population expressed in parts per million (ppm)

*LC_{95} - Lethal concentration which kills 95% of exposed population expressed in parts per million (ppm)

* SE = Standard Error

*Relative Resistance ratio (RR) = LC_{50} of the least susceptible population /LC_{50} of the most susceptible population

population was most susceptible to cyantraniliprole with LC_{50} value of 2.7 ppm. Population from Kolhapur showed reduced susceptibility to flubendiamide (16. 2 ppm), whereas Madurai population were most susceptible with LC_{50} value of 2.3 ppm. Resistance ratios (RR_{50}) varied from 1.1-fold to 7-fold, indicating slightly more variation among populations than for chlorantraniliprole and cyantraniliprole (Table 1).

The slope values for tomato leaf miner population collected from different location when tested against indoxacarb ranged between 1.1 and 2.2 with an average of 1.61 (SE = 0.1) (Table 1). The most susceptible population among ten different locations to indoxacarb was from Solapur with an LC_{50} value of 2.8 ppm followed by Coimbatore (3.8 ppm), Kurnool (4.6 ppm), Chittoor (5.2 ppm), Raichur (5.4 ppm), Chikkamagaluru (6.1 ppm), Davanagere (6.5 ppm), Chamarajanagar (6.6 ppm) and Madurai (7.7 ppm), while the most tolerant strain of *P. absoluta* to indoxacarb was Kolhapur population (12.2 ppm) resulting in a four-fold increase in the resistance ratio (Table 1).

Indoxacarb affects insects from direct exposure and through ingestion of treated foliage/fruit which leads to immediate feeding cessation. It kills pests by binding to a site on sodium channels and blocking the flow of sodium ions into nerve cells. The result is impaired nerve function, feeding cessation, paralysis, and death. It may take days for insects to die (Brugger, 1997).

The slope of response line to spinosad ranged from 1.7 to 2.6 with an average of 2.16 (SE = 0.2). The LC_{50} value for *P. absoluta* population from different location ranged from 2.1 to 9.3 ppm. Raichur population showed least susceptibility to spinosad with an LC_{50} value of 9.3 ppm resulting in four-fold resistance to the insecticide. Madurai population had an LC_{50} value of 2.1 ppm indicating most susceptibility to Spinosad. To this insecticide LC_{95} values ranged from 13.4 to 51.6 ppm which was lower than the recommended field dose (Table 1).

Spinosad has very low toxicity for mammals, birds, insect predators and the greatest effect of spinosad is on the Lepidoptera, Diptera, Thysanoptera, Coleoptera and Orthoptera (Toews *et al.,* 2003). Spinosad has two unique modes of action, acting primarily on the insect nervous system at the nicotinic acetylcholine receptor and exhibiting activity at the GABA receptor (Watson *et al.,* 2010).

Acetamiprid and imidacloprid belonging to neonicotinoid group were selected to examine the toxicity of these insecticides against second instar larvae of *P. absoluta* population collected from different locations from Karnataka, Maharashtra, Tamil Nadu and Andhra Pradesh.

The slopes of acetamiprid varied from 1.7 to 2.7 with an average of 2.06 (SE = 1.9) (Table 1). Median lethal concentration values tested to acetamiprid among *P. absoluta* population of different locations ranged from 2.9 to 22.9 ppm resulting in eight-fold resistance, highest resistance ratio among all insecticides tested. Solapur field strains showed more susceptibility to acetamiprid with an LC_{50} value of 2.9 ppm followed by Kurnool (6.1 ppm), Kolhapur (7.0 ppm), Chittoor (13.7 ppm), Coimbatore (14.2 ppm), Chamarajanagar (14.3 ppm), Raichur (16.6 ppm), Madurai (17.2 ppm) and Chikkamagaluru (17.4 ppm). Davanagere population showed least susceptibility to tested insecticide with an LC_{50} value of 22.9 ppm (Table 1).

With respect to another neonicotinoid *i.e.,* imidacloprid, the slope of dose response line ranged from 1.8 to 2.4 with an average of 2.13 (SE = 0.2). Among all tested insecticides so far against *P. absoluta* population from various locations, imidacloprid exhibited least toxicity for tomato leafminer with an LC_{50} value ranging from 26.4 to 106.0 ppm resulting in four-fold difference in resistance among different population. Kurnool population were more susceptible with LC_{50} value of 26.4 ppm and the most tolerant one was recorded from Kolhapur (LC_{50} 106.0 ppm) indicating less susceptibility to imidacloprid when compared to other populations and also LC_{95} values of all the populations (149.1 to 595.2 ppm) exceeded the recommended field dose (60 ppm) (Table 1).

Insecticides belonging to different group like diamides, oxadiazines, spinosyns and neonicotinoids having different mode of action exhibited varied level of toxicity across the populations collected from different locations of Karnataka, Maharashtra, Tamil Nadu and Andhra Pradesh. Among diamides, *viz.,* flubendiamide, chlorantraniliprole, cyantraniliprole, variation in relative toxicity against *P. absoluta* populations were observed. The present study revealed that *P. absoluta* population from different locations had high susceptibility to chlorantraniliprole (LC_{50} values 1.4 to 4.7 ppm) followed by cyantraniliprole (LC_{50} values 2.7 to 9.2 ppm) and flubendiamide (LC_{50} values 2.3 to 16.2 ppm). These results are in conformity with findings of Kumar *et al.* (2020) that LC_{50} values of chlorantraniliprole and flubendiamide to *P. absoluta* population from five districts of Tamil Nadu ranged between 0.27 to 0.60 ppm and 1.01 to 2.25 ppm respectively, indicating lower LC_{95} values than the recommended label rate for both chlorantraniliprole and flubendiamide, suggesting that the particular diamide insecticides would provide the expected control of *P. absoluta* infestation. Similar results were also recorded by Prasannakumar *et al.* (2020) LC_{50} values for cyantraniliprole ranged from 10.0 (Bangalore population) to 29.4 ppm (Anantapur population) and for flubendiamide it was 5.1 (Kolar population) to 32.3 ppm (Anantapur population) indicating that, to both the insecticides Anantapur population showed reduced susceptibility compared to populations from other location. which necessitates the judicious use of chemicals for its management. Therefore, regular monitoring the susceptibility of different population exposed to distinct active ingredients is essential. Similarly, Roditakis *et al.* (2012) reported that *P. absoluta* population from Greece showed susceptibility to both chlorantranilliprole and flubendiamide with an LC_{50} values ranging between 0.12 to 0.53 mg/L and 0.31 to 1.31 mg/L respectively. The LC_{50} values varied from 3.17 to 29.64 µg/L and 94 to 230 µg/L for chlorantraniliprole and flubendiamide with nine-fold and three-fold resistance ratios (Campos *et al*., 2014b).

Results of present study and also reports of Kumar *et al.,* 2020 and Prasannakumar *et al.* (2020) from India have demonstrated that development of resistance to diamides have not been reported in India as compared to native place of this pest, resistance ratio ranged between two to ten-fold, which indicates low level of resistance status in *P. absoluta* populations from different location of South India. From our study, it can be concluded that, this pest with 8 to 10 generations per year, combined with wide spread and intensive use of flubendiamide and chlorantraniliprole to control *P. absoluta,* may end up with development of resistance in a relatively shorter period of time in the field where populations are large and selection pressures can be much higher than in the laboratory and also due to continuous spraying of over dosages of insecticides, ignorance or a lack of concern in dealing with usages of insecticides. (Guillemaud *et al*., 2015). Also, inter-regional differences with respect to median lethal concentrations among tested populations may be due to insecticide use patterns wherein during initial days of introduction of this pest, majority of farmers were unaware about nature of damage, economic importance and early identification of the pest and also certain farmers had abandoned infested fields for cattle grazing and hence incurred complete crop loss. Hence, alternative management approaches, such as the preservation of natural enemies, the destruction of crop remains and the application of economic injury levels, would help to delay diamide resistance. More crucially, farmers should be encouraged to adopt insecticide rotation, which is likely one of the most feasible strategies. Silva *et al.* (2016a) found that the majority of *P. absoluta* are susceptible to spinosyns, which might be utilised as a substitute for diamides. Additionally, absence of cross-resistance between spinosyns and diamides have been demonstrated by Campos *et al.* (2014a) which suggests an interesting alternative for managing resistance for this pest and even molecules such as abamectin, chlorfenapyr, indoxacarb, and metaflumizone, may be used as alternative chemicals to manage *P. absoluta* populations.

With respect oxadiazines insecticide indoxacarb, present study revealed that there was variation in median lethal concentration values of *P. absoluta* populations from different locations of South India wherein LC_{50} values ranged between 2.8 (Solapur population) to 7.7 ppm (Madurai population) with a resistance ratio of 1.3-to-4.3-fold resistance. These results are in conformity with the findings of Silva *et al.* (2016) that the LC_{50} values varied among eight field populations of Brazil to indoxacarb, wherein LC_{50} value varied from 0.92 (Tiangu) to 2.89 (Pelotas) mg/L, with resistance ratio values between 1.1 and 3.3 times when compared with susceptible population LC_{50} value which was 0.86 mg /L from Iraquara, showing that all the populations are susceptible to this insecticide. Also, LC_{50} values to indoxacarb was found to be 17.5 ppm, which indicated that Brazilian population is more resistant to indoxacarb than Indian population of *P. absoluta*, implying that the pest was first detected in Brazil before being introduced to India. However, the LC_{95} was always lower than the recommended label rate and no cases of control failures were detected (Roditakis *et al.,* 2012). Variability in responses to indoxacarb have also been reported by Kumar *et al.* (2020) in populations from Tamil Nadu, with LC_{50} ranging from 0.82 to 6.38 ppm resulting in an eight-fold difference; Prasannakumar *et al.* (2020) in populations from Bangalore, Kolar, Madurai, Salem, Coimbatore and Anantapur wherein Kolar (22.8 ppm), Salem (16.00 ppm) and Anantapur (33.21 ppm) population showed reduced susceptibility to indoxacarb than the susceptible population (11.13 ppm).

P. absoluta population exhibited variation in LC_{50} when they were treated with spinosad insecticide with Madurai population showing more susceptibility (LC_{50} value of 2.1 ppm) and Raichur population showing least susceptibility (LC_{50} value of 9.3 ppm). Similar results were observed when relative toxicity of spinosad against second instar larvae of *P. absoluta* from Urmia city in Iran was evaluated by Hosseinzadeh *et al.* (2019). LC_{50} values at 72h after treatment indicated second instar larva was more susceptible to spinosad (1453.0 ppm) but the LC_{50} values recorded in this study are higher than those observed in our results. This is due to the fact that pest was noticed for the first time in the year 2010 in Iran earlier than India and probably pesticide usage pattern and availability of host and number of generations per year may be the reason for having higher LC_{50} values. *P. absoluta* population from nine distinct locations in Greece showed LC_{50} value ranging from 0.08 to 0.26 ppm, resulting in a three-fold difference and also high slopes of the response line to spinosad were observed, ranging from 1.45 to 2.28 and resulting in an average of 1.83 (Roditakis *et al.,* 2012). LC_{50} values were lower than present results which may be due to less cropping area, temperate country and low pesticide usage intensity.

Among seven insecticides tested against *P.* absoluta in the present study Neonicotinoids (imidacloprid and acetamiprid) were found to be least toxic to the test insect with LC_{50} values ranging from 2. 9 to 22.9 ppm for acetamiprid and 26.4 to 106.0 ppm for imidacloprid. These results are in line with findings of Sallam *et al.* (2015) who have repored that imidacloprid was inferior

and least toxic to fourth instar larvae of *P. absoluta* with an LC_{50} value of 2115.70 ppm. Present results are in harmony with Kandil *et al.* (2020) that emamectin benzoate, chlorantraniliprole and spinetoram showed more toxicity with LC_{50} values of 0.26, 0.46 and 0.59 ppm, respectively whereas imidacloprid (40.23 ppm) has least toxicity to *P. absoluta* population from Egypt. Although imidacloprid have not been registered for *P. absoluta* control in India, its potency against this new pest have been investigated and as per literature, it is also effective against micro-lepidopterans in its early stage. High variability in the LC_{50} values was observed for imidacloprid. When compared with the recommended field rate, very low toxicity levels were detected. Plots treated with imidacloprid suffered greater damage by the pests with low marketable and total yield. Poor performance by reportedly effective insecticides for controlling *P. absoluta* with imidacloprid suggest that the *P. absoluta* population which invaded Ethiopia is not resistant only to the older group of insecticides such as organophosphates (*e.g*., profenofos), but also relatively to the new molecules (Ayalew, 2015). Ramesh and Ukey (2007) and Kay (2006) found that imidacloprid were weak and ineffective insecticide against tomato leaf miner. In our study, populations of *P. absoluta* showed comparatively low levels of resistance to chlorantraniliprole, cyantraniliprole, flubendiamide, Spinosad and indoxacarb, whereas neonicotinoid insecticides showed higher LC_{50} values (acetamiprid 2.9 to 22.9 ppm and imidacloprid 25.4 to 106.0 ppm) and variation of resistance ratio values for acetamiprid was higher than for the other assessed insecticides, showing heterogeneity of susceptibility among *P. absoluta* populations. The first case of insecticide resistance development in *P. absoluta* were reported for organophosphates and pyrethroids in Chile (Salazar and Araya, 2001) and to avermectins and nereistoxin derivatives in both Brazil (Siqueira *et al.,* 2000). Thereafter, silva *et al.* (2011) reported high levels of resistance in some Brazilian states to insect growth regulators, indoxacarb, *B. thuringiensis*, bifenthrin and permethrin in this pest (>100 times), which were associated with control failures. Resistance to spinosad was also reported (Campos *et al.,* 2014b; Reyes *et al.,* 2012), which caused cross-resistance to spinetoram (Campos *et al.,* 2014a). In this scenario, diamides have played a major role in the control of *P. absoluta*, to which no resistance in the open field has been reported yet, despite the first reported cases in European greenhouses (Roditakis *et al.,* 2015). Additionally, use of diamides may have impacted the susceptibility status of *P. absoluta* to other products such as indoxacarb, abamectin, cartap as well as to the novel insecticides chlorfenapyr and metaflumizone, because their exclusive mode of action could eliminate previous existing mechanisms of resistance in field populations.

Differences in population dynamics, pesticide usage pattern and migratory behaviour are thought to be the most likely explanations for the differential resistance profile of insects. In an ideal world, insecticides should control their target pest without impacting the existence of others they should be 'deadly to the pest and harmless to the rest'. Thus, the reduced susceptibility of *P. absoluta* to various field collected population thus indicates a development of resistance to various insecticides necessitating the judicious use of chemicals for its management. Although insecticidal resistance in *P. absoluta* has not been reported from India, already existing resistance reports from different parts of the world indicate, resistance management strategies must take place immediately for effective results before control failures occur in the field. The resistance management should be components of IPM, which minimize pesticide usage through the application of alternative tactics such as cultural control and conservation of natural control through selective insecticides. Therefore, regular monitoring the susceptibility of different population exposed to distinct active ingredients is essential. Insecticide resistance programs should be implemented to conserve current efficient insecticides, used for management of *P. absoluta.*

REFERENCES

Ayalew, G. 2015. Efficacy of selected insecticides against the South American tomato moth, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) on tomato in the Central Rift Valley of Ethiopia. *African Entomology,* **23**: 410-417.

Campos, M. R., Rodrigues, A. R. S., Silva, W. M., Silva, T. B. M., Silva, V. R. F., Guedes, R. N. C. and Siqueira, H. A. A. 2014a. Spinosad and the tomato borer *Tuta absoluta*: A bioinsecticide, an invasive pest threat, and high insecticide resistance. *PLoS One*, **9**(8): 1-12.

Campos, M. R., Silva, T. B. M., Silva, W. M., Silva, J. E. and Siqueira, H. A. A. 2014b. Susceptibility of *Tuta absoluta* (Lepidoptera: Gelechiidae) Brazilian populations to ryanodine receptor modulators. *Pest Management Science,* DOI 10.1002/ps.3835

Chang, P. E. C. and Metz, M. A. 2021. Classification of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera:

Gelechiidae: Gelechiinae: Gnorimoschmini) based on cladistic analysis of morphology. *Proceedings of the Entomological Society of Washington*, **123**(1): 41-54.

Deshmukh, S., Pavithra, H. B., Kalleshwaraswamy, C. M., Shivanna, B. K., Maruthi, M. S. and Mota-Sanchez, D. 2020. Field efficacy of insecticides for management of invasive fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) on maize in India. *Florida Entomologist*, **103**(2): 221-227.

Desneux, N., Wajnberg, E., Wyckhuys, A. G., Burgio, G. and Arpaia, S. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of Pest Sci*ence, **83**:197–215.

EPPO. 2005 European and Mediterranean Plant Protection Organization. *Tuta absoluta*. Data sheets on quarantine pests. *EPPO Bulletin*., **35**: 434-435.

Guillemaud, T., Blin, A., Goff, I. L., Desneux, N., Reyes, M., Tabone, E., Tsagkarakou, A., Nino, L. and Lombaert, E. 2015. The tomato borer, *Tuta absoluta*, invading the Mediterranean Basin, originates from a single introduction from Central Chile. *Scientific Reports***,** **5**: 8371 | DOI: 10.1038/srep08371

Hosseinzadeh, A., Aramideh, S. and Ghassemi-Kahrizeh, A. 2019. Efficacy of bio-insecticides on *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in laboratory and field conditions. *Agriculture Engineering International CIGR Journal,* **21**(3): 164-170.

Kandil, M. A., Sammour, E. A., Abdel-Aziz, N. F., Agamy, E. A. E. M., EL-Bakry, A. M. and Abdelmaksoud, N. M. 2020. Comparative toxicity of new insecticides generations against tomato leafminer *Tuta absoluta* and their biochemical effects on tomato plants. *Bulletin of the National Research Centre*, **44**:126.

Kalleshwaraswamy, C.M., Shankara Murthy, M., C.A. Viraktamath, C.A. and N.K. Krishna Kumar, 2015. Occurrence of Tuta absolute (Lepidoptera: Gelechiidae) in the Malnad and Hyderabad-Karnataka region of Karnataka, India. Florida Entomologist, **98:**970-971.

Kay, I. R. 2006. Testing insecticides against *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelichiidae) using a tomato plantbioassay. *Plant Protection Queterly,* **21**: 20-24.

Kumar, J. S., Jayaraj, J., Shanthi, M., Theradimani, M., Balasubramani, V., Irulandi, S. and Prabhu, S. 2020.Toxicity of insecticides to tomato pinworm, *Tuta absoluta* (Meyrick) populations from Tamil Nadu. *Indian Journal Agricultural Research,* **54** (5):585-591.

Leite, G. L. D., Picanco, M., Jham, G. N. and Marquini, F. 2005. Intensity of *Tutaabsoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) and *Liriomyza* spp. (Diptera: Agromyzidae) attacks on *Lycopersicumesculentum* Mill. leaves. *Ciencia Agrotecnologia,* **28** (1): 42-48.

Pimentel, D., Mcnair, S., Janecka, J., Wightman, J., Simmonds, C., Connell, C., Wong, E., Russel, L., Zern, J., Aquino, T. and Tsomondo, T. 2001. Economic and environmental threats of alien plant, animal and microbe invasions. *Agriculture Ecosystem and Environment,* **84**:1–20.

Prasannakumar, N. R., Jyothi, N., Saroja, S. and Ram Kumar, G., 2020. Relative toxicity and insecticide resistance of different field population of tomato leaf miner, *Tuta absoluta* (Meyrick). *International Journal of Tropical Insect Science,* https://doi.org/10.1007/s42690-020-00334-1.

Ramesh, R. and Ukey, S. P. 2007. Bio-efficacy of botanicals, microbials and newer insecticides in the management of tomato leafminer, *Liriomyza trifolii* burgess. *International Journal of Agricultural Science,* **3**: 154-156.

Reyes, M., Rocha, K., Alarcón, L., Siegwart, M. and Sauphanor, B. 2012. Metabolic mechanisms involved in the resistance of field populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) to spinosad. *Pesticide Biochemistry and Physiology,* **102**: 45–50.

Roditakis, E., Skarmoutsou, C. and Staurakaki, M. 2012b. Toxicity of insecticides to populations of tomato borer *Tuta absoluta* (Meyrick) from Greece. *Pest Management Science,* **69**: 834-840.

Salazar, E. R. and Araya, J. E. 2001. Respuesta de la polilla del tomate, Tuta absoluta (Meyrick), a insecticidas en Arica. *Agricultura Técnica*, **61**(4): 429-435.

Sallam, A. A., Soliman, M. A. and Mohamed, A. Khodary, M. A. 2015. Effectiveness of certain insecticides against the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Advances in Applied Agricultural Science,* **03**(02): 54-64.

Shashank, P. R., Chandrashekar, K., Naresh, M. and Sreedevi, K. 2015. Occurrence of *Tuta absoluta* (Lepidoptera: Gelechiidae): An invasive pest from India. *Indian Journal Entomology,* **77**(4): 323-329.

Silva, G. A., Picanco, M. C., Bacci, L., Crespo, A. L., Rosado, J. F. and Guedes, R. N. C. 2011. Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta. Pest Management Science,* **67**: 913–920.

Silva, J. E., Assis, C. P. O., Ribeiro, L. M. S. and Siqueira, H. A. A. 2016a. Field-evolved resistance and cross-resistance of Brazilian *Tuta absoluta* (Lepidoptera: Gelechiidae) populations to diamide insecticides. *Journal of Economic Entomology,* **109**(5):2190–2195

Siqueira, H. A. A., Guedes, R. N. C. and Picanco, M. C. 2000. Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agricultural and Forest Entomology*, **2**: 147–153.

Sridhar, V., Chakravarthy, A. K., Asokan, R., Vinesh, L. S., Rebijith, K. B. and Vennila, S., 2014, New record of the invasive South American tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in India, *Pest Management in Horticultural Ecosystems*, **20**(2):148-154.

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Management of rugose spiraling whitefly (RSW), *Aleurodicus rugioperculetus* Martin with biopesticides on coconut with a note on its natural enemies

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ABSTRACT: Studies on the evaluation of biopesticides *viz., Beauveria bassiana, Isaria fumosorosea* NBAIR *pfu*-5*,Metarhizium anisopliae, Lecanicillium lecanii,* Azadirachtin 10000 ppm, soapnut along with jet water spraywere undertakenat SKPP Horticultural Polytechnic College, Ramachandrapuram and Horticultural Research Station (HRS), Ambajipeta, Andhra Pradesh during 2020-21 and 2021-22 with an objective of examining their impact onthe management of rugose spiraling whitefly (RSW), *Aleurodicus rugioperculetus* Martin in coconut (*Cocos nucifera* L.) palms variety East Coast Tall (ECT).The overall and pooled results during the seasons 2020-21 and 2021-22, revealed that, Azadirachtin 10000 ppm @ 1 ml/l had recorded with lowest number of RSW nymphs per leaflet and *I. fumosorosea* NBAIR pfu-5 @ 5 g/l (T_2) recorded with lowest number of adults, incidence and intensity under high incidence (> 20 spirals per leaflet) of RSW. The natural enemies mainly predators *viz.,* spiderswere documented during the study.

Keywords: Coconut, RSW incidence, bio pesticides, spraying, management, East Coast Tall (ECT)

INTRODUCTION

Coconut palm (*Cocos nucifera*) is often described as "Kalpavriksha" due to its multifarious use and play important role in world coconut export trade (Ahuja *et al.,* 2014). It is cultivated for oil, tender water and raw materials used in the coir industry.India stands first in world coconut trade with 31.46 per cent production. Bulk of coconut production, in India comes from Kerala, Karnataka, Tamil Nadu, Maharashtra followed by Andhra Pradesh sharing 90 per cent of area about 1.15 lakh ha with a production of 1,377.53 m nuts. However, the production and productivity of coconut is often limited by incidence of several pests and diseases (Chowdappa *et al.,* 2018 and Neeraja *et al.,* 2020). Recently, invasive rugose spiraling whitefly (RSW), *Aleurodicus rugioperculetus* Martin (Aleyrodidae: Hemiptera) was reported on coconut palm for the first time during August-September, 2016 at Pollachitaluk, Coimbatore district in Tamil Nadu (Chandrika *et al.,* 2017) and Palakad taluk in Kerala. In Andhra Pradesh, it was first reported at Kadiyapulanka nursery gardens, East Godavari district during late December 2016 (Chalapathi Rao *et al.,* 2018). Very recently, studies of Raghuteja *et al.* (2023) for the first time reported that East Coast Tall (ECT) variety of coconut palms infested with low, medium and high incidence of invasive *A. Rugioperculetus* resulted in nut dropping of 4.06, 22.33 and 28.51% at Ambajipeta, while it was 4.68, 23.49 and 30.58% at Kalavalapalli coconut plantations. It was reported that infestation of RSWreflects nut yield loss up to 6.61% and 22.45% in ECT palms withlow and medium RSW incidence, while comparativelygreater yield loss of 27.59% inECT palms with high incidence respectively (Raghuteja *et al.,* 2023).

The study on incidence of RSW is required to understand the behaviour of the pest and find its peak infestation period, so that the farmers could adopt eco-friendly techniques for managing this insect pest at the farm level. Effective management of RSW is critical in maximizing coconut yield. Over reliance on pesticides and its indiscriminate use over last four decades has resulted in many negative consequences, *viz.,* Resurgence, Resistance and Residual aspects (Raghuteja *et al.,* 2020). Botanical pesticides which are non-toxic to man and also environmentally friendly can be used as alternatives to the synthetic pesticides. Insecticides and neem oil have been found effective against the pest in several countries. In India, tobacco extract, neem oil, pongamia oil, rosin soap and detergent solution in addition to various entomopathogenic fungal isolates are effective (Gundappa *et al.,* 2013; Boopathi *et al.,* 2015; Srinivasan *et al.,* 2017). Hence, keeping in view the

present investigation was designed with an objective of evaluation of various biopesticides against RSW under high incidence (> 20 spirals per leaflet) infesting coconut palms along with documentation of natural enemies.

MATERIALS AND METHODS

The efficacy of different bio-pesticides was evaluated against RSW in East Coast Tall (ECT) variety of 7 years age-old palms with high RSW incidence as per the damage rating scale during 2020-21 and 2021-22 at SKPP Polytechnic college, Ramachandrapuram (16°83'72"NL and 82°03'25" EL) and HRS, Ambajipeta (16°59'38"NL and 81°95'36" EL). Evaluation of bio pesticides was carried out at Ramachandrapuram, horticultural polytechnic college working under the aegis of Dr. YSR Horticultural University as the plantations were found suitable for carrying out the experimentation with the desired pest load during 2020-21. The documentation of different predatory spiders was also carried out in the study.

The numbers of treatments of biopesticides were eight replicated thrice and statistically analysed by simple randomised block design (RBD). The observations on RSW incidence were made at weekly intervals starting from 7 days after imposing the treatments and continued up to 28 days. The data pertaining to number of RSW nymphs and adults wererecorded on four randomly selected pest infested leaflets per leaf per palm from the top, middle and lower whorl representing four directions (total of 4 leaves/palm) was worked out and expressed as mean number of leaflet/leaf/palm (total of 4 leaflets/ leaf) (16 leaflets/palm) at 1 day before spraying (DBS), 7, 14, 21 and 28 days after spraying (DAS). Estimation of RSW incidence and intensity (%) were also calculated using the following formulae

$$\textbf{RSW Incidence (\%)} = \frac{\textit{Number of leaves infested by RSW}}{\textit{Total number of leaves per palm}} \times 100$$

$$\textbf{RSW Intensity (\%)} = \frac{\textit{Number of leaflets infested by RSW}}{\textit{Total number of leaflets per leaf}} \times 100$$

The randomly selected four leaflets/ leaf/ palm for each treatment were marked carefully, sealed in a polythene cover and immediately brought to the laboratory. The data was collected on population of RSW nymphs under Nikon SMZ18 13.5 x stereomicroscope and adults on visual basis.

Statistical Analysis

The statistical analysis of data was done by using OPSTAT software. The data was transformed by arc sine and square root transformations before the data subjecting for analysis. After the analysis the data was tabulated for interpretation of results.

RESULTS AND DISCUSSION

Spraying of bio pesticides *viz., B. bassiana*, *I. Fumosorosea* NBAIR pfu-5**,** *M. anisopliae, L. lecanii*, Azadirachtin 10000 ppm, soapnut powder and Jet water spray were undertaken to evaluate efficacy against RSW infested coconut palms with high (> 20 spirals per leaflet) incidence as per the damage rating scale developed by Srinivasan *et al.,* (2016) during 2020-21 and 2021-22.

The pooled analysis (2020-21 and 2021-22) of data indicated that, significant difference was observed among different treatments of bio pesticides against RSW incidence from 7th day and continued till 28th day. *I. fumosorosea* NBAIR pfu-5 @ 5 g/l (T_2) recorded least incidence with 82.98, 78.37, 76.47 and 71.89 per cent throughout the experimental period and found to be promising followed by Azadirachtin 10,000 ppm @ 1 ml/l (T_5) with 83.80, 78.87, 77.12 and 72.82 per cent followed by soap nut powder @ 3 g/l (T_6) with 85.18, 80.25, 77.62 and 73.79 per cent. The highest incidence of 90.49, 91.71, 93.34 and 95.57 per cent was recorded in control (Table 1).

The pooled analysis (2020-21 and 2021-22) of data showed that, significant difference was observed among different treatments of bio pesticides against RSW intensity from 7th day and continued till 28th day. *I. fumosorosea* NBAIR pfu-5 @ 5 g/l (T_2) recorded least intensity with 88.81, 84.44, 81.79 and 76.83 per cent throughout the experimental period and found to be promising followed by Azadirachtin 10,000 ppm @ 1 ml/l (T_5) with 88.81, 84.44, 81.79 and 76.83 per cent.

The pooled analysis of data (2020-21 and 2021-22) indicated that, significant difference was observed among different treatments against RSW nymphs from 7th day and continued till 28th day after spraying. Treatment (T_5) Azadirachtin 10000 ppm @ 1 ml/l recorded least number (42.63, 37.04, 33.11 and 24.00 nymphs) with 30.18 per cent reduction after spraying, 44.82 per cent reduction over control and proved to be superior over remaining treatments followed by *I. fumosorosea* NBAIR pfu-5 @ 5 g/l (T_2) (43.68, 36.98, 34.51 and 26.89 nymphs) with 27.53 per cent reduction and 42.67 per cent reduction over control followed by soapnut powder treatment (T_6) with 45.90, 39.46, 37.72 and 28.36 nymphs (24.46 per cent reduction and 38.89 per cent reduction over control). The highest population of 50.75, 57.29, 61.32 and 64.31 nymphs per leaflet was recorded in control plots (Table 2).

The pooled analysis of data (2020-21 and 2021-22) indicated that, significant difference was observed among different treatments against RSW adults. *I. fumosorosea* NBAIR pfu-5 @ 5 g/l (T_2) recorded lowest number (36.42, 35.25, 33.59 and 29.11 adults) with 12.55 per cent reduction after spraying, 15.52 per cent reduction over control and proved to be superior followed by Azadirachtin 10,000 ppm @ 1 ml/l (T_5) with 36.46, 35.14, 33.10 and 30.90 adults (11.16 per cent reduction and 16.63 per cent reduction over control) followed by soapnut powder @ 3 g/l with 36.99, 35.72, 35.01 and 33.21 adults (8.28 per cent reduction and 13.35 per cent reduction over control). *L. lecanii*@ 5 g/l (T_4) recorded with 7.77 per cent reduction and 12.74 per cent reduction over control (37.10, 35.91, 34.78 and 34.14 adults).

Table 1. Efficacy of bio pesticides against incidence and intensity of RSW, *A. rugioperculetus* under high incidence palms (>20 spirals per leaflet) (Pooled data of 2 years)

Tr. No.	Treatments	Before Spraying (B.S)		7 Days after Spraying (7 DAS)		14 Days after Spraying (14 DAS)		21 Days after Spraying (21 DAS)		28 Days after Spraying (28 DAS)	
		Incidence (%)	Intensity (%)	Incidence (%)	Intensity (%)	Incidence (%)	Intensity (%)	Incidence (%)	Intensity (%)	Incidence (%)	Intensity (%)
T_1	*B. bassiana*	85.71 (68.01)	91.02 (73.13)	85.12 (67.28)	90.58 (72.09)	82.05 (64.91)	87.37 (69.16)	80.12 (63.49)	85.39 (67.50)	77.98 (61.99)	82.44 (65.19)
T_2	*I. fumosorosea* NBAIR pfu-5	86.42 (69.12)	91.74 (73.26)	82.98 (65.61)	88.81 (70.43)	78.37 (62.26)	84.44 (66.74)	76.47 (60.96)	81.79 (64.71)	71.89 (57.96)	76.83 (61.19)
T_3	*M. anisopliae*	89.00 (70.75)	94.33 (76.61)	87.15 (68.96)	92.42 (73.99)	84.87 (67.08)	90.19 (71.72)	82.97 (65.60)	88.28 (69.97)	81.39 (64.42)	85.38 (67.49)
T_4	*L. lecanii*	87.34 (71.07)	92.05 (73.68)	84.29 (66.62)	89.79 (71.33)	81.07 (64.18)	86.39 (68.32)	78.85 (62.59)	84.17 (66.53)	76.90 (61.26)	82.41 (65.17)
T_5	Azadirachtin 10000 ppm	86.11 (68.24)	91.27 (72.86)	83.80 (66.24)	89.39 (70.96)	78.87 (62.61)	84.14 (66.50)	77.12 (61.39)	82.39 (65.17)	72.82 (58.55)	77.76 (61.83)
T_6	Soapnut powder	88.59 (70.64)	93.91 (76.98)	85.18 (67.33)	90.49 (72.02)	80.25 (63.59)	85.57 (67.45)	77.62 (61.74)	82.94 (65.58)	73.79 (59.18)	79.23 (62.86)
T_7	Jet water spray	89.58 (71.27)	94.90 (77.37)	86.67 (68.56)	91.98 (73.53)	83.97 (66.37)	89.29 (70.87)	81.64 (64.60)	86.96 (68.80)	79.48 (63.04)	84.79 (67.03)
T_8	Control	88.53 (70.29)	93.53 (75.52)	90.49 (72.02)	94.49 (76.40)	91.71 (73.23)	95.71 (78.01)	93.34 (75.01)	97.34 (80.57)	95.57 (77.82)	98.41 (83.15)
	S.E (m)	3.41	2.65	0.004	0.15	0.002	0.08	0.24	0.28	0.15	0.62
	C.D at 5 %	N.S	N.S	0.01	0.46	0.006	0.25	0.73	0.85	0.46	1.89
	C.V	8.43	6.12	0.009	0.21	0.005	0.20	0.64	0.70	0.42	1.60

Table 2. Efficacy of bio pesticides against nymphs of RSW, *A. Rugioperculetus* under high incidence palms (>20 spirals per leaflet) (Pooled results of 2 years)

Tr. No.	Treatments	Before Spraying (B.S)	7 Days after Spraying (7 DAS)	14 Days after Spraying (14 DAS)	21 Days after Spraying (21 DAS)	28 Days after Spraying (28 DAS)	Per cent Reduction	Per cent Reduction over control
T_1	*B. bassiana* commercial	53.22 (7.36)	50.68 (7.19)	43.74 (6.69)	41.56 (6.52)	34.56 (5.96)	19.88	31.18
T_2	*I. fumosorosea* NBAIR pfu-5	49.01 (7.07)	43.68 (6.68)	36.98 (6.16)	34.51 (5.96)	26.89 (5.28)	27.53	42.67
T_3	*M. anisopliae* commercial	51.15 (7.22)	49.67 (7.12)	42.73 (6.61)	41.07 (6.49)	33.53 (5.88)	18.38	32.62
T_4	*L. lecanii* commercial	52.60 (7.32)	48.29 (7.02)	41.49 (6.52)	38.77 (6.31)	32.58 (5.79)	23.42	34.99

T_5	**Azadirachtin 10000 ppm**	**48.97 (7.07)**	**42.63 (6.60)**	**37.04 (6.16)**	**33.11 (5.83)**	**24.00 (4.99)**	30.18	44.82
T_6	Soapnut powder	50.12 (7.15)	45.90 (6.85)	39.46 (6.36)	37.72 (6.22)	28.36 (5.42)	24.46	38.89
T_7	Jet water spray	52.92 (7.34)	49.27 (7.09)	43.17 (6.65)	40.87 (6.47)	33.74 (5.89)	21.09	32.60
T_8	Control (No sprayings)	49.46 (7.10)	50.75 (7.19)	57.29 (7.83)	61.32 (7.89)	64.31 (8.08)	-	-
	S.E (m)	0.09	0.11	0.10	0.11	0.12	-	-
	C.D at 5 %	N.S	0.35	0.31	0.33	0.36	-	-
	C.V	2.25	2.82	2.67	2.86	3.44	-	-

Table 3. Efficacy of bio pesticides against adults of RSW, *A. Rugioperculetus* under high incidence palms (>20 spirals per leaflet) (Pooled Results of 2 years)

Tr. No.	Treatments	Before Spraying (B.S)	7 Days after Spraying (7 DAS)	14 Days after Spraying (14 DAS)	21 Days after Spraying (21 DAS)	28 Days after Spraying (28 DAS)	Per cent Reduction	Per cent Reduction over control
T_1	*B. bassiana* commercial	38.41 (6.28)	36.99 (6.16)	36.22 (6.10)	35.51 (6.04)	34.71 (5.98)	6.64	11.81
T_2	*I. fumosorosea* NBAIR pfu-5	**39.28 (6.35)**	**36.42 (6.12)**	**35.25 (6.02)**	**33.59 (5.88)**	**29.11 (5.75)**	12.55	15.52
T_3	*M. anisopliae* commercial	39.13 (6.33)	38.44 (6.28)	37.58 (6.21)	37.19 (6.18)	35.82 (6.07)	4.78	8.36
T_4	*L. lecanii* commercial	38.47 (6.28)	37.10 (6.17)	35.91 (6.08)	34.78 (5.98)	34.14 (5.93)	7.77	12.74
T_5	**Azadirachtin 10000 ppm**	38.16 (6.25)	36.46 (6.12)	35.14 (6.01)	33.10 (5.84)	30.90 (5.65)	11.16	16.63
T_6	Soapnut powder	38.41 (6.28)	36.99 (6.16)	35.72 (6.06)	35.01 (6.00)	33.21 (5.85)	8.28	13.35
T_7	Jet water spray	39.35 (6.35)	37.89 (6.24)	37.31 (6.19)	36.65 (6.14)	35.88 (6.07)	6.15	9.17
T_8	Control (No sprayings)	38.20 (6.26)	38.72 (6.30)	40.98 (6.48)	41.15 (6.49)	41.79 (6.54)	-	-
	S.E (m)	0.07	0.03	0.05	0.02	0.03	-	-
	C.D at 5 %	N.S	0.08	0.16	0.06	0.09	-	-
	C.V	1.92	0.74	1.45	0.59	0.88	-	-

*Mean of three replicates; DAS: Days after spraying, Figures in the parenthesis are $\sqrt{x} + 0.5$ transformed values

The findings imply that *I. fumosorosea* was more effective in the field than *B. bassiana*, *M. anisopliae* or *L. lecanii* in controlling the exotic*A. rugioperculetus*. To physically infiltrate the host and suppress its regulatory system, *I. fumosorosea* releases chitinase, chitosanase and lipase (Ali *et al.,* 2010). These results are in line with those of Boopathi *et al.* (2013), Boopathi *et al.* (2015) and Chalapathi Rao *et al.* (2020). *I. fumosorosea* NBAIR Pfu-5 reduced the early nymphal instars of RSW by 52-68 per cent and 35-40 per cent in Godavari Ganga hybrid and Gauthami Ganga variety of coconut, according to Chalapathi Rao *et al.* (2020). Selvaraj *et al.*(2020) identified *I. Fumosorosea* NBAIR Pfu-5 as promising strain and observed overall reduction of 72.20-73.83 per cent and 74.26-75.83 per cent in RSW population in Karnataka and Andhra Pradesh with two sprays at 15 days interval in coconut and oil palm.

Dipcolonic acid, hydroxy carboxylic acid and cyclosporine are released by *L. lecanii* and elevate the pH of the haemolymph, causing clotting and ending the haemolymph's circulation in the insect. Similar findings were obtained against *A. dispersus* by Boopathi *et al.* (2013). Elango and Nelson (2020) discovered that 1 x 10^8 conidia/ml of *L. lecanii* (NBAIR VL-15 strain) caused up to 50 per cent RSW mortality.

The current investigation indicated that Azadirachtin 10,000 ppm was efficient at massacring and preventing the growth of invasive RSW nymphal stages at high incidence. Azadirachtin, a major active element isolated from *Azadirachtaindica* seeds, works as a growth regulator, anti feedant and insect repellant against insects of various genera, including those that feed on plant fluids (Copping and Duke, 2007) by inhibiting the activity of ecdysone-20-monooxygenase in the haemolymph, which converts ecdysone to 20-hydroxyecdysone (active form of moulting hormone). The findings are supported with those of Elango and Nelson (2020), Alagar *et al*. (2021) and Krishnarao and Chalapathi Rao (2019) who found that Azadirachtin 10,000 ppm was effective against RSW nymphs. In the current study, the powdered soapnut, which contains active components such as triterpenoid saponins (I) and sesquiterpene glucoside (II), was found to have stronger larvicidal and pupicidal effects, resulting in the death of all *A. rugioperculetus* developmental stages. Koodalingam *et al.* (2009). Who explained the superiority of soapnut powder against stages of the *A. aegypti* mosquito, confirmed the findings.

***I. fumosorosea* NBAIR, *pfu*-5 infested nymph and adult**

Plate 1. Mycosis of Entomo pathogenic fungi against RSW nymphs and adults

Gasteracantha geminata
Family: Araneidae

***Oxyopes* spp.**
Family: Oxyopidae

***Plexippus* spp.**
Family: Salticidae

***Tetragnatha* spp.**
Family: Tetragnathidae

Carrhotusviduus
Family: Salticidae

Brettuscingulatus
Family: Salticidae

***Peucetia* spp.**
Family: Oxyopidae

***Phintelloides* spp.**
Family: Salticidae

Telamoniadimidiata
Family: Salticidae

Argiopeanasuja
Family: Araneidae

***Gasteracantha* spp.**
Family: Araneidae

Hyllus semicupreus
Family: Salticidae

Plate 2. Documentation of natural enemies (Spiders)

Thus, considering all aspects in the present study azadirachtin 10,000 ppm or *I. Fumosorosea* are recommended but in view of low cost of *I. Fumosorosea* and possibility of natural epizootics during favourable conditions provides us the best option for the management of RSW.

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REFERENCES

Ahuja, S. C., Ahuja, U. and Ahuja, S. 2014. Coconut - history, uses, and folklore. *Asian Agricultural History,* **18**(3): 221-244.

Alagar, M., Siva, K.V., Praneetha, S., Chinnadurai, K., Josephraj, K. A. and Maheswarappa, H. P. 2021. Ecofriendly Management of rugose spiralling whitefly, *Aleurodicus rugioperculetus* Martin infesting coconut. *Indian Journal of Entomology,* 1-4.

Ali, S., Huang, Z. and Ren, S. 2010. Production of cuticle degrading enzymes by *Isari fumosorosea* and their evaluation as a biocontrol agent

against diamondback moth. *Journal of Pest Science,* **83**: 361-370.

Boopathi, T., Palaniappan, K., Soibam, B. S., Manickavasagam, K., Mohankumar, S. and Madhaiyan, R. 2015. Microbial control of the invasive spiralling whitefly on cassava with entomopathogenic fungi.*Brazilian Journal of Microbiology,* **46**: 1077–1085.

Chalapathi, N. B. V. R., Rakshith, R. D., Krishna, R. G. and Ramanandam, G. 2018. A review on rugosespiralling whitefly, *Aleurodicus rugioperculetus* Martin (Hemiptera: Aleyrodidae) in India. *Journal of Pharmacognosy and phytochemistry,* **7**(5): 948-953.

Chalapathi, N. B. V. R., Ramani, B. S. L., Roshan, R. D. and Bhagavan, B. V. K. 2020. Biocontrol management options for invasive white flies on Coconut. *Indian Coconut journal,* **63** (4): 19-24.

Chandrika, M., Josephraj, K.A., Anjali, A.S., Merin, B. and Hegde, V. 2017. Invasive rugose spiralling whitefly infesting coconut palms in India.

Chowdappa, P., Hedge, V., Chandrika, M., Josephraj, K.A. and Merin, B. 2018. Pest and disease free coconut. *Indian Coconut Journal,* **61** (2): 24-28.

Copping, L. G. and Duke, S. O. 2007. Natural products that have been used commercially as crop protection agents a review. *Pest Management Sciences,* **63**: 524-554.

Elango, K. and Nelson, J. S. 2020. Efficacy of Biopesticides against coconut rugose spiralling whitefly, *Aleurodicus rugioperculetus* Martin under Laboratory Conditions. *Biopesticides International,* **16**(1): 21-26.

Gundappa, Kamala, P. D. J. and Abraham, V. 2013. Management of spiralling whitefly, *Aleurodicus dispersus* (Russel) in guava, *Psidium guajava* L. *Pest Management in Horticultural Ecosystems*, **19** (1): 102-105.

Koodalingam, A., Periasamy, M. and Munusamy, A. 2009. Antimosquito activity of aqueous kernel extract of soapnut, *Sapindus emarginatus*: impact on various developmental stages of three vector mosquito species and non-target aquatic insects. *Parasitology Research,* **105**: 1425–1434.

Krishnarao, G. and Chalapathi, N. B. V. R. 2019. Surveillance and eco-friendly management of new invasive alien pest, rugose spiralling whitefly, *Aleurodicus rugioperculetus* Martin: Inherent Menance. *Journal of Applied Zoological Research.* **30**(2): 148-158.

Neeraja, B., Chalapathi, N. B. V. R. and Bhagavan, B. V. K. 2020. Occurrence of leaf blight– a new concern to coconut plantations in Andhra Pradesh. *The Indian Coconut Journal,* **63** (4): 28-29.

Raghuteja, P.V., Chalapathi, N. B. V. R., Padma, E. And Kireeti, A. 2023. Immature nut fall of coconut and incidence of Rugose Spiraling Whitefly, *Aleurodicus rugioperculetus* Martin. *Indian Journal of Entomology,* 1-4.

Raghuteja, P.V., Chalapathi, N. B. V. R., Padma, E., Kireeti, A., Umakrishna, K. and Rao, G. K. 2023. Yield loss in coconut variety East Coast Tall (ECT) owing to rugose spiralling whitefly (RSW), *Aleurodicus rugioperculetus* M. infestation. *Phytoparasitica*. **51**(3).

Raghuteja, P.V., Emmanuel, N., Sujatha, A., Umakrishna, K., Viji, C.P. and Patro, T.S.S. K. 2020. Impact of IPM and non-IPM practices on sucking pest complex and natural enemies of okra (*Abelmoschus esculentus* L.) pests. *Journal of Entomology and Zoology Studies*, **8**(4): 487-495.

Selvaraj, K., Sumalatha, B.V., Ramanujam, B., Poornesha, B., Kandan, A., Amala, U. and Bakthavatsalam, N. 2020. *Isarea fumosorosea*: a Potential Biocontrol Agent for management of Invasive Rugose Spiralling Whitefly in coconut and oil palm. ICAR: NBAIR publication. 11/2020.

Srinivasan, T., Rajamanickam, K., Ramjegathesh, R. and Venkatesan, K. 2017. Eco friendly management of invasive coconut rugose spiralling whitefly in Tamil Nadu. *Indian Coconut Journal*, **60** (8): 21-23.

Srinivasan, T., Saravanan, P. A., Josephraj, K. A., Rajamanickam, K., Sridharan, S., David, P. M. M., Natarajan, N. and Shoba, N. 2016. Invasion of the rugose spiralling whitefly, *Aleurodicus rugioperculetus* Martin (Hemiptera: Aleyrodidae) in Pollachi tract of Tamil Nadu, India. *Madras Agricultural Journal*, **103**: 349-353.

Thangavel, B., Palaniappan, K., Manickavasagam Pillai, K., Subbarayalu, M. and Madhaiyan, R., 2013. Pathogenicity, ovicidal action, and median lethal concentrations (LC50) of entomopathogenic fungi against exotic spiralling whitefly, Aleurodicus dispersus Russell. *Journal of Pathogens*, 393787.

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Effect of root-knot nematode infestation on growth and biochemical parameters of *Plectranthus rotundifolius* (Poir.) Spreng

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ABSTRACT: Studies were conducted to assess the crop loss in Coleus, *Plectranthus rotundifolius,* a short duration tuber yielding vegetable, due to infestation of root knot nematode, *Meloidogyne incognita.* Plants inoculated with 5000 J_2 showed 50.78% reduction in the yield of coleus. Five local collections (Alur, Mankada, TCM-9, M-131 and Pulamanthol) and two improved varieties (Sree Dhara and Nidhi) were screened for relative tolerance to *M. incognita*. Local collection, Alur showed significant superiority in reducing the nematode population in soil and root. Lowest root-knot index (1.00) and reproduction factor (0.39) was recorded by the Alur collection compared to improved varieties. Defence enzyme activity and phenolic content in the resistant variety Alur collection was significantly higher than the improved varieties. Considering the ability to resist nematodes and yield potential, the local variety Alur collection can be used in breeding programmes to develop high yielding nematode resistant varieties suitable for cultivation in Kerala.

Keywords: *Plectranthus rotundifolius*, *Meloidogyne incognita*, growth parameters, yield, biochemical parameters, microplot studies.

INTRODUCTION

Chinese potato or coleus, *Plectranthus rotundifolius* (Poir.) Spreng is an under exploited tuber yielding vegetable, with high marketing potential. The tubers are rich in carbohydrates (18-21%), minerals like calcium and iron, vitamins like thiamine, riboflavin, niacin and ascorbic acid. In Kerala, it is mainly cultivated in Northern districts, but nowadays the demand for tubers fuelled the cultivation in Southern district also. The incidence of pests *viz.*, plant parasitic nematodes, rodents, stem borer and leaf folder are major constraints in the cultivation of coleus. The crop losses caused by nematodes in tuber crops are more severe than other cultivated crops as nematodes on these crops not only reduce their yield but also affect quality of the tubers as nematodes feed directly on the tubers. Infested tubers are smaller in size, often malformed with irregular wart like protuberances on the tuber surfaces and all of these reduce the marketability of the tubers. Besides, nematodes continue to multiply inside tuber after harvest during transportation and storage. Root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 infestation was reported in *P. rotundifolius* from Kerala by Sathyarajan *et al.* (1966) and among the pests, *M. incognita* found to be the serious limiting factor in the production of *P. rotundifolius.* Due to the attack of *M. incognita* in *P. rotundifolius*, conspicuous galls like swellings are formed in the roots and tubers. Heavily infested tubers start rotting while less infested ones shrunk and develop more prominent galls during storage (Mohandas and Ramakrishnan, 1998).

Since coleus is a short duration crop and tubers being the consumable part, application of chemical pesticides in soil results in very high level of pesticide residues in tubers, contamination of ground water and adverse effect on non-target organisms. Crop loss assessment and identification of nematode resistant varieties is highly essential for formulation of environment friendly management strategy. Hence the present study was undertaken to assess the crop loss in *P. rotundifolius* due to *M. incognita,* screen coleus cultivars against *M. incognita* and to estimate the biochemical changes in tolerant and susceptible cultivars due to *M. incognita* infestation.

MATERIALS AND METHODS

Identification of *M. incognita*

M. incognita was identified by preparing the perineal pattern of female nematode ((Taylor and Netscher, 1974) and observing under stereo microscope. Root-knot nematode infected plants were collected from Department of Nematology, College of Agriculture, Vellayani and the roots were gently washed with water. Mature female nematodes (25 no's) were collected from the galls in roots using sterilized forceps and kept in 45% lactic acid. The anterior part of the nematode body was

cut off by using a scalpel and gently pressed to remove the inner tissues. The posterior part of the nematode body was cut and the cut portion was kept in 45% lactic acid. The posterior cuticular part was trimmed into a square shape with the perineal pattern in the centre. The perineal pattern was transferred onto a microscope slide in a small drop of glycerine and was aligned as anus oriented downward. A coverslip was placed over it. The perineal pattern was observed and the species was identified with the help of identification keys given by Eisenback (1985).

Maintenance of pure culture of *M. incognita*

M. incognita juveniles used in this study was collected from pure culture maintained in tomato plants (variety-Vellayani Vijay) kept in the glass house of Department of Nematology, College of Agriculture, Vellayani. Viable egg masses adhering on the root surface were hand-picked from the infested roots and transferred to a beaker containing sterile water. Second stage juveniles (J_2) hatched were collected after 3 to 5 days and inoculation in the root zone was done as per the method of Venkitesan and Sethi (1977). Sub culturing of nematode was done periodically for maintaining the pure culture.

Effect of different inoculum levels of *M. incognita* on crop loss

A microplot experiment was conducted at Department of Nematology, College of Agriculture, Vellayani to study the effect of different inoculum levels of *M. incognita* on growth, yield and quality parameters of *P. rotundifolius*. Microplots of size 1mx1m were filled with denematised potting mixture prepared by mixing soil, sand and farm yard manure in 2:1:1 ratio. Cuttings of coleus raised in nursery were transplanted at a spacing of 30 cm between rows and plants. Fifteen days after planting newly hatched *M. incognita* juveniles (J_2) were inoculated to the rhizosphere of the cuttings @100, 500, 1000 and 5000. Each treatment was replicated four times and the experiment was laid out in Completely Randomized Design. Uninoculated plants served as control. The results were assessed in terms of biometric characters, yield and quality parameters. Growth parameters *viz*. plant height, plant spread, number of branches and leaves were recorded at 1, 2, 3, 4 and 5 months after inoculation (MAI). Yield parameters *viz.* number of tubers/plant (total and marketable), weight of tubers/plant (total, marketable and edible portion), size of tubers in term of diameter and total yield/plot were recorded at the time of harvest. The quality parameters *viz.* protein, starch, sugar and crude fibre of tubers infested with different inoculum levels of *M. incognita* were estimated adopting standard procedures. The tubers were dried in a hot air oven at 70^0C and were ground to pass through 0.5 mm mesh in a willey mill. The protein and starch content were estimated by modified micro-kjeldahl method (Jackson, 1973) and potassium ferry cyanide method (Pigman, 1970). The standard procedure suggested by A.O.A.C (1969, 1975) were followed to estimate sugar and crude fibre content.

Screening of coleus cultivars against *M. incognita*

Five local collections of coleus (Alur, Mankada, TCM-9, M-131, Pulamanthol), one KAU released variety (Nidhi) and one variety released from Central Tuber Crops Research Institute, Sreekaryam (Sree Dhara) were screened against *M. incognita* under pot culture condition in Department of Nematology, College of Agriculture, Vellayani during 2022-2023. Well-developed mature healthy disease-free tubers were selected and washed in running water to remove the soil particles. The cuttings from plants raised in nursery were transplanted in pots containing 5 kg denematized potting mixture prepared by mixing red soil, sand and farm yard manure in 2:1:1 ratio. The trial was laid out in completely randomized design replicated thrice with five plants in each replication. Egg masses of *M. incognita* (pure culture maintained in tomato plants) were picked from roots using needle and transferred to a beaker containing distilled water. The hatched second stage juveniles @2/g soil were inoculated to the rhizosphere of the cuttings fifteen days after transplanting. The plants were watered regularly and kept in glass house at 27-30^0C. Three months after nematode inoculation, the plants were uprooted and observations on number of galls, females, egg masses, eggs/egg mass and nematode population were recorded. Root -knot indexing was done in 0-5 scale of Taylor and Sasser (1978). Nematode population in soil was estimated by Cobb's sieving and decanting technique followed by modified Baermann's funnel technique (Southey, 1986). Five-gram root was collected from each plant and stained using acid fuchsin lactophenol method (Franklin and Goodey, 1949) and number of females present were counted. The number of egg masses in the root (5g) was estimated by immersing the roots in Phloxine B solution (0.15 g Phloxine B in IL water) for 15 minutes to stain egg masses and number of eggs per egg masses were estimated by following the

method of Byrd *et al*. (1983). The reproduction factor (RF) was calculated according to Oostenbrink's formula (RF=Pf/Pi ; Pf-final population; Pi-Initial population) (Oostenbrink, 1966)

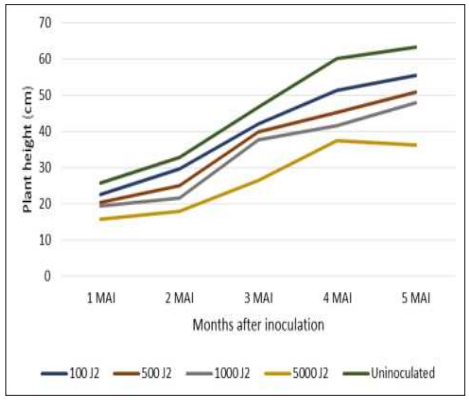
Assessment of biochemical response of coleus cultivars to *M. incognita* infection

Biochemical basis of resistance in the resistant and susceptible cultivars was assessed by estimating changes in total phenols, peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) in the roots and leaves of plants three months after inoculation of nematode. The phenol content was estimated by adopting standard method described by Bray and Thorpe (1954) using Folin- Ciocalteu reagent and measuring absorption at 650 nm in a spectrophotometer. PO activity was assessed using spectrophotometric method described by Srivastava (1987). PPO and PAL activity was assayed according to the procedure described by Mayer *et al.* (1966) and Dickerson *et al.* (1984) respectively. The data generated were subjected to analysis of variance (ANOVA) (Cochran and Cox, 1965).

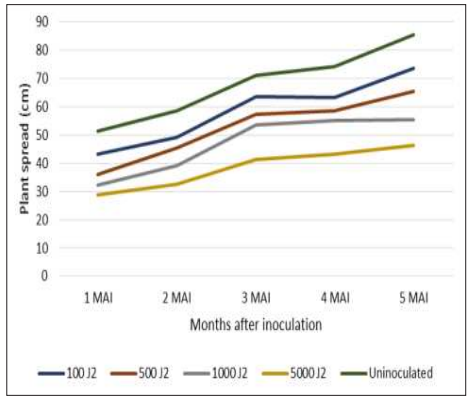
RESULTS AND DISCUSSION

Effect of different inoculum levels on crop loss

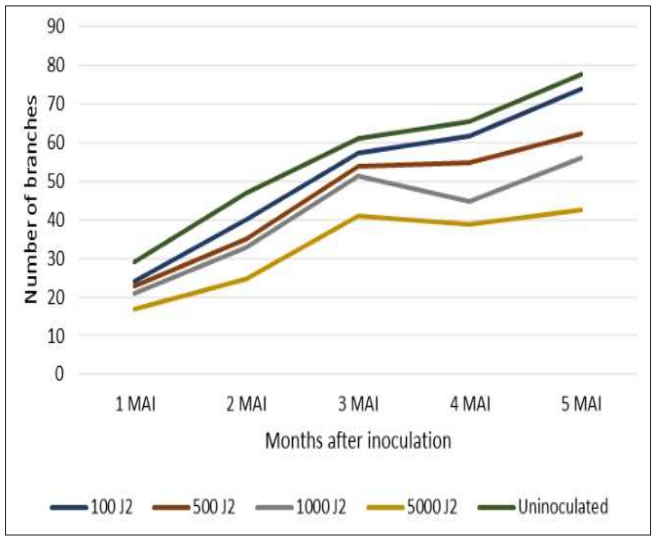
The effect of different inoculum levels of *M. incognita* on the growth parameters (plant height, plant spread, number of branches and leaves) of *P. rotundifolius* showed significant variation compared to the uninoculated control plants (Fig 1). There was significant reduction in plant height from 3 to 5 MAI at lowest inoculum level of 100 J_2. During the second and third month, there was significant reduction in plant height at 500 J2 ranging from 14.44 to 24.24. Highest reduction (37.78 to 45.45 %) was recorded by 5000 J_2 inoculated plants from one to five MAI. This was in accordance with the findings of Abbasi and Hisamuddin (2014) who reported significant reduction in plant height with increasing inoculum levels of *M. incognita* in green gram. Kumar (2004) reported 17.00 to 25.00 % reduction in hight of plants of *Plumbago rosea* at 10000 J_2 level of *M. incognita* six months after inoculation onwards. In this study, even at lowest inoculum of 100 and 500 J_2 significant reduction in plant height was observed indicating the high susceptibility of *P. rotundifolius* to *M. incognita* infestation. Regarding plant spread also, progressive reduction was observed with increase in inoculum levels. At lowest nematode inoculum level of 100 J_2, the plant spread was 43.25 cm at 1MAI while plants inoculated with 5000 J_2 recorded plant spread of 28.75 cm. Similar trend was observed in 2, 3, 4 and 5 MAI also with inoculum levels of 100 J_2 (49.25 to 73.75 cm), 500 J_2 (45.50 to 65.50 cm), 1000 J_2 (39.25 to 55.50 cm) and 5000 J_2 (28.75 to 46.25 cm). Highest reduction in plant spread was recorded in plants inoculated with 5000 J_2 (41.75 to 45.91% over uninoculated) from 1 to 5 MAI. With respect to number of branches and leaves also an increase in percent reduction was recorded from 1 to 5 MAI with increase in inoculum levels. Highest reduction in number of leaves was observed in 5000 J_2 inoculated plants (15.67 to 47.16%). The mean number of branches ranged from 24.25 to 74.00 at lowest inoculum level of 100 J_2 while at highest inoculum level (5000 J_2) it ranged from 16.75 to 42.50. Kumar (2004) reported 24.00 per cent reduction in number of branches of *Plumbago rosea* at 100 J_2 inoculum level at the time of harvest. At 1MAI, lowest number of leaves (275.00) was recorded in plants inoculated with 5000J_2, while in plants inoculated with lowest inoculum level (100 J_2) mean number of leaves was 358.50. Similar findings were reported by Nalinakumari *et al.* (1995) and Kumar (2004) in betel vine (57 to 68 %) and chethikoduveli (16 to 29 %) respectively.



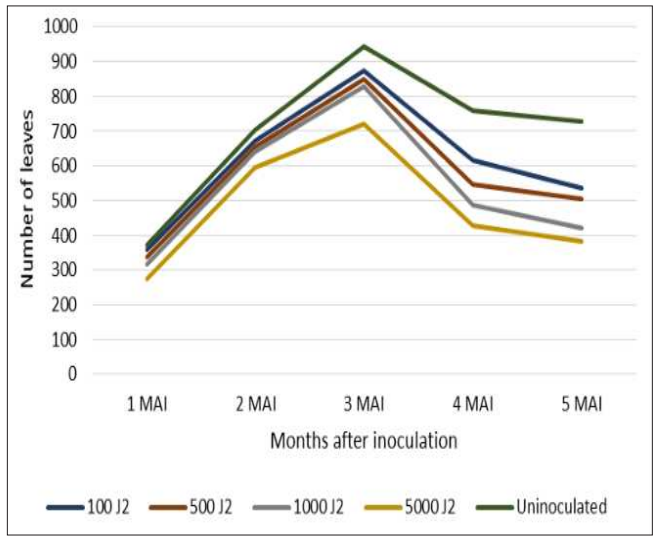
A. Effect on plant height



B. Effect on plant spread



C. Effect on number of branches



D. Effect on number of leaves

Fig.1. Effect of different levels of *M. incognita* on the growth parameters of *P. rotundifolius* at different intervals after inoculation

A progressive decrease in yield parameters was noticed with increase in inoculum levels of *M. incognita*. Statistically significant difference was observed in number and weight of tubers (total and marketable) at different inoculum levels (100, 500, 1000 and 5000). The percentage reduction over uninoculated in number and weight of total and marketable tubers at different inoculum levels ranged from 15.77 to 61.41. The size of tubers also showed significant variation at different inoculum levels. The tuber size in plants inoculated with 100 J_2 was 9.50 cm while in uninoculated it was 15.25 cm. The weight of edible portion ranged from 195.00 to 312.50 g/plant at different inoculum levels while in uninoculated it was 388.75g. Per plot yield was lowest in 5000 J_2 inoculated plants (2.51 kg) while in plants inoculated with lowest inoculum level of 100 J_2 it was 4.71 kg (Table 1). These findings are in accordance to Mohandas and Ramakrishnan (1997) who reported significant reduction in yield of *Dioscorea rotundata* at an inoculum level of 100 J_2.

The quality parameters of tubers (protein, starch, sugar and crude fibre) also differed significantly in tubers of plants inoculated with different inoculum levels of *M. incognita*. An increase in protein content in tubers was recorded with increased inoculum level of *M. incognita*. Highest protein content was observed in tubers of plants inoculated with 5000 J_2 (8.49 g/100 g dry weight of tuber) while in uninoculated control plants it was 7.42 g. The increase in protein content of tubers in different levels of inoculum ranged from 12.94 to 14.42%. This may be due to the function of defense mechanism of the plant. This finding was in line with Paulson and Webster (1972), who reported increased protein synthesis in hypersensitive cells in tomato galls. Arya and Tiagi (1982) reported increased protein content in infested cells of carrot. Higher rate of protein synthesis by plants during invasion of nematodes as means of defence response in bitter gourd was reported by Gautam and Podder (2014). There was significant variation in the starch content of *P. rotundifolius* tubers at different inoculum levels compared to the uninoculated (18.36 g/100 g dry weight of tubers). The effect of 5000, 1000, 500 and 100 J_2 levels was statistically independent with mean starch content of 12.24, 15.62, 16.54 and 17.70 g/100 g dry weight of tuber respectively (Table 1). This decrease in starch content due to increase in inoculum level of *M. incognita* could be due to the increased amylase activity. Orion and Bronner (1973) reported localized strong amylase activity and decreased starch content within the giant cells of the tomato galls. Mahapatra and Nayak (2019) reported significant reduction in starch content of bitter gourd due to *M. incognita* infestation. In the case of sugar content of tubers, there was statistically significant variation between different inoculum levels (3.07 to 3.42 g/100g dry weight of tuber) and uninoculated (3.72 g/100g dry weight of tuber). The tubers of 500 and 100 J_2 inoculated plants showed 15.86 and 8.06% reduction in sugar content over the uninoculated and these effects were statistically on par. The percentage reduction in sugar content at 1000 and 5000 J_2 levels was 17.20 and 17.47 respectively. Roy (1979) reported the localization of invertase in the oesophagus and the intestine of the

Table 1. Effect of different levels of *M. incognita* on yield and quality parameters of *P. rotundifolius* at harvest

Levels of inoculum	Yield parameters (per plant)							Quality parameters (g/100g dry weight of tuber)			
	Total number of tubers	Total number of marketable tubers	Size of tubers (cm)	Weight of total tubers (g)	Weight of total marketable tubers (g)	Weight of edible portion of tubers (g)	Yield per plot (Kg)	Protein	Starch	Sugar	Crude fibre
100 J_2	74.75[b]	60.25[b]	9.50b	425.00[b]	340.00[b]	312.50[b]	4.71[b]	8.38[a]	17.70[b]	3.42[b]	1.28[b]
500 J_2	62.50[c]	51.00[c]	13.50[a]	376.25[c]	320.00[c]	261.75[c]	3.70[c]	8.41[a]	16.54[c]	3.13[c]	1.01[c]
1000 J_2	53.75[d]	39.00[d]	14.75[a]	357.50[d]	260.00[d]	227.50[d]	2.60[d]	8.42[a]	15.62[d]	3.08[c]	0.67[d]
5000 J_2	44.75[e]	28.75[e]	15.50[a]	290.00[e]	223.75[e]	195.00[e]	2.51[d]	8.49[a]	12.24[e]	3.07[c]	0.60[d]
Uninoculated	88.75[a]	74.50[a]	15.25[a]	527.50[a]	405.00[a]	388.75[a]	5.10[a]	7.42[b]	18.36[a]	3.72[a]	1.58[a]
CD 0.05	3.036	3.622	2.767	7.603	6.778	10.256	0.232	0.168	0.083	0.140	0.087

*Mean of four replications

nematode parasite and suggested the possibility of its secretion by the nematode into the host tissue resulting in changed carbohydrate metabolism during the course of host parasite interaction. The finding of these experiments was supported by Pandey *et al.* (2017) who reported significant reduction in total sugar content in green gram due to the infestation of *M. incognita*. Regarding crude fibre content also, there was significant reduction with increase in inoculum levels and the percentage reduction over uninoculated varied from 18.99 to 62.03 %. The decrease in crude fibre content of tubers with increase in inoculum level of *M. incognita* may be due to the poor absorption and storage of nutrients by infested plant roots. This finding was in tune with Sunilkumar (2016) who reported per cent reduction of 5.70, 5.49, 10.71 and 19.01 in the crude fibre content of ginger rhizome after inoculation with 100, 500, 1000 and 10000 J_2 of *M. incognita* respectively.

Screening of coleus cultivars against *M. incognita*

Data on reaction of cultivars of *P. rotundifolius* to *M. incognita* in terms of nematode population characteristics showed statistically significant variation (Table 2). The performance of local cultivar Alur collection was significantly superior to all other cultivars with lowest number of *M. incognita* juveniles in soil (128.33/200cc) and root (10.67/5g). Total nematode population also varied significantly between different cultivars of *P. rotundifolius*. Regarding number of females, eggs and egg masses also, Alur collection recorded lowest number and showed significant superiority to other cultivars. Lowest root-knot index (2.00) and reproduction factor (0.39) was recorded in Alur collection while in susceptible cultivar Pulamanthol collection it was 4.00 and 2.75 respectively. Based on root-knot index and reproduction factor, Alur collection was rated resistant and Sree Dhara, TCM-9 and M-131 were rated as moderately resistant to *M. incognita*. Ankita (2019) evaluated 30 genotypes of *P. rotundifolius* and reported Kenichira local, Suphala, CP-8 and Edayur as resistant to *M. incognita.* Resistance of Alur collection to *M. incognita* infestation was reported first time in this study. The potential of Alur collection to resist the attack of *M. incognita* was directly reflected in the biometric characters and yield also. Regarding plant height, no. of leaves, no. of branches, weight of shoot and root, Alur collection outperformed the other cultivars. Highest number and weight of tubers was recorded in Alur collection with 27.24 to 28.82% increase over susceptible cultivar, Pulamanthol collection (Table 3). This observation in this study is in line with Kanakam *et al*. (2019). The significant difference in nematode population, biometric characters and yield observed between the local collection, Alur and other cultivars may be due to the presence of resistance traits in the resistant cultivar.

Table 2. Population build-up of *M. incognita* in different cultivars of *P. rotundifolius*

Cultivar	Final Nematode Population			Total nematode population	Reproduction factor	No. of egg masses (5g root)	No. of eggs/ egg mass	Gall index	Reaction
	Soil (200cc)	Root (5g)	Females (5g root)						
Nidhi	683.67 (26.09)^{b}	88.67 (9.41)^{a}	90.00 (9.49)^{a}	862.33 (29.33)^{b}	2.16^{b}	32.00 (5.64)^{a}	191.33 (13.83)^{b}	4	S
Alur collection	128.33 (11.33)^{e}	10.67 (3.25)^{d}	16.67 (4.08)^{d}	155.67 (12.48)^{f}	0.39^{e}	8.33 (2.87)^{d}	115.53 (10.74)^{f}	2	R
Pulamanthol collection	921.33 (30.34)^{a}	91.33 (9.56)^{a}	91.67 (9.57)^{a}	1101.00 (33.17)^{a}	2.75^{a}	31.67 (5.62)^{a}	203.33 (14.26)^{a}	4	S
Sree Dhara	775.00 (27.83)^{b}	62.67 (7.91)^{b}	35.67 (5.95)^{b}	873.33 (29.54)^{b}	2.19^{b}	22.67 (4.74)^{bc}	167.00 (12.92)^{c}	3	MR
Mankada	550.67 (23.43)^{c}	93.67 (9.68)^{a}	96.67 (9.83)^{a}	741.00 (27.19)^{c}	1.85^{c}	28.33 (5.32)^{ab}	197.33 (14.05)^{ab}	4	S
TCM-9	361.67 (18.98)^{d}	61.67 (7.85)^{b}	33.33 (5.77)^{b}	456.67 (21.34)^{d}	1.14^{d}	21.67 (4.64)^{bc}	155.33 (12.46)^{d}	3	MR
M131	278.33 (16.65)^{d}	47.33 (6.88)^{c}	22.67 (4.76)^{c}	348.30 (18.64)^{e}	0.87^{d}	17.67 (4.19)^{c}	128.00 (11.31)^{e}	3	MR
CD (0.05)	(2.348)	(0.510)	(0.583)	(2.119)	0.291	(0.833)	(0.402)		

Figures presented in the paranthesis are square root transformed

Table 3. Growth and yield parameters of different coleus cultivars infested with *M. incognita*

Cultivar	*Plant height (cm)	*No. of leaves	*No. of branches	*Weight of shoot (g)	*Root weight (g)	*No. of tubers / plant	*Weight of tubers/plant (g)
Nidhi	35.67^{f}	509.67^{g}	33.00^{e}	208.00^{f}	12.33^{d}	51.67^{d}	370.00^{g}
Alur collection	67.33^{a}	676.67^{a}	49.33^{a}	490.67^{a}	24.33^{a}	73.00^{a}	540.33^{a}
Pulamanthol collection	39.00^{ef}	541.33^{f}	36.00^{d}	232.67^{f}	13.00^{d}	56.67^{c}	424.67^{f}
Sree Dhara	47.67^{d}	597.67^{d}	38.67^{cd}	338.00^{d}	17.00^{bc}	60.33^{bc}	493.00^{d}
Mankada	42.33^{e}	567.33^{e}	37.33^{d}	296.33^{e}	16.33^{c}	58.33^{c}	472.33^{e}
TCM-9	52.67^{c}	614.00^{c}	40.33^{c}	393.00^{c}	17.67^{bc}	63.00^{b}	505.33^{c}
M131	60.33^{b}	638.67^{b}	44.33^{b}	423.67^{b}	19.67^{b}	64.33^{b}	518.33^{b}
CD (0.05)	3.462	8.316	2.849	25.099	3.024	4.317	10.863

*Mean of three replications

Table 4. Variation in phenol, peroxidase, poly phenol oxidase and phenyl alanine ammonia lyase in leaves and roots of different cultivars of *P. rotundifolia* infested with *M. incognita*

Cultivars	Phenol content (mg of catechol/ g tissue)*		Peroxidase (PO) (min/g/fresh weight) *		Phenylalanine Ammonia Lyase (PAL) * (µg of cinnamic acid g/ fresh weight)		Polyphenol oxidase (PPO) * min/g/ fresh weight	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Nidhi	0.63 ^{d}	2.07^{d}	5.81 ^{d}	6.08 ^{d}	12.75 ^{d}	13.01^{d}	6.70 ^{b}	7.88^{e}
Alur collection	1.32 ^{a}	6.50 ^{a}	9.07^{a}	9.64 ^{a}	15.85^{a}	17.44^{a}	9.26 ^{b}	11.30^{a}
Pulamanthol collection	0.47 ^{e}	1.82 ^{d}	5.76 ^{d}	6.01 ^{d}	12.16 ^{d}	12.79^{d}	6.61^{b}	8.41^{de}
Sree Dhara	1.05 ^{b}	5.00^{b}	6.87 ^{c}	7.32^{c}	14.88 ^{b}	15.58^{bc}	7.37^{a}	9.43 ^{c}
Mankada	0.84 ^{c}	3.18^{c}	6.77 ^{c}	7.09 ^{c}	14.21^{bc}	14.65^{c}	7.19 ^{b}	8.89 ^{b}
TCM-9	0.82 ^{c}	3.12^{c}	6.76 ^{c}	7.07 ^{c}	14.09 ^{c}	14.65^{c}	7.01 ^{c}	9.12 ^{cd}
M131	1.21 ^{a}	6.39 ^{a}	7.97 ^{b}	8.28 ^{b}	15.61 ^{a}	16.28^{b}	8.31^{c}	10.34 ^{b}
CD 0.05	0.150	0.334	0.449	0.503	0.715	1.127	0.430	0.869

*Mean of three replications

The biochemical basis of resistance was assessed in terms of phenol content and defence enzyme activity. Alur collection recorded highest phenol content both in leaf (1.32 mg of catechol/ g tissue) and root (6.50 mg of catechol/ g tissue) and it was statistically on par with moderately resistant cultivar M-131 giving 1.21 and 6.39 mg of catechol/ g tissue respectively (Table 4). Regarding defence enzymes viz. PO, PPO and PAL, Alur collection recorded highest activity both in leaf and root compared to other varieties. The percentage increase over the susceptible cultivar, Pulamanthol collection ranged from 21.19 to 60.40. Similar findings were reported Das *et al.* (2011) in banana who reported increased activity of PO, PAL and PPO in roots of resistant banana hybrids compared to susceptible ones.

The present investigation revealed that *M. incognita* infestation in coleus have a significant impact on the growth and yield characteristics of the plant and also on the quality parameters of its tubers. Screening of different cultivars of coleus against *M. incognita* revealed that Alur collection is the resistant variety which performed better in reducing the multiplication of nematodes and increasing the yield and quality parameters. This study concludes that *M. incognita* infestation in coleus could result in significant crop loss and quality deterioration of tubers by directly or indirectly affecting its growth and biochemical parameters. The phenol and defence enzyme activities were found higher in roots and leaves of Alur collection compared to susceptible cultivar, Pulamanthol collection. Alur collection can be utilized in breeding programmes for developing high yielding varieties.

REFERENCES

A.O.A.C. 1969. *Official and Tentative Methods of Analysis*. Tenth edition. Association of Official Agricultural Chemists, Washington, D. C., p. 145.

A.O.A.C. 1975. *Official and Tentative Methods of Analysis*. Twelfth edition. Association of Official Agricultural Chemists, Washington, D. C., p. 136.

Abbasi and Himayuddin. 2014. Effect of different inoculum levels of *Meloidogyne incognita* on growth and biochemical parameters of *Vigna radiata*. *Asian Journal of Nematology,* **3** (1):15-20.

Ankitha, M. O. 2019. Genetic variability in chinese potato (*Solenostemon rotundifolius* (Poir) J. K. Morton) for yield and nematode tolerance. M. Sc. (Ag.) thesis. Kerala Agricultural University. Thiruvananthapuram, Vellayani, 150p.

Arya, M. and Tiagi, B. 1982. Reaction of some carrot cultivars to root knot nematode, *Meloidogyne incognita*. *Indian Journal of Nematol*ogy, **12**: 397.

Bray, H. G. and Thorpe, W. V. 1954. Analysis of phenolic compounds of interest in metabolism. *Methods of Biochemical Analysis*. Vol. 1, Intersience Publishers, New York, **1**: 27-52.

Byrd, D.W., Ferris, H. and Nusbaum, C. J. 1972. A method for estimating numbers of eggs of *Meloidogyne* spp. in soil. *Journal of Nematol*ogy, **4**(4): 266-269.

Cochran, W.G. and Cox, G.M. 1965. Experimental design (2nd ed.), John Wiley and Sons Inc, New York, pp. 182.Cochran & Cox https://doi.org/10.1002/bimj.19650070313

Das, S. C., Balamohan, T. N., Poornima, K., Velalazan, R., and Seenivasan, N. 2011. Screening of banana hybrids for resistance to *Meloidogyne incognita*. *Indian Journal of Nematol*ogy, **41**(2): 189-196.

Dickerson, D . P., Pascholati, S. F., Hagerman, A. E., Butler, L. G., and Nicholson, R. L. 1984. Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. *Physiological Plant Pathol*ogy, **25**: 111-123.

Eisenback, J. D. 1985. Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.) In: Sasser, J. N. and Carter, C. C. (eds), *An Advanced Treatise on Meloidogyne Biology and Control*. North California State University, Raleigh, USA, pp. 95-112.

Franklin, M. T. and Goodey, J. B. 1949. A cotton blue-lactophenol technique for mounting plant-parasitic nematodes. *Journal of Helminthology*, **23** (3-4): 175-178.

Gautam, S. K. and Poddar, A. N. 2014. Study on protein and sugar content in *Meloidogyne incognita* infested roots of bitter gourd. *International Journal of Current Microbiology and Applied Sciences,* **3**(5): 470-478

Jackson, M. L. 1973. *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi, p. 498.

Kankam, F., Sowley, E. N. K., Adomako, J. and Boateng, A. 2019. Variations in the level of resistance to root-knot nematodes (*Meloidogyne* spp.) infestation among ten cowpeas (*Vigna unguiculata* L. Walp.) genotypes. *Ghana Journal of Agricultural Science,* **54**(2): 68-78.

Kumar, S. T. 2004. Host parasite relationship and management of important nematodes associated with chethikoduveli, *Plumbago rosea* L. Ph.D. thesis, Kerala Agricultural University, Thrissur, p. 192.

Mahapatra, M. and Nayak, D. K. 2019. Biochemical and physiochemical changes in susceptible and resistant bitter gourd cultivars/varieties as influenced by root knot nematode, *Meloidogyne incognita*. *Journal of Entomology and Zoology Studies*, **7**(3): 80-87.

Mayer, A. M., Harel, E. and Ben-Shaul, R. 1966. Assay of catechol oxidase- A critical comparison of methods. *Phytochemistry,* **5:** 783-789.

Mohandas, C. and Ramakrishnan, S. 1997. Pathogenic effect of root-knot nematode, *Meloidogyne incognita* on African white yam, *Dioscorea rotundata. Indian Journal of Nematology,* **27**:233-236

Mohandas, C. and Ramakrishnan, S. 1998. Fluctuation in the population of root-knot nematode in chinese potato tubers during storage. *Journal of Root Crops,* **24**: 55-57

Nalinakumari, T., Sheela, M. S., Hebsybai and Kuryan, K. J. 1995. Comparative effect of root-knot and reniform nematode on growth and yield of betel vine. *Indian Journal of Nematology*, **25**: 14-15.

Orion, D. and Bronner, R. 1973. The localization of starch, amylase and invertase in *Meloidogyne javanica* galls, *Nematologica*, **19**: 401-402.

Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Meited. Landbhoogesch Wageningen, **66**: 1

Pandey, R. K., Nayak, D. K. and Kar, R. K. 2017. Effects of nematode infection on sugar and starch. *Journal of Global Biosciences*, **6**(4): 4935-4939.

Paulson, R. E. and Webster, J. M. 1972. Ultrastructure of the hypersensitive reaction in roots of tomato, *Lycopersicon esculentum* L. to infection by the root-knot nematode, *Meloidogyne incognita*. *Journal of Plant Physiology and Pathology*, **2**: 227-234.

Pigman, W. 1970. *Analytical methods for carbohydrates*. The carbohydrates vol. II B. Academic Press, New York, pp. 101-145.

Roy, T. K. 1979. Histochemical studies of hydrolytic enzymes in *Meloidogyne incognita* (Nematoda: Tylenchidae) and infected host *Lycopersicon esculentum* and their role in host parasite relationship. *Indian Journal of Experimental Biology,* **17**: 1357-1362.

Sathyarajan, P. K., Das, N. M. and Nair, M. R. G. K. 1966. Root-knot nematode as a pest of *Coleus parviflorus* in Kerala. *Agriculture Research Journal of Kerala*, **4:** 144-145.

Southey, J. F. 1986. Extract of *Meloidogyne* egg masses. In: Southey, J. F. (ed.), *Laboratory Methods for Work with Plants and Soil Nematodes*. HMSO, London, pp. 42-44.

Srivastava, S. 1987. Peroxidase and polyphenol oxidase in *Brassica juncea* plants infected with *Macrophomina phaseolina* (Tassai) Goid. and their implication in disease resistance. *Journal of Phytopathol*ogy, **120:** 249-254.

Sunilkumar B. C. 2016. Biochemical changes due to root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in ginger (*Zingiber officinale* Roscoe). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p. 158.

Taylor and Netscher, C. 1974. An improved technique for preparing perineal patterns of *Meloidogyne* spp. *Nematol*ogica, **20 (2**): 268-269.

Taylor, A. L. and Sasser, J. N. 1978. *Biology, identification and control of root-knot nematode, Meloidogyne incognita,* North Carolina State University, and United States Agency for International Development Raleigh NC, USA, 3p.

Venkitesan, T. S. and Sethi, K. G. H. 1977. Pathogenicity of *Radopholus similis* to black pepper, *Piper nigrum*. *Indian Journal of Nematol*ogy, **7**: 17-26.

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Effect of soil properties and temperature on the nematode antagonistic potential of bacterial bioagent, *Pasteuria penetrans* against root knot nematode, *Meloidogyne incognita*

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ABSTRACT: The nematode antagonistic bacterium, *Pasteuria penetrans* is one of the proven and highly efficient biocontrol agents. A study was conducted to understand the influence of various abiotic factors. Five different soil types *viz*., alluvial, black, red, laterite and sandy were filled in paper cups (200g/cup) and freshly hatched second stage juvenile (J2) of root knot nematode, *M. incognita* (1000 J2/cup) along with 10 mg of root powder (1.84 x 10^6 spores/g) was thoroughly mixed and incubated at 25°C for 48 h. The parasitization was higher (95.5%) in sandy soil with a clay content of 6.0 per cent. Spore attachment of *P. penetrans* at four levels of temperatures *viz*., 15, 20, 25 and 30°C in red loamy soil was tested. Significant influence of temperature was observed in parasitization of second stage juvenile under 25^0C (90.2%). A glasshouse experiment was conducted to study the influence of nematicides. Application of carbofuran along with *P. penetrans* increased the rate of parasitisation which was 83.1 per cent as against 61.0 per cent in *P. penetrans* treatment only. Influence of root exudates of host and non-host plants of *M. incognita* was tested on the parasitizing ability. The parasitizing ability significantly increased by 4.5 per cent in tomato root exudates and by 4.0 per cent in coleus. The host plant had influence over the number of spore attachment on J2. The results of these experiments paved way to exploit the nematode bacterial hyper parasite, *P. penetrans* for managing plant parasitic nematodes under varied soil environmental conditions.

Keywords: Root knot nematode, *Pasteuria penetrans*, soil type, temperature, nematicides, spore attachment, root exudates

INTRODUCTION

Plant parasitic nematodes are one of the limiting factors in crop productivity. Most of the horticultural crops are susceptible to nematodes and cause 10 – 15% yield loss. Chemical nematicides are widely used for nematode management. Some of the major nematicides were withdrawn from market due to their harmful effects to soil and environment. *Pasteuria penetrans* is a potential bacterial hyper parasite of nematodes. Biological control potential of this bacterium has been proved by many Nematologists in India (5, 11) and abroad. The endospores of *P. penetrans* can tolerate various temperature regimes, moisture levels, pH and chemicals. This research paper describes the parasitisation potential of *P. penetrans* on root knot nematode, *Meloidogyne incognita* under various abiotic stresses.

MATERIALS AND METHODS

Soil Type

Influence of soil type on the activity of *P. penetrans* was carried out in five different type *viz*., alluvial, black, red, laterite and sandy. Plastic cups (250g capacity) were filled with sterilized soils (200g/cup) of the above mentioned types separately. Freshly hatched juveniles (J2) of *M. incognita* (1000 J2/cup) along with 10 mg of root powder (*P. penetrans* @ 1.84 x 10^6 spores / g) was thoroughly mixed with soil and incubated at 25ºC for 48 h. Soil was maintained with 25 per cent moisture level by adding 50 ml water. Each treatment was replicated six times. The nematodes in each replicate were extracted by using a combination of sieving and Baermann funnel technique and the number of spores attached/J2 and percentage of parasitisation were assessed. The soil texture, field capacity, pH, EC and organic matter were analysed as per standard methods.

Temperature

Interaction between *P. penetrans* and *M. incognita* was determined under four levels of temperatures *viz*., 15, 20, 25 and 30ºC on red loamy soil. Plastic cups were filled with sterilized soil (200 g/cup) and thoroughly mixed with 100 J2 of *M. incognita* along with 10 mg root power / cup (1.84 x 10^6 spores/g) and incubated at the above mentioned temperatures for 48 h. Each level of temperature was replicated eight times. The spore attachment percentages on J2 were recorded after extraction of nematodes from soil.

Nematicides

Glasshouse experiment

A glasshouse experiment was conducted to study the influence of nematicides on *P. penetrans*. Sterilized soil was filled in 2.5 kg clay pots and 3-week-old tomato cv. Co 3 seedlings were transplanted. The following treatments were given three days after transplanting.

T1- *M. incognita* 5000 J2 / pot
T2 - *M. incognita* 5000 J2 / pot + *P. penetrans* root power 10 mg /pot @1.84 x 10^6 spores / g
T3 - *M. incognita* 5000 J2 / pot + carbofuran 3 mg / pot
T4 - *M. incognita* 5000 J2 / pot + *P. penetrans* root power 10 mg @1.84 x 10^6 spores / g+ carbofuran 3 mg/ pot

Each treatment was replicated five times and completely randomised. Plant biometric observations, soil and root nematode population, number of J2 parasitized and number of spores attached per J2 were recorded at 60 days after transplanting.

Root exudates

Root exudates of tomato *(Lycopersicon esculentum* Mill), Chilli *(Capsicum annuum L.),* Cowpea *(Vigna unglciculata L.)***,** coleus (*Coleus blumi,* Benth)**,** marigold (*Tagetes patula* L.), maize *(Zea mays L.)., (Sorghum vulgare L.)* and sunflower *(Helianthus annus L.)* were collected 35-40 days after germination. Root exudates of each plant species was taken individually in 5 cm Petr idish (5 m l / dish). Freshly hatched 500 J2 of *M. incognita* and 10 mg root powder (1.84 x 10^6 Pp spores / g) per dish were added and gently mixed with the root exudates and incubated at 25º C for 48 h. Tap water was used as control. A completely randomised block design with eight replicates was adopted. The percentage of individual J2 parasitized and the numbers of spores attached per J2 were recorded.

RESULTS AND DISCUSSION

Influence of different abiotic factors and root exudates on parasitization of bacterial parasite, *P. penetrans* is elaborated below.

Soil type

The texture of soils varied from sandy to clay loam with the clay content varying from 6 per cent to 25.9 per cent. The percentage of parasitisation was high in sandy soil (95.5) with a clay content of only 6.0 per cent and the least in laterite soil (71.0) with a clay content of 25.9 per cent (Table 1 & 2). The relationship

Table 1. Properties of different types of soil used for parasitisation study

Soil types	Coarse sand (%)	Fine Sand (%)	Silt (%)	Clay (%)	Field Capacity	pH	EC dSm-1	OM (%)
Alluvial (sandy loam)	20.9	32	26	19.5	28.5	9.1	1.05	0.83
Black cotton (clay loam)	20.2	43.8	3.5	22.5	30.2	8.3	0.52	1.1
Red (sandy loam)	41.6	26.4	17	12	14.7	7.4	0.48	0.38
Laterite (sandy clay loam)	32.1	22.7	18.2	25.9	20	4.9	0.24	2.18
Sandy	40.3	51.2	2.5	6	5	8	0.05	-

Table 2. Effect of Different soil types on the interaction of *M. incognita* and *P. penetrans*

Soil Types	Parasitization (%)	Number of spores/J2
Alluvial	86.9 (68.78)^c	8.0^c
Black	81.3 (64.38)^d	7.0^d
Red	90.7 (72.24)^b	9.5^b
Laterite	71.9 (57.99)^e	6.3^e
Sandy	95.5 (77.75)^a	10.7^a
CD (P=0.05)	3.5	0.47

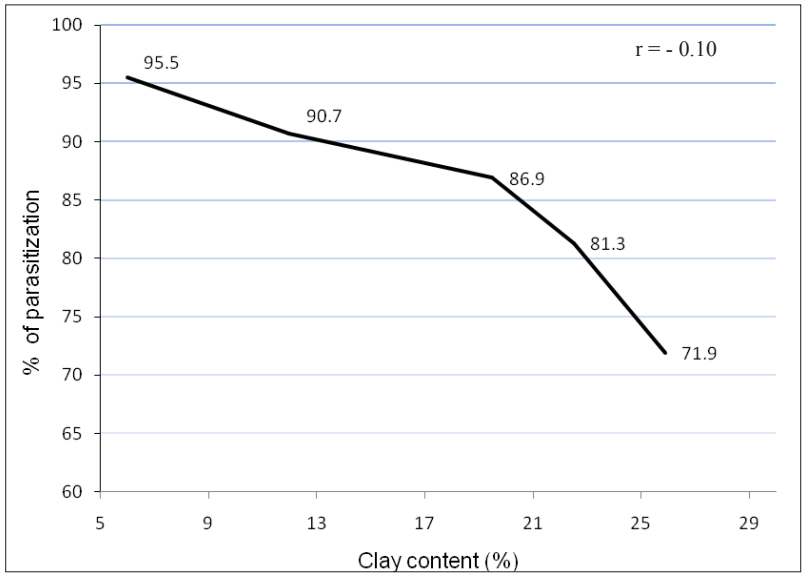

Fig. 1. Influence of clay content on percentage of parasitization of *P. penetrans* on *M. incognita*

between the clay content and extent of parasitization was statistically negatively correlated (r = - 0.10) (Fig. 1) The clay content also influenced the spore load carried by individual J2 which varied from 6.3 in laterite soil to 10.7 in sandy soil. Textural and physical characters of soils were studied for interaction of *M. incognita* and *P. penetrans*.

The spore attachment on *M. incognita* and the percentage of parasitization varied significantly in soils of different textures. Soil texture and other factors such as salinity, pH, organic content etc., that influence the nematode movement in soil (5) are likely to influence the *P. penetrans* activity. The parasitizing ability of *P. penetrans* was high in sandy soil and low in laterite soil. The higher parasitizing ability of the hyper-parasite is thus attributable to the more rapid and easy dispersal of nematode in sandy and sandy loam soils when compared to heavy laterite soil. Spaull (7) observed that *P. penetrans* occurred more frequently in sand and loamy sand than in other types in sugarcane fields of South Africa. The soil structure and EC have also been reported to affect the dispersal of *P. penetrans* spores in soil with loose texture (2). Physical and chemical soil properties might also affect the bacterial establishment and dispersal as has been shown for other nematode-antagonist systems. Small soil pores existed in soil containing high percentage of clay which may restrict the movement of *P. penetrans* spores. Verdjo-Lucas (12) discussed that spore may adhere to clay particles and would not be free to attach themselves to nematodes. This factor also could have influenced the reduced level of parasitization observed in the heavy soils in the present investigation.

Temperature

Significant influence of temperature was observed in the extent of parasitisation of J2 by *P. penetrans* which was 90.5 per cent under 25ºC and 20.7 per cent under 15 ºC. The same trend was seen in the spore load carried by individual J2 which ranged from 3.4 under 15ºC to 7.5 under 25ºC (Table 3). The results of present experiment agreed with the findings of Anjukamra and Dhavan (1). Greater spore attachment was observed on *M. javanica* at

Table 3. Influence of temperature on per cent parasitization of *P. penetrans*

Temperature	Percentage of parasitization	Number of spores attached/J2
15^0C	20.7^{d} (26.0)	3.4^{d}
20^0C	64.4^{b} (53.4)	4.3c
25^0C	90.59^{a} (71.9)	7.5^{a}
30^0C	55.6^{c} (48.0)	5.8^{b}
CD (P=0.05)	0.7	0.2

Table 4. Effect of nematicides on the interaction of *M. incognita* and *P. penetrans* in tomato

Treatments	Number of nematodes /200 g soil	Number of galls /g root	Number of egg masses/ 2g root	Percentage of J2 parasitized/ 200 g soil	Number of spores attached/ J2	Fresh weight of shoot (g)	Fresh weight of root (g)
M. incognita alone	320.0[a]	28.8[a]	40.4[a]	-	-	31.4[c]	19.2[abc]
M. incognita + *P. penetrans* (10mg)	147.4[b]	22.6[b]	18.6[b]	61	8.3	41.6[b]	19.8[abc]
M. incognita + carbofuran 6 mg	122.3[c]	20.6[bc]	16.0[bc]	-	-	47.6[b]	21.2[ab]
M. incognita + *P. penetrans* (10mg) + carbofuran 6 mg	107.0[d]	18.0[bc]	11.4[c]	83.1	11	57.4[a]	22.8[a]
CD (P=0.05)	14	4.8	5.4			7.9	7.0

Table 5. Effect of root exudates on the parasitizing ability of *P. penetrans*

Treatments	Percentage of parasitization	Number of Spores/ J2
Tomato	97.2 (80.37)[a]	180[a]
Coleus	96.7 (79.53)[b]	17.8[b]
Cowpea	91.1 (72.64)[d]	14.1[c]
Sorghum	50.5 (45.29)[f]	5.0[e]
Maize	47.3 (43.45)[g]	4.6[ef]
Sunflower	69.8 (56.66)[e]	9.0[d]
Tagetes	26.0 (29.33)[h]	3.0[ef]
Tap water	92.8 (74.44)[c]	15.5[c]
CD P=0.05)	1.14	2.2

22.5 – 30.0°C (8). Wallace (13) reported that the optimum temperature for *M. incognita* J2 migration is 25°C. Thus the optimum temperature for spore attachment corresponds with the optimum temperature for migration of *M. incognita* J2 in soil. The data further substantiates that the higher parasitizing ability of *P. penetrans* was probably due to greater nematode mobility at 25°C.

Nematicides

Glasshouse experiment

The nematicide and *P. penetrans* when applied together reduced the number of galls, egg masses and soil population to the highest level, which was followed by individual application of carbofuran and *P. penetrans* (Table 4). Application of carbofuran along with *P. penetrans* increased the rate of parasitisation which was 83.1 per cent as against 61.0 per cent in *P. penetrans* treatment only. The number of spores encumbered was also higher when *P. penetrans* was applied along with carbofuran (Table 4).The growth of plant assessed through fresh weight of shoot and root indicated that the application of the bio control agent along with the nematicides gave the best results than the application of either carbofuran or *P. penetrans* alone (Table 5). Application of a combination treatment consisting of

nematicide and P. *penetrans* reduced the number of galls, egg and soil nematode population in a significantly better manner than either component applied individually.

The nematicides carbofuran and aldicarb had no detrimental effect on *P. penetrans* (9). The possibility of increased spore attachment by *P. penetrans* in the presence of nematicide resulting in synergistic reduction of root galling by *M. javanica* was suggested by Brown *et al*. (3). Enhanced activity of the juvenile due to toxic effect of carbamate and organophorphate nematicides resulting in more frequent encounter with the spores has been suggested as reason for increased spore encumbrance and level of parasitism by *P. penetrans* (14).

Root exudates

The influence of root exudates of host and non-host plants of *M. incognita* was tested on the parasitizing ability of *P. penetrans***.** The parasitizing ability significantly increased over control in tap water in tomato by 4.5 per cent and coleus by 4.0 per cent. In the other plants tested namely cowpea, sunflower, maize and tagetes, the parasitizing ability decreased and ranged from 91.1 per cent to 26.0 per cent, while in control it was 92.8 per cent. The difference between exudates of different host plants on the degree of parasitisation by P. penetrans was statistically significant. The host plant had influence over the number of spores which attach to the J2 In tap water control it was 15.5/J2 while in tomato and coleus it was 18.0 and 17.8/J2, respectively. In the exudates of other plant species tested the spore load varied from 3.0 tagetes to 14.1 in cowpea (Table 6). Madula *et al*. (4) reported a high level of infection (75 per cent) of *M. javanica* with *P. penetrans* occurred under continuous cropping with tomato and lower level (25 per cent) under continuous cropping with tobacco and suggested that the action of root exudates could increase the multiplication of *P. penetrans*. The chemical components of root exudates probably influenced the differences in *P. penetrans* parasitization. O'Brien (6) reported that the carbohydrate related lectins are primary factors for spore attachment. So that the carbohydrates present in root exudates may be involved in spore adherence on juvenile cuticle. The parasitization by *P. penetrans* was very low in the exudates of the nematode antagonistic plant, tagetes. Nematicidal property of tagetes also reduce mobility of juveniles and this probably resulted in lower parasitization of J2 under this treatment, since the attachment of spores on J2 cuticle depends on chance contact which increases with nematode migration (8).

CONCLUSION

The bacterial hyper parasite, *Pasteuria penetrans* is a potential bio control agent for nematode management. The findings of the present research work proved that *P. penetrans* can withstand wide range of temperatures and pH ranges. Similarly, the bacterium is compatible with nematicides. Root exudates of nematode host plants enhance the parasitisation per cent of *P. penetrans*.

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REFERENCES

Brown, S. M. and Nordmeyer, D. 1985. Synergistic reduction in root galling by Meloidogyne javanica with Pasteuria penetrans and nematicides.

Kamra, A. and Dhawan, S. C. 1994. Effect of storage temperature on viability of Pasteuria penetrans spores infecting Heterodera cajani. *Indian Journal of Nematology*, **24**(2): pp.116-119.

Madulu, J. D., Trudgill, D. L. and Phillips, M. S. 1994. Rotational management of Meloidogyne javanica and effects on Pasteuria penetrans and tomato and tobacco yields. *Nematologica*, **40**(1-4): pp.438-455.

Mohan, S., Mauchline, T. H., Rowe, J., Hirsch, P. R. and Davies, K. G. 2012. P asteuria endospores from H eterodera cajani (Nematoda: Heteroderidae) exhibit inverted attachment and altered germination in cross-infection studies with G lobodera pallida (Nematoda: Heteroderidae). *FEMS microbiology ecology*, **79**(3): pp.675-684.

Norton, D. C. 1978. *Ecology of plant-parasitic nematodes*, John Wiley & Sons, Inc..

O'Brien, P. C. 1981. Studies on parasitism of *Meloidogyne javanica* by *Bacillus penetrans*.

Spaull, V.W. 1984. Observations on *Bacillus penetrans* infecting *Meloidogyne* in sugarcane fields in South Africa. *Rev. Nematol*, **7**(3): pp.277-282.

Stirling, G. R. 1981. Effect of temperature on infection of Meloidogyne javanica by *Bacillus penetrans.*

Stirling, G. R. 1984. Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. *Phytopathology*, **74**(1): pp.55-60.

Swarnakumari, N. and Sivakumar, C.V. 2012. Bioefficacy of obligate bacterial Parasite, *Pasteuria penetrans* against root-knot nematode, *Meloidogyne incognita* infestation in chilli. *Indian Journal of Nematology*, **42**(1): pp.42-45.

Verdejo, S. and Mankau, R. 1986. October. Culture of pasteuria-penetrans in meloidogyne-incognita on oligoxenic excised tomato root culture. *Journal of Nematology* 18(4): pp. 635-635. 3012

Wallace, H. R. 1966. The influence of moisture stress on the development, hatch and survival of eggs of Meloidogyne javanica. *Nematologica*, **12**(1): pp.57-69.

Wright, D. J. 1981. Nematicides: mode of action and new approaches to chemical control. *Plant parasitic Nematodes*, **3**: pp.421-449.

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Varietal reaction of onion cultivars against *Alternaria porri* causing purple blotch and its management

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ABSTRACT: Ten onion cultivars were evaluated during *kharif* 2021-22 and 2022-23 for their reaction to *Alternaria porri* under field conditions. Of them, three varieties *viz*., Arka Kalyan, Arka Niketan and Arka Bheema showed resistant reaction while two varieties *viz.,* Rampura and Satara local were moderately resistant and rest were moderately susceptible. Among 10 fungicides, 10 botanicals and six bio agents evaluated *in vitro* against *A. porri*, 0.1%Azoxystrobin-23EC, 0.1%Tebuconazole-25EC, *Allium sativum* (15%) and *Trichoderma harzianum* recorded the maximum inhibition of mycelial growth of *Alternaria porri*. The field evaluation of different fungicides and botanicals during indicated that 0.1%Tebuconazole-25EC was recorded minimum PDI of 22.68 and yield of 271.00q/ha. 0.1% Azoxystrobin-23EC,0.1%Difenconazole-25EC,0.2%Mancozeb-75WP and *Allium sativum* cloves extract were next best treatments in reducing the disease intensity by recording PDI of 24.00, 26.00, 28.00 and 46.00 and yield of 268.00q/ha, 262.65q/ha, 264.00q/ha and 234.65q/ha respectively. During *kharif* 2022-23 also 0.1%Azoxystrobin-23EC was found to be the effective treatment followed by 0.1%Tebuconazole-25EC.

Keywords: *Alternaria porri,* fungicides, management, onion, purple blotch

INTRODUCTION

Onion (*Allium cepa* L.) is an important bulbous vegetable crop of global importance and it is used as vegetable, salad and spice in the daily diet by large population. It has been reported to be rich in phytochemicals especially flavonols which are medicinal (Javadzadeh *et al*., 2009). Onion is regarded as highly export oriented crop and earn valuable foreign exchange for the country. Several factors have been identified for the low productivity of onion in India. The most important factor is the purple blotch disease caused by *Alternaria porri* (Ellis) C if. Which affects both bulb and seed crop throughout India. The yield loss of onion in India due to this disease under favorable conditions varies from 5.0 to 96.5per cent (Gupta and Pathak, 1988). In earlier studies, Sharma. (1997) Chethana *et al.* (2011) and Behera *et al.* (2013) were reported the screening of some varieties to locate the tolerance and resistant types. In the present studies, an attempt has been made to test Onion varieties against purple blotch disease and its management. Spraying of broad spectrum fungicides has been recommended for management of disease. Control achieved by these chemicals is inadequate. Therefore, it is thought worthwhile to test the efficacy of more promising chemicals like Propineb-50WP, Metiram-50WP, Hexaconazole-5EC, Azoxystrobin-23EC, Tebuconazol-25EC, Myclobutanil-10WP, and Difenconazole - 25EC against fungus. Not much light has been shed on biological control, botanicals which are effective against *Alternaria porri*. Hence, an attempt has been made to test commonly available botanicals and bio agents against the pathogen.

MATERIALS AND METHODS

Reaction of Onion cultivars

An experiment was conducted at College of Horticulture, Bidar, Karnataka during *Kharif* 2021-22 and 2022-23 in Randomized Complete Block Design with a plot size of 3.6 m x 1.8 m. Ten cultivars viz., Satara Local, Rampura, Arka Kalyan, Agripound Dark Red, Ballary Red, Arka Niketan, Nasik Red, Telagi Red, Kumata and Arka Bheema seedlings were planted at a spacing of 15 cm x 10 cm (row to row x plant to plant) and all the recommended agronomic practices were followed to raise a good crop except fungicidal spray to avoid the killing of fungal pathogen (Anonymous, 2017). As there was heavy incidence of purple blotch during both the years, the cultivars were scored for the disease incidence under natural field conditions without artificial inoculation. At bulb development stage, disease score was measured on ten randomly selected plants from each plot at fortnightly intervals by using 0 to 5 point rating scale (Sharma, 1986).

Scale	Severity
0	No disease symptoms
1	A few spots towards tip covering 1 to 10 per cent leaf area
2	Several dark purplish brown patch covering 11 to 20 percent leaf area
3	Several patches with paler outer zone covering 21 to 40 percent leaf area
4	Leaf streaks covering 41 to 75 per cent leaf area and breaking of the leaves from center
5	Leaf streaks covering more than 76 percent leaf area followed by complete drying and breaking of the leaves from the center.

Percent Disease Index was worked out as follows.

$$\text{Percent Disease Index (PDI)} = \left[\frac{\text{Sum of individual ratings X 100}}{\text{Number of plants or leaves examined X maximum disease grade}} \right]$$

Table 1. Performance of onion cultivars against *Alternaria porri* under field conditions

Cultivar	Reaction	Percent Disease Index		Yield (q/ha)	
		kharif 2021-22	*kharif 2022-23*	*kharif 2021-22*	*kharif 2022-23*
Satara Local	MR	24.65^{e}	22.00^{d}	227.00^{fg}	226.00^{e}
Rampura	MR	24.00^{e}	24.00^{d}	182.65^{h}	183.65^{g}
Arka Kalyan	R	08.67^{f}	10.00^{e}	315.00^{b}	316.32^{b}
Agripound Dark Red	MS	36.00^{d}	34.65^{c}	298.66^{d}	297.32^{c}
Ballary Red	MS	38.00^{cd}	40.00^{b}	187.32^{h}	184.65^{g}
Arka Niketan	R	09.34^{f}	08.67^{e}	307.34^{c}	312.32^{b}
Nasik Red	MS	44.00^{b}	42.00^{b}	222.67^{g}	220.32^{f}
Telagi Red	MS	48.00^{a}	50.00^{a}	231.00^{f}	236.65^{d}
Kumata	MS	38.00^{cd}	40.00^{b}	238.00^{e}	236.67^{d}
Arka Bheema	R	10.00^{f}	09.32^{e}	331.00^{a}	332.00^{a}

Note: 1. R = Resistant, MR = Moderately Resistant, MS = moderately susceptible, S=Susceptible HS = highly susceptible

2. In the vertical columns means followed by same letters are not different statistically by DMRT (P=0.05).

***In vitro* evaluation of fungicides**

Ten fungicides with different modes of action at recommended dose were evaluated in the laboratory for their efficacy against *Alternaria porri* by the poisoned food technique (Nene and Thapliyal, 1979). Each treatment was replicated 3 times. The molten sterilized PDA was used as nutrient medium and required quantity of each fungicide was added separately so as to get a required concentration of that fungicide. The fungicides were thoroughly mixed by stirring and about 15 ml poisoned medium was poured to each of the 90mm

petri dishes and allowed for solidification. The actively growing periphery of 9 day old culture of *Alternaria porri* was carefully cut by using a gel cutter and transferred aseptically to centre of each petri dish containing the poisoned solid medium. Suitable control was maintained by growing the cultures on PDA without the fungicides. The plates were incubated at 27±1^{O}C for 9 days and the colony diameter was recorded 9 days after growth (Table-2). The percent inhibition of mycelial growth over control was calculated using the formula of Vincent (1947).

$$I = \frac{C-T}{C}$$

I = per cent inhibition of mycelial growth
C = radial growth of fungus in control
T = radial growth of fungus in treatment.

***In vitro* evaluation of botanicals**

Healthy plants were selected from which the fresh leaves and other parts were obtained and thoroughly washed with tap water then air dried. Aqueous plant extract was prepared by grinding 100g leaves/other parts with 100ml distilled water (w/v) using a blender and filtrate was collected by passing through double layered muslin cloth. The supernatant was taken as standard plant extract solution (100%). All the extracts obtained were passed through filter paper used for assay. The poisoned food technique (Nene and Thapliyal, 1979) was followed to evaluate the efficacy of botanicals in laboratory against *Alternaria porri* at 15% concentration (Table-3). Each treatment was replicated 3 times. The method followed for conducting the experiment was same as that used for fungicide evaluation.

***In vitro* evaluation of bio-agents**

Dual culture technique (Dennis and Webster, 1971) was followed to study interaction of six antagonists in the laboratory. Six bio-agents with a control treatment were used for evaluation. Pour 20ml of PDA into 90mm petri dishes and allowed for solidification. Discs measuring 5 mm of *Alternaria porri* was taken from 9 day old culture and was placed at one end of the petri dish then respective antagonistic organisms were inoculated at the opposite side (Table-4). A control was maintained by inoculating only *Alternaria porri* at one end in case of fungal antagonistic. In case of bacterial antagonistic *Alternaria porri* was placed at both ends of petri plates and bacterial culture was inoculated at centre of the petri plate, control was maintained by inoculating *Alternaria porri* at the both the ends of the petri plates. Each treatment was replicated four times and incubated for 6 days at 27 ± 1^{0}C. The activity of antagonistic organisms

Table 2. *In vitro* evaluation of fungicides against *Alternaria porri*

Treatments	Fungicides	Concentration (%)	Percent inhibition of mycelia growth
T1	Propineb -50WP	0.2	80.08 ^{e}
T2	Metiram-50WP	0.2	57.62^{h}
T3	Mancozeb-75WP	0.2	82.63^{d}
T4	Chlorothalonil-75WP	0.2	47.37^{i}
T5	Copper oxy chloride-50WP	0.3	72.42^{g}
T6	Myclobutanil-10WP	0.1	73.40^{g}
T7	Azoxystrobin-23EC	0.1	92.58^{a}
T8	Difenconazole-25EC	0.1	87.05^{c}
T9	Tebuconazole-25EC	0.1	88.87^{b}
T10	Hexaconazole-5EC	0.1	77.47^{f}

Note: In the vertical columns means followed by same letters are not different statistically by DMRT (P=0.01).

Table 3. *In vitro* evaluation of botanicals against *Alternaria porri*

Treatments	Botanicals	Plant Parts used	Concentration (%)	Percent inhibition of mycelia growth
T1	*Allium cepa*	Bulbs	15	28.62[i]
T2	*Allium sativum*	Cloves	15	65.37[a]
T3	*Clerodendron inerme*	Leaves	15	57.36[b]
T4	*Azadirachta indica*	Leaves	15	39.50[e]
T5	*Lantana camera*	Leaves	15	35.44[f]
T6	*Aloe vera*	Leaves	15	55.30[c]
T7	*Ocimum sanctum*	Leaves	15	33.24[g]
T8	*Glyricidia maculata*	Leaves	15	26.27[j]
T9	*Eucalyptus globes*	Leaves	15	48.20[d]
T10	*Durantha repens*	Leaves	15	30.50[h]

Note: In the vertical columns means followed by same letters are not different statistically by DMRT (P=0.01).

Table 4. Effect of different antagonists on growth of *Alternaria porri*

Treatments	Antagonists	Percent inhibition of mycelia growth
T1	*Trichoderma harzianum*	54.00[a]
T2	*Trichoderma viride*	52.25[a]
T3	*Trichoderma virens*	41.00[c]
T4	*Trichoderma konnigii*	48.25[b]
T5	*Pseudomonas fluorescence*	20.25[e]
T6	*Bacillus subtilis*	31.50[d]

Note: In the vertical columns means followed by same letters are not different statistically by DMRT (P=0.01).

were recorded by measuring the colony diameter of *Alternaria porri* in each treatment and compared with control.

Management of purple blotch, *Alternaria porri*

The field experiment was laid out in RCBD with 13 treatments and 3 replications during *Kharif* 2021-22 and 2022-23 at College of Horticulture, Bidar, Karnataka. Healthy Telagi Red seedlings were planted in the field with 15cm X 10cm (row to row X plant to plant) spacing in plot size of 3.6m X 1.8m. All other cultural practices and pest control practices were followed as recommended in package of practices (Anonymous, 2017). The first spraying was carried out as soon as first symptom of disease was noticed in the field. 4 sequential sprays of fungicides and botanicals were taken at an interval of 15 days (Table-5). Disease severity was recorded on ten randomly selected plants in each plot, just one day before each spraying and fifteen days after last spraying. An observation on severity of disease on foliage was recorded by using 0 to 5 point scale and PDI was worked out. The bulb yield in each plot was recorded and computed to hectare basis, the percent increase over control was calculated.

RESULTS AND DISCUSSION

Reaction of onion cultivars

All the screened genotypes were categorized for their reaction on the basis of PDI values. Those with 1- 10 PDI value were considered as resistant, while those with 11-25 PDI as moderately resistant, 26-50 PDI as

Table 5. Effect of different fungicides and botanicals on purple blotch of onion caused by *Alternaria porri*

Treatment	Mean PDI		Bulb yield (q/ha)		Percent yield increase over control	
	Kharif 2021-22	*Kharif 2022-23*	*Kharif 2021-22*	*Kharif 2022-23*	*Kharif 2021-22*	*Kharif 2022-23*
T1- 0.3% Copper oxy chloride-50WP	35.34^{f}	34.65^{e}	247.32^{f}	246.67^{e}	28.59	29.15
T2-0.2% Metiram-50WP	39.32^{e}	41.34^{d}	247.65^{f}	249.65^{d}	28.76	30.70
T3-0.2% Mancozeb-75WP	28.00^{hi}	29.32^{f}	264.00^{b}	264.67^{b}	37.26	38.57
T4- 0.2%Propineb -50WP	32.67^{g}	31.34^{ef}	252.00^{e}	250.65^{d}	31.02	31.23
T5-0.1%Hexaconazole-5EC	30.00^{h}	30.64^{f}	259.34^{c}	260.00^{c}	34.83	36.12
T6- 0.1%Azoxystrobin- 23 EC	24.00^{ij}	22.66^{g}	268.00^{a}	270.67^{a}	39.34	41.71
T7-0.1%Tebuconazole-25EC	22.68^{j}	24.00^{g}	271.00^{a}	267.00^{ab}	40.89	39.79
T8-0.1%Difenconazole-25EC	26.00^{i}	24.00^{g}	262.65^{b}	268.00^{a}	36.55	40.31
T9-0.1% Myclobutanil-10WP	33.34^{fg}	34.00^{e}	255.32^{d}	250.00^{d}	32.74	30.89
T10-15%*Allium sativum*	46.00^{d}	48.00^{c}	234.65^{g}	233.32^{f}	21.99	22.15
T11-15% *Aloe vera*	49.32^{c}	48.65^{c}	227.32^{h}	230.34^{g}	18.18	20.59
T12- 15% *Clerodendron inerme*	52.00^{b}	53.32^{b}	229.67^{h}	225.32^{h}	19.40	17.96
T13-Control	62.00^{a}	64.00^{a}	192.34^{i}	191.00^{i}	-	-

Note: In the vertical columns means followed by same letters are not different statistically by DMRT (P=0.05).

moderately susceptible, 51-75 PDI as susceptible and more than75 PDI as highly susceptible (Mishra *et al.,* 2009). Observations indicated that out of ten varieties evaluated, none of them were found highly resistant. However, three varieties viz., Arka Kalyan, Arka Niketan and Arka Bheema showed resistant reaction. Whereas, two varieties viz., Rampura and Satara local showed moderately resistant reaction and rest of them showed moderately susceptible reaction against purple blotch (Table1). At the time of harvest, bulb yield was recorded and computed to hectare basis. No relationship between disease severity and bulb yield was observed, it might be due to genetic potential of cultivars. Utilization of resistant cultivars in farming system is the most simple, effective and economical method in the management of plant diseases. Besides this, the resistant cultivars conserve the natural resource and reduce the cost, time and energy when compared to the other methods of disease management. Similar kind of work done by earlier workers who reported that the variety Arka Kalyan was found moderately resistant (Chethana *et al*., 2011 and Kavitha *et al*., 2017). The results are in agreement with Bal *et al*. (2019) who reported that the variety Arka Niketan was found resistant reaction.

***In vitro* evaluation of fungicides**

The results indicated that significant difference among fungicides in inhibiting the growth of the *Alternaria porri.* Among ten fungicides were evaluated, Azoxystrobin-23EC(92.58%) recorded maximum inhibition of mycelia growth of pathogen followed by Tebuconazole-25EC(88.87%), Difenconazole-25EC(87.05%),Mancozeb-75WP(82.63%), Propineb-50WP(80.08%), Hexaconazole-5EC(77.47%) and least inhibition was observed in Chlorothalonil-75WP (47.37%)(Table-2). *In vitro* evaluation of fungicides provides useful and preliminary information regarding efficacy of fungicides against pathogen within a short period and it is very much necessary before they are planned to be used under field experiments. Similar observations were reported by Priyanka *et al*. (2017) who studied the efficacy of six fungicides under *in vitro*

against *A. porri* at three different concentrations (0.1, 0.2 and 0.3 %) and found that 100 per cent mycelia growth inhibition with propiconazole, difenoconazole and tebuconazole at all the concentrations tested. Triazoles are the potent group of fungicides having a strong ergosterol synthesis inhibitory action which blocks the cytochrome P-450 dependant enzyme and C-14 alpha de-methylase which are needed to convert lanosterol to ergosterol. The biosynthesis of these ergosterols is critical to the formation of cell walls of fungi. Lack of normal sterol production slows or stops the growth of the fungus and preventing further infection and/or invasion of host tissues. The results on the efficacy of Mancozeb-75WP are in conformity with Chethana *et al.* (2011). The results are in agreement with Arunakumara K.T. (2006) who reported propineb-50 WP as effective fungicide against *A. solani* causing early blight of tomato. Chlorothalonil-75 WP was less effective against *A.porri* (Chethana *et al.,* 2012).

***In vitro* evaluation of botanicals**

The results revealed that effect of plant extracts on the fungal growth was significant. The *Allium sativum* cloves extract was found effective in inhibiting the mycelia growth (65.37%) followed by *Clerodendron inerme* (57.36%), *Aloe vera* (55.30%), *Eucalyptus globes* (48.20%) and least inhibition was observed in *Glyricidia maculata* (26.27%) (Table3). The results are in conformity with Prasad and Naik (2003) and Mesta *et al.,* (2011) where Garlic clove extract was found effective in inhibiting the mycelial growth of *Alternaria solani* and *Alternaria helianthi* respectively. The effectiveness of Garlic clove extract as a pesticide is due to volatile oil which contains dialyl disulphide, diallyl trisulphide and sulphodoxides derived from allicin (Vijayalakshmi *et al.,* 1999). Pramodkumar (2007) reported *Clerodendron* leaf extract as one of the best plant extract in inhibiting the mycelial growth of *Alternaria porri.*

In vitro* evaluation of antagonists against *Alternaria porri

All the *Trichoderma* sp inhibited the growth of *Alternaria porri* effectively. Among these antagonists *Trichoderma harzianum* showed highest inhibition (54.00%) followed by *Trichoderma viride* (52.25%) (Table4). This may be due to higher competitive ability of *Trichoderma* sp. either by mycoparasitism, antibiosis and also due to possibilities of existence of microbial interactions *viz*., stimulation, inhibition, mutual intermingling of growth of antagonistic isolate over test pathogen. Vinale *et al*. (2008) reported that *Trichoderma* sp. produced secondary metabolites such as 6-pentyl-alpha-pyrone (6pp), iso-cyanide derivatives, acids (heptelidic and koningic acid), peptaibols and cell wall degrading enzymes (CDWE) which are involved in the growth inhibition of many phytopathogenic fungi. Imtiaz and Lee (2008) reported *Trichoderma harzianum* and *Trichoderma virens* are most effective in inhibiting the growth of *Alternaria porri.*

Management of purple blotch, *Alternaria porri*

In subsequent sprays all the fungicides and botanicals treated plots recorded significantly less percent disease index over control. During *Kharif*-2021-22, among fungicides 0.1%Tebuconazole-25EC was significantly effective in reducing the disease intensity by recording a PDI of 22.68 and yield of 271.00q/ha (Table5). 0.1% Azoxystrobin-23EC,0.1% Difenconazole - 25EC, 0.2% Mancozeb-75WP, 0.1%Hexaconazole - 5EC and 0.2% Propineb-50WP were next best treatments found effective in reducing the disease intensity by recording a PDI of 24.00,26.00,28.00,30.00 and 32.67 and yield of 268.00q/ha, 262.65q/ha, 264.00q/ha, 259.34q/ha and 252.00q/ha respectively. Among botanicals tested, minimum PDI of 46.00 and yield of 234.65q/ha was recorded in *Allium sativum* cloves extract (15%) and maximum PDI of 62.00 and yield of 192.34q/ha was recorded in the control plot (Table5). Again during *Kharif* - 2022-23, among fungicides 0.1% Azoxystrobin - 23EC was significantly effective in reducing the disease intensity by recording a PDI of 22.66 and yield of 270.67q/ha (Table6). 0.1% Tebuconazole-25EC, 0.1% Difenconazole-25EC, 0.2% Mancozeb-75WP, 0.1% Hexaconazole-5EC and 0.2% Propineb were next best treatments found effective in reducing the disease intensity by recording a PDI of 24.00, 24.00, 29.32, 30.64 and 31.34 and yield of 267.00q/ha, 268.00q/ha, 264.67q/ha, 260.00q/ha and 250.65q/ha respectively. Among botanicals tested, PDI of 48.00 and yield of 233.32q/ha was recorded in *Allium sativum* cloves extract (15%) and maximum PDI of 64.00 and yield of 191.00q/ha was recorded in the control plot (Table5). Studies conducted by Wangikar *et al.* (2012) on management of purple blotch of onion in Marathawada region of Maharashtra revealed that lowest disease severity of purple blotch with spray of Mancozeb-75WP at 0.25%, Hexaconazole-5EC at 0.1% and Difenconazole-25EC at 0.05%. Gupta et al. (2012) reported that systemic fungicides Tebuconazole-25EC at 0.1% and Azoxystrobin-23EC at 0.1% effectively controlled purple blotch disease of Garlic. Chethana *et al.* (2015) reported that Mancozeb-75WP at 0.25% effectively controlling purple blotch of Onion. The

results on the effectiveness of foliar application of *Allium sativum* cloves extract in the management of *Alternaria* blight are in conformity with Nashwa and Abo-Elyousr (2012).

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REFERENCES

Anonymous, 2017. *Package of Practices*, University of Horticultural Sciences, Bagalkot, Karnataka, India pp. 120-124.

Arunakumara, K.T. 2006. Studies on *Alternaria solani* (Ellis and Martin) Jones and Grout causing early blight of Tomato. M.Sc. (Agri.) Thesis. Uni. Agric. Sci. Dharwad India.

Bal, S., Maity, T. K., Sharangi, A. B. and Maji, A. 2019. Screening of onion (*Allium cepa* L.) germplasm against purple blotch disease. *J. Pharmacogn. Phytochem*, **8**(6): 546-548.

Behera, S., Santra, P., Chattopadhyay, S., Das, S. and Maity, T. K. 2013. Variation in onion varieties for reaction to natural infection of *Alternaria porri* (ellis) ciff. and *Stemphylium vesicarium* (wallr.) *The Bioscan*, **8**(3): 759-761.

Chethana, B. S., Ganeshan, G. and Manjunath, B. 2011. Screening of genotypes and effect of fungicides against purple blotch of onion. *Journal of Agricultural Technology*, **7**(5): pp.1369-1374.

Chethana, B. S., Ganeshan, G., Archana, S. and Bellishree, K. 2015. Integrated disease management of purple blotch disease of onion. *The Bioscan*, **10**(4), pp.1729-1733.

Dennis, C. and Webster, J. 1971. Antagonistic properties of species of group *Trichoderma* production and non-volatile antibiotics. *Transactions of British Mycological Society,* **57:** 25-39.

Gupta, R. B. L. and Pathak, V. N. 1988. Yield losses in onions due to purple blotch disease caused by Alternaria porri. *Phytophylactica*, **20**(1): pp.21-24.

Gupta, R. C., Pandey, N. K. and Gupta, R. P. 2014. Management of purple blotch (Alternaria porri) disease of garlic (Allium sativum L.).

Imtiaj, A. and Lee, T. S. 2008. Antagonistic effect of three Trichoderma species on the *Alternaria porri* pathogen of onion blotch. *World Journal of Agricultural Sciences,* **4**(1):13-17.

Javadzadeh, A., Ghorbanihaghjo, A., Bonyadi, S., Rashidi, M. R., Mesgari, M., Rashtchizadeh, N. and Argani, H. 2009. Preventive effect of onion juice on selenite - induced experimental cataract. *Indian journal of ophthalmology*, **57**(3): pp.185-189.

Kavitha, S., Veeraghanti, B., Gangadhara Naik and Hegde, K. T. 2017. Screening of onion genotypes against purple blotch. *Int. J. Pure App. Biosci*, **5**(1): 221-223.

Mesta,V. I., Benagi, Kulkarni, S. and Shankargoud. 2011. *In vitro* evaluation of fungicides and plant extracts against *Alternaria helianthi* causing blight of sunflower. *Karnataka Journal of Agricultural Sciences,* **22**(1):111-114.

Mishra, R. K., Verma, A., Singh, S. and Gupta, R. P. 2009. Screening of Garlic lines against purple blotch and *Stemphylium* blight. *Pest Management in Horticultura Ecosystems*, **15**(2): 138-140.

Nashwa, S. M. and Abo-Elyousr, K. A. 2012. Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. *Plant Protection Science*, **48**(2): pp.74-79.

Nene, Y. L. and Thapliyal, P. N. 1979. Fungicides in plant disease control. *Fungicides in plant disease control*, (Ed. 2).

Pramodkumar, T. 2007. Biological management of Alternaria blight of onion. *M.Sc. (Agri.) Thesis*. University of Agricultural Sciences, Dharwad (India).

Prasad, and Naik, M. K. 2003. Evaluation of genotypes, fungicides and plant extracts against early blight of Tomato caused by *Alternaria solani*. *Indian Journal of Plant Protection,* **31**(2): 43-49.

Priyanka, J., Mali, B. L. and Meena, M. K. 2017. Effective management of purple blotch of onion caused by *Alternaria porri* (Ellis) through host resistance, fungicides and botanicals. *Int. J. Curr. Microbiol. App. Sci*, **6**(5): 1737-1745.

Sharma, S. R**.** 1986. Effect of fungicidal sprays on purple blotch and bulb yield of Onion. *Indian Phytopath*ology, **39**(1): 72-82.

Sharma, I. M. 1997. Screening of onion varieties/lines against purple blotch caused by *Alternaria porri* under field conditions. *Plant Disease Research,* **12**(1): 60-61.

Vijayalakshmi, K., Subhashini, B. and Shivani, K. 1999. Plants in Pest Control. Series of Booklets Centre for Indian Knowledge Systems, Chennai.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Barbetti, M. J., Li, H., Woo, S. L. and Lorito, M. 2008. A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiol. Mol. Plant Pathol*, **72:** 80-86.

Vincent, J. M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. **59:** 850.

Wangikar, A. A., Dandnaik, B. P., Falke, A. R. and Khandare, P. M. 2012. Management of purple blotch of onion caused by *Alternaria porri* in Marathwada region. In: Abstract, IV National Symposium on Plant protection in horticultural crops: Emerging challenges and sustainable pest management organized at Indian Institute of Horticultural Research, Bangalore held on 25-28 April, pp.112.

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Survival and host range of *Phytophthora capsici* Leon under Karnataka conditions

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ABSTRACT: *Phytophthora capsici* Leonian is a destructive pathogen of sweet pepper worldwide. In this study, survival and host range of *P. capsici* under Karnataka conditions was studied. Slender amaranth (*Amaranthus viridis*), black nightshade (*Solanum nigrum*) and spurge (*Euphorbia geniculata*) were found as weed hosts for the pathogen. The pathogen was infective on fruits and leaves of watermelon, cucumber, gherkin, squash, pumpkin, ridge gourd, round melon, snake gourd and sponge gourd. On bottle gourd, it was infective on fruits while non infective on leaves and root. Bitter gourd and ash gourd were not hosts without any foliar, fruit and root infection. Among solanaceous crops, tomato, chilli and potato leaves, fruits and root were infected. On brinjal, only fruit infection was recorded. The isolate was weakly pathogenic on cruciferous vegetables cabbage and cauliflower with only leaf infection. None of the leguminous vegetable tested showed root infection. Leaf and pod infection was observed on French bean and cowpea. On malvaceous vegetable crop okra, only fruit infection was recorded. In Piperaceae crops, only leaf infection was observed on black pepper and betel vine. Eight millets tested were found non-hosts for the Kadur isolate of *P. capsici*. The pathogen survived up to 90 days when infected fruit was buried in soil irrespective of three soil textures; sandy loam, sandy clay and sandy clay loam.The information on survival and host range generated would help in developing disease management strategy against *P. capsici* in Karnataka.

Keywords: *Phytophthora capsici*, soil survival, host range, Karnataka

INTRODUCTION

Phytophthora blight incited by *P. capsici* Leon is the most devastating field diseases of sweet peppers worldwide. *P. capsici* is an oomycete pathogen of vegetable crops worldwide, causing crop losses exceeding 50% (Sanogo and Ji, 2012). The pathogen has reportedly caused severe epidemics in Central and South America, Europe, Asia, and United States where susceptible vegetables are grown (Granke *et al*., 2012a). Globally the disease and pathogen is widely distributed and has been reported in all the major pepper production areas (CABI, 2022).

Phytophthora blight has been reported to be destructive disease causing marketable yield loss in hot and sweet pepper (*C. annuum* L) in India. In India, the disease is of economic interest on sweet pepper in Himachal Pradesh, Karnataka and Tamil Nadu (Sharma and Bhardwaj, 1976; Chaudhary and Banyal, 2013b; Chowdappa *et al*., 2014; Singh, 2015).

P. capsici is reported as a broad host pathogen infecting cultivated crops, ornamentals and native plants belonging to more than 15 plant families (Satour and Butler, 1967; Erwin and Ribeiro, 1996; Hausbeck and Lamour, 2004; Tian and Babadoost, 2004; French-Monar *et al*., 2006; Granke *et al*., 2012). In India, this pathogen has been reported on plantation crops, black pepper (Sharma and Anandaraj, 1997), cocoa (Chowdappa *et al*., 1993) and betel vine (Kumar *et al*., 2004), however there are no reports on pathogen city on different cultivated and weed hosts existing in sweet pepper agro ecosystem. This information is required as sound disease management strategies rely on knowledge of pathogen host range.

Review of research done in India on *P. capsici* points out to gaps in knowledge on survival and host range. Keeping in view the above knowledge and technology gaps, the present study was undertaken with an objective to determine the survival and experimental host range of *P. capsici* Kadur isolate.

MATERIALS AND METHODS

Source and maintenance of culture

A highly virulent, previously characterized isolate of *Phytophthora capsici* Leon (GenBank accession number MZ474494) maintained in fungal pathology laboratory was used in this work (Kumar G. M. S, 2022). The pathogen was isolated from infected fruit of sweet pepper sourced from Kadur region of Karnataka. Working culture of the isolate was maintained by periodical

culture on carrot agar medium with incubation at 25±2 ºC in dark for three to five days. For long term storage, agar plugs removed from the colony margin were placed in sterile water in 1.5 ml screw capped bottles and stored at room temperature in dark conditions. Virulence and aggressiveness of the isolate was maintained by inoculation and re isolation from sweet pepper fruits at regular intervals.

Survival of *P. capsici* in different soils

An experiment was conducted to study the survival of *P. capsici* in three soils of different textures in pots under glass house conditions. Soil samples for this study were obtained from different sweet pepper growing areas in Karnataka (Table 1). Three representative soil samples from Belgaum, Haveri and Bengaluru were selected based on soil colour (black soil, red soil and loamy soil) and texture by feel method.

Soil texture was determined by mechanical analysis using the international pipette method (Piper, 1966) at the soil testing laboratory of ICAR-National Bureau of Soil Survey and Land Use Planning (NBSS & LUP), Regional centre, Bengaluru. After computing the relative percentage of different size groups namely clay, silt and sand, the textural class of the soils was determined using the triangular textural diagram given by the USDA.

Table 1. Source of soils used in the study

Sl. No.	Sample code	GPS coordinates	Place
	Haveri	14º36¹36.55’’N 75 º 28¹01.32’’E	Kollapur village, Byadagi taluk, Haveri district, Karnataka
	Belgaum	15º61¹01.75’’N 74 º 63¹72.13’’E	KadatanBagewadi village, Khanapur taluk, Belgaum district, Karnataka
	Bengaluru	13º08¹10.43’’N 77 º 29¹53.79’’E	Sixth block of Hesaraghatta farm of ICAR- IIHR, Bengaluru, Karnataka

The pathogen survival was studied in sterile soil at a moisture regime of 100% water holding capacity. One hundred gram of infected fruit with mycelium and sporangia was buried in soil as inoculum. Soil was sterilized before inoculation. Soil moisture was maintained at 100% WHC throughout the study period. The survival of the pathogen was assessed at monthly intervals up to six months by baiting with sweet pepper seedlings. The survival was confirmed by isolation of the pathogen from infected seedlings and morphological observations (Larkin *et al*., 1995).

Host range of *P. capsici*

In this study, the experimental host range of *P. capsici* Kadur isolate was determined by pathogenicity test on 8 weed plants and 36 crops commonly found in sweet pepper agro- ecosystem. The tested weeds are known to occur commonly in sweet pepper fields in Karnataka region of India. The crops tested are cultivated in rotation after sweet pepper crop / widely cultivated in fields adjacent to sweet pepper fields in Karnataka. Plants of 3-5 leaf stage were inoculated with zoospore suspension to test for root rot symptoms (Bosland and Lindsey, 1991). Pathogenicity on detached leaves and fruits were also tested by placing five mm diameter mycelia plug (Foster and Hausbeck, 2010; Chowdappa *et al.,* 2014).

RESULTS AND DISCUSSION

Survival of *P. capsici* in soil

The results of soil texture analysis of three representative soils used in the pathogen survival study are presented in the Table 2.

The results revealed that the three soils were of different texture with varied percentage of sand, clay and silt content. Soil from Belgaum belonged to sandy clay textural class with 45.86% sand, 35.40% clay and 18.74% silt. The texture of soil from Haveri was sandy loam with 68.96% sand, 19.50% clay and 11.54% silt. Bengaluru soil belonged to sandy clay loam texture with

Table 2.Texture of soils used in soil survival study*

Sample Code	Very course sand	Coarse Sand	Medium sand	Fine sand	Very fine sand	Sand	Clay	Silt	Coarse silt	Fine silt	Texture
Haveri	20.04	15.31	13.16	13.57	6.89	68.96	19.50	11.54	5.04	6.50	Sandy loam
Belgaum	5.84	9.13	10.51	13.16	7.22	45.86	35.40	18.74	7.75	10.99	Sandy clay
Bengaluru	7.47	15.47	14.42	11.68	7.26	56.32	26.63	17.05	6.76	10.29	Sandy clayloam

* All values are in %

Table 3. Survival of *P. capsici* in infected fruit tissue buried in soil of different textures

Soil texture	Duration (months)					
	1	2	3	4	5	6
Sandy loam	+	+	+	-	-	-
Sandy clay	+	+	+	-	-	-
Sandy clay loam	+	+	+	-	-	-

+ Detection, - No detection of *P. capsici* Kadur isolate with sweet pepper seedling baiting based on the re-isolation.

56.32% sand, 26.63% clay and 17.05% silt. Among the three soils, Haveri soil was moderately coarse textured whereas Belgaum and Bengaluru soils were moderately fine textured soils.

An experiment was conducted to study the survival of *P. capsici* in three different soils types. The result of the survival of *P. capsici* buried in soil is presented in the Table 3. In the current investigation, the pathogen was detected by baiting up to three months in all the three different textured soils assessed. The pathogen was not detected after third month irrespective of soil type.

The findings of present study are consistent with the observation of previous workers on soil survival of *P. capsici* in crop residue. It is reported that many *Phytophthora* species do not survive for extended period without their host. Mycelia, sporangia and zoospores of most *Phytophthora* species survive for few weeks (Ansani and Matsuoka, 1983; Schlub, 1983; Erwin

and Riberio, 1996; Roberts *et al*., 2005). Ansani and Matsuoka (1983) have reported that the mycelium of *P. capsici* survived less than 120 days in infected root tissue burried in soil, while zoospores and sporangia survived for fewer than 75 days. In a study by Roberts *et al*. (2005), sporangia, zoospores and mycelia of *P. capsici* were found to survive for 57 days in Florida at 30 °C and 100% soil moisture-holding capacity when buried inside soil. Contrary to these works, Schlub (1983) could not isolate *P. capsici* from inoculated leaf tissue two days after being placed on the soil surface in a field, but was easily isolated after 14 days if buried.

Oospores play an important role in the disease cycle of *P. capsici* as overwintering dormant spores. They are dormant in soil and plant tissue. Oospores survive in soil from few months up to several years in different types of soils (Turkensteen *et al.*, 2000; French-Monar *et al*., 2007; Babadoost and Pavon, 2013). In the present study, mycelial and sporangial survival on infected fruit tissue buried in soil was investigated. No work on oospore survival was carried out as the Kadur isolate used in the current study was heterothallic and was not able to produce oospore in the culture medium when paired with other available isolates on carrot agar medium. Pairing work indicated that the isolates tested belonged to one mating type, either A1 or A2. Further, reference tester isolates of known mating type was not available to induce oospore production. *P. capsici* of hot and sweet pepper isolates in Karnataka were reported to be of A1 mating type (Chowdappa *et al.*, 2014).

It can be concluded from the present findings that the pathogen survives for few months when buried in soil as infected fruit with mycelium and sporangia. Mechanical collection and destruction of crop residue or infected plant part has to be carried out as a good practice to prevent burial and soil survival of inoculum. Residue management should be recognized as a component in integrated management programme against *Phytophthora* blight of sweet pepper.

P. capsici host range

Weeds and native plants should be considered when endeavoring to manage and control plant pathogens of cultivated plants. Whether as a pest itself, vector of a pathogen, or reservoir of a pathogen or its vector, weeds could significantly influence disease incidence. The relationship between these factors plays a critical role in determining disease incidence and impact (Wisler and Norris, 2005). In this study, pathogenicity experiments were conducted to examine experimental host range of Kadur isolate of *P. capsici*. Pathogenicity of Kadur isolate was tested on eight weed hosts known to occur commonly in sweet pepper agroecosystem in Karnataka (Table 4).

Out of eight weeds tested (Table 4), slender amaranth (*Amaranthus viridis* L.), spurge (*Euphorbia geniculata* Ortega) and black nightshade (*Solanum nigrum* L.) were found as weed host for Kadur isolate of *P. capsici*. The isolate was infective on leaves of spurge, slender

Table 4. Pathogenicity on common weeds found in sweet pepper ecosystem

Common Name	Scientific Name	Pathogenicity	
		Leaf	Root
Slender amaranth	*Amaranthus viridis* L.	+	-
Lantana	*Lantana camara* L.	-	-
Spurge	*Euphorbia geniculata* Ortega	+	-
Congress grass	*Parthenium hysterophorus* L.	-	-
Black nightshade	*Solanum nigrum* L.	+	+
Goat weed	*Ageratum conyzoides* L.	-	-
Balloon vine	*Cardiospermum halicacabum*L.	-	-
Madras pea pumpkin	*Cucumis maderaspatanus* L.	-	-

amaranth and black nightshade. Root infection was observed only in black nightshade plant. Congress grass (*Parthenium hysterophorus* L.), lantana (*Lantana camara* L.), goat weed (*Ageratum conyzoides* L.), balloon vine (*Cardiospermum halicacabum* L.) and Madras pea pumpkin (*Cucumis maderaspatanus* L.) were not found to be host for this isolate of *P. capsici*.

In previous host range and characterization studies, black nightshade (*Solanum nigrum*) is reported as a weed host of *P. capsici* (Tamietti and Valentino, 2001; Tian and Babadoost, 2004; French-Monar *et al*., 2006). In literature other weeds that are reported to harbor *P. capsici* includes; common Carolina geranium (*Geranium carolinianum*), American black nightshade (*Solanum americanum*) and common purslane (*Portulaca oleracea*) (Ploetz and Haynes, 2000; Ploetz *et al*., 2002). In addition, slender amaranth (*Amaranthus viridis* L.) and spurge (*Euphorbia geniculata* Ortega) were found host for *P. capsici* in this study. These two weeds have not been previously reported as host of *P. capsici*.

The results of the present study validate that weeds can act as alternative host of *P. capsici* and contribute to its survival. Weeds as alternative hosts have epidemiological implications. In, sweet pepper growing regions of South India, oospores production of *P. capsici* in natural fields was not reported. This is due to non-existence of opposite mating types in the same field or geography. In the absence of oospore production, the pathogen has to over winter as mycelia, sporangia and zoospore propagules in infected plant debris buried in soil. However this survival is also for short duration. The identified weeds have the potential of serving as additional type of pathogen survival during offseason in addition to other survival strategies. Weed hosts and crop hosts should be considered when developing disease management strategy against *P. capsici*. Weed control should form essential component of *P. capsici* management strategy.

Strategies to manage plant disease from use of resistant varieties to crop rotation, elimination of reservoirs, landscape planning, surveillance, quarantine, risk modeling, and anticipation of disease emergences all rely on knowledge of pathogen host range (Morris and Moury, 2019). In this current study, pathogenicity of *P. capsici* Kadur isolate was tested on 36 crops commonly cultivated in sweet pepper agroecosystem in Karnataka. The results are presented in the Table 5.

The differential pathogenic response was observed in cucurbitaceous crops tested as experimental hosts (Table 5). The pathogen was infective on fruits and leaves of watermelon, cucumber, gherkin, squash, pumpkin, ridge gourd, round melon, snake gourd and sponge gourd. On

Table 5. Pathogenicity of *P. capsici* (Kadur isolate) on crops commonly cultivated in sweet pepper agroecosystem

Crop	Genotype	Scientific Name	Pathogenicity		
			Leaf	Fruit	Root
Leguminous vegetables					
Garden pea	Arka Karthik	*Pisum sativum* var. *hortense*Neilr.	-	-	-
Yard long bean	Arka Mangala	*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.	-	-	-
Cow pea	Arka Samrudhi	*Vigna unguiculata* (L.) Walp.	+	+	-
French bean	Arka Sharath	*Phaseolus vulgaris* L.	+	+	-
Velvet beans	Arka Shubra	*Mucuna pruriens* (L.) DC.	-	-	-
Solanaceous vegetables					
Tomato	NS501	*Solanum lycopersicum* L.	+	+	+
Chilli	Arka Lohith	*Capsicum annum* L.	+	+	+
Brinjal	Arka Anand	*Solanum melongena* L.	-	+	-
Potato	Kufri Jyoti	*Solanum tuberosum* L.	+	+	+

Cucurbitaceous vegetables						
	Cucumber	NS 404	*Cucumis sativus* L.	+	+	-
	Gherkin	Chandini RZF1	*Cucumis sativus* L.	+	+	-
	Bottle gourd	Arka Bahar	*Lagenaria siceraria* (Molina) Standl.	-	+	-
	Ash gourd	Local	*Benincasa hispida* (Thunb.) Cogn.	-	-	-
	Ridge gourd	Arka prasan	*Luffa acutangula* (L.) Roxb	+	+	-
	Sponge gourd	Local	*Luffa aegyptiaca* Mill.	+	+	-
	Snake gourd	Local	*Trichosanthescucumerina* L.	+	+	-
	Bittergourd	Arka Harit	*Momordica charanita* L.	-	-	-
	Pumpkin	Arka Suryamukhi	*Cucurbita moschata* Duchesne	+	+	-
	Squash	SQ-14	*Cucurbita pepo* L.	+	+	+
	Watermelon	Arka Muthu	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	+	+	+
	Musk melon	Arka Siri	*Cucumis melo* L.	-	-	-
	Round melon	Arka Tinda	*Praecitrullusfistulosus* (Stocks) Pangalo	+	+	-
Malvaceous vegetable						
	Okra	Arka Nikita	*Abelmoschus esculentus* (L.) Moench	-	+	-
Cruciferous vegetables						
	Cauliflower	White Wonder	*Brassica oleracea* var. *botrytis* L.	+		-
	Cabbage	Golden Acre	*Brassica oleracea* var. *capitata* L.	+		-
Piperaceae						
	Black pepper	Panniyur 1	*Piper nigrum* L.	+	-	-
	Betel vine	Mitha Paan	*Piper betle* L.	+	-	-
Millets						
	Foxtail millet	SiA 3156	*Setariaitalica*(L.) P.Beauv.	-	-	-
	Little millet	DHLM 36-3	*Panicum sumatrense* Roth ex Roem. & Schult.	-	-	-
	Finger millet	CFMV 1	*Eleusine coracana* (L.) Gaertn.	-	-	-
	Proso millet	TNAU 202	*Panicum miliaceum* L.	-	-	-
	Barnyard millet	DHBM 93-2	*Echinochloafrumentacea* (ROXB.) LINK	-	-	-

Kodo millet	RK 390-25	*Paspalum scrobiculatum* L.	-	-	-
Pearl millet	HHB67 Improved	*Pennisetum glaucum* (L.) R.Br.	-	-	-
Sorghum	CSV27	*Sorghum bicolor* (L.) Moench	-	-	-
Maize	Local	*Zea mays* L.	-	-	-

+ = Infective, - = Non infective

bottle gourd it was infective on fruits while non infective on leaves and root. Bitter gourd and ash gourd were not hosts without any foliar, fruit and root infection. Among 13 cucurbitaceous hosts tested, only watermelon and squash took root infection.

Among solanaceous crops tested, the isolate was pathogenic on tomato, chilli and potato with infection on leaves, fruits and root. On brinjal, it was not infective on leaves and root but infective on fruits. The isolate was weakly pathogenic on cruciferous vegetables cabbage and cauliflower where only leaf infection was observed. None of the leguminous vegetables tested showed root infection. Leaf and pod infection was observed on French bean and cowpea. On malvaceous vegetable crop okra, only fruit infection was recorded. In piperaceae crops, black pepper and betel vine, only leaf infection was observed. All the eight millets tested were found non-hosts for the Kadur isolate of *P. capsici*.

In other parts of the world, *P. capsici* is reported as a broad host range pathogen infecting cultivated crops, ornamentals and native plants belonging to more than 15 families with major threat to cultivated crop plant families cucurbitaceae, fabaceae and solanaceae (Satour and Butler, 1967; Erwin and Ribeiro, 1996; Hausbeck and Lamour, 2004; Tian and Babadoost, 2004; French-Monar *et al*., 2006; Granke *et al*., 2012b). In India, other than sweet pepper and hot pepper, black pepper, cocoa, betel vine and coconut are reported as cultivated hosts of *P. capsici* (Anandaraj *et al*., 1989; Chowdappa *et al*., 1993; Kumar and Kumar, 2004; Jonathan *et al*., 2006; Prathibha, *et al*., 2018).

Survival of plant pathogen inoculums determines the nature of disease initiation, dispersion and epiphytotics development. Knowledge on survival of plant pathogen on host and soil is required to break infection chain by devising suitable management interventions that reduce the source of inoculum and subsequent disease development. *Phytophthora* blight in sweet pepper is an emerging disease in India. The information on experimental host range generated in this study will help in risk analysis of further range expansion of this pathogen in the country. Crop sequence patterns should take in to account this host range information so as to reduce pathogen survival and perpetuation. Inter-cropping, mixed-cropping and sequence cropping of sweet pepper with solanaceous, cucurbitaceous, cruciferous and leguminous vegetables should be avoided. Cropping sequence with non-host crops like millets has to be practiced in areas where the disease is reported to be widely prevalent.

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REFERENCES

Ansani, C. V. and Matsuoka, K. 1983. Sobrevivencia de *Phytophthora capsici* Leonian no solo (Survival of *Phytophthora capsici* Leonian in soil). *Fitopatologia. Brasileira.*, **8**: 269-276.

Babadoost, M. and Pavon, C. 2013. Survival of oospores of *Phytophthora capsici* in soil. *Plant Diseases,* **97**: 1478–1483.

Bosland, P. W. and Lindsey, D. L. 1991. A seedling screen for *Phytophthora* root rot of pepper, *Capsicum annuum*. *Plant Diseases*, **75**:1048-1050.

Chaudhary, J. and Banyal, D. 2013b, Status and distribution of *Phytophthora* leaf blight and fruit rot of capsicum in Himachal Pradesh and its management through fungicides. *Indian Phytopathology*, **66**(2): 135- 139.

Chowdappa, P., Madhura, S., Nirmal Kumar, B. J., Mohan Kumar, S. P. and Hema, K. R. 2014. *Phytophthora boehmeriae* revealed as the dominant pathogen responsible for severe foliar blight of *Capsicum annuum* in South India. *Plant Disease*, **98**: 90-98.

Chowdappa, P., Mohanan, R. C. and Ramanujam, B. 1993. Occurrence of *Phytophthora capsici* on cocoa in Kerala. *Indian Phytopathology*, **46** (1): 92-93.

Erwin, D. C. and Ribeiro, O. K. 1996. *Phytophthora* disease worldwide. *The American Phytopathological Society*: St Paul, MN, USA.

Foster, J. M. and Hausbeck, M. K. 2010, Managing *Phytophthora* crown and root rot in bell pepper using fungicides and host resistance. *Plant Disease,* **94** (6): 697-702.

French-Monar, R. D., Jones, J. and Roberts, P. D. 2006. Characterization of *Phytophthora capsici* associated with root and weeds on Florida vegetable farms. *Plant Disease,* **90:** 345–350.

French-Monar, R. D., Jones, J. B., Ozores-Hampton, M. and Roberts, P. D. 2007. Survival of inoculums of *Phytophthora capsici* in soil through time under different soil treatments. *Plant Disease*, **91**: 593-598.

Granke, L. L., Quesada-Ocampo, L., Lamour, K. and Hausbeck, M. K 2012a. Advances in research on *Phytophthora capsici* on vegetable crops in the United States. *Plant Disease*, **96** (11): 1588–1600.

Granke, L. L., Quesada-Ocampo, L. M. and Hausbeck, M. K. 2012b Differences in virulence of *Phytophthora capsici* isolates from a worldwide collection on host fruits. *European Journal of Plant Pathology*, **132:** 281-296.

Hausbeck, M. K. and Lamour, K. H. 2004. *Phytophthora capsici* on vegetable crops: Research progress and management challenges. *Plant Disease,* **88** (12): 1292-1303.

Jonathan, E. I., Umamaheswari, R. and Bommaraju, P. 2006. Bio efficacy of native plant growth promoting rhizobacteria against *Meloidogyne incognita* and *Phytophthoracapsici* disease complex in betelvine. *Indian Journal of Nematology*, **36** (2): 217-223.

Kumar, C. P. C. and Kumar, C. S. K. 2004. Epidemiological studies on *Phytophthora* foot rot disease of betelvine. *Indian Journal of Plant Protection*, **32** (2): 100-101.

Larkin, R. P., Ristaino, J. B. and Campbell, C. L. 1995. Detection and quantification of *Phytophthora capsici* in soil. *Phytopathology*, **85**: 1057- 1063.

Morris, C. E. and Moury, B., 2019, revisiting the concept of host range of plant pathogens. *Annual Review of Phytopathology*, **57**: 63–90.

Piper, C. S. 1966. Soil and Plant Analysis. Hans Publishers. Bombay, India, 368 pp.

Ploetz, R. C. and Haynes, J. L. 2000. How does *Phytophthora capsici* survive in squash fields in southeastern Florida during the offseason? *Proceedings of the Florida State Horticulture Society,* **113**: 211- 215.

Ploetz, R. C., Heine, G., Haynes, J. and Watson, M. 2002. An investigation of biological attributes that may contribute to the importance of *Phytophthora capsici* as a vegetable pathogen in Florida. *Annals of Applied Biology*, **140**: 61- 67.

Prathibha, V. H., Hegde, V., Sharadraj, K. M., Rajesh, M. K., Rachana, K. E. and Chowdappa, P. 2018. Differentiation of *Phytophthora* species associated with plantation crops using PCR and high-resolution melting curve analysis. *Journal of Plant Pathology*, **100**: 233–240.

Roberts, P. D., Urs, R. R., French-Monar, R. D., Hoffine, M. S., Seijo, T. E. and McGovern, R. J. 2005. Survival and recovery of *Phytophthora capsici* and oomycetes in tail water and soil from vegetable fields in Florida. *Annals of Applied Biology*, **146**: 351–359.

Kumar G. M. S. 2022. Epidemiology and management of *Phytophthora* leaf blight and fruit Rot in sweet pepper *Capsicum annuum* L. Ph.D. Thesis, University of Agricultural Sciences, Bangalore, P. 279

Sanogo, S. and Ji, P. 2012. Integrated management of *Phytophthora capsici* on solanaceous and cucurbitaceous crops: current status, gaps in knowledge and research needs. *Canadian Journal of Plant Pathology*, **34** (4): 479-492.

Satour, M. M. and Butler, E. E. 1967. A root and crown rot of tomato caused by *Phytophthora capsici* and *P. parasitica*. *Phytopathology*, **57**: 510–515.

Schlub, R. 1983. Epidemiology of *Phytophthora capsici* on bell pepper. *Journal of Agricultural Sciences,* **100** (1): 7-11.

Sharma, S. L. and Bhardwaj, S. S. 1976. A fruit rot and leaf blight of bell pepper caused by *Phytophthora nicotianae* var. *nicotianae*. *Indian Phytopathology*, **29**: 93-94.

Sharma, Y. R. and Anandaraj, M. 1997. *Phytophthora* foot rot of black pepper. In : Management of threatening plant diseases of national importance. [(Eds.) Singh, D.V., Agnihotri, V. P. and Sarbhoy, A. K., *Malhotra Publishing House*, New Delhi. pp. 237-348.

Singh, A. K. 2015. Epidemiology and management of *Phytophthora* leaf blight and fruit rot of bell pepper. *M.Sc. Thesis,* Dr. Yashwant Singh Parmar University of Horticulture and Forestry Nauni, Solan.

Tamietti, G. and Valentino, D. 2001. Physiological characterisation of a population of *Phytophthora capsici* Leon. from northern Italy. *Journal of Plant* Pathology, **83**: 199-205.

Tian, D. and Babadoost, M. 2004. Host range of *Phytophthora capsici* from Pumpkin and pathogenicity of Isolates. *Plant Diseases*, **88** (5):485-489.

Turkensteen, A, L. J., Fliera, W. G., Wanningena, R. and Mulder, A. 2000. Production, survival and infectivity of oospores of *Phytophthora infestans*. *Plant Pathology*, **49**: 688-696

Wisler, G. and Norris, R. 2005. Interactions between weeds and cultivated plants as related to management of plant pathogens. *Weed Science*, **53** (6): 914 – 917.

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Pre-and post-harvest management of sooty blotch disease of mango

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ABSTRACT: The field survey conducted in Odisha state during 2014-18 to document major and new diseases of mango, a kind of superficial smudgy fungal blemishes were observed on fruits of late maturing mango varieties. The new type of superficial fungal blackening referred as '*Sooty blotch'* disease wherein, group of fungi colonize on waxy layer of fruit cuticle which downgrades the fruit quality and eye appeal by spoiling green fresh look of fruits. To manage the disease, series of studies were undertaken under field condition as well as after harvest as post-harvest dip treatments. Under field condition, among various protective fungicides evaluated, spray of 0.3 % copper oxychloride-based formulation thrice at fifteen days interval (by scheduling the first spray before monsoon) provided satisfactory control by inhibiting the colonization of sooty blotch fungi. Subsequently, various post-harvest dip treatments were also evaluated to remove the superficial blackening of fruits after harvest. Among them, dipping of blemished fruits in Arka mango wash for 10-15 minutes, followed by washing in water twice removed 99% of fungal blemishes from sooty blotch infected mangoes without any peel injury and post-harvest quality of fruits.

Keywords: Mango, sooty blotch, field spray, fungicide, post-harvest dip treatment

INTRODUCTION

Number of factors affect the visual appearance of the fruits which may be physical, physiological, pathological or entomological. In market, the color of fruit, its appearance, flavor, texture and nutritional value are major attributes determining the consumer preference. In mango, anthracnose, bacterial canker, scab and sooty mould are the major diseases that affect the quality of the fruit and contributing to decreased quality in addition to insect pests and physical damages. New group of fungi referred as sooty blotch, colonise the waxy layer of mangoes, survive on fruit leachates and cause superficial skin blackening of fruits which is different from sooty mould fungi growing on sugary excretions of sap sucking insects. It causes a kind of cosmetic damage to the fruit without affecting the inside pulp quality. But its residual effect of skin blackening severely affects the consumer preference and marketability of fruits which in turn cause severe financial loss to the mango growers and traders. During survey, the sooty blotch symptoms were also recorded on banana, bael, anola, star goose berry, aou and many tree plants bordering the orchards. Skin blotching caused by sooty blotch cannot be easily removed by any water wash as that of sooty mould. In addition sooty blotch infection cannot be ignored as fresh and clean fruits are preferred for local or distant urban markets. The mid and late season mango varieties whose maturity coincides with wet summer season were more prone to incidence of sooty blotch. In India occurrence of sooty blotch on mango was confirmed from several states viz., Odisha, Tamil Nadu, Karnataka, Uttar Pradesh, Andaman and Nicobar and West Bengal.

Although there is a considerable knowledge of sooty blotch and flyspeck on other crops worldwide (Williamson and Sutton, 24; Gleason *et al*., 10), little is known about the etiology and control of sooty blotch in mango and no fungicide is having any label claim for its control in mango. In apple sooty blotch (SB) and Flyspeck (FS), are reported as major diseases. (Williamson and Sutton, 24; Batzer *et al*., 3; Diaz-Arias *et al*., 7). They grow on the epicuticular wax layer of apples (Nasu *et al*., 18; Ocamb-Basu and Sutton, 19; Johnson *et al*., 13) and complex of SBFS fungi often coexist on the same fruit in the same orchards (Johnson and Sutton, 14; Williamson and Sutton, 24). In the United States, SBFS epidemics in apple lead to downgrading fruit from premium market grade to processing use (Sutton & Sutton, (23); Williamson and Sutton, (24).Gleason *et al*.(10) reported an elaborate list of more than 50 fungi associated with SBFS fungal complex in apple. In mango, blotch has been reported to be caused by fungi like *Zasmidium* sp., *Pseudocercospora* sp., *Mycosphaerella* sp under Odisha condition (ICAR-IIHR Annual report, 2016-2017). However, fungi colonizing SBFS from other parts of the

country has to be established since it may vary according to the prevailing climatic condition as reported in apple (Gleason *et al*., 10).

In apple, strategic SBFS management strategies have been suggested by various workers based on cultural practices, chemical methods (Brown and Sutton, 4; Rosenberger *et al*., 21; Duttweiler *et al*., 8) and post-harvest treatments (Hendrix, 11; Batzer *et al*., 2). In the regions of southeastern United States where high rainfall and relative humidity prevails, weekly application of fungicide was found inadequate to completely control this disease, resulting in a 5 to 10% reduction in marketable apples (Mainand Gurtz, 16). Therefore, growers experience sporadic control failures related to poor fungicide coverage, inadequate pruning, and environmental conditions that are highly favorable for SBFS development (Cooley *et al*., 6). It has been reported that sodium hypochlorite dips reduce the population of *Botrytis cinerea*, *Mucor piriformis* and *Penicillium expansum* on fruits and vegetables (Spotts and Peters, 22; Jones and Sutton, 15; Williamson, and Sutton, 24 and reduce the severity of SBFS (Colby, 5; Hendrix, 11; Winsiewsky *et al*. (25).

For management of sooty blotch infection in mango, as such no control measures are available to control sooty blotch either at field level or after harvest. In order to adequately quantify the benefits of various post-harvest treatments, reliable and cost-efficient assessment methods are needed. Keeping the above points in view, the current study was inducted with the objective to evaluate the efficacy of various fungicides to manage the sooty blotch infection at field level and to evaluate post-harvest dip treatments to remove sooty blotch signs on mango fruits after harvest.

MATERIALS AND METHODS

Protective fungicide spray for managing sooty blotch infecting mango under field conditions

The field trial was conducted during 2016-17 in Rayagada district of Odisha on the existing mango orchard on cv. Amrapali. Rayagada district has latitude of 26^{o} N and a longitude of 94^{o} 20'E with the average rainfall of approximately 1340.0mm. The fungicides which are already in use to manage mango diseases were taken for current study. The experiments were conducted using randomized block design with three replicates. All spraying schedule were carried out in line with standard commercial orchard practice. Each replicate consisted of 5-trees. Fungicides were applied @ 7-8 L per tree targeting mainly on fruits *i.e.,* sprays were applied to runoff. Three sprays were given with an interval of 15 days from May end with two consecutive sprays at 15 days interval with fungicides as per detail given in Table 1. Ten secondary branches at mid- height (2 m) of each tree were marked, and all fruit on these branches (app. 50 per tree) were observed once in a week from the day in which disease signs were first visually detected by the naked eye in the unsprayed check. These fruits were evaluated for sooty blotch incidence on the tree itself i.e., *in situ* for symptoms of sooty blotch(proportion of affected fruit in total number of fruits observed). At harvest, 100 fruits samples were arbitrarily collected across each treatment for assessing the disease severity and brought to laboratory by maintaining replication and treatment details. In the laboratory, disease severity (percentage of fruit portion covered by symptoms) was visually estimated for individual fruit with the self-made severity scores of 0-5 scale, 0–Healthy; 1:<1% fruit surface covered with sooty blotch; 2: 1-10% fruit surface covered sooty blotch; 3: 11-25% fruit surface covered with sooty blotch; 4:26-50% fruit surface covered with sooty blotch; 5:>50% fruit surface covered with sooty blotch signs.

Post-harvest dip treatment for removal of fungal blackening caused by sooty blotch

To remove the superficial black fungal growth of mango fruits caused by sooty blotch, initial screening was carried out with certain treatments including IIHR-Pongamia soap and ordinary detergent soap. Brushing of infected fruits with detergent soap with the help of cotton or muslin cloth followed by washing with water yielded good result by removing 80-85 percent blackening but it was very laborious and tedious process. However, brushing could lead to formation of minor wounds on fruit surface. Hence some improved methods were tried subsequently. Among hence various compounds tried to remove the blackening caused by sooty blotch, a selected treatment combination showed good results in removing the fungal blackening. Therefore, it was taken for detailed evaluation at various concentration (500, 750, 1000 ppm), dipping time (5, 10, 15 mins) and pH (5.5, 6.5.7.5) with all possible combinations with five replication @20 fruits per replication. After post-harvest treatment, the fruits were washed with water twice and subjected sooty blotch severity scoring on 0-5 scale as described above.

Phytotoxicity studies of standardized technology

To know the potential effect of prolonged dipping time of selected treatment on mango skin, an experiment was

conducted on cv. Amrapali by dipping the sooty blotch infected mangoes in 1000ppm solution (with standard pH of 6.5) for 15, 30, 45, 60, 75, 90, 120 and 150 mins. After respective dipping time, fruits were washed with water and analyzed visually for peel injury. Similarly, to know the potential effect higher concentration of selected treatment (other than the recommended concentration of 1000 ppm) on peel, higher concentration 2000, 3000 and 4000 ppm with 15- and 30-mins dipping time (with standard pH of 6.5) were evaluated on cv. Amrapali and fruits were washed with water and analyzed visually for peel injury.

Evaluation of standardized post-harvest dip treatment on post-harvest quality parameters of mango

Those treatment showed best results were studied for their role on post-harvest quality parameters of mango. At IIHR-CHES, Bhubaneswar, fruits of Amrapali and Neelum were subject to analysis (after the post-harvest dip treatment with the standardized technology) for the parameters such as fruit firmness, fruit shrinkage, peel color, pulp colour and sensory evaluation for its acceptability, percent weight loss and physico-chemical parameters such as acidity (%) and TSS (^{o}Brix). Further, at Post Harvest Technology laboratory of ICAR-IIHR, Bengaluru, three mango varieties namely Alphonso, Amrapali, and Totapuri were subject to elaborate study (after the post-harvest dip treatment with the standardized technology) on ripening rate, TSS, fruit spoilage, and organoleptic quality during storage at room temperature.

RESULTS AND DISCUSSION

Sooty blotch is a new disease infecting mango fruits which was documented during recent years in many states of India. Sooty blotch (SB) colonies appear as circular to irregular, olive-green to dull black fungal growth on a waxy layer of near maturing mango fruits (Fig 1A and B). Flyspecks (FS) can be recognized by the presence of black shiny round fungal dots in groups that resemble the fly excreta (Williamson and Sutton, 24). Both SB and FS were found on the same fruits and produce respective colonies. However, the occurrence of flyspeck is negligible in Odisha even though seen in other mango growing regions (data not shown). Sooty mould is fundamentally different from sooty blotch, as sooty mold grows on sugary excretions produced by sap-sucking tiny insects and produce fungal encrustation over the sugary excretions. Sooty blotch significantly reduces the market value of mangoes (Fig 2A) due to moisture loss and shrinkage of affected area (Fig 2B). Mango twigs act as a major source of inoculum. In addition sooty blotch fungi also colonizes the twigs of jack fruit, sapota, star gooseberry, acacia, simaruba, fig, piasala and Calatropis *etc*., and other avenue trees.

Fig1. Sooty blotch incidence on mangoes (A), close up view of sooty blotch on a mango fruit (B)

Fig. 2. A. Sooty blotch infected mango fruits affects market value (2B) Moisture loss

Table 1. Impact of fungicides against sooty blotch disease severity under field condition

Fungicide treatment[#]	Sooty blotch disease severity (0-5 scale)
Copper oxychloride (0.2%)	1.91[c]
Copper oxychloride (0.3%)	0.97[a]
Thiophanate methyl (0.2%)) + Captan (0.3%))	1.31[b]
Captan (2 gm/L) + Thiophanate methyl (0.1%)	2.20[d]
Thiophanate methyl (0.2%)	3.20 [e]
Control	3.79 [f]

[#]Three spray, Replication: 3 (@5 trees/replication)
In a column means followed by a common letter are not significantly different at 5 per cent (P=0.05) level by DMRT

Different fungicides were evaluated to manage sooty blotch at field level in Kasipur block, Rayagada Dt of Odisha, on cv Amrapali. All five fungicide spray treatments significantly reduced sooty blotch severity compared to unsprayed control (Table 1). The highest sooty blotch control was achieved with a spray of 0.3% copper oxychloride thrice at an interval of 15 days starting from the end of May (Table 1; Fig 3). Besides, fruits received copper oxychloride (COC) spray, post-harvest decay was very minimal till the end of shelf-life period as against unsprayed control were partial to complete decay was observed (Fig 4 &5). The efficiency of fungicides may vary according to the dominant pathogen species involved in causing sooty blotch in a particular region/location. Further, the time of spray has to be decided according to the local weather condition and expected period of rain. To increase the efficiency of sooty blotch control, the fungicide coverage has to be improved by increasing the volume of water applied per unit area, reducing travel speed of sprayer and including a surfactant, if possible, to enhance fruit wetting. However, the time of spray (May/June/July) has to be decided for other regions/states of our country according to the expected period of rain and maturity period of mango. Therefore, region specific evaluation of fungicide regime is required for SBFS control (Duttweiller *et al*., 2008).

The better approach for sooty blotch management is to protect the mangoes with prophylactic fungicides in the field itself, knowing the fact that sooty blotch spores are available in the field (in mango twigs and reservoir hosts) and it may initiate the infections on fruits at any time if the trees are exposed to an accumulated wetting period of 5-7 days. In apple, trifloxystrobin or carbendazim applied at pre-blossom and flowering stage reduced the incidence of flyspeck at harvest by an average of 70% compared with the untreated control (McKenna *et al*., 2012)**.** In the mid western and northeastern United States, the strobilurin fungicides kresoxim-methyl and trifloxystrobin controlled SBFS in apple orchards as effectively as standard treatment of thiophanate-methyl plus captan (Babadoost *et al*., 1).

Fig. 3. Fruits received COC (0.3%) as pre harvest spray (left) and fruits without any spray (right)

Fig. 4. Fruits received copper oxychloride 0.3% spray (left) after harvest and one week after storage at room condition (right)

Fig. 5. Fruits didn't receive copper oxychloride 0.3% spray (left) after harvest and one week after storage at room condition (right)

Evaluation of post-harvest dip treatments for removal of blackening caused by sooty blotch

Efforts were intensified to find an alternate method to remove fungal blackening caused by sooty blotch on a waxy layer of fruits. Initial screening with several organic compounds and Generally Regarded As safe (GRAS) compounds were conducted including detergent soap and IIHR-Pongamia soap (data not shown). Among the various GRAS compounds evaluated, certain treatment combination gave good results in terms of removing the fungal blackening. Hence it was taken for detailed evaluation at a various concentration (500, 750, 1000 ppm), dipping time (5, 10, 15 mins) and pH (5.5, 6.5.7.5). The interaction between the concentration selected treatment and dipping time revealed 15 mins dip recorded with lesser disease grade of 1.17 among other interactions (Table 2). Interaction between pH and dipping time revealed 15 mins dip with 6.5 pH with lesser disease grade of 2.08 (Table 3). Interaction among the varied concentration and pH evaluated, 1000ppm of selected treatment with pH 6.5 resulted in a lesser disease grade of 1.00 (Table 4).

Table 2. Two-way interaction between the concentration of selected best treatment and dipping time in reducing sooty blotch disease severity grade on fruits of cv Amrapali

	Sooty blotch disease severity grade (0-5 scale)[#]		
	Dipping time		
Conc.	5 mins	10 mins	15 mins
500ppm	3.58[g]	3.04[e]	2.53[d]
750ppm	3.18[f]	2.46[d]	1.80[b]
1000ppm	2.03[c]	1.88[b]	1.17[a]
Control	5.00[h]	5.00[h]	5.00[h]
CD(0.05)	0.122		
CD(0.01)	0.160		

[#] Mean of five replication @ 20 fruits per replication

In a column means followed by a common letter are not significantly different at5 per cent (P=0.05) level by DMRT

Table 3. Two-way interaction between pH of mango wash and dipping time in reducing sooty blotch disease severity grade on fruits of cv Amrapali

	Sooty blotch disease severity grade (0-5 scale)[#]		
	pH of mango wash		
Dipping time	**5.5**	**6.5**	**7.5**
5mins	3.47[c]	3.18[d]	3.70[f]
10mins	3.11[cd]	2.75[b]	3.43[e]
15 mins	2.74[b]	2.08[a]	3.05[c]
CD(0.05)	0.105		
CD(0.01)	0.139		

[#] Mean of five replication @ 20 fruits per replication
In a column means followed by a common letter are not significantly different at5 per cent (P=0.05) level by DMRT

Table 4. Two-way interaction between mango wash concentration and pH in reducing sooty blotch disease severity grade on fruits of cv Amrapali

	Sooty blotch disease severity grade (0-5 scale)[#]		
	pH of mango wash		
Conc	**5.5**	**6.5**	**7.5**
500ppm	2.98[f]	2.76[e]	3.41[g]
750ppm	2.61[d]	1.92[b]	2.91[f]
1000ppm	1.83[b]	1.00[a]	2.25[c]
Control	5.00[h]	5.00[h]	5.00[h]
CD(0.05)	0.121		
CD(0.01)	0.160		

[#]Mean of five replication @ 20 fruits per replication
In a column means followed by a common letter are not significantly different at5 per cent (P=0.05) level by DMRT

Table 5. Post-harvest evaluation of dip treatment for removal blackening caused by sooty blotch in the varied concentration of mango wash, dipping time and pH

	Sooty blotch disease severity grade (0-5 scale)[#]		
	pH of mango wash		
Conc. x Dipping time	**5.5**	**6.5**	**7.5**
500ppm x 5 min	3.56[k]	3.36[k]	3.84[l]
500ppm x 10 min	2.88[ij]	2.76[hij]	3.48[k]
500ppm x 15 min	2.52[fg]	2.16[e]	2.92[j]
750ppm x 5 min	3.36[k]	2.68[ghi]	3.52[k]
750ppm x 10 min	2.56[fgh]	2.02[de]	2.82[ij]

750ppm x 15 min	1.92[d]	1.08[b]	2.40[f]
1000ppm x 5 min	1.98[de]	1.68[c]	2.44[f]
1000ppm x 10 min	2.00[de]	1.24[b]	2.42[f]
1000ppm x 15 min	1.52[c]	0.25[a]	1.90[d]
Control	5.00[m]	5.00[m]	5.00[m]
CD(0.05)	0.211		
CD(0.01)	0.278		

[#]Mean of five replication @ 20 fruits per replication
In a column means followed by a common letter are not significantly different at5 per cent (P=0.05) level by DMRT

Table 6. Analysis of variance for sooty blotch disease grade for 4x3x3 factorial experiment

Sources of variation	df	Mean square
Treatment	35	9.215**
Concentration (Conc.)	3	89.258**
Dipping time	2	10.279**
pH	2	7.922**
Conc. x Dipping time	6	1.424**
Dipping time x pH	4	0.307**
Conc. x pH	6	1.200**
Conc. x Dipping time x pH	12	0.116**
Err	144	0.028
Total	179	1.825

** Significant at 1% level.

The results of three-way interactions revealed mango wash concentrations of 1000ppm, having pH 6.5- and 15-mins dipping time recorded the least disease grade of 0.25 among the varied concentration, pH and dipping time evaluated. This above-said combination resulted in 95 % removal of mango blackening caused by sooty blotch colonization on fruit skin (Table 5; Fig 6). The final analysis of the variance table revealed that among the treatments involving the varied concentration of mango wash, pH and dipping time and their interaction were all significant at 1% level (Table 6).

Fig. 6. Mangoes infected with sooty blotch infection. Fruits before (left) and after (right) post-harvest dip treatment

Effect of range of dipping time and concentration on mango fruits cv. Amrapali

Mango wash was tested for maximum dipping time which may have the potential to cause peel injury of mangoes. For that, fruits were dipped in 1000ppm of mango wash having pH 6.5 with varied dipping time (15, 30, 45, 60, 90, 120, and 150 mins). There was no peel injury on mangoes up to 90 mins of dipping time. However, 120 mins dipping time resulted in 10 % peel injury and 150 mins dipping time resulted in 25 -50% peel injury of mangoes (Table 7).

Table 7. Effect of dipping time in 1000pppm of mango wash on mango fruit scv. Amrapali

Dipping time (in mins) in 1000 ppm of mango wash- 6.5 pH	15	30	45	60	90	120	150
Percent tissue damage[≠]	0	0	0	0	0	1 – 10	25-50

[≠]Mean of five replication @ ten fruits per replication

Table 8. Effect of high concentration of mango wash on mango fruits cv. Amrapali

	Conc. of mango wash (ppm)							
	1000		**2000**		**300**		**4000**	
Dipping time (min)	15	30	15	30	15	30	15	30
Percent tissue damage[≠]	0	0	0	0	0	0	0	10-25

[≠] Mean of five replication @ ten fruits per replication.

Mango wash was tested at the higher range of concentration to know the potential effect of mango wash to cause peel injury on mangoes. This can serve as the precautionary note to the growers. Mango fruits cv. Amrapali was dipped in 1000, 2000, 3000 and 4000ppm (with standard pH of 6.5) of mango wash for 15 and 30 mins respectively resulted in no peel injury up to 3000ppm. However dipping of mangoes in 4000ppm for 30 mins resulted up to 25% of peel injury (Table 8).

Validation of technology on various mango varieties

This final standardized technology was validated in other varieties of mangoes such as Alphonso, Neelum, Maylepelian (rootstock) and Totapuri including Amrapali at IIHR, Bengaluru, Karnataka state. In all variety, the above said treatment involving mango wash concentration of 1000ppm, having pH 6.5- and 15-mins dipping time resulted in 100 percent removal of mango blackening caused by sooty blotch (Table 9). However, mango wash had nil effect on fruits having spots or symptoms caused by other fungi as evidenced with totapuri fruits during experimentation at IIHR, Bengaluru.

Table 9. Experimentation of Arka mango wash at IIHR, Bengaluru on different varieties of mango

Sooty blotch Disease grade	**Mango varieties**				
	Alphonso	**Neelum**	**Amrapali**	**Totapuri**	**Maylepelian**
Before dip treatment	3	3	3	2	4
After dip treatment	0	0	0	0	0

Standardized Methodology

The required quantity of solution A has to be added in 10 L of potable water immediately before treatment in a plastic container to make 1000ppm and with pH of 6.5 by adding solution B. The sooty blotch infected mango fruits had been dipped in the solution for a maximum of

15 mins and washed twice in the water to remove the residual effect of chemicals.

Post-harvest quality studies of mangoes dipped in Standardized 'Arka mango wash'

Mango fruit varieties of Amrapali and Neelum were subjected to analysis of post-harvest quality parameters such as fruit firmness, fruit shrinkage, peel color, pulp colour and sensory evaluation for its acceptability, percent weight loss and physico-chemical parameters of mangoes such as acidity (%) and TSS (°Brix) at IIHR-CHES, Bhubaneswar. It was concluded that standardized 'Arka mango wash' had no adverse effect on fruit quality and physico-chemical properties of treated fruits of tested varieties and retained all desired parameters. (Table 9&10).

Table 9. Evaluation of standardized 'Arka Mango wash' on fruit firmness and quality parameters of mangoes

Treatment	Amrapali		Neeum	
	Treated fruits	Untreated fruits	Treated fruits	Untreated fruits
Fruit firmness (N)	20	15	6.5	6.2
Fruit shrinkage	Nil	Nil	Nil	Nil
Peel colour*	3	3	5	5
Pulp colour	Deep orange	Deep orange	Yellow	Yellow
Sensory evaluation for overall acceptability#	6	6	6	6

*Assessment of fruit colour: [Index 1-6 Dull Green-1; Light green-2; Greenish Yellow-3; Yellowish Green-4; Light Yellow-5; Yellow orange-6 (Fama, 2006)]
#Sensory evaluation for overall acceptability based on flavor, taste, and acceptance [Scale 1-10: 9-10- Excellent; 6-8 Good; 4-5 Fair; 1-3 Poor].

Table 10. Evaluation of standardized 'Arka Mango wash' on percent weight loss and physico-chemical parameters of mangoes

Treatment	Var. Amrapali				Var. Neelam			
	Treated fruits		Untreated fruits		Treated fruits		Untreated fruits	
	6d	8d	6d	8d	6d	8d	6d	8d
Percent weight loss	3.74	9.6	3.7	10.6	3.3	4.8	3.5	5.9
Acidity (%)	0.35	0.21	0.32	0.16	0.19	0.16	0.19	0.16
TSS (°Brix)	15.6	17.1	15.9	17.6	18.4	18.6	18.6	19.3

Further, three mango varieties namely Alphonso, Amrapali, and Totapuri were subjected to elaborate study at Post Harvest Technology laboratory, ICAR-IIHR, Bengaluru to know the effect of standardized 'Arka mango wash' on ripening rate, TSS, fruit spoilage, and organoleptic quality of mangoes during storage at room temperature. The ripening rate (Table 11), TSS (Table 12), organoleptic quality (Table 13), and fruit spoilage (Table 14) of all three mango varieties were found no undesirable effect on fruits subjected with 'Arka mango wash' and further it was also noted that this technology could also increase the shelf life of treated fruits by delaying the fruit spoilage compared to untreated mangoes.

Table 11. Effect of standardized 'Arka mango wash' on the ripening rate of three mango varieties

Treatment	Ripening rate (1-5 scale)						
	Alphonso						
	3d	5d	7d	9d	12d	14d	16d
Treated	1.88	2.61	3.17	4.40	4.28	4.54	4.65
Control	1.99	2.61	3.24	4.10	4.31	4.68	4.61
	Amrapali						
	3d	5d	7d	9d	12 d	14 d	16 d
Treated	1.08	1.29	2.02	4.20	4.65	4.83	5.00
Control	1.00	1.15	2.12	4.27	4.73	4.97	5.00
	Totapuri						
	3d	5d	7d	9d	12d	14d	16d
Treated	1.02	1.08	1.13	1.41	1.67	2.88	3.54
Control	1.00	1.00	1.10	1.34	2.42	3.03	3.74

(1-5 scale: 1-unripe, 2-quarter ripe; 3- half ripe; 4-3/4th ripe; 5-completely ripe)

Table 12. Effect of standardized 'Arka mango wash' on TSS of three mango varieties during storage at RT

Treatment	TSS (^{o}Brix) taken on respective days after treatment			
	9 d	**12 d**	**14 d**	**17 d**
	Alphonso			
Treated	17.17	-	18.05	-
Control	18.24	-	18.65	-
	Amrapali			
Treated	22.11	20.75	-	-
Control	23.30	22.20	-	-
	Totapuri			
Treated	-	-	16.36	17.00
Control	-	-	16.58	17.23

Table 13. Effect of standardized 'Arka mango wash' on the organoleptic quality of three mango varieties during storage at RT

Treatment	Organoleptic quality*scale of 1-5$^{\#}$				
	Appearance	**pulp colour**	**pulp texture**	**Taste**	**off flavour**
	Alphonso				
Treated	3.15	3.79	3.62	3.94	nil
Control	2.46	3.71	3.62	3.79	nil

	Amrapali				
Treated	4.17	4.04	3.75	4.19	nil
Control	3.69	4.00	3.44	3.62	nil
	Totapuri				
Treated	3.65	3.58	3.02	3.42	nil
Control	**3.33**	4.08	3.38	3.65	nil

***Evaluated with a panel of 13 members #Scale 1-5: 1- very poor; 2- poor; 3- average 4- good; 5- very good**

Table 14. Effect of standardized 'Arka mango wash' on fruit spoilage of three mango varieties during storage at RT

Treatment	**Percentage of fruit spoilage**		
	Alphonso		
	7 days	**9 days**	
Treated	0.0	15.41	
Control	0.0	29.7	
	Amrapali		
	7 days	**9 days**	
Treated	0.0	3.92	
Control	0.0	7.78	
	Totapuri		
	7days	**12 days**	**14days**
Treated	0.0	8.37	12.35
Control	0.0	10.05	15.14

Table 15. Validation of standardized 'Arka mango wash' at Experimental farm of Directorate of Horticulture (DERAS farm), Odisha farm on the bulk quantity of mango var. Amrapali

Sooty blotch disease grade of mango var. Amrapali (0-5 scale)		**Percent disease reduction over control**
Before Treatment	**After Treatment**	
2	0	100
3	0	100
4	0	100
5	0.25	95

Fig. 7. Mango fruits cv. Amrapali with disease grade 4 before and after treatment at Experimental farm of Directorate of Horticulture (DERAS farm), Odisha

The standardized 'Arka mango wash' has been validated on around 80 tonnes of Amrapali mangoes (received from the Tribal sub-plan (TSP) villages of Rayagada district of Odisha) at Experimental farm of Directorate of Horticulture, Odisha (DERAS farm) and at farmer's orchard at Kasipur, Rayagada. The received infected mangoes were separated into groups based in their disease grades. Fruits up to disease grade of 4 resulted in 100% removal of mango blackening after treatment and disease grade 5 fruits resulted in 95% removal after treatment compared to control groups (Fig 7, 8and 9). After removal of sooty blotch blemishes, these mangoes were sold at a fair price as appearance looks good when compared to untreated blackened mangoes. The untreated blackened mangos fetches for poor price, wherein buyers and traders were reluctant to purchase them from the farmers.

Fig. 8. Large scale validation of 'Arka mango wash technology' on var. Amrapali at Experimental farm of Directorate of Horticulture, Odisha (DERAS farm), Bhubaneswar.

Fig. 9. 'Arka mango wash technology' at a farmer orchard, Rayagada, Odisha

Economics of standardized 'Arka mango wash'

Rate on 1 kg of healthy mango cv. Amrapali = Rs. 20/-;

Rate of 1 kg of mango with blackened appearance = less than Rs. 10/-

The cost involved for chemicals to treat one tonne of mango = Rs. 750/-

Labour charge for treating one tonne of mango = Rs 250/-

The total cost involved for treating one tonne of mangoes = Rs.1000/- (Rs. 1/kg of fruits)

Precautions to be noted

To ensure the availability of sufficient concentration of chemical, the water condition has to be monitored, and it is advised to change the water after every 3-4 washes. The water used for the treatment should be of potable water quality (water quality parameters have to be as per drinking water guideline mainly concerning pH, hardness, turbidity, dissolved solids, sodium, chlorine, etc.,) to get the desirable result of this above technology. As the deep bore well water having increased dissolved solids and undesirable parameter that interfere in achieving the desirable results as observed in our course of experiments too. Therefore, before initiating the treatment on large scale, the treatment has to be done on small scale to ensure the desired results as the treatment largely depends on the use of good quality water.

In the case of apple, five-to-seven-minute dip in 500

ppm chlorine, followed by brushing and a freshwater rinse, reduced incidence of SB from 100 to 0% and FS from 100 to 27% (Hendrix, 11). Batzer *et al*. (2) reported that a 7-min dip of apple infected with SBFS in 800 ppm chlorine resulted in a mean increase from 25 and 55% to 100% Extra Fancy grade for 'Jonathan' and 'Golden Delicious' apples, respectively, and increased market value by 31 and 14%, respectively.

For holistic sooty blotch management in mango, the orchard should always have full sunlight, good air circulation, with proper water drainage. Further, a regular pruning schedule has to be followed as per recommendation, which will eliminate unnecessary plant growth and excessive shading thereby increase the air movement. Further in the author's view, controlling of fungal blackening caused by sooty blotch infection at field level found to be the better option to retain the freshness of mangoes as the post-harvest treatment removes the waxy layer available on the fruit along with the fungal growth because sooty blotch fungi colonize on the waxy layer to utilize the fruit leachates leaked out of the fruit. Hence the mangoes will lose the glossy appearance even though the blackening is removed. However, the Arka Mango wash technology can be used as a feasible option for sooty blotch removal to increase the retail value of the fresh-market fruits, if the grower missed to take the advantage of fungicide management program.

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REFERENCES

Babadoost, M., Gleason, M. L., McManus, P. S., and Helland, S. J. 2004. Evaluation of a wetness-based warning system and reduced-risk fungicides for management of sooty blotch and flyspeck of apple. *Horticulture Technology,* **14**:27-33.

Batzer, J. C., Gleason, M. L., Harrington, T. C. and Tiffany, L. H. 2005. Expansion of the sooty blotch and flyspeck complex on apples based on analysis of ribosomal DNA gene sequences and morphology. *Mycologia,* **97**: 1268-1286.

Batzer, J. C., Gleason, M. L., Weldon, B., Dixon, P. M., and Nutter, F. W. 2002. Evaluation of post harvest removal of sooty blotch and flyspeck on apples using sodium hypochlorite, hydrogen peroxide with peroxyacetic acid, and soap. *Plant Diseases,* **86:** 1325-1332.

Brown, E. M. and Sutton, T. B. 1986. Control of sooty blotch and flyspeck of apple with captan, mancozeb, and mancozeb combined with dinocap in dilute and concentrate applications. *Plant Diseases,* **70** :281-284.

Colby, A. S. 1920. Sooty blotch of pomaceous fruits. *Trans III. Academy of science*, **13**:139-179.

Cooley, D. R., Gamble, J. W., and Autio, W. R. 1997. Summer pruning as a method for reducing flyspeck disease on apple fruit. *Plant Diseases,***81**:1123-1126.

Diaz -Arias, M. M., Batzer, J. C., Harrington, T. C., Wong, A.W., Bost, S. C., Cooley, D. R., Ellis, M. A., Hartman, J. R., Rosenberger, D. A., Sundin, G.W., Sutton, T. B., Travis, J.W., Wheeler, M. J., Yoder, K. S. and Gleason, M. L. 2010. Diversity and biogeography of sooty blotch and flyspeck fungi on apple in the Eastern and Midwestern United States. *Phytopathology,***100**: 345-355.

Duttweiler, K. B., Gleason, M. L., Dixon, P. M., Sutton, T. B., Mcmanus, P. S. and Monteiro, J. E. B. A. 2008. Adaptation of an apple sooty blotch and flyspeck warning system for the Upper Midwest United States. *Plant Diseases,* **92**: 1215-1222.

Giraldo, L. F., R. A. Forero., C. R. Salazar and R. Torres. 1977. The effect of packaging and potassium permanganate on the storage of tomatoes under room conditions. *Ravista Institute. Collanbiano Agropecuario*, **12**: 393-405.

Gleason, M. L., Batzer, J. C., Sun, G., Zhang, R., Díaz Arias, M. M., Sutton, T. B.,Crous, P.W., Ivanović, M., Mcmanus, P. S., Cooley, D. R., Mayr, U., Weber, R. W. S., Yoder, K. S., Del Ponte, E. M., Biggs, A. R. and Oertel, B. 2011. A new view of sooty blotch and flyspeck. *Plant Diseases,* **95**:368-383.

Hendrix, F. F., Jr. 1991. Removal of sooty blotch and flyspeck from apple fruit with a chlorine dip. *Plant Diseases,* **75**:742-743.

ICAR-IIHR Annual Report 2016-17, ICAR-Indian Institute of Horticultural Research, Bengaluru-560089, 57.

Johnson, E. M., and Sutton, T. B. 2000. Response of two fungi in the apple sooty blotch complex to temperature and relative humidity. *Phytopathology,* **90**:362-367.

Johnson, E. M., Sutton, T. B. and Hodges, C. S. 1997. Etiology of apple sooty blotch disease in North Carolina. *Phytopathology,* **87**:88-95.

Jones, A. L. and Sutton, T. B. 1996. Diseases of Tree Fruits in the East. *Michigan State University Extension Publications*, E154.

Main, C. E. and Gurtz, S. K. 1988. 1987 Crop Losses due to Plant Disease and Nematodes. N.C. State University. *Department of Plant Pathology Special Publications*, 8

Nasrin, M. M., Molla, M., Alamgir Hossae, M. S. Alamand Yasmin, L. 2008. Effect of postharvest treatments on shelf life and quality of tomato. *Bangladesh Journal of Agricultural Research,* **33**: 579-58.

Nasu, H. and Kunoh, H. 1987. Scanning electron microscopy of flyspeck of apple, pear, Japanese persimmon, plum, Chinese quince, and pawpaw. *Plant Diseases,* **71**:361-364.

Ocamb-Basu, C. M. and Sutton, T. B. 1988. The effects of temperature and relative humidity on germination, growth, and sporulation of *Zygophialajamaicensis*. *Phytopathology,* **78**:100-103.

Ritenour, A., Sargent, S. A. and Bartz. J. A 2014. Chlorine Use In Produce Packing Line**s**. Publication .HS761/CH160 accessed https://edis.ifas.ufl.edu/pdffiles/CH/CH16000.pdf .

Rosenberger, D. A., Engleberger, F. W. and Meyer, F. W. 1996. Effects of management practices and fungicides on sooty blotch and flyspeck diseases and productivity of Liberty apples. *Plant Diseases, 80:798*-803.

Spotts, R. A. and Peters, B. B. 1980. Chlorine and chlorine dioxide for control of d'Anjou pear decay. *Plant Diseases,* **64**:1095-1097.

Sutton, A. L. and Sutton, T. B. 1994. The distribution of the mycelial types of *Gloeodespomigea* on apples in North Carolina and their relationship to environmental conditions. *Plant Diseases,* **78:668**-673.

Williamson, S. M. and Sutton, T. B. 2000. Sooty blotch and flyspeck of apple: Etiology, biology, and control. *Plant Diseases,* **84**:714-724.

Winsiewsky, M. A., Glatz, B. A., Gleason, M. L. and Reitmeier, C. A. 2000. Reduction of Escherichia coli O157:H7 counts on whole fresh apples by treatment with sanitizers. *Journal of Food Protection,* **63**:703-708.

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Screening of F_1 intergeneric hybrid progenies of papaya for papaya ringspot virus (PRSV) resistance

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ABSTRACT : The present investigation was undertaken to develop papaya ringspot virus (PRSV) resistant papaya hybrids through intergeneric hybridization. Intergeneric hybridization was done involving nine *Carica papaya* cultivars as female and *Vasconcellea cauliflora* as male. Intergeneric F_1 hybrids were artificially inoculated. Artificial screening for papaya ringspot virus was carried out 27 days after sap inoculation. Out of twenty nine F_1 hybrid plants of CO 7 x *V. cauliflora,* only six plants (CO 7V1 to CO 7V6) were found free from PRSV symptoms. Similarly, out of fifty five F_1 hybrid plants of Pusa Nanha x *V. cauliflora* only twenty three (PNV1 to PNV23) were found free from the symptoms and seventy plants (CPV1 to CPV70) out of 335 plants of CP50 x *V. cauliflora* were found free from PRSV symptoms. Molecular markers ISSR markers were used to check and verify the hybridity. The resistance of the hybrids and parents were subjected to DAS ELISA test. ELISA titre value varied from 0.216 to 0.927. Among the parents, the resistant male parent *V. cauliflora* had recorded the lowest titre value of 0.216. However, the susceptible female parent CO 7 recorded the highest titre value of 0.972 followed by Pusa Nanha 0.952 and CP50 0.942. Among the hybrids ELISA titre value ranged from 0.218 to 0.29.

Keywords: *Carica papaya*, *Vasconcellea cauliflora*, intergeneric hybrids, papaya ringspot virus, resistance

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most important fruits of tropical and subtropical regions of the world and belongs to family Caricaceae. India is the largest producer of papaya in the world. In India, it is commercially cultivated in Andhra Pradesh, Gujarat, Maharashtra, Karnataka, West Bengal, Assam, Orissa, Madhya Pradesh, Manipur, Tamil Nadu and Bihar and certain extent in Kerala. Papaya is affected by a number of diseases caused by various pathogens and viruses. Now a days the most destructive disease of *C. papaya* worldwide is papaya ring spot caused by papaya ring spot virus (PRSV) -type P Litz, (1984), Manshardt, (1992), a definitive potyvirus species in the *Potyviridae* (Shukla *et al*., 1994). PRSV is grouped into two types, Type P (PRSV – P) infects cucurbits and papaya and type W (PRSV-W) infects cucurbits but not papaya (Gonsalves, 1998). Almost all cultivated varieties are highly susceptible. *Carica cauliflora* J., a wild species having non-edible fruits is known to be resistant for this viral disease (Jimenez and Horovitz, 1957). Now the species *cauliflora* has been grouped under the genera *Vasconcellea* (Vegas *et al*., 2003).

Incidence of PRSV has been reported to be more than 90 per cent in India (Varma, 1996; Jagadish Chandra and Samuel, 1999) and rendering papaya orchards economically unviable (Hema and Theertha Prasad, 2003). The results of the roving survey for papaya ringspot incidence in Karnataka revealed the presence of the disease in all the districts ranging from 75 to 100 per cent except Udupi, Hassan and Kodaku (Kunkalilkar Suresh and Byadgi, 2004). In Tamil Nadu, the disease was first noticed in Coimbatore during 2003 (Jyoti Sharma *et al.,* 2004). The present study was conducted to evaluate intergeneric F_1 hybrids of papaya for their resistance to PRSV.

MATERIALS AND METHODS

Plant materials

Seedlings were artificially inoculated with papaya ring spot virus through artificial inoculation method. The seedlings showing initial resistance alone were taken to field for further evaluation. The details of the parents and F_1 seedlings are presented in Table 1.

Mechanical inoculation of PRSV toparents, F_1 progenies

One gram of infected leaves was ground in a pre-chilled mortar and pestle using 1 ml of 0.1M chilled sodium phosphate buffer (pH 7.2) containing β-mercaptoethanol and 0.01 M EDTA. The sap was rub inoculated using the pestle or glass rod on the young

Table 1. Scale of disease incidence and intensity score

Reaction	Intensity score	Symptom
Apparently healthy (AH)	0-1	0 = No disease symptoms
Moderately resistant (MR)	1-2	1 = Slight mosaic on leaves 2 = Mosaic patches and / or necrotic spots on leaves
Moderately susceptible (MS)	2-3	3 = Leaves near apical meristem deformed slightly, yellow, and reduced in size
Susceptible (S)	3-4	4 = Apical meristem with mosaic and deformation
Highly susceptible (HS)	>4	5 = Extensive mosaic and serious deformation of leaves, or plant death).

leaves of seedlings at 3 leaves stage previously dusted with carborundum powder 600 meshes. After 5 minutes, the excess sap was washed off by distilled water. The disease incidence and intensity score was given using the scale developed by Dhanam (2006). Details of the disease incidence and intensity score scale are presented in APPENDIX I.

Transplanting

Experiment was laid out in a Randomized Block Design with three replications. Forty five day old healthy seedlings along with parents (6 seedlings each) were planted at a spacing of 1.8 × 1.8 m and standard package of practices were followed during the period of study.

Hybridity confirmation using ISSR markers

To confirm the hybridity of these intergeneric progenies, ISSR marker analysis was carried out using six CO 7 x *V. cauliflora,* twenty three Pusa Nanha x *V. cauliflora* and seventy plants of CP50 x *V.cauliflora*. DNA from leaves of parents and F_1 was carried out following CTAB method (Doyle and Doyle, 1987). PCR reaction was performed using 6 (ISSR) primers. PCR reaction was carried out in total volume of 10 µl in 96 tubes PCR plates. Following were the master mix of solution for one reaction. For ISSR primers, reagents of 10 X Taq buffer + $MgCl_2$ (15 mM) on 1.0 µl, dNTP (2 mM)on 1.0 µl, Primers 10µM 1.0 µl (0.5µl each for combination), Taq polymerase (3 IU / µl) on 0.1 µl, Sterile double distilled water on 4.9 µl and Template DNA 10 ng / µl on 2 µl. Cycling profile- Touch down protocol was followed for all the primers. PCR cycles included initial denaturation at 94°C for 3 min followed by 19 cycles of 30 Sec (-0.5°C) denaturation at 94°C, annealing at 63°C for 30 sec and 1 min in extension at 72°C. Again 19 cycles of 15 Sec denaturation at 94°C, annealing at 55°C for 30 sec, 1 min in extension at 72°C, 10 min in final extension at 72°C and infinitive final hold at 4°C. Electrophoresis was performed in 1.5 per cent agarose with 120V for 2 hours.

Source of antiserum and positive sample: Antibody for PRSV and their positive samples were provided from DSMZ, Braunschweing, Germany. DAS-ELISA was performed for the detection of PRSV by following the manufacturer's instructions (DSMZ Gmbh, Braunschweig, Germany). Purified IgG was diluted in coating buffer (1:1000) and 200 µl was added to each well of a micro titer plate (Grainer). The plates were then incubated at 37°C for 2 to 4 hours and thereafter plates were washed with PBS-T using wash bottle, soaked for a few minutes and repeat washing for twice. Plates were blotted by tapping upside down on tissue paper. 200 µl aliquots of the test sample (extracted in sample extraction buffer) were added to duplicate wells. The plates were incubated overnight at 4°C. The plates were washed as in earlier and added with 200 µl of the anti-virus conjugate (1:500) to each well and incubated at 37°C for 2 hours. Then the plates were washed three times as done earlier. Finally, 200 µl of freshly prepared substrate (10 mg ρ-nitro phenyl phosphate (Sigma 104-105) dissolved in 10 ml of freshly prepared substrate buffer) was added to each well and incubated in dark at room temperature for 20 to 45 minutes or as long as necessary to obtain clear reactions. Spectrometric measurement of absorbance was then read at 405 nm (EL 800, BIO-TEK Instrument Inc., and USA). The reaction was stopped by adding 50 µl of 3 M NaOH. Buffer served as negative control.

RESULTS AND DISCUSSION

Screening of F_1 progenies through artificial inoculation against PRSV under glass house conditions

In a perennial crop like papaya, field screening for diseases is very difficult since, it requires a larger area for

planting. Hence, screening in glass houses in the nursery stage proved quick and rapid method. Observation for PRSV was done 27 days after inoculation. A total number of 29 seedlings in CO 7 x *V.cauliflora,* 55 plants in Pusa Nanha x *V.cauliflora*and 335 plants in CP50 x *V.cauliflora* were artificially inoculated with papaya ringspot virus through sap inoculation method. Typical PRSV symptom of mottling of leaves and water soaked lesions on stems were observed in the susceptible parents and the hybrids. Regarding the female parents, all were found to exhibit the virus symptoms uniformly after sap inoculation. Symptom free F_1 hybrids were transplanted in the main field for further evaluation. The failures of PRSV symptoms to develop on the manually inoculated hybrid plants indicate the incorporation of genes resistant to PRSV (Table 1). Further, the wild genus *V. cauliflora* was found to be completely resistant to the strain PRSV prevalent in Coimbatore area of Tamil Nadu, India (Manoranjitham *et al*., 2008).

Hybridity confirmed intergeneric hybrids

Three intergeneric hybrids of CO 7 x *Vasconcellea cauliflora* crosses out of six, eight intergeneric hybrids of Pusa Nanha x *Vasconcellea cauliflora* crosses out of 23 and seven intergeneric hybrids of CP 50 x *Vasconcellea cauliflora* crosses out 70 were tested for hybridity. The primer UBC - 856 produced unique banding patterns in*Vasconcellea cauliflora* (male parent) in which five bands were prominent, out of which third and fifth were absent in female parent (Fig. 1) but present in CO 7 x*Vasconcellea cauliflora* (CO7V3). The same primer produced distingusible band between Pusa Nanha x*Vasconcellea cauliflora* (PNV9) which was used for the identification of true hybrid (Fig. 2). In case of UBC-807 primer, one prominent band was observed in male parent which was absent in female parent but present in CP 50 x*Vasconcellea cauliflora* (CPV23) hybrid (Fig. 3). These primers were helpful to identify F_1's in cross CO 7 x*Vasconcellea cauliflora,* Pusa Nanha x *Vasconcellea cauliflora*and CP 50 x *Vasconcellea cauliflora.* The hybridity confirmed F_1 plants were forwarded to F_2.

Ruas *et al*. (2003) used Inter-simple sequence repeat (ISSR) markers and successfully evaluated the genetic divergence among the eight *Coffea* species. To confirm the hybridity of intergeneric hybrids involving *Carica papaya* x *V. cauliflora*, Praveen (2005) also used ISSR markers and confirmed successfully.

ELISA titre value for parents and F_1 hybrids

The Enzyme Linked Immunosorbent Assay (ELISA), a powerful immunological test (Clark and Adams,

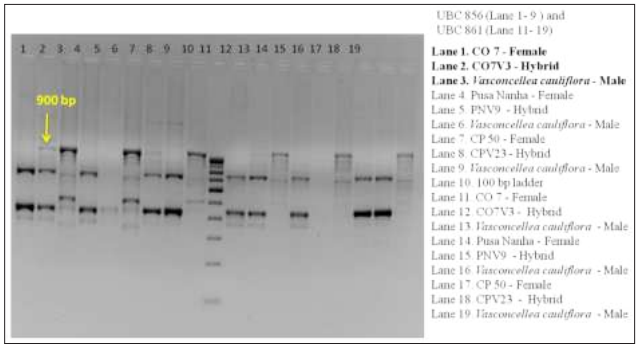

Fig. 1. ISSR Marker profile of parents and F_1s

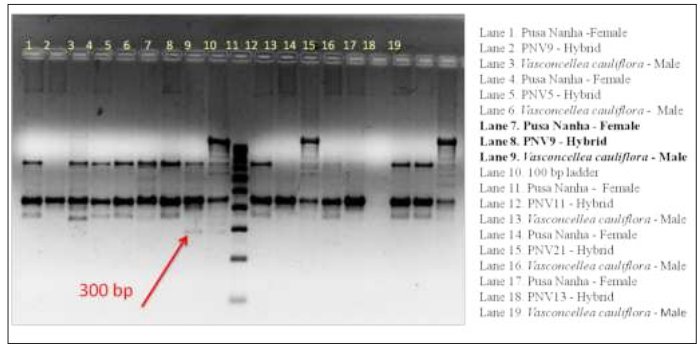

Fig. 2. ISSR marker UBC 856 profile of parents and F_1s

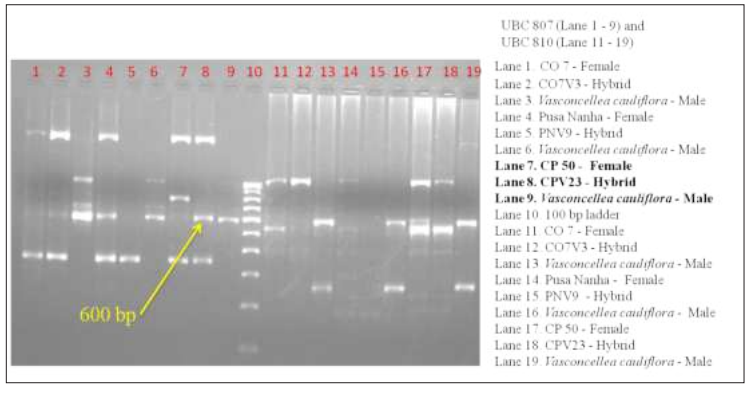

Fig. 3. ISSR marker profile of parents and F_1s

1977), is extensively used for detecting, identifying and quantifying viruses in many plant species (Clark, 1994). This test could be a component of a reliable method for screening *C. papaya* x *C. cauliflora* hybrid plants for PRSV resistance. In this study, the resistance of the hybrids and parents was assessed by serological test. Parents and their hybrids *viz*., CO 7 x *V.cauliflora*, Pusa Nanha x *V.cauliflora* and CP50 x *V.cauliflora* were subjected to DAS- ELISA test. Parents and F_1 progenies involving CO 7 and *Vasconcellea cauliflora* were subjected to DAS- ELISA test. ELISA titre value varied from 0.216 to 0.972. Among the parents, the resistant male parent *Vasconcellea cauliflora* had recorded the lowest titre value of 0.216. However, the susceptible female parent CO 7 recorded the highest titre value of 0.972, followed by PusaNanha (0.952) and CP 50 (0.942). Among the hybrids involving CO7 and *Vasconcellea cauliflora*, ELISA titre value varied from 0.243 to 0.266 (Table 3). Among them, the cross combination CO7V3, confirmed hybrid through molecular markers, was found to record the lowest titre value of 0.243 followed hybrid CO7V5 (0.245) and CO7V6 (0.247).

Among the hybrids involving PusaNanha x *V.cauliflora*, ELISA titre value varied from 0.218 to 0.286 **(Table 2).**Among the hybrid combinations, the combinations PNV3 and PNV9 recorded the lowest titre value of 0.218 followed by PNV1 (0.219), PNV6 (0.220), PNV11 (0.220), PNV8 (0.222) and PNV13 (0.223). All the above said hybrid combinations were confirmed as true hybrids through molecular marker studies.

Among the hybrids involving CP50 x *V.cauliflora*, ELISA titre value varied from 0.218 to 0.299 (Table 3). Among them, the cross combination CPV23 a confirmed hybrid through molecular markers, was found to record the lowest titre value of 0.218 followed by other confirmed hybrids *viz*., CPV56 (0.219), CPV39 (0.220), CPV31 (0.221), CPV1 (0.222), CPV26 (0.226) and CPV12 (0.232).

The results revealed that the lowest value of 0.216 was recorded by the resistant male parent.*V. cauliflora* however; all the female parents used for this study recorded very high titre values proving their susceptibility. Manoranjitham *et al.* (2008) reported that *V.cauliflora* registered the lowest titre value which clearly indicated its natural resistance to PRSV. They also reported that *V.cauliflora* is resistant to all the strains of PRSV which are prevalent in Coimbatore conditions.

Among the parents, the gynodioecious female parent CO7 was found to be highly susceptible than the other two dioecious female parents *i.e*. Pusa Nanha and CP 50.Thirugnanavel (2010) also reported that tolerant genotypes recorded the lower ELISA absorbance value than

Table 2. Screening of F_1 progenies through artificial inoculation against PRSV under glass house conditions

Parents / Hybrids	Total number of plants inoculated	Disease scoring (number of plants in each category)						Number of plants without symptom 27 days after inoculation
		0	1	2	3	4	5	
CO 7	5	0	0	0	0	0	5	0
Pusa Nanha	5	0	0	0	0	0	5	0
CP 50	5	0	0	0	0	0	5	0
Vasconcellea cauliflora	5	5	0	0	0	0	0	5
CO 7 x *Vasconcellea cauliflora*	29	6	0	0	0	10	13	6
Pusa Nanha x*Vasconcelleacauliflora*	55	23	0	0	0	15	17	23
CP 50 x *Vasconcellea cauliflora*	335	70	0	0	0	100	165	70

Table 3. ELISA titre value for parentsand F_1 population involving CO7 (apparently free from PRSV after inoculation)

Parents and their hybrids	OD value at 405nm	Parents and their hybrids	OD value at 405nm	Parents and their hybrids	OD value at 405nm
Vasconcellea cauliflora	0.216	*Vasconcellea cauliflora*	0.216	*Vasconcellea cauliflora*	0.216
CO 7	0.972	Pusa Nanha	0.952	CP 50	0.942
Buffer	0.102	Buffer	0.102	Buffer	0.102
CO7V3	0.243	PNV1	0.219	CPV1	0.222
CO7V5	0.245	PNV3	0.218	CPV12	0.232
CO7V6	0.247	PNV6	0.220	CPV23	0.218
		PNV8	0.222	CPV26	0.226
		PNV9	0.218	CPV31	0.221
		PNV11	0.220	CPV39	0.220
		PNV13	0.223	CPV56	0.219

Fig. 4. Field view of intergeneric F_1 hybrids

the susceptible ones. Among the genotypes tested, tolerant genotype CP 50 recorded the lowest value of 0.187 at flowering and 0.198 at harvest.

In the present study, the cross combinations *viz*., C7V3, CO7V5 and CO7V6 were recorded the lowest titre values. Similarly the crosses namely PNV1, PNV3, PNV6, PNV8, PNV9, PNV13 and PNV21 were observed the lowest titre values. CP50 x *V.Cauliflora* progenies *viz*., CPV1, CPV12, CPV23, CPV26, CPV31, CPV39 and CPV56 were recorded the lowest titre values proved their tolerance to this virus . This observation confirms the earlier report of Manshardt (1992) *who studied the intergeneric hybrids involving C.cauliflora x C.papaya* hybrids. Similar studies using ELISA test had been conducted previously to identify PRSV-P infected *C. papaya* (Gonsalves and Ishii, 1980; Thomas and Dodman, 1993).

Reaction of parents and F_1 hybrids after transplanting under field conditions

The study revealed varied levels of tolerance for PRSV by the parents and their hybrids (Figure 4). All the hybrids which were artificially inoculated with PRSV, but not showing virus symptoms, and their parents the male parent *V.Cauliflora* was not showed the PRSV symptoms but the female parents CO7 , Pusa Nanha and CP50 showing virus symptoms in the main field. This may be due to the fact that tolerance is affected by many factors including inherent genetics, time of infection and climatic conditions (Vimla Singh *et al*., 2005).

CONCLUSION

Based on the disease intensity score, reaction to the papaya ringspot virus and yield performance, selected F_1 combinations *viz*., CO 7 x *Vasconcellea cauliflora* (CO7V3), Pusa Nanha x *Vasconcellea cauliflora* (PNV9) and CP 50 x *Vasconcellea cauliflora* (CPV23) were advanced to F_2 generations.

REFERENCES

Clark, M.F., Adams, A. N.1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology*, **34**: 475-83.

Clark. 1994. Immunodiagnosis methods using polyclonal and monoclonal antibodies. In: The identification and characterization of pest organisms. (Hawksworth, D.L., ed). Wallingford, UK; CAB International, 377-393.

Dhanam, S. 2006. Studies on papaya ringspot disease (*Carica papaya* L.). A part of M.Sc. Thesis**.** Tamil Nadu Agricultural University, Coimbatore.

Doyle, J.J. and Doyle, J. L.1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin***, 19**: 11-15.

Gonsalves, D. 1998. Control of papaya ringspot virus in papaya: a case study. *Annual Review of Phytopathology***, 36**: 415-437.

Gonsalves, D. 1994. Papaya ringspot. **In:** Compendium of Tropical Fruit Diseases. (Ploetz, R.C, ed). MN, USA: APS Press.67.

Gonsalves, D. and Ishii, M. 1980. Purification and serology of papaya ringspot virus. *Phytopathology***, 70:** 1028-32.

Hema, M.V. and Theertha Prasad, D. 2003. Management of papaya ringspot virus-need for transgenic approach. *Indian Journal of Agricultural Biochemistry***, 16**: 1-11.

Jagadish Chandra, K. and Samuel, L. D. K. 1999. Viral and phytoplasmal diseases of papaya in India. **In:** Diseases of Horticultural Crops –Fruits. (Ed.) Verma, L.R. and Sharma, R.C. Indus Publishing Co., New Delhi.

Jimenez, H. and Horovitz, S. 1957. Cruzabilidad entree species de Carica. *Agronomy Tropical***,7:**207-215.

Jyoti Sharma, Jain, R. K. and Varma, A. 2004. Detection of papaya ringspot virus in naturally infected papaya plants by reverse transcription polymerase chain reaction. *Indian Phytopathology,* **57:** 237-239.

Kunkalilkar, S. and Byadgi, A. S. 2004. Molecular characterization, cloning of coat protein gene, epidemiology and management of papaya ring spot virus. *Indian Phytopathology,* **57:** 330-331.

Litz, R.E, 1984. Papaya. **In:** Evans DA Sharp WR, Ammirato PV & Yamada Y,eds. Handbook of Plant Cell Culture, Vol 2. New York, NY, USA: Macmillan, 349-368.

Manshardt, R.M. 1992. Papaya. **In:** Hammerschlag FA, Litz FA & Litz RE, eds. biotechnology of perennial fruit crops. Wallingford, UK; CAB International, 489-511.

Manoranjitham, S.K., Auxcilia, J., Balamohan, T. N., A. Thirugnanavel and Rabindran, R. 2008. Confirmation of PRSV resistance in wild type *Vasconcelleacauliflora* through sap inoculation studies. S-II: Genetic resources and crop Improvement. Second International Symposium on Papaya. 9-12 December, Madurai India

Praveen, K.S. 2005.Interspecific hybrid progeny evaluation in papaya (*Carica papaya* L.). M.Sc. Thesis. University of Agricultural Sciences, Bangalore.

Ruas, P.M., Raus, C. F., Rampim, V.P. Carvalho, E.A. Raus and Sera, T. 2003. Genetic relationship in *Coffea* species and parentage determination of interspecific hybrids using ISSR (Inter-simple sequence repeat) markers. *Genetics and Molecular Biology*, **26** (3):345-349.

Shukla, D.D, C.W. Ward and A.A. Brunt.1994. The polyviridae. Wallingford, UK: CAB International.

Thirugnanavel, A. 2010. Breeding for PRSV resistance in papaya (*Carica papaya* L.) through germplasm screening and intergeneric hybridization. Ph. D Thesis. Tamil Nadu Agricultural University Coimbatore.

Thomas, J.E. and Dodman, R. L. 1993. The first record of papaya ring spot virus –type P from Australia. *Australasian Plant Pathology*, **22**, 1-7.

Varma, A.K. 1996. Viral and Mycoplasmal diseases of papaya (*Carica papaya* L.) Disease Scenario in Crop Plants, Vol. I-Fruits and Vegetables (Eds). Agnihort, V.P., Om Prakash, Ram Krishnan and Mishra, A.K. International Books and Periodical Supply Service, New Delhi.

Vegas, A.,Trujillo, G., Sandrea, Y. and Mata, J. 2003. Obtention, regeneration and evaluation of intergeneric hybrids between *Carica papaya* and *Vasconcellea cauliflora*. *Intersciencia*, **28**(12):710-714.

Vimla Singh, G.P., Rao and Shukla, K. 2005. Response of commercially important papaya cultivars to papaya ring spot virus in eastern U.P. conditions. *Indian Phytopathology*, 58: 212-216.

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Symptomatological, morphological and molecular validation of *Colletotrichum gloeosporioides* (Penz.) Sac. associated with leaf spot disease of arecanut

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ABSTRACT: The outbreak of leaf spot disease of arecanut caused by *Colletotrichum* spp. created havoc in hill and coastal zones of Karnataka. *Colletotrichum* is one among the top ten widespread plant-pathogenic fungi. The symptoms which are predominant on leaves were also noticed on nuts with circular or oblong to irregular brownish spots surrounded by larger yellow halo. Cultural characteristic of the isolated pathogen on potato dextrose agar produced dense, cottony, whitish to slight greyish mycelium with even margin without any zonation. Microscopic observation revealed that conidia with single celled, hyaline and cylindrical with rounded ends besides that length of the conidia varied from 12.06 to 12.30 µm and width 3.8 to 4 µm having 2-3 oil globules. The ITS based sequence analysis revealed that *Colletotrichum gloeosporioides* is associated with leaf spot disease of arecanut. It is concluded that the *C. gloeosporioides* was found predominant among the isolated cultures and sole responsible for epidemic of leaf spot disease.

Keywords: Arecanut, *Colletotrichum,* identification, leaf spot, symptoms

INTRODUCTION

Arecanut (*Areca catechu* L.) is an important plantation crop of India belongs to the family Arecaceae. Arecanut industry forms the economic backbone of a substantial number of farm families (Balasimha and Rajagopal, 2004). It is extensively used in India by all sections of the people as masticatory and in several social and religious ceremonies (Bhat *et al.,* 2021). Areca nut is extensively cultivated in the plains and foothills of Western Ghats and North Eastern regions of India. Presently it is cultivated in 9.38 lakh ha with a production of 13.68 lakh tones and average productivity is 1.46 MT/ha. In Karnataka it is grown in an area of 4.71 lakh hectares with a production of 7.03 lakh tonnes and 1.49 MT/ha. The major area under cultivation is confined to Karnataka, Kerala and Assam. Among the states Karnataka stands first in area, production and productivity (Anon., 2022). Arecanut palm is affected by a number of diseases at different stages of growth and development. About 20 diseases, causing varying degrees of damage to the palm have been recorded in India (Bavappa, 1982). Among the fungal disease, leaf spot cause more catastrophic yield loss up to 60 percent (Hedge, 2018). In recent year's leaf spot disease is epidemic in Karnataka and Kerala. Leaf spot of arecanut though a minor disease in the past, has nowbecome a major disease especially during rainy season. The disease began as circular to irregular spots which enlarged as the disease progressed. Later, the spots were light to dark brown in color having ash grey center, surrounded by dark brown margins and yellow halo. In severe cases, the adjacent spots eventually coalesced to form large irregular patches leading to blighted appearances and finally covered the entire leaf lamina turning the leaf color to pale yellow. Fungus produces the conidia within 3 to 5 days at 30 °C and at 90% relative humidity. Survival of conidia, and conidia in infected leaf debris was studied in soil maintained at different soil moisture levels. Survival of conidia declined rapidly under moist conditions (≥12% moisture, vol/wt.), but under dry conditions, viable conidia could be detected up to 12 months after incorporation into soil (Hartung *et al.*, 1981; Mohanan *et al.*, 1989; Salotti *et al.*, 2022). Sudden outbreak of disease in traditional growing areas made impact on socioeconomic status and livelihood of farming community. Hence, early detection based on field symptoms and prophylactic spray should be followed for effective management. Therefore, present investigation was more focused on symptomatology of leaf spot disease in different growth stages of arecanut and molecular confirmation of etiology based on ITS sequence analysis.

MATERIALS AND METHODS

Collection of disease samples: As result of epidemic of leaf spot disease in hill zone and coastal zones, the roving survey was conducted to know the disease severity. The diseased leaf samples of arecanut palm showing typical leaf spot symptoms were collected during survey from Shivamogga and Chikkamagaluru districts.

Isolation of pathogen: The samples were brought to the Arecanut Research Centre, Shivamogga. The standard

tissue isolation technique was used to isolate the fungus. The infected portion of the leaf bits were thoroughly washed and cut into small pieces (1 mm) in such a way that each piece consisted of infected along with surrounding portion of healthy green tissues. The pieces were surface sterilized with 1% sodium hypochlorite solution for 30 seconds followed by three successive washing with sterilized distilled water to remove the traces of chemical if any and then left for drying. After drying, the sterilized pieces were transferred to autoclaved Petri plates containing 20ml PDA media under aseptic conditions. These Petri plates were then placed in BOD incubator at 27±1°C for 9 days.

Morphological identification: A loopful of pure culture was taken from the nine days old culture and placed it on the slide and mixed thoroughly with lactophenol to obtain uniform spread. A cover slip was placed over it. Length and breadth of the conidia were measured under high power objective lens of Lawrence and Mayo binocular microscope (LM-52-1803-S) and TC capture 3.9.0 software and drivers were installed for image acquisition, managing and processing. Further, the morphological characters such as hyphae, conidial shape, size and oil globules were documented.

Molecular identification of the pathogen: The genomic DNA was extracted using Cetyltrimethyl ammonium bromide (CTAB) protocol given by Doyle and Doyle (1987). ITS1/ITS4 5′-TCCGTAGGTGAACCTGCGG-3′ and 5’-TCCTCCGCTTATTGATATGC-3’ primers were used in the experiment and PCR conditions were followed as initial denaturation (94°C; 5 min.), denaturation (94°C; 1 min.), annealing (54°C; 1 min.), extension (72°C; 2 min.), final extension (72°C; 10 min.) for 35 cycles. Separation of amplified product on 1.5% agarose gel. PCR product were purified using protocol of QIAquick PCR Purification Kit. Purified PCR product sequenced by Barcode Biosciences Pvt. Ltd. Bengaluru. Homology of obtained sequence was accomplished through NCBI (National Centre for Biotechnology Information) BLAST (Basic Local Alignment Search Tool) (http://blast.ncbi.nlm.nih.gov) and the sequences were submitted to NCBI database as OQ948330.

Phylogenetic analysis: A multiple-sequence alignment was carried out using comparable reference sequences of other *Colletotrichum* species which are retrieved from NCBI database and multiple sequence aligned using CLUSTAL W (Edgar and Batzoglou, 2006) algorithm of MEGA 6.0 software to check the genetic diversity

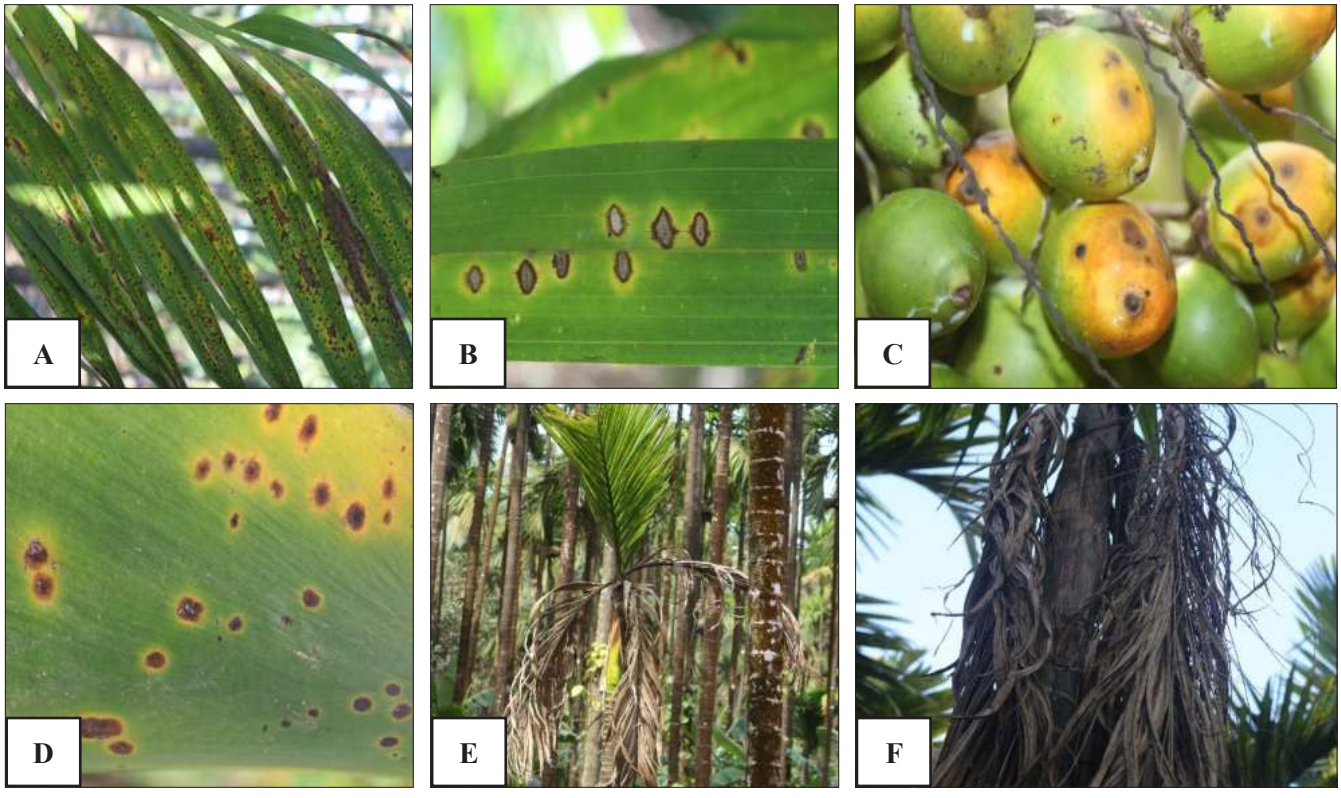

Fig. 1. Symptomatology of arecanut leaf spot disease incited by *C. gloeosporioides* A-B. Indicates small, circular or oblong to irregular brownish spots on leaves. C. Circular brown spots on thenuts. D. Brownish spot on leaf sheath. E-F. Severely blighted symptoms of leaves lead to drooping.

among different *Colletotrichum* isolates (Tamura *et al.* 2013). Phylogenetic relationships were analyzed by the distance methods. The distance matrix for the aligned sequences was calculated using Kimura's two parameter model (Kimura 1980), and analyzed with the neighbor-joining (NJ) method (Saitou and Nei 1987) using MEGA (Molecular Evolutionary Genetics Analysis program) version 6, excluding positions with gaps. The reliability of the inferred tree was estimated by bootstrap analysis (Felsenstein, 1985). The final trees were displayed using Clustal Omega multiple alignment package (Sievers and Higgins, 2021).

RESULTS AND DISCUSSION

Symptomatology of leaf spot disease of arecanut: The initial symptoms of the leaf spot disease appeared as small, circular or oblong to irregular brownish spots (Figure 1). The center of the spot was grey or straw color surrounded by yellow halo. In the advanced stages, spots coalesced to give a blighted appearance to the leaves.Similar symptoms were observed by Hegdeand Hegde(1986) in anthracnose disease of arecanut. Based on survey it was observed that fungal infection was also noticed even on nuts.

Morphological characterization of isolated culture on potato dextrose agar: The cultural characteristic of the isolated pathogen on Potato dextrose agar produced dense, cottony, whitish to slight greyish mycelium with even margin without any zonation (Figure 2). Microscopic observation (40X Microscopic field) revealed that acoenocytic, hyaline hyphae with profuse branching habit. Conidia with single celled, hyaline and cylindrical with rounded ends besides that length of the conidia varied from 12.06 to 12.30 µm and width 3.8 to 4 µm having 2-3 oil globules. Microscopic observations are in confirmatory with literatures of Weir *et al.* (2012) and Hassan *et al.* (2018) in citrus anthracnose from New Zealand and persimmon anthracnose from South Korea, respectively.

Fig. 2. Pathogen isolated on PDA media and microscopic view of conidia (40X)

DNA sequences of obtained isolates were compared using bioinformatics tool like NCBI (National Centre for Biotechnological Information) blast programme. Based on sequence comparison, nucleotide sequences of the ITS1/ITS4 region of the ribosomal DNA of isolates had 100% homology with *C. gloeosporioides* isolates available in the NCBI. Thus, obtained isolates were confirmed as *Colletotrichum gloeosporioides*.

Phylogenetic analysis: The Cladogram (Figure 3) obtained from MEGA 6.0 software showed that obtained sequence grouped with *Colletotrichum gloeosporioides* and compared with reference sequences of different species which fell in different group when it was rooted with *Colletotrichum xanthorrhoeae.* The results obtained are in agreement with the results obtained by earlier workers Serra *et al*. (2011) and Zivkovic (2017).

CONCLUSION

The pathogen causing leaf spot disease of arecanut was isolated and identified based on symptomatology and ITS based molecular conformation as *Colletotrichum gloeosporioides*. However, on the basis of available literature, this is the recent report from Karnataka.

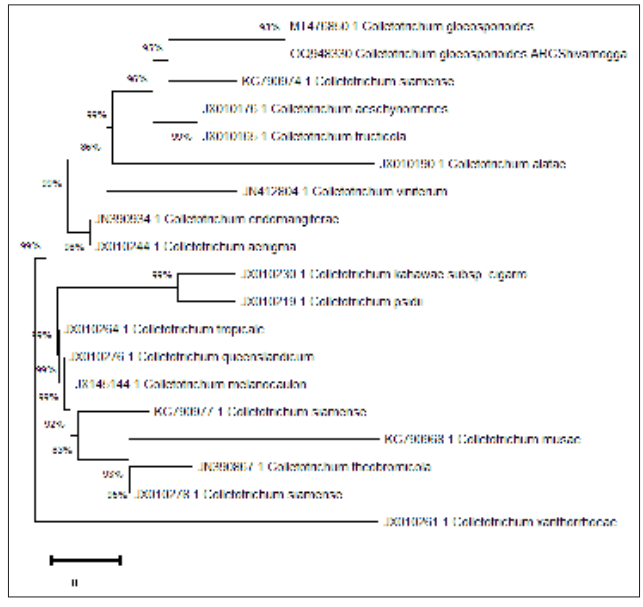

Fig. 3. Phylogenetic analysis of different isolates of *C. gloeosporioides*

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REFERENCES

Anonymous, 2022. Directorate of Arecanut and Spice Development, Calicut, Kerala.

Balasimha, D. and Rajagopal, V. 2004. Introduction in Arecanut. (Eds: Balasimha D, Rajagopal V). Central Plantation Crops Research Institute. Kasaragod. Kerala. India: 1-6.

Bavappa, K. V. A. 1982. The Areca nut palm. CPCRI, Kasaragod.

Bhat, S. K. Bhat, S. and Kalladka, P. K. 2021. Effects of arecanut (*Areca catechu* L.). chewing on human health: misleading titles lead to wrong notions. *International Journal of Science and Research Methodology,* **17(3)**: 95-109.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin,* **19**:11-15.

Edgar, R. C. and Batzoglou, S.2006. Multiple sequence alignment. *Current opinion in structural biology*, **16(3)**: 368-373.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution,* **39(4)**: 783-791.

Hartung, J. S. Burton, C. L. and Ramsdell, D. C. 1981. Epidemiological studies of blueberry anthracnose disease caused by *Colletotrichum gloeosporioides*. *Phytopathology,* **71**(4): 449-453.

Hassan, O., Jeon, J. Y., Chang, T., Shin, J. S., Oh, N. K. and Lee, Y. S. 2018. Molecular and morphological characterization of *Colletotrichum* species in the *Colletotrichum gloeosporioides* complex associated with persimmon anthracnose in South Korea. *Plant Disease,* **102(5)**:1015-1024.

Hegde, G. M. 2018. Field efficacy of fungicides to manage leaf spot of areca nut. *Advance Plants Agricultural Research,* **8(6)**:496-498.

Hegde, Y. R. and Hegde, R. K. 1986. Studies an anthracnose of arecanut (*Areca catechu* L.) caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. *Plant Pathology Newsletter,* **4**: 24-27.

Kimura, M. A. 1980. Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal Molecular Evolution,* **16**: 111-120.

Mohanan, R. C., Kaveriappa, K. M. and Nambiar, K. K. N. 1989. Epidemiological studies of *Colletotrichum gloeosporioides* disease of cocoa. *Annals of Applied Biology,* **114(1)**: 15-22.

Saitou, N. and Nei, M.1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evaluation,* **4(4)**: 406-425.

Salotti, I,Ji, T. and Rossi, V. 2022.Temperature requirements of *Colletotrichum* spp. belonging to different clades. *Front Plant Science,* **22(13)**.

Serra, I.M.R.D.S., Menezes, M., Coelho, R.S.B., Ferraz, G.M.G., Montarroyos, A.V.V. and Martins, L.S.S. 2011. Molecular analysis in the differentiation of *Colletotrichum gloeosporioides* isolates from the cashew and mango trees. *Brazilian Archives of Biology and Technology,* **54(1)**:1099-1108.

Sievers, F. and Higgins, D. G. 2021. Multiple sequence alignment: Methods and protocols. The clustal omega multiple alignment package. *Humana Press publisher*: 3-16.

Tamura, T., Stecher, G., Peterson, D., Filipski, A. and Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution,* **30(12)**: 2725-2729.

Weir, B. S., Johnston, P. R. and Damm U. 2012. The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology,* **73**:115-180.

Zivkovic, S., Trkulja, N., Popovic, T., Oro, V. and Ivanovic, Z. 2017. Morphological and molecular identification of *Colletotrichum gloeosporioides* from *Citrus reticulata*. In International Symposium: Current Trends in Plant Protection-Proceedings. *Institute for Plant Protection and Environment, Belgrade (Serbia)*:**21**.

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A new casing formulation for enhancing yield of milky mushroom, *Calocybe indica*

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ABSTRACT: The global popularity of milky mushrooms, *Calocybe indica*, is increasing due to their impressive size, appealing milky appearance, delicious taste, attractive colour, excellent shelf life, and unique texture. The casing layer is crucial for inducing fructification in milky mushrooms, providing moisture, physical support, and facilitating gas exchange. The present study evaluated various casing material combinations to find suitable options for commercially cultivation of milky mushroom *C. indica*. Different raw materials, *viz*., raw coir pith, Arka Fermented Cocopeat (AFC), MnSO4, milky mushroom grown substrate, and sandy, loamy soil, were evaluated individually and in different combinations. It was observed that the combination of AFC and sandy, loamy soil in a ratio of 60:40 resulted in a higher milky mushroom yield of 156.90g/kg wet substrate. Similarly, casing sandy loamy soil treated with MnSO4 (150 ppm) resulted in a higher yield of 198.83g/kg wet substrate. Additionally, the combination of casing soil and milky mushroom colonized substrate in a ratio of 90:10 led to a higher mushroom yield of 174g/kg wet substrate. The casing combination of the mushroom colonized substrate (10%), MnSO4 (100 ppm), AFC (600g), and sandy loam soil (400g) exhibited the highest mushroom yield of 241.2g/kg wet substrate.

Keywords: AFC, *Calocybe indica,* casing, milky mushroom, MnSO4, spawn, CAC-ing.

INTRODUCTION

The milky mushroom, *Calocybe indica*, also known as the white summer mushroom or *Dudh Chhatta*, is gaining global popularity due to its impressive size, appealing milky appearance, delicious taste, attractive color, excellent shelf life, and unique texture (Chadha and Sharma, 1995). It is now India's third most commercially grown mushroom, following the white button (*Agaricus bisporus*) and oyster (*Pleurotus* spp.) mushrooms. Initially collected in the wild from West Bengal, India, by Purkayastha and Chandra in 1974, the production technology for *Calocybe indica* was introduced by Purkayastha and Nayak in 1979 and further improved in 1981. The ICAR-IIHR has standardized its cultivation technology. Milky mushrooms are becoming increasingly prevalent worldwide due to their robust size, attractive milky appearance, delicious taste, appealing color, excellent shelf life, unique texture, and sustainable yield (Chang, 2007; Alam et al., 2010). Unlike most edible mushrooms, which require low temperatures (< 25°C) for commercial cultivation, the milky mushroom thrives in warmer climates (30–35°C), making it suitable for hot, humid regions throughout the summer (Pani, 2010; Kumar et al., 2012). This adaptability addresses the challenge of cultivating mushrooms in warm weather without the need for expensive infrastructure (Thakur and Singh, 2014).

In commercial cultivation, casing is a crucial agronomic practice for milky mushrooms. The casing layer, applied after the germination phase, stimulates the transition from vegetative to reproductive growth (Pardo et al., 2004). Effective casing materials must have high water-holding capacity, good air-space ratio, porosity, and bulk density (Yadav, 2006). Researchers have used various materials for casing, including peat moss, loam soil, spent mushroom substrate, coconut coir, biogas slurry, and farmyard manure (Krishnamoorthy et al., 2000). The CAC-ing technique (Compost Added at Casing) involves adding small amounts of fully colonized mushroom mycelium substrate to the casing soil, potentially increasing mushroom yield (Ratnoo and Anila Doshi, 2012). This promising technology, currently used in button mushroom cultivation, could be experimented with for milky mushrooms. The quality of the casing layer is influenced by factors such as texture, compactness, pH, structure, water holding capacity, porosity, and bulk density. Traditionally, growers have used locally available soils, well-decomposed spent compost, and farmyard manure as casing materials. However, exploring new alternative combinations can lead to consistent and improved biological efficiency. The present study focuses on developing new casing material formulations (different casing combinations, CAC-ing experiments, and micronutrients) to achieve consistent higher biological efficiency and improved cultivation methods for *C. indica* in tropical regions.

MATERIALS AND METHODS

Studies were conducted during 2017-21 at ICAR-

Indian Institute of Horticultural Research, Bengaluru. Various raw materials were used during experimentation, including raw coir pith, Arka fermented cocopeats, MnSO4, mushroom-grown substrate, and soil. Standard mycological techniques were followed, such as pure culture production through tissue culture, maintaining pure cultures through sub-culture, and using low-temperature and sterile water storage methods. Cultivation experiments were conducted on sterile paddy straw, raw coir pith, commercial coco peat, and Arka fermented cocopeats (sterilized at 121°C, 15-18 psi pressure, for 25 minutes to 2 hours, depending on the substrate). Spawn was prepared using sterile sorghum grains (sterilized at 121°C, 15-18 psi pressure, for 3 hours). Polypropylene (PP) bags of the required sizes were used as containers for both spawn production and mushroom cultivation. Cultures were inoculated under laminar airflow conditions and incubated at 30±2^{0}C. The cultivation experiments were inoculated with 5% spawn in a spawning room and incubated in a spawn-running room under ambient conditions. After the complete spawn run, the bags were cased according to treatment requirements, and necessary humidity, light and ventilation were maintained using a high-pressure pump fogger.

Casing material preparation: Casing substrate mixing was prepared as required, and mixed cased material with 60-70% moisture was mixed with chalk powder (8%) to bring the pH to 7.5-8. It was then pasteurized at 80^{o}C for 2 hours.

RESULTS

The results indicated that the highest yields were achieved with soil (100%), raw coir pith + soil (60:40), and raw coir pith + soil (20:80), recording 139.67g, 133.97g and 128.40g per kg of wet substrate, respectively (Table 1). Raw coir pith contains lignin, which inhibits mushroom fungus growth, resulting in poor yields. Therefore, the highest yields were obtained from soil alone (Control), raw coir pith + soil (60:40), and raw coir pith + soil (20:80). Similar findings of lower yields with coir pith were reported by Chinara and Mahapatra (2022). Compared to other substrates, the poor growth of *C. indica* mycelium in coir pith likely influenced the mushroom production in the present study. It can be concluded from this study that fresh coir pith delays the growth of *C. indica*. Pani (2012) also observed a gradual reduction in fruiting bodies and productivity with delayed casing soil application.

Table 1. Study of different combinations of raw coconut coir pith along with soil for production of *C. indica* on paddy straw substrate

Treatment	2017-18		2018-19		Average	
	Yield (g)	BE (%)	Yield (g)	BE (%)	Yield (g)	BE (%)
Raw coir pith (100%)	102.7	29.34	83.8	23.94	92.10	26.36
Raw coir pith + Soil (80:20)	103.8	29.66	106.2	30.34	128.40	36.78
Raw coir pith + Soil (60:40)	69.7	19.91	114.2	32.63	133.97	38.39
Raw coir pith + Soil (40:60)	94.7	27.06	131	37.43	86.27	24.67
Raw coir pith + Soil (20:80)	140.7	40.20	130.2	37.20	121.60	34.79
Soil (100%)	161.3	46.09	129.9	37.11	139.67	39.97
CV	4.68		5.34			
CD(0.01)	4.24		3.93			

During 2017-18 and 2018-19, six different combinations of commercial cocopeat pith and soil were evaluated as casing materials for the growth and yield of *C. indica* on a paddy straw substrate. The results showed that casing with soil (100%) and commercial coco pith + soil (60:40) yielded higher results, with 138.10g and 126.05g per kg of wet substrate, respectively (Table 2). Applying casing materials provides physical support to the milky mushrooms in the bags and induces sporophore formation. Additionally, Kerketta et al. (2018) found

that various casing materials positively affected the growth and yield of milky mushrooms. Casing soil protects and supports mushrooms against pests and diseases, aids in developing sporophores, and facilitates gaseous exchange necessary for mushroom growth and development (Krishnamoorthy, 2016). Similar results were also reported by Satish et al. (2022) and Amin et al. (2010).

Table 2. Study of different combinations of commercial cocopeat along with soil for production of *C. indica on* paddy straw substrate

Treatment	2017-18		2018-19		Average	
	Yield (g)	BE (%)	Yield (g)	BE (%)	Yield (g)	BE (%)
Commercial coco pith (100%)	139.3	39.46	91.3	26.09	115.30	32.78
Commercial coco pith + Soil (80:20)	79.5	22.52	126.9	36.26	103.20	29.39
Commercial coco pith + Soil (60:40)	116.9	33.12	135.2	38.63	126.05	35.88
Commercial coco pith + Soil (40:60)	78.8	22.32	142.8	40.80	110.80	31.56
Commercial coco pith + Soil (20:80)	94.4	26.74	137	39.14	115.70	32.94
Soil (100%)	146.3	41.44	129.9	37.11	138.10	39.28
CV						
CD(0.01)						

During 2017-18 and 2018-19, six combinations of Arka fermented cocopeat (AFC) and soil were evaluated as casing materials for the growth and yield of *C. indica* on a paddy straw substrate. The results indicated that AFC + soil (60:40) and AFC + soil (80:20) yielded higher mushroom yield, with 156.90g and 146.25g per kg of wet paddy straw substrate, respectively, compared to the other treatments. The lowest yield was recorded with control soil (100%) at 113.45g per kg wet substrate (Figure 1). Pradeep Singh Shekhawat et al. (2023) also reported similar results: commercial cocopeat combination treatments obtained the highest mushroom yields.

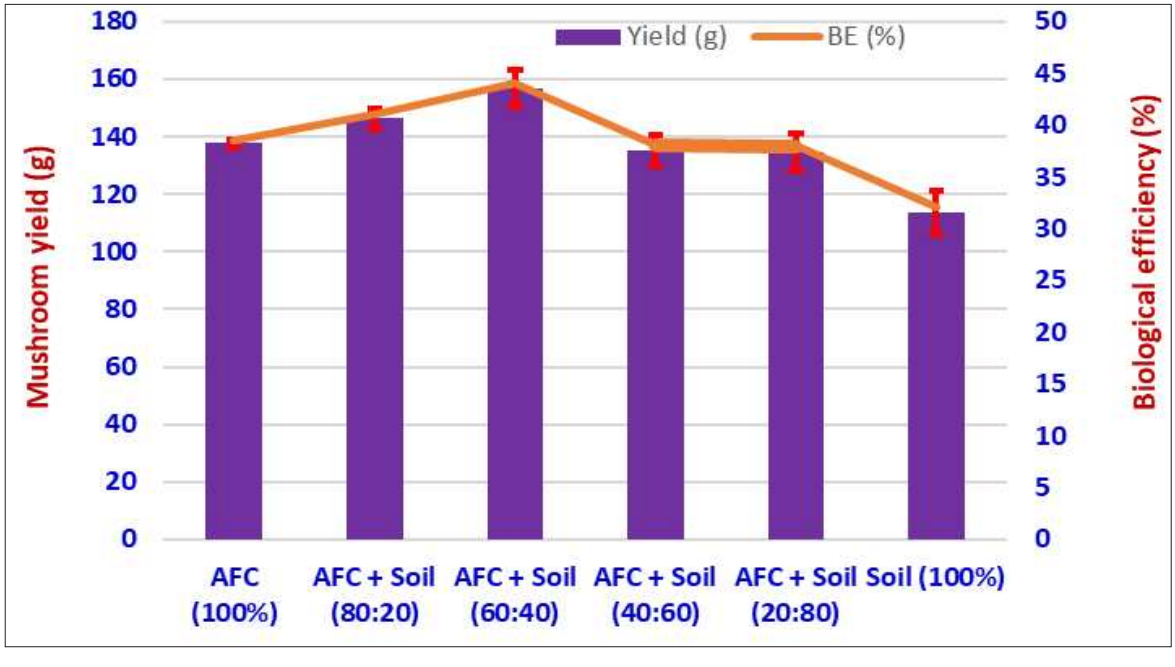

Fig. 1. Effect of different combinations of Arka fermented Cocopeat (AFC) along with soil on production of *C. indica* with paddy straw substrate

During 2019-20 and 2020-21, five different concentrations of MnSO4 and soil were tested as casing materials for the growth and yield of *C. indica* on a paddy straw substrate. The results showed that casing soil treated with 150 ppm MnSO4 recorded the highest yield at 198.83g per kg of wet substrate, followed by casing soil treated with 100 ppm MnSO4, which yielded 193.77g per kg. The lowest yield was recorded in the control group (soil), with 135.55g per kg wet substrate (Table 4). MnSO4 content was analyzed in all harvested mushroom samples, and the results indicated that the MnSO4 treated casings had significantly higher MnSO4 content than the untreated control (Figure 2). Similar findings were reported by David et al. (2006), who observed that adding manganese to casing soil increased button mushroom yield by 9.6% to 11.8% compared to the control. Saheb and Golnoosh (2023) also reported that applying manganese and iron to casing soil increased button mushroom yield by 11.2% compared to the control.

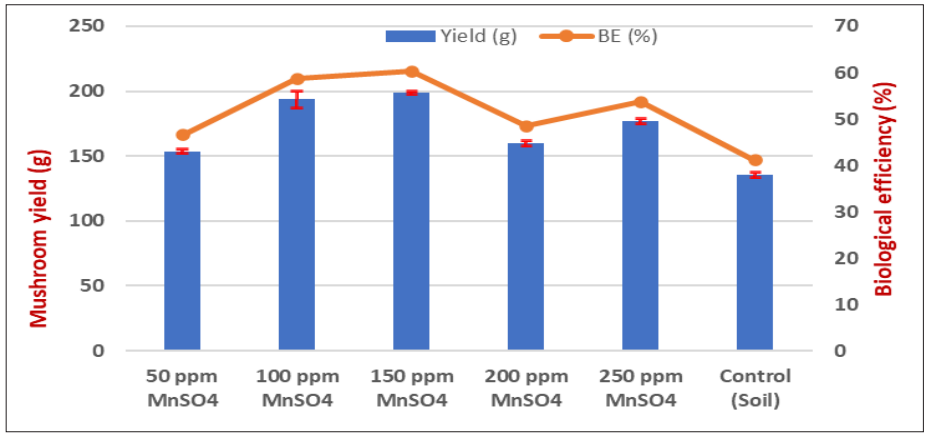

Fig. 2. Evaluation of different concentration of $MnSO_4$ along with soil as a casing for growth and yield of milky mushroom

Five combinations of casing soil and mushroom-colonized substrate were evaluated for their impact on the growth and yield of *C. indica* grown on a paddy straw substrate. The results showed that the combinations of casing soil + mushroom-colonized substrate at ratios of 90:10 and 92:8 recorded higher mushroom yields, producing 174 g and 169 g per kg of wet substrate, respectively. These yields were comparable and outperformed the other treatments and the control. The control group recorded the lowest yield, with 135.85 g per kg wet substrate (Fig 3). The CAC-ing technique was evaluated in this study and resulted in significant yield increases of 36.13% to 43.01% over a 32-day harvest phase. The increased yields from the CAC-ing technique may be attributed to the higher population of Pseudomonas and improved aeration due to the addition of crushed colonized compost in the casing layers (Nair and Hayes, 1975).

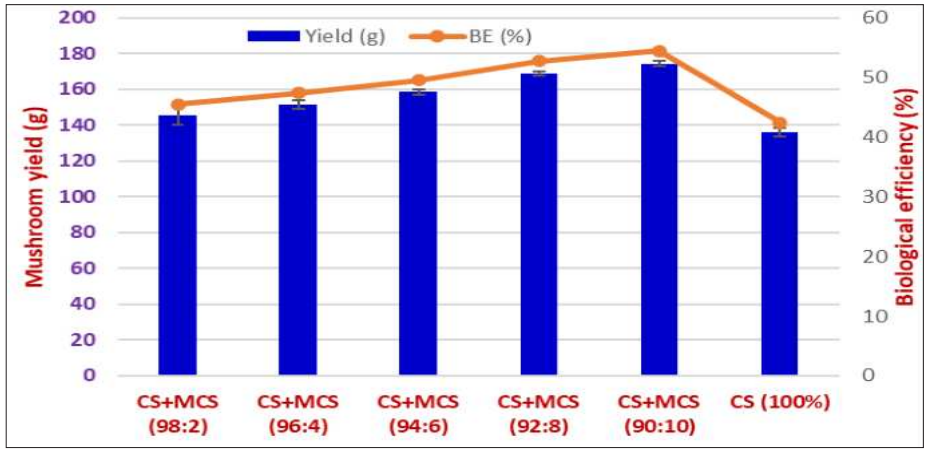

Fig. 3. Evaluation of Mushroom colonized substrate (CAC-ing) for production of milky mushroom *C. indica*

Six combinations of mushroom-colonized substrate, MnSO4, AFC+ soil, and control (soil) were evaluated as casing materials for the growth and yield of milky mushroom *C. indica*. Results showed that the casing combination of mushroom-colonized substrate (10%) + MnSO4 (100 ppm) + AFC (600 g) + soil (400 g) recorded the highest mushroom yield of 241.2 g/kg wet substrate. The combination of the mushroom colonized substrate (8%) + MnSO4 (150 ppm) + AFC (600 g) + soil (400 g), which yielded 208.6 g/kg wet substrate. The control treatment recorded the lowest yield of 174.4 g/kg wet substrate (Table 3). Similar results were obtained in previous studies that examined the effects of these treatments individually. However, this is the first time the combined effect of mushroom-colonized substrate, MnSO4, AFC, and soil as a casing formulation has been evaluated, and the combination significantly increased the milky mushroom yield. The varied production potential of different casing materials might be attributed to their distinct physical properties and nutritional compositions. Additionally, Kerketta et al. (2018) discovered that using various casing materials positively impacted the growth and yield of milky mushrooms. Casing soil protects mushrooms from pests and diseases, supports developing sporophores, and facilitates gaseous exchange, essential for growth and development (AS Krishnamoorthy, 2016).

Table 3. Combination of Arka fermented cocopeat, Cacing and micronutrients along with casing soil on the growth and yield of milky mushroom *C. indica*

Treatments	Yield (g/kg wet substrate)	Biological efficiency
Mushroom colonized substrate (8%) + 100 ppm $MnSO_4$ + AFC + Soil (60:40)	185.6	58.00
Mushroom colonized substrate (10%) + 100 ppm $MnSO_4$ + (AFC + Soil (60:40))	241.2	75.38
Mushroom colonized substrate (8%) + 150 ppm $MnSO_4$ + AFC + Soil (60:40)	208.6	65.17
Mushroom colonized substrate (10%) + 150 ppm $MnSO_4$ + AFC + Soil (60:40)	200.3	62.59
Mushroom colonized substrate (8%) + 100 ppm $MnSO_4$ + AFC + Soil (40:60)	206.3	64.48
Mushroom colonized substrate (10%) + 150 ppm $MnSO_4$ + AFC + Soil (40:60)	199	62.19
Casing soil (100%)	174.4	54.51
CV	5.89	
CD(0.01)	4.62	

*AFC- Arka fermented cocopeat

CONCLUSION

The casing layer plays a crucial role in milky mushroom yield by containing microorganisms that induce environmental changes, aiding the transition from the vegetative to the reproductive stage. Additionally, this layer provides necessary moisture and physical support and facilitates gas exchange during fruiting body formation. Meeting all these parameters with soil or coco peat alone is challenging. Based on the present investigation, the tested casing materials, combined with the mushroom-colonized substrate, MnSO4, AFC, and soil, have potential commercial applications for achieving improved quality and higher yields of milky mushrooms in India.

REFERENCES

Alam, N., Amin, R., Khair, A. and Lee, T. S. 2010. Influence of different supplements on the commercial cultivation of milky white mushroom. *Mycobiology*, **38** (3): 184–188.

Anurag Kerketta, Nand Kishore Pandey, H. K. Singh and C. S. Shukla. 2018. Effect of Straw Substrates and Casing Materials on Yield of Milky Mushroom (Calocybe indica P&C.) Strain CI-524. *International Journal of Current Microbiology and Applied Sciences*, **7**(2): 317-322.

Chadha, K. L. and Sharma, S. R. 1995. Mushroom Research in India: History, Infrastructure and Achievements. Advances in Horticulture, **13**: 1-12.

Chang, S. T. 2007. Mushroom cultivation using the ZERI principle: Potential for application. *Micologia Aplicada International*, **19**(2): 33–34.

David. A. Weil., Robert Beelman and David. M. Beyer. 2006. Manganese and other micronutrient additions to improve yield of Agaricus bisporus. Bioresource Technology, **97**(8):1012-7.

Diego Cunha Zied, Marli Teixeira Almeida Minhoni, J. Emilio Pardo-González, and Arturo Pardo-Giménez. 2010. *American Society for Horticultural Science*, **45**(11): 1649-1653.

Krishnamoorthy, A. S., Muthuswamy, M. T. and Nakkeeran, S. 2000. Technique for commercial production of milky mushroom Calocybe indica P&C. *Indian Journal of Mushroom*, 18, 19-23.

Kumar, R., Singh, G., Mishra, P. and Singh, R. 2012. Effect of different organic supplements and casing mixtures on yield of two strains of milky mushroom (*Calocybe indica*). *Indian Phytopath*, 65, 399-403.

Chinara, N. and Mahapatra, S.S. 2022. Evaluation of casing materials on productivity of milky mushroom (Calocybe indica P&C). *Mushroom Research*, **31**(2):167-170.

Pani, B. K. 2010. Evaluation of some substrates for cultivation of white summer mushroom (Calocybe indica). *Research Journal of Agricultural Sciences*, **1**(4): 357–359.

Pardo, A., Gonzalez, J. E. P., Pardo, J. and D.E. Juan, A. J. 2004. Assessment of different casing materials for use as peat alternatives in mushroom cultivation: Evaluation of quantitative and qualitative production parameters. *Spanish Journal of Agricultural Research*, **2**(2): 267-272.

Ratnoo, R.S. and Doshi, A. 2012. Evaluation of different casing materials and cacing in Agaricus bisporus cultivation. *International Journal of Plant Protection*, **5**:136-140.

Ruhul Amin., Abul Khair., Nuhu Alam and Tae Soo Lee. 2010. *Mycobiology*. 2010 Jun; **38**(2): 97-101.

Saheb Soodaee Mashaee and Golnoosh Banitalebi. 2023. Effect of Manganese, Iron and Growth Promoting Bacteria on Some Quantitative and Qualitative Characteristics of Button Mushroom (Agaricus bisporus), A Study of Compost Added to a Casing Technique in *Agaricus bisporus* Cultivation from Phase III Bulk Compost, **54**(3):369-382.

Thakur, M. P. and Singh, H. K. 2014. Advances in the cultivation technology of tropical mushrooms in India. *JNKVV Research Journal*, **48**(2): 120–135.

Yadav, R. S. 2006. Use of vermiproducts in the cultivation of milky mushroom (*Calocybe indica*). Doctoral dissertation. Dharwad, India: UAS.

Yaddanapudi Satish., Renuka Devi Sri.P., Dr. Krishna Mohan .K., Pranitha .G., Divya .V., Niharika, P., Swetha, P., Maheshwari, K., Sowmya, T., Kavya Sree,G., Rejuslin Bruitjo. R. S. and Varsha Reddy Patlolla. 2022. Impact of different casing materials for production of milky mushroom (*Calocybe indica*). International Journal for Research in Applied and Natural Science, **8**(10): 202-205.

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An optimized substrate combination for cultivating exotic King oyster mushroom, *Pleurotus eryngii*

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ABSTRACT: *Pleurotus eryngii*, commonly known as the 'King oyster mushroom', is one of the large and edible fungi native to Europe and Asia. In the present study, different agricultural and lignocellulosic waste materials were evaluated for optimizing cultivation of king oyster mushrooms at ICAR- Indian Institute of Horticultural Research, Bengaluru, India. The highest mushroom yield (146.08 g/kg wet substrate) was achieved with a substrate mixture of sawdust (37.5%), corn cob powder (37.5%), rice bran (12.5%), and wheat bran (12.5%) using a 5% spawn dose. Increasing the spawn dose to 8% further improved the yield, with the same substrate mixture yielding 179.8 g/kg wet substrate. The best yield (211.5 g/kg wet substrate) was obtained with the same substrate mixture at a higher spawn dose, demonstrating a significant improvement in biological efficiency. These findings support the potential for using locally available agricultural waste for sustainable mushroom cultivation, contributing to organic recycling and economic efficiency.

Keywords: Agricultural waste, corn cob powder, King oyster mushroom, *Pleurotus eryngii,* saw dust

INTRODUCTION

Oyster mushrooms (*Pleurotus* spp.) rank second in global mushroom production, following shiitake mushrooms. The king oyster mushroom (*Pleurotus eryngii*), a member of the oyster mushroom family, is edible, basidiomycetic, and saprophytic (Lewinsohn et al., 2002). It is considered the best among *Pleurotus* species due to its excellent cap and stem consistency, culinary qualities, and most extended shelf life among oyster mushrooms (Yildiz et al., 2002). The *P. eryngii* has been commercially cultivated in China, Japan, and Taiwan because of its excellent texture and flavor appeal to consumers (Eguchi et al., 1999; Peng, 1996, 1998; Royse, 1999: Melanouri et al., 2022). *Pleurotus eryngii* is a giant, edible mushroom native to Europe and Asia. While rare in the wild, it is cultivated worldwide and is famous for its buttery flavor and eggplant-like texture, especially in specific Asian cuisines. The species has many common names, including king oyster mushroom, French horn mushroom, king trumpet mushroom, and trumpet royale. Using scientific names helps avoid confusion. The name "king oyster" hints at its close genetic relationship to the popular oyster mushrooms (including *Pleurotus ostreatus*), a group of species native to North America. However, the king oyster does not resemble its relatives much. The *P. eryngii* has a thick, vertical white stem (or "stipe") and a relatively small tan or gray cap, unlike the big white cap and all-but-absent horizontal stipe familiar to American mushroom hunters (Mahbuba et al., 2010; Melanouri et al., 2022).

P. eryngii possesses various medicinal properties, including an anti-inflammatory protein called PEP, which has demonstrated its ability to inhibit the growth of colon cancer cells in both human tissue cultures and live mice without harming healthy cells. Utilizing lignocellulosic waste presents an economical means of organic recycling and supports mushroom cultivation. Different agricultural and lignocellulosic waste materials are employed for this purpose worldwide. In the present study, ICAR-Indian Institute of Horticultural Research has conducted evaluations using locally available agricultural substrates and varying spawning rates to cultivate king oyster mushrooms successfully.

MATERIAL AND METHODS

Various locally available agricultural wastes were evaluated in different combinations and with varying doses of spawn to determine their effect on king oyster mushroom yield. Further, the best substrate combinations were tested with different doses of spawn.

Source of King oyster mushroom and culture preparation

This investigation utilized commercially grown Arka PE-2 obtained from the ICAR-Indian Institute of Horticultural Research, Bengaluru. The pure cultures of various strains were prepared on malt extract agar (MEA) medium. The inoculated Petri dishes were incubated in a growth chamber at 25 ± 2°C in the dark for about ten days until the mycelium had fully developed. This culture was then used to inoculate the mother culture.

The mother culture medium was prepared by boiling sorghum grains, mixing with 4-5 % calcium carbonate, and maintaining a moisture level 48-50%. Glass bottles measuring 550g capacity were filled with 250 g of the mixture, tightly packed, with the necks plugged with non absorbant cotton and covered with butter paper. The bottles were sterilized in an autoclave at 121°C and 15 kg/cm² pressure for one hour. The *P. eryngii* inoculated bottles were placed on a rack in the laboratory for incubation at 25±2°C. The substrate of the mother culture was colonized by mycelial growth within 15–20 days after inoculation. The fully colonized bottles were then used for spawning.

Spawn preparation

White jowar was used to produce the spawn. The jowar was first boiled to a half-cooked stage, and the excess water was drained. The grains were then cooled to 55°C, and CaCO3 was mixed in at a rate of 5% of the wet-boiled grain basis. This substrate mixture was filled into autoclavable polypropylene bags (8x16 inches) plugged with nonabsorbent cotton plugs with autoclavable neck rings. The bags were sterilized at 121°C for 3 hours under 1 kg/cm² pressure. After cooling to room temperature, the sterilized bags were inoculated with the Generation-2 spawn of the selected strains to be tested separately. These inoculated bags were incubated for 45 days in a dark room at 25 ± 2°C for mycelium growth.

Cropping and harvesting

After the mycelial growth was complete (45 days after spawn inoculation), slits were made in the fully colonized substrate bags, which were then moved to the cropping room. The temperature, relative humidity, and light were maintained at 18°C, 85%, and approximately 500 lux, respectively. Mushrooms were harvested when the cap surfaces were flat to slightly up-rolled at the margins. Two to three flushes of mushrooms were harvested from each bag. The yield and various quality parameters of the mushrooms were recorded regularly.

Statistical analysis

The experiment was conducted using a completely randomized design with ten replications (n = 10). Data were analyzed and graphs were created using the statistical program SPSS 12.0 and Microsoft Excel.

RESULTS AND DISCUSSION

The growth and yield patterns of the *P. eryngii* strain cultivated on various combinations of locally available substrates are presented in Table 1. The highest mushroom yield (146.08 g/kg wet substrate) was achieved with a substrate mixture of sawdust (37.5%), corn cob powder (37.5%), rice bran (12.5%), and wheat bran (12.5%), which was significantly different from all other treatments and the control. The second highest yield (127.2 g/kg wet substrate) was obtained with a substrate mixture of coir pith (40%), sawdust (40%), rice bran (10%), and wheat bran (10%). This was followed by a yield of 110.58 g/kg wet substrate using a mixture of sawdust (40%), wood chips (40%), rice bran (10%), wheat bran (10%), and CaCO3 (3%).

Table1. Evaluation of different locally substrate for king oyster mushroom with 5% spawn dose

Treatment	Yield (g/kg wet substrate)	Biological efficiency (%)	Average fruit body weight (g)
T1- Coir pith (40%), Saw dust (40%), Rice bran (10%), Wheat bran (10%)	127.2	36.29	35.65
T2- Sawdust (40%), Wood chips (40%), Rice bran (10%), Wheat bran (10%), CaCO3 (3%)	110.58	31.57	25.65
T3- Sawdust (40%), paddy straw powder (40%), RB(10%), WB(10%)	33.5	9.57	19.8
T4- Sawdust (26.7%), Paddy straw powder (26.7%), Corn cob powder (26.7%), Rice bran (10%), Wheat bran (10%)	101.62	28.74	26.52
T5- Sugar Cane pith (80%), Rice bran (20%), CaCO3 (3%)	44.98	12.86	25.65
T6-PSP (40%), Sugarcane pith 40%), Rice bran (10%), WB(10%)	55.58	16.00	24.52

T7-paddy straw powder (80%), Rice bran (10%), Wheat bran (10%)	15.02	4.29	19.58
T8- Sawdust (37.5%), Corn cob powder (37.5%), Rice bran (12.5%), Wheat bran (12.5%)	146.08	41.71	49.50
CV	5.27		
CD (0.01)	3.83		

The highest mushroom yield (179.8 g/kg wet substrate) was achieved with a substrate mixture of sawdust (37.5%), corn cob powder (37.5%), rice bran (12.5%), and wheat bran (12.5%), which differed significantly from all other treatments and the control (Table 2). The second highest yield (149.2 g/kg wet substrate) was obtained with a substrate mixture of coir pith (40%), sawdust (40%), rice bran (10%), and wheat bran (10%). Following this, a yield of 138.5 g/kg wet substrate was recorded with a mixture of sawdust (40%), wood chips (40%), rice bran (10%), wheat bran (10%), and CaCO3 (3%). Mahbuba et al. (2010) reported similar findings, showing greater biological efficiency with sawdust substrates compared to rice straw-based substrates. Amin et al. (2007) observed the highest number of fruiting bodies of various oyster mushroom species on sawdust compared to rice straw. While king oyster mushrooms produce fewer fruiting bodies, their texture and shelf life are superior to other *Pleurotus* species. Comparable results were also noted with shiitake mushrooms (Sarker et al., 2009).

Table2. Evaluation of different locally substrate for king oyster mushroom with 8% spawn dose

Treatment	Yield (g/kg wet substrate)	Biological efficiency (%)	Average fruit body weight (g)
T1- Coir pith (40%), Saw dust (40%), Rice bran (10%), Wheat bran (10%)	149.2	42.6	40.58
T2- Sawdust (40%), Wood chips (40%), Rice bran (10%), Wheat bran (10%), CaCO3 (3%)	138.5	39.6	31.65
T3- Sawdust (40%), paddy straw powder (40%), RB (10%), WB (10%)	112.5	32.1	25.6
T4- Sawdust (26.7%), Paddy straw powder (26.7%), Corn cob powder (26.7%), Rice bran (10%), Wheat bran (10%)	125.6	35.9	32.52
T5- Sugar Cane pith (80%), Rice bran (20%), CaCO3 (3%)	112.6	32.2	31.56
T6-PSP (40%), Sugarcane pith 40%), Rice bran (10%), WB (10%)	99.8	28.5	29.5
T7-paddy straw powder (80%), Rice bran (10%), Wheat bran (10%)	124.5	35.6	26.5
T8- Sawdust (37.5%), Corn cob powder (37.5%), Rice bran (12.5%), Wheat bran (12.5%)	179.8	51.4	53.6
CV	1.82		
CD (0.01)	4.59		

The growth and yield patterns of the *P. eryngii* strain cultivated on various combinations of locally available substrates using an 8% spawn dose were recorded (Table 3). The highest mushroom yield (211.5 g/kg wet substrate) was achieved with a substrate mixture of sawdust (37.5%), corn cob powder (37.5%), rice bran (12.5%), and wheat bran (12.5%), which differed significantly from all other treatments and the control. The second highest yield (152.5 g/kg wet substrate) was obtained with a substrate mixture of coir pith (40%), sawdust (40%), rice bran (10%), and wheat bran (10%). Following this, a yield of 145.5 g/kg wet substrate was recorded

with a mixture of sawdust (40%), wood chips (40%), rice bran (10%), wheat bran (10%), and CaCO3 (3%). Rashid et al. (2016) assessed five types of sawdust for cultivating the white oyster mushroom *Pleurotus florida*. They found that the raintree sawdust substrate yielded the highest biological efficiency (212.8%) compared to the other sawdust types. Sarita et al. (2021) studied the optimal spawn rate for maximum yield and biological efficiency in oyster mushrooms. They cultivated two oyster mushroom strains (PL-19-05 and PL-19-06) using wheat straw as the substrate and wheat grain spawns at different rates (3%, 4%, 5%, and 6%). Among these rates, the highest yield and biological efficiency were observed at the 6% spawn rate, indicating its suitability for achieving higher yields under humid conditions in the Udaipur region. Alananbeh et al. (2014) highlighted that increasing the spawning rate significantly enhances yield, biological efficiency, and the total number of fruiting bodies. Similarly, Deora et al. (2021) observed a proportional increase in yield with the spawn rate up to a certain threshold, beyond which higher spawn rates also led to increased bag temperatures.

CONCLUSION

King oyster is considered the best among *Pleurotus* species due to its excellent cap, stem consistency, culinary qualities, and most extended shelf life among oyster mushrooms. This mushroom is being cultivated on many substrate combinations throughout the world. In the present studies, a substrate combination of sawdust, corn cob powder, rice bran, and wheat bran with a 10% spawn dose has potential commercial applications for achieving improved quality and higher yields of King oyster mushrooms in India.

Table 3. Evaluation of different locally substrate for king oyster mushroom with 10% spawn dose

Treatment	Yield (g/kg wet substrate)	Biological efficiency (%)	Average fruit body weight (g)
T1- Coir pith (40%), Saw dust (40%), Rice bran (10%), Wheat bran (10%)	152.5	43.6	41.52
T2- Sawdust (40%), Wood chips (40%), Rice bran (10%), Wheat bran (10%), CaCO3 (3%)	145.5	41.6	33.65
T3- Sawdust (40%), paddy straw powder (40%), RB(10%), WB(10%)	125.5	35.9	27.5
T4- Sawdust (26.7%), Paddy straw powder (26.7%), Corn cob powder (26.7%), Rice bran (10%), Wheat bran (10%)	135.5	38.7	34.65
T5- Sugar Cane pith (80%), Rice bran (20%), CaCO3 (3%)	128.5	36.7	35.65
T6-PSP (40%), Sugarcane pith 40%), Rice bran (10%), WB (10%)	129.5	37.0	34.65
T7-paddy straw powder (80%), Rice bran (10%), Wheat bran (10%)	135.6	38.7	31.52
T8- Sawdust (37.5%), Corn cob powder (37.5%), Rice bran (12.5%), Wheat bran (12.5%)	211.5	60.4	56.5
CV	1.94		
CD (0.01)	4.87		

REFERENCES

Alananbeh, K.M., Bouqellah, N.A. and N. S. Al Kaff. 2014. Cultivation of oyster mushroom *Pleurotus ostreatus* on date-palm leaves mixed with other agro-wastes in Saudi Arabia. *Saudi Journal of Biological Sciences*, **21**:616-625.

Amin, S.M., Rahman, M.M., Hossain, M.M., Haque, M.M. and Sarker, N.C. 2007. Effect of different substrates on the growth and yield of five selected oyster mushrooms. *Bangladesh Journal of Mushroom,* **1**: 21-25.

Deora, A. 2021. Cultivation of Kabul Dhingri (*Pleurotus eryngii*) mushroom by standardizing protocols in subtropical zones of world. *Scientific Reports*, **11**(1):14692.

Eguchi, F., Watanabe, Y., Sudo, K. and Higaki, M. 1999. Pharmacological effects of *Pleurotus eryngii* on the hyperlipemia. In: Proceedings of 3rd International Conference on Mushroom Biology and Mushroom Products. University of Western Sydney, Hawkesbury, 333-339.

Eirini-Maria Melanouri, Marianna Dedousi and Panagiota Diamantopoulou. 2022. *Carbon Resources Conversion,* **5**: 52-60.

Gyorfi, J. and Hajdu, C. S. 2007. Casing-material experiments with *P. eryngii.* International *Journal of Horticultural Science*, **13**: 33-36.

Lewinsohn, D., Wasser, S. P., Reshetnikov, S. V., Hadar, Y. and Nevo, E. 2002. The Pleurotus eryngii species complex in Israel: distribution and morphological description of a new takson. *Mycotaxon*, **81**: 51-67.

Mahbuba Moonmoon, Md. Nazim Uddin., Saleh Ahmed., Nasrat Jahan Shelly and Md. Asaduzzaman Khan. 2010. Cultivation of different strains of king oyster mushroom (*Pleurotus eryngii*) on saw dust and rice straw in Bangladesh. *Saudi Journal of Biological Sciences,* **17**: 341-345.

Peng, J.T. 1996. Research on the Automatic Production of Pleurotus eryngii (DC.:Fr.) Quel (in Chinese). In: A Report on Agricultural Research in the Republic of China on Taiwan (Crops) 1992–1996, Council of Agriculture, Executive Yuan, ROC. 89- 91.

Peng, J. T. 1998. Research on the Automatic Production of King Oyster Mushroom (in Chinese). In: Proceedings of 1998 Agricultural Science and Technology Exhibition. Council of Agriculture, Executive Yuan, ROC, 44-46.

Md.Harun-or-Rashid, Debu Kumar Bhattacharjya, Ratan Kumar Paul, Md. Samsur Rahaman, Md. Saifur Rahaman, Md. Nuruddin Miah and Kamal Uddin Ahmed. 2016. Effect of different saw dust substrates on the growth and yield of Oyster Mushroom (*Pleurotus florida*). *Bioresearch Communications*, **02 (**01): 193-199.

Royse, D. J. 1999. Yield stimulation of king oyster mushroom, *Pleurotus eryngii*, by brewer's grain and spawn mate IISE supplementation of cottonseed hull and wood chip substrates. Mushroom News, 47: 4-8.

Sarita, S.S., Sharma, Somdutt, Chirag Gautam and Roop Singh. 2021. *The Pharma Innovation Journal*. **10**(9): 1783-1787.

Sarker, N.C., Ahmed, S., Hossain, K., A. Jahan and N.M.M. Quddus, 2009. Performance of different strains of shitake mushroom (Lentinus edodes) on saw dust. *Bangladesh Journal*, Mushroom, **3**: 1-7.

Yildiz, S., Yildiz, U.C., Geze, E. D. and Temiz, A. 2002. Some lignocellulosic wastes used as raw material in cultivation of the Pleurotus ostreatus culture mushroom. *Process Biochem*, **38:** 301-306.

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RESEARCH NOTE

Efficacy of newer insecticides against pomegranate thrips and natural enemies

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ABSTRACT: The present experiment was carried out at Agroforestry Research Station, S. D. Agricultural University, Sardarkrushinagar during 2019-21 to evaluate the newer insecticide molecules against the pomegranate thrips. The pooled results of three years revealed that the two spray of cyantraniliprole 10.26% OD @ 0.30 ml/L at 15 days interval during the *hastbahar* recorded lowest thrips population (1.95 thrips/ twig) and found significantly superior over rest of the treatments. It was followed by spinosad 45 % SC @ 0.4 ml/L (2.58 thrips/ twig). The pooled results on occurrence of spider revealed that untreated control treatment recorded maximum spider population (0.79 spider/ twig). It was followed by cyantraniliprole 10.26% OD @ 0.20 ml/L and neem oil 1500 ppm whereas the plants treated with lambda cyhalothrin 5% EC 1 ml/L recorded lowest number of spider (0.24 spider/ twig).

Keywords: Pomegranate, thrips, newer molecule, spider, cyantraniliprole

Pomegranate (*Punica granatum* L.) locally known as *anar*, *dadam* or *dalim* is an important fruit crop grown in tropical and subtropical regions of the world. Maharashtra, Gujarat, Uttar Pradesh, Andhra Pradesh, Karnataka, Rajasthan and Tamil Nadu are the major pomegranate growing states of India. Various abiotic and biotic stress played vital role in reducing the fruit yield of pomegranate. The insect pests are a major limiting factor and about 90 species of insects are reported to feed on pomegranate (Gaikwad *et al.,* 2023). Among them, thrips, *Scirtothrips dorsalis* Hood is an economically important pest. Both, nymphs and adults rasp the leaves, tender shoot, immature developing floral parts, fruit and suck the oozing cell sep resulting in to corky appearance of the damaged parts. It can affect any stage of development of the plant thus it is crucial to manage at appropriate time (Balikai *et al*., 2011). Some insecticides are found to be not very effective to manage thrips effectively. Hence the present inspection was carried out to examine the efficacy of new insecticide molecule against pomegranate thrips and their effect on natural enemies.

The pomegranate was raised with recommended agricultural practices. The two spray of insecticides were carried out during the *hastha bahar*. The spray volume was standardized by spraying control treatment with sole application of water. The first spray was carried out at the 50% flowering and second spray was carried out 15 days after first spray. Spraying was done using a knapsack sprayer fitted with a hollow cone nozzle. The thrips population was recorded before spray and 1st, 3rd, 7th, 10th and 15th days after each spray. There were nine treatments including untreated control (Table 1) laid out in a randomized block design.

The nymph and adult populations of thrips were recorded during the vegetative/fruiting stage of the crop from five randomly selected twigs/ plant by tapping the shoot on the black paper and counting the number of thrips during before spray and 1st, 3rd, 7th, 10th and 15th days after each spray. The population of natural enemies (spider) were also recorded during the before spray and 10th and 15th days after each spray. The recorded data were subjected to statistical analysis.

Effect of treatments on thrips

The pooled results on thrips incidence during year 2019-20 presented in table 1 revealed that minimum thrips population (1.66 thrips/ twig) was recorded in plants treated with cyantraniliprole 10.26% OD @ 0.30 ml/L. It was remained at par with spinosad 45 % SC @ 0.4 ml/L (2.09 thrips/ twig), spinosad 45 % SC @ 0.25 ml/L (2.52 thrips/ twig), lambda cyhalothrin 5% EC@ 1.0 ml/L (2.59 thrips/ twig) and cyantraniliprole 10.26% OD @ 0.20 ml/L (2.64 thrips/ twig). The control treatment recorded maximum population of thrips (7.17 thrips/ twig).

The perusal data on pooled results of thrips incidence on pomegranate during year 2020-21 is presented in table 1. The result revealed that lowest thrips population was observed (2.15 thrips/ twig) with cyantraniliprole 10.26% OD @ 0.30 ml/L. It was followed by spinosad 45 % SC @ 0.4 ml/L (3.38 thrips/ twig). The highest thrips population (8.02 thrips/ twig) was observed in untreated

Table 1. Efficacy of insecticides against thrips infesting pomegranate (Pooled over year)

Treatment	Dose (ml/L)	No. of thrips /twig			
		2019-20	2020-21	2021-22	Pooled
Cyantraniliprole 10.26% OD	0.20	1.77^{abc} (2.64)	2.19^{c} (4.30)	2.20^{c} (4.33)	2.05^{d} (3.71)
Cyantraniliprole 10.26% OD	0.30	1.47^{a} (1.66)	1.63^{a} (2.15)	1.59^{a} (2.04)	1.56^{a} (1.95)
Lambdacyhalothrin 5% EC	0.50	1.83^{bcd} (2.86)	2.39^{d} (5.22)	2.23^{c} (4.45)	2.15^{e} (4.12)
Lambdacyhalothrin 5% EC	1.0	1.76^{abc} (2.59)	2.16^{c} (4.17)	1.81^{ab} (2.77)	1.91^{c} (3.14)
Fipronil 5% SC	1.0	1.96^{cd} (3.35)	2.46^{d} (5.53)	2.19^{c} (4.29)	2.20^{e} (4.35)
Spinosad 45% SC	0.25	1.74^{abc} (2.52)	2.09^{bc} (3.87)	2.04^{bc} (3.68)	1.96^{c} (3.33)
Spinosad 45% SC	0.4	1.61^{ab} (2.09)	1.97^{b}(3.38)	1.68^{a} (2.34)	1.75^{b}(2.58)
Neem oil 1500ppm	5.0	2.11^{d} (3.95)	2.50^{d}(5.74)	2.51^{d} (5.79)	2.37^{f} (5.13)
Untreated control	--	2.77^{e} (7.17)	2.92^{e} (8.02)	3.25^{e} (10.07)	2.98^{g} (8.38)
S. Em. ±		0.10	0.06	0.09	0.02
CD (p = 0.05)		0.32	0.21	0.30	0.05
C. V (%)		8.09	8.03	8.10	8.09

* Figures in parenthesis are retransformed values of $\sqrt{x+0.5}$ transformation
Treatment means with the letter(s) in common are not significant by DMRT at 5% level of significance

control. The observation on results of thrips incidence on pomegranate during year 2021-22 presented in table 1 revealed that all the treatments were found significantly superior over control (10.07 thrips/twig/ plant). The least incidence of thrips was observed in plants treated with cyantraniliprole 10.26% OD @ 0.30 ml/L (2.04 thrips/ twig/ plant) and found at par with spinosad 45 % SC @ 0.4 ml/L (2.34 thrips/twig/ plant) and lambda cyhalothrin 5% EC@ 1.0 ml/L (2.77 thrips/twig/ plant).

The perusal data on pooled result of thrips incidence in pomegranate during three years are presented in table 1. The plant treated with cyantraniliprole 10.26% OD @ 0.30 ml/L exhibited lowest population of thrips (1.95 thrips/ twig) and found significantly superior over all other treatments. It was followed by spinosad 45 % SC @ 0.4 ml/L (2.58 thrips/ twig). In the past, Solankar *et al.* (2021) concluded that spray of cyantraniliprole 10.26% OD @ 0.9 ml/ L exhibited lowest thrips population (1.89 thrips/ twig) at Rahuri, Maharashtra. At Prabhani, Maharashtra, Gaikwad *et al*. (2023) noted that plants treated with cyantraniliprole 10.26% OD @ 15 ml/ 10 L recorded lowest number of thrips (1.68 thrips/ twig) which was followed by spinosad 45% SC @ 3.2 ml/ 10 L (1.75 thrips/ twig). From Karnataka, Satyanarayana *et al.* (2023) noted that two spray of Cyantraniliprole 10.26% OD @ 70g.a.i/ha recorded lowest population of thrips (1.22 thrips/ twig) among the seven treatments. The present results on effect of newer molecule on thrips population are in close conformity with the above worker.

Effect of treatments on spiders

The perusal data on occurrence of spider during the 2019-20 presented in table 2 revealed that the maximum number of spider was observed in plant treated with cyantraniliprole 10.26% OD @ 0.20 ml/L (0.84 spider/ twig) while lowest population was observed in plant treated with lambda cyhalothrin 5% EC 1 ml/L (0.17 spider/ twig).

The control plants recorded highest number of spider (0.80 spider/ twig) while plants treated with lambda cyhalothrin 5% EC 1 ml/L recorded lowest number of spider (0.28 spider/ twig) during the year 2020-21 (Table 2). The results on spider population during the year 2021-22 presented in table 2 revealed that maximum population of spider was observed in untreated control

Table 2. Effect of newer molecule on occurrence of spider on pomegranate (Pooled over year)

Treatment	Dose (ml/L)	No. of spider /twig			
		2019-20	2020-21	2021-22	Pooled
Cyantraniliprole 10.26% OD	0.20	1.16^{a} (0.84)	0.97^{b} (0.45)	1.01^{bcd} (0.51)	1.05^{b} (0.59)
Cyantraniliprole 10.26% OD	0.30	1.00^{abc} (0.50)	0.96^{b} (0.43)	0.86^{e} (0.23)	0.94^{ef} (0.39)
Lambdacyhalothrin 5% EC	0.50	0.90^{c} (0.32)	0.94^{b} (0.38)	0.95^{cde} (0.40)	0.93^{ef} (0.36)
Lambdacyhalothrin 5% EC	1.0	0.82^{c} (0.17)	0.88^{b} (0.28)	0.89^{de} (0.28)	0.86^{f} (0.24)
Fipronil 5% SC	1.0	0.93^{bc} (0.36)	0.89^{b} (0.29)	0.92^{de} (0.34)	0.91^{ef} (0.33)
Spinosad 45% SC	0.25	1.01^{abc} (0.52)	1.02^{ab} (0.54)	1.05^{abc} (0.60)	1.03^{bcd} (0.55)
Spinosad 45% SC	0.4	0.96^{bc} (0.41)	1.03^{ab} (0.56)	0.91^{de} (0.32)	0.96^{bde} (0.43)
Neem oil 1500ppm	5.0	1.00^{abc} (0.50)	1.01^{ab} (0.53)	1.12^{ab} (0.75)	1.05^{b} (0.59)
Untreated control	--	1.11^{ab} (0.73)	1.14^{a} (0.80)	1.16^{a} (0.84)	1.14^{a} (0.79)
S. Em. ±		0.06	0.05	0.04	0.03
CD (p = 0. 05)		0.16	0.14	0.12	0.08
C. V (%)		19.38	18.03	14.80	17.52

* Figures in parenthesis are retransformed values of $\sqrt{x + 0.5}$ transformation
Treatment means with the letter(s) in common are not significant by DMRT at 5% level of significance

(0.84 spider/ twig) while minimum population of spider was observed in plants treated with cyantraniliprole 10.26% OD @ 0.30 ml/L (0.23 spider/ twig). The data on pooled over year presented in table 2 revealed that highest population of spider (0.79 spider/twig) was observed in untreated control which was followed with cyantraniliprole 10.26% OD @ 0.20 ml/L and neem oil 1500 ppm. The plants treated with lambda cyhalothrin 5% EC 1 ml/L recorded lowest number of spider (0.24 spider/ twig).

From the above discussion it can be concluded that two spray of cyantraniliprole 10.26% OD @ 0.30 ml/L at 15 days interval found significantly superior for reducing the thrips incidence in pomegranate over the rest of the treatments. Regarding to the spider population, the control plants recorded maximum spiders which was followed cyantraniliprole 10.26% OD @ 0.20 ml/L and neem oil 1500 ppm.

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REFERENCES

Balikai, R. A., Kotikal, Y. K. and Prasanna, P. M. 2011. Status of Pomegranate Pests and Their Management Strategies in India. *Acta Horticulture*, 569-584.

Gaikwad, D. S., Lad, A. G., Fand, D. N., Deokar, B. D. and Khedkar, P. B. 2023. Efficacy of newer insecticides against pomegranate thrips *(Scirtothrips dorsalis/ Rhipiphorothrips cruentatus)*. *The Pharma Innovation Journal,* **12**(12S): 1511-1514.

Satyanarayana, C., Nadaf, A. M. and Thoke, S. 2023. Bio efficacy of different insecticides against pomegranate insect pests and their impact on natural enemies. *Emergent Life Sciences Research,* **9**(2): 111-119.

Solankar, R. D. Walvnj, A. R., Kabre, G. B. and More, P. E. 2021. Bioefficacy and compatibility of new insecticides molecules against sucking pests of pomegranate. *The Pharma Innovation Journal*, **11**: 669–672.

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RESEARCH NOTE

Report on the incidence of apple and nut borer, *Citripestis eutraphera* (Meyrick) (Lepidoptera: Pyralidae) in cashew, *Anacardium occidentale* L. (Anacardiaceae) in South Gujarat

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ABSTRACT: This paper reports the incidence of mango fruit borer, *Citripestis eutraphera* (Meyrick) (Lepidoptera: Pyralidae) on cashew as apple and nut borer in South Gujarat. The moth lays eggs on rough areas of the fruits near the peduncle. Larvae after hatching scrapped the skin of the fruit or young nut and then bore into an immature apple as well as nuts and feeds on inner content. The damage was more frequent where two or more fruits touched each other. By considering the importance of this pest in cashew, there is need of further studies on its biology, seasonal incidence, natural enemies and management practices in Gujarat.

Keywords: *Citripestis eutraphera,* cashew, apple and nut borer

Cashew (*Anacardium occidentale*) is an important plantation crop with high export potential in India. Presently cashew is cultivated in 17 states of the country. The total area under cashew cultivation is 11.92 lakh ha with production of 7.81 lakh tonnes. In Gujarat area under cashew is 11.84 thousand ha with production of 7.01 thousand MT and 802 kg/ha productivity (Femina, 2024). The successful cultivation of cashew is constrained by number of biotic and abiotic factors. Among biotic factors, more than 190 species of insect and mite pests have been listed on cashew occurring in different cashew growing countries of the world (Sundararaju, 1984).

Invasive Alien Species (IAS) poses a serious threat to the vegetation of the area being invaded. They proliferate very quickly away from their native land probably due to the abundance of food and/or absence of a natural enemy they had in their native land. The mango fruit borer, *Citripestis eutraphera* is one of the IAS, which was earlier confined to Andaman Islands. According to records, *C. eutraphera,* described by Edward Meyrick in 1933 and was found restricted to the Andaman Islands on local endemic mango species, *Mangifera andamanica* L. belonging to the family Anacardiaceae (Bhumannavar, 1991). Later, Jacob *et al*. (2004) reported that *C. eutraphera* also became a major pest on cashew in the Andaman Islands. This borer was probably restricted to these Islands for almost 23 years until 2014 when it was reported from mainland India by Jayanthi *et al.* (2014) on cultivated mango species, *M. indica.* This species recently invaded and spread to mainland India and infested mango in Karnataka, Tamil Nadu, Kerala, Gujarat, parts of Maharashtra and Odisha (Krull, 2004; Jayanthi *et al.,* 2014; Hiremath *et al*., 2017; Bana *et al.*, 2018) and recently in Punjab (Singh *et al*., 2021). It also infested seedlings and grafts of cashew, *A. occidentale* in Kerala (Hiremath *et al*., 2017). Recorded infestation levels on mango fruits ranged from 2.46% to 64.00% in India during fruiting period in the surveyed region of Karnataka and Tamil Nadu (Soumya *et al*., 2016; Jayanthhi *et al.,* 2014).

Recently, the mango fruit borer, *C. eutraphera* became a pest on cashew as apple and nut borer in Gujarat. Mango is the main fruit crop of South Gujarat. The infestation of *C. eutraphera* was reported in Gujarat, where it caused significant damage in mango (Bana *et al*., 2018) and became a major pest. Now, *C. eutraphera* preferred cashew as additional host in South Gujarat ecosystem. The moth lays eggs (less than 1 mm in diameter) on rough areas of the fruits (Fig. B) and near the pedicels which was white in colour when first laid but change to red (Fig. A). Moreover the eggs were also observed near the attachment of apple and nut. The full grown larva measured about 20 mm in length (Fig. D). Pre-pupa observed light green in colour (Fig. E). Larvae pupate either in soil in form of earthen cocoon (Fig. G) or in fallen fruit (Fig. F). The site of pupation in soil was confirmed by laboratory rearing of infested fruit (Fig. W). Forewing of *C.eutraphera* was ground colour yellowish-grey, veins black scaled, with creamy white scales, with rusty red, cream, black fringe; hind wing ground colour dirty white with black scaling along veins anal area less black veined scales but with long dark, white hairs (Fig. H, I).

The nature of damage of the larvae of *C. eutraphera* was studied in respect to its feeding habit both under field and laboratory condition. Larvae after hatching scrapped the skin of the apple (Fig. C) and then bore an

immature apple as well as nuts (Fig. M, N), and feeds on internal content of apple (Fig. K) as well as on young developing nuts. Mostly the damage was seen at the attachment of apple and nut whereinlarva made entry by tunnelling along the jointed parts of the fruit (Fig. L). Larva completely devoured inner content of nut (Fig. S, T). Larva also entered from pedicel (Fig. P). The damage was more frequent where two or more fruits touched each other (Fig. O).

The infested apple and nuts become partially unfilled and dries up before full development and maturity of nuts. In infested fruits, bored holes filled with frass (Fig. M, N)and found blackened around the bored area (Fig. V).Generally 1 to 3 caterpillars were seen feeding either in the apple or nut, but there are reports stating that up to five larvae can occur in cashew apples and three in developing nuts. The exit hole allows ants, beetles, and occasionally microorganisms to enter the fruits and becomes rotted (Fig. U). Therefore, the damage caused by *C. eutraphera* in cashew caused a total loss of apple and nut quality and also made loss of seed germination.

Recently Kori Nagaraj *et al*. (2022) reported *C. eutraphera* as apple and nut borer in cashew, in maidan parts of Karnataka, India wherein they reported the extent of damage ranges from 10 to 16 percent on developing young cashew apples. According to them the moth lays eggs on tender vegetative shoots of cashew and after hatching the neonate larva initially bores into the terminal tender shoots, and seedlings/grafts in nursery. The larva damages vascular bundles inside the tender

Fig. A to W. A-Eggs (microscopic view), **B**-Eggs laid on rough surface;**C**-Early instar larva; **D**-Mature larva; **E**-Pre-pupa; **F**-Pupa; **G**- Earthen cocoon; **H**- Adult in resting stage; **I**- Adult (wing spanned); **J,K**- Damage in mature fruit (apple); **L**- Infestation mostly mostly observed at the point of attachment of apple and nut; **M-N** Bored holes filled with frass; **O**- Infestation at jointed fruit; **P**- infestation near peduncle; **Q**-Bored hole seen in mature nut; **R**- Larva tunneling in mature nut; **S**- Cross section of damaged nut; with larva inside; **T**- Cross section of healthy and damaged mature nut; **U**- Infested mature apple showed subsequent rotting; **V**- Blackened portion observed around the bored/scrapped area; **W**-Laboratory rearing of larvae.

shoots by excessive tunneling, throwing frass material and their excreta from the bored holes. This affects uptake of water and nutrient to upper terminal canopy, resulting in yellowing, drying of leaves and wilting of terminal shoots. The peak infestation of *C. eutraphera* as apple and nut borer of cashew was found during February to May, which coincides with apple and nut formation stage. These observations are in agreement with the findings of Kori Nagaraj *et al*. (2020), who reported that peak infestation of apple and nut borer, *C. eutraphera* was during peak summer months in Bangalore condition and also in maidan parts of Karnataka (Aswathanarayana Reddy *et al*., 2016). By considering the importance of this pest in cashew, there is need of further studies on its biology, seasonal incidence, natural enemies and management practices in Gujarat.

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REFERENCES

Aswathanarayana Reddy, N., Subramanyam. B., Vasudeva, K. R. and Rajendra, B. N. 2016. Population dynamics and management of cashew apple and nut borer, *Thylecoptilapanerosema* (Lepidoptera: Pyralidae) in maidan parts of Karnataka. In: Proceedings of National Seminar on Strategies for Development of Cashew, held on 19-20 February at Regional Fruit Research Station, Vengurle, Maharashtra. 63.

Bana, J. K., Kumar, S. and Sharma, H. 2018. Diversity and nature of damage of mango insect-pests in South Gujarat ecosystem. *Journal of Entomology and Zoology Studies*, **6**(2): 274-278.

Bhumannavar, B. S. 1991. Record of *Citripestis eutraphera* Meyrick (Pyralidae: Lepidoptera) on Mangiferaandamanica in India. *Journal of Bombay Natural History Society*, **88**(2): 299.

Femina. 2024. Innovative developmental strategies for sustainable cashew production. In: Souvenir of National Conference on Cashew on "Sustainable Cashew Production: Challenges and Opportunities" to be held at Goa during June 14-15, 2024.

Hiremath, S. R., Kumara, S. A. and Prathapan, K. D. 2017. First report of the mango fruit borer, *Citripestis eutraphera* (Meyrick) (Lepidoptera: Pyralidae) as a seedling borer of cashew, *Anacardium occidentale* L. (Anacardiaceae). *Journal of Lepidopterists Society*, **71**(2):115 116.

Jacob, T. K., Veenakumari, K. and Bhumannavar, B. S. 2004. Insect pests of cashew in the Andaman Islands. *Cashew*, **18**(4): 25 28.

Jayanthi, P. D. K., Abraham Verghese, P. R. Shashank and Vivek Kemparaj, 2014. Spread of indigenous restricted fruit borer, *Citripestis eutraphera* (Meyrick) (Lepidoptera :Pyralidae) in mango: Time for domestic quarantine regulatory reforms. *Pest Management in Horticultural Ecosystems,* **20**(2): 227-230.

Kori Nagaraj., N. Aswathanarayana Reddy., Subramanyam, B. and Ramegowda G. K. 2022. Report of incidence of mango fruit borer, *Citripestiseutraphera* (Meyrick) (Lepidoptera: Pyralidae) as apple and nut borer in cashew, *Anacardium occidentale* L. (Anacardiaceae) in maidan parts of Karnataka, India. *Insect Environment*, 25 (1).

Kori Nagaraj., Ramegowda, G. K., Aswathanarayana Reddy, N., Narabenchi, G.B. and Vishuvardhana. 2020. Studies on apple and nut borer, *Citripestis eutraphera* Meyrick (Lepidoptera: Pyralidae) in cashew. In: XVII AZRA International Conference on Frontier Research in Applied Zoology and Insect Pest management Strategies: A way forward for Food and Nutritional from 12-14th, February, 2020 at UAS, Raichur, Karnataka, 82-83.

Krull, S. M. 2004. Studies on the mango-ecosystem in Papua New Guinea with special reference to the ecology of *Deanolis sublimbalis* Snellen (Lepidoptera: Pyralidae) and to the biological control of *Ceroplastes rubens* Maskell (Homoptera: Coccidae). *Thesis Ph.D*., Justus-Liebig Universitat Gieben, 190.

Singh, S., Shashank, P., Singh, V. and Kaur, R. 2021. Occurrence of indigenously restricted fruit borer, ***Citripestis eutraphera***on mango in Punjab and its damage potential. *Indian Journal of Plant Protection,* **49**(1): 09-13.

Soumya, B. R., Abraham Verghese., Kamala Jayanthi and S. K. Jalali. 2016. Need to strengthen quarantine between Andaman and Nicobar Islands and mainland India. *Current Science*, **111** (11): 1753 - 1756.

Sundararaju, D. 1984. Studies on cashew pests and their natural enemies in Goa. *Journal of Plantation Crops*, **12:** 38-46.

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RESEARCH NOTE

Field efficacy of some newer insecticides along with biopesticides against major insect pests in broccoli

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ABSTRACT: An experiment was conducted to test the efficacy of certain new insecticides and biopesticides against major pests of broccoli at College of Agriculture, Rajendranagar, Hyderabad, India. There were nine treatments *viz*., acetamiprid 20% SP @ 0.2 g/L, *Bacillus thuringiensis* subsp. *kurstaki* @ 2 g/L, *Beauveria bassiana* @ 5 g/L, cyantraniliprole 10.26% OD @ 1.2 ml/L, chlorantraniliprole 18.50% SC @ 0.3 ml/L, diafenthiuron 50% WP @ 1.2 g/L, emamectin benzoate 5% SG @ 0.4 g/L, fipronil 5% SC @ 2 ml/L and *Metarhizium anisopliae* @ 5 g/L along with untreated control replicated thrice. The treatment cyantraniliprole 10.26% OD @ 1.2 ml/L was the most effective in management of *Plutella xylostella*, *Crocidolomia pavonana* and *Spodoptera litura* population followed by chlorantraniliprole 18.50% SC @ 0.3 ml/L and emamectin benzoate 5% SG @ 0.4 g/L. In case of *Myzus persicae*, acetamiprid 20% SP @ 0.2 g/L proved to be the most effective treatment showing maximum reduction in *M. persicae* population followed by cyantraniliprole 10.26% OD @ 1.2 ml/L and chlorantraniliprole 18.50% SC @ 0.3 ml/L.

Keywords: Aphid, acetamiprid, broccoli, chlorantraniliprole, cyantraniliprole, diamondback moth

Broccoli (*Brassica oleracea* L. var. *italica*) is a popular exotic vegetable with rich source of potassium, phosphorus, sulfur, and magnesium. Anticancer and antioxidant properties are also found in broccoli (Podsedek, 2007). Broccoli, which originated in the Mediterranean region, is widely grown in China, the USA, Spain, Mexico, India, and Italy. In India, it is cultivated in Uttar Pradesh, Himachal Pradesh, and the hilly areas of the Nilgiri Hills, Jammu and Kashmir, and the northern plains. The productivity of broccoli is influenced by many factors, of which insect pests such as lepidopterans and aphids are the most important. Farmers resort to repeated sprays of multiple pesticides to manage pests. However, the repeated use of conventional pesticides threatens the environment and natural enemies, and also causes problems of insecticide resistance. As completely doing away with chemical pesticides is not feasible, especially when insect pests load is high, using pesticides that are more selective and effective is imperative to minimize the damage (Jemimah *et al*., 2021). Hence, the present study was undertaken to evaluate some newer insecticides and biopesticides which will be more effective and have less impact on the environment and human health.

The present experiment was conducted at Rajendranagar, Hyderabad during *rabi* 2022-23 to study the efficacy of different insecticides and biopesticides against major insect pests infesting broccoli (Shishir F1 hybrid; Known-you seed). The experiment was laid out in randomized block design with 10 treatments including control replicated thrice with an individual plot size of 20 m^2 (5 m x 4 m). All the insecticides and biopesticides selected were applied as per the recommended dosages as foliar spray. Two sprayings were given during the crop period using a power sprayer at an interval of 15 days by initiating the first spray when the pest reached ETL. The observations on major insect pests were recorded one day before spraying as pretreatment counts and post treatment counts were recorded on 3rd, 5th and 7th day after spraying. Population of *Myzus persicae* (Sulzer) was recorded from 3 leaves *viz*., each one from top, middle and bottom plant canopy and represented as numbers per leaf per plant. The population of lepidopterans *i.e*., *Plutella xylostella* (Linnaeus), *Crocidolomia pavonana* (Fabricius) and *Spodoptera litura* (Fabricius) were recorded as larval counts and represented as numbers per plant. The observations were taken from 5 randomly

selected plants in each plot and represented as numbers per plant.The mean population data obtained from different treatments was transformed into square root values before analysis. The modified data was then subjected to an analysis of variance (ANOVA). To differentiate the means of treatments that showed significant differences ($P < 0.05$), Duncan's Multiple Range Test (DMRT) was applied.The level of significance was fixed at $\alpha = 0.05$. All these statistical procedures were conducted utilizing WASP software.

The data regarding efficacy of different insecticides and biopesticides against major insect pests infesting broccoli after first and second spray are presented in table 1.

Diamondback moth (*Plutella xylostella*)

After 1st spray, a significantly less number of *P. xylostella* larvae were recorded by cyantraniliprole 10.26% OD @ 1.2 ml/L (0.76 larvae/plant) followed by chlorantraniliprole 18.50% SC @ 0.3 ml/L (1.11 larvae/plant), emamectin benzoate 5% SG @ 0.4 g/L (1.42 larvae/plant) and *Bacillus thuringiensis* subsp. *kurstaki* @ 2 g/L (2.40 larvae/plant). The plots treated with *Beauveria bassiana* @ 5 g/L (2.82 larvae/plant), acetamiprid 20% SP @ 0.2 g/L (2.96 larvae/plant) and diafenthiuron 50% WP @ 1.2 g/L (2.96 larvae/plant) were statistically on par with each other. Fipronil 5% SC @ 2 ml/L treated plots had 3.40 larvae/plant statistically equivalent in effectiveness with *Metarhizium anisopliae* @ 5 g/L (3.44 larvae/plant). After 2nd spray,the treatment cyantraniliprole 10.26% OD @ 1.2 ml/L recorded lowest mean population of *P. xylostella* larvae (0.51 larvae/plant) followed by chlorantraniliprole 18.50% SC @ 0.3 ml/L (0.84 larvae/plant), emamectin benzoate 5% SG @ 0.4 g/L (1.24 larvae/plant), *Bacillus thuringiensis* subsp. *kurstaki* @ 2 g/L (1.76 larvae/plant) and *Beauveria bassiana* @ 5 g/L (1.92 larvae/plant) (both on par with each other).The plots treated with acetamiprid 20% SP @ 0.2 g/L (2.24 larvae/plant), diafenthiuron 50% WP @ 1.2 g/L (2.40 larvae/plant), fipronil 5% SC @ 2 ml/L (2.44 larvae/plant) and *Metarhizium anisopliae* @ 5 g/L (2.51 larvae/plant) were statistically at par in effectiveness. All treatments showed superiority over untreated control where 4.22 larvae/plant was noted. These findings are in concurrence with Chowdary and Sharma (2019), who reported 81.02 per cent reduction in *P. xylostella* larval population on cabbage sprayed with chlorantraniliprole @ 10 g a.i./ha. Similarly, Beena and Selvi (2022) reported 77-99.5 per cent larval reduction by spraying with cyantraniliprole 10.26% OD @ 60 g a.i./ha on cauliflower.

Leaf webber (*Crocidolomia pavonana*)

After 1st spray, the treatment cyantraniliprole 10.26% OD @ 1.2 ml/L recorded significantly lower population of *C. pavonana* larvae (0.69 larvae/plant) followed by chlorantraniliprole 18.50% SC @ 0.3 ml/L (0.96 larvae/plant), emamectin benzoate 5% SG @ 0.4 g/L (1.36 larvae/plant) and *Bacillus thuringiensis* subsp. *kurstaki* @ 2 g/L (2.87 larvae/plant). Acetamiprid 20% SP @ 0.2 g/L (3.33 larvae/plant), *Beauveria bassiana* @ 5 g/L (3.38 larvae/plant) and diafenthiuron 50% WP @ 1.2 g/L (3.42 larvae/plant) were statistically at par in effectiveness. The treatments fipronil 5% SC @ 2 ml/L (3.87 larvae/plant) and *Metarhizium anisopliae* @ 5 g/L (4.00 larvae/plant) were statistically on par with each other. After 2nd spray, the treatment cyantraniliprole 10.26% OD @ 1.2 ml/L showed the lowest mean population of 0.47 larvae/plant followed by chlorantraniliprole 18.50% SC @ 0.3 ml/L (0.78 larvae/plant), emamectin benzoate 5% SG @ 0.4 g/L (1.20 larvae/plant), *Bacillus thuringiensis* subsp. *kurstaki* @ 2 g/L (1.69 larvae/plant) and acetamiprid 20% SP @ 0.2 g/L (1.74 larvae/plant) (both on par with each other).The treatments *Beauveria bassiana* @ 5 g/L (2.09 larvae/plant), diafenthiuron 50% WP @ 1.2 g/L (2.20 larvae/plant) and fipronil 5% SC @ 2 ml/L (2.24 larvae/plant) were statistically on par with each other in their efficacy against *C. pavonana*. The treatment *Metarhizium anisopliae* @ 5 g/L harbored highest larval population of 2.53 larvae/plant. However, all the treatments were superior to untreated contro l4.38 larvae/plant.The present study is in line with Sambathkumar (2020) who recorded 100 per cent reduction (*in vitro* efficacy study) in *C. pavonana* population by spraying chlorantraniliprole 18.5 % SC @ 0.3 ml/L. Similarly, Jemimah *et al*. (2021) also reported that chlorantraniliprole 18.5 % SC @ 0.3 ml/L showed 84.14 per cent reduction in *C. pavonana* population on cabbage.

Tobacco caterpillar (*Spodoptera litura*)

After 1st spray, the lowest mean population of *S. litura* larvae were recorded in cyantraniliprole 10.26% OD @ 1.2 ml/L (0.62 larvae/plant) followed by chlorantraniliprole 18.50% SC @ 0.3 ml/L (0.98 larvae/

Table 1. Efficacy of different insecticides and biopesticides against major insect pests infesting broccoli during *rabi* 2022-23

Treatments	Dose (g or ml/L)	Mean number of larvae per plant									Mean number of *M. Persicae* per leaf per plant		
		P. xylostella			*C. pavonana*			*S. litura*					
		DBS	1st spray	2nd Spray	DBS	1st spray	2nd spray	DBS	1st spray	2nd spray	DBS	1st spray	2nd spray
Acetamiprid 20% SP	0.2 g	4.00 (1.99)	2.96 (1.72)	2.24 (1.50)	4.80 (2.19)	3.33 (1.82)	1.74 (1.32)	4.27 (2.06)	2.98 (1.71)	2.27 (1.50)	61.53 (7.84)	9.98 (3.15)	6.18 (2.48)
*Bacillus thuringiensis*subsp. *kurstaki*	2 g	4.07 (2.01)	2.40 (1.54)	1.92 (1.38)	5.20 (2.28)	2.87 (1.69)	1.69 (1.30)	4.60 (2.14)	2.64 (1.63)	1.58 (1.25)	59.80 (7.72)	43.29 (6.57)	29.44 (5.42)
Beauveria bassiana	5 g	3.87 (1.96)	2.82 (1.68)	2.02 (1.42)	5.00 (2.23)	3.38 (1.84)	2.09 (1.44)	4.53 (2.12)	2.93 (1.71)	2.11 (1.44)	59.40 (7.70)	49.27 (7.01)	33.44 (5.78)
Cyantraniliprole 10.26% OD	1.2 ml	4.27 (2.06)	0.76 (0.87)	0.51 (0.73)	5.07 (2.25)	0.69 (0.83)	0.47 (0.67)	4.00 (2.00)	0.62 (0.77)	0.58 (0.77)	61.93 (7.86)	13.40 (3.65)	8.47 (2.90)
Chlorantraniliprole 18.50% SC	0.3 ml	4.20 (2.04)	1.11 (1.04)	0.84 (0.91)	4.87 (2.20)	0.96 (0.98)	0.78 (0.89)	3.93 (1.98)	0.98 (0.98)	0.93 (0.96)	63.93 (7.98)	17.42 (4.17)	11.49 (3.38)
Diafenthiuron 50% WP	1.2 g	4.00 (2.00)	2.96 (1.72)	2.40 (1.54)	4.93 (2.22)	3.42 (1.85)	2.20 (1.48)	4.20 (2.04)	3.07 (1.74)	2.40 (1.54)	61.93 (7.86)	21.80 (4.66)	16.89 (4.10)
Emamectin benzoate 5% SG	0.4 g	4.13 (2.03)	1.42 (1.19)	1.24 (1.12)	5.13 (2.26)	1.36 (1.16)	1.20 (1.07)	4.27 (2.06)	1.42 (1.18)	1.31 (1.13)	60.60 (7.77)	43.38 (6.58)	28.17 (5.30)
Fipronil 5% SC	2 ml	4.07 (2.01)	3.40 (1.84)	2.44 (1.55)	5.07 (2.25)	3.87 (1.96)	2.24 (1.49)	4.33 (2.08)	3.27 (1.80)	2.46 (1.56)	60.53 (7.77)	36.67 (6.05)	22.29 (4.72)
Metarhizium anisopliae	5 g	3.80 (1.94)	3.44 (1.85)	2.51 (1.58)	4.93 (2.19)	4.00 (2.00)	2.53 (1.62)	4.40 (2.09)	3.33 (1.83)	2.98 (1.72)	59.67 (7.71)	49.97 (7.06)	33.76 (5.80)
Untreated Control	-	4.33 (2.08)	4.38 (2.09)	4.22 (2.04)	5.20 (2.28)	5.07 (2.25)	4.38 (2.08)	4.60 (2.14)	4.40 (2.09)	3.87 (1.97)	60.87 (7.79)	60.36 (7.75)	46.20 (6.79)
CD at 5%	-	NS	0.110	0.147	NS	0.110	0.124	NS	0.135	0.148	NS	0.394	0.324

DBS- Days before spraying, NS- Non significant**,** Figures on parentheses are square root transformed values of original data.

plant) and emamectin benzoate 5% SG @ 0.4 g/L (1.42 larvae/plant). The plots treated with *Bacillus thuringiensis* subsp. *kurstaki* @ 2 g/L (2.64 larvae/plant), *Beauveria bassiana* @ 5 g/L (2.93 larvae/plant), acetamiprid 20% SP @ 0.2 g/L (2.98 larvae/plant) and diafenthiuron 50% WP @ 1.2 g/L (3.07 larvae/plant) were statistically on par with each other. Fipronil 5% SC @ 2 ml/L (3.27 larvae/plant) treated plots were statistically equivalent in effectiveness with *Metarhizium anisopliae* @ 5 g/L (3.33 larvae/plant). After 2nd spray, a significantly less number of *S. litura* larvae were recorded incyantraniliprole 10.26% OD @ 1.2 ml/L (0.58 larvae/plant) followed by chlorantraniliprole 18.50% SC @ 0.3 ml/L (0.93 larvae/plant), emamectin benzoate 5% SG @ 0.4 g/L (1.31 larvae/plant) and *Bacillus thuringiensis* subsp. *kurstaki* @ 2 g/L (1.58 larvae/plant) (both on par with each other). The treatments *Beauveria bassiana* @ 5 g/L (2.11 larvae/plant), acetamiprid 20% SP @ 0.2 g/L (2.27 larvae/plant), diafenthiuron 50% WP @ 1.2 g/L (2.40 larvae/plant) and fipronil 5% SC @ 2 ml/L (2.46 larvae/plant) were statistically on par with each other. The treatment *Metarhizium anisopliae* @ 5 g/L (2.98 larvae/plant) harbored highest *S. litura* population. However, all the treatments were significantly superior over untreated control (3.87 larvae/plant).The present study is more or less in accordance with Reddy *et al*. (2017) who reported 63 per cent reduction in *S. litura* population by spraying emamectin benzoate 5% SG @ 0.4 g/L on cabbage. Similarly, Kamde *et al*. (2018) recorded 61.72-75.38 per cent reduction in *S. litura* population on cabbage by spraying cyantraniliprole 10.26% OD @ 1.2 ml/L.

Aphid (*Myzus persciae*)

After 1st spray, the treatment acetamiprid 20% SP @ 0.2 g/L showed maximum efficacy, with lowest *M. Persicae* population (9.98 aphids/leaf/plant) followed by cyantraniliprole 10.26% OD @ 1.2 ml/L (13.40 aphids/leaf/plant), chlorantraniliprole 18.50% SC @ 0.3 ml/L with (17.42 aphids/leaf/plant), diafenthiuron 50% WP @ 1.2 g/L (21.80 aphids/leaf/plant), fipronil 5% SC @ 2 ml/L (36.67 aphids/leaf/plant), *Bacillus thuringiensis* subsp. *kurstaki* @ 2 g/L (43.29 aphids/leaf/plant) and emamectin benzoate 5% SG @ 0.4 g/L (43.38 aphids/leaf/plant) (both on par with each other).The treatments *Beauveria bassiana* @ 5 g/L (49.27 aphids/leaf/plant) and *Metarhizium anisopliae* @ 5 g/L (49.97 aphids/leaf/plant) were statistically equal in efficacy against *M. Persicae* population. The plots treated with acetamiprid 20% SP @ 0.2 g/L showed the lowest mean population (6.18 aphids/leaf/plant) after 2nd spray followed by cyantraniliprole 10.26% OD @ 1.2 ml/L (8.47 aphids/leaf/plant), chlorantraniliprole 18.50% SC @ 0.3 ml/L with (11.49 aphids/leaf/plant), diafenthiuron 50% WP @ 1.2 g/L (16.89 aphids/leaf/plant), fipronil 5% SC @ 2 ml/L (22.29 aphids/leaf/plant), emamectin benzoate 5% SG @ 0.4 g/L (28.17 aphids/leaf/plant) and *Bacillus thuringiensis* subsp. *kurstaki* @ 2 g/L (29.44 aphids/leaf/plant) (both on par with each other). *Beauveria bassiana* @ 5 g/L (33.44 aphids/leaf/plant) and *Metarhizium anisopliae* @ 5 g/L (46.20 aphids/leaf/plant) were statistically at par in effectiveness. All the treatments selected for efficacy study were significantly effective over untreated control (46.20 aphids/leaf/plant).Srivastava *et al*. (2016) reported acetamiprid 20% SP @ 150 g/ha caused 83.05 per cent reduction in aphid population on cabbage. Bhede *et al*. (2018) revealed that cyantraniliprole 10.26% OD @ 600 ml/ha caused 96.27 per cent reduction in aphid population on cauliflower. The present results are in line with the above researchers.

Based on the findings, it can be concluded that cyantraniliprole 10.26% OD @ 1.2 ml/L and chlorantraniliprole 18.50% SC @ 0.3 ml/L can be used for effective management of insect pests in broccoli ecosystem.

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REFERENCES

Beena, R. and Selvi, V. 2022. Bioefficacy of insecticides used against diamondback moth and their potential impact on natural enemies in cauliflower. *Journal of Applied and Natural Science,* **14**(4): 1240-1245.

Bhede, B.V., Bhosle, B. B., More, D. G. and Zanwar, P. R. 2018. Bioefficacy of different insecticides against aphid *Brevicoryne brassicae* on cauliflower. *Journal of Entomological Research*, **42**(2): 211-215.

Chowdary, N. M. and Sharma, P. C. 2019. Bioefficacy of newer insecticides against *Plutella xylostella*

(L.) infesting cabbage. *Himachal Journal of Agricultural Research*, **45**(1): 46-50.

Jemimah, N., Sridevi, G., Anitha, V., Devi U.G. and Kumar, M.V. N. 2021. Bioefficacy of insecticides against leaf webber in cauliflower. *The Journal of Research PJTSAU*, **49**(13): 81-87.

Kamde, N., Upadhyay, S. N., Meena, L. K., Jain, P. and Birle, K. B. H. 2018. Bio-efficacy of some newly evolved novel insecticides against diamondback moth and tobacco caterpillar in cabbage. *Journal of Entomology and Zoology Studies*, **6**(2): 741-747.

Podsedek, A. 2007. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *Food Science and Technology*, **40**(1):1-11.

Reddy, M. S. S., Singh, N. N. and Mishra, V. K. 2017. Efficacy of insecticides against *Spodoptera litura* infesting cabbage. *Annals of Plant Protection Sciences*, **25**(1): 215-230.

Sambathkumar, S. 2020. *In vitro* efficacy of newer insecticide molecules against diamondback moth, *Plutella xylostella* (Linnaeus) and leaf webber, *Crocidolomia binotalis* Zeller in cabbage. *Journal of Entomology and Zoology Studies*, **8**(4): 365-371.

Srivastava, R., Shankara, S., Tanuja, P. and Ajaykumar, K. 2016. Bioefficacy of acetamiprid 20% SP against aphid, *Brevicoryne brassicae* (L.) (Aphididae: Homoptera) in cabbage. *Bioscan*, **11**(4):2177-2180.

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RESEARCH NOTE

Hibiscus arnottianus (Family: Malvacea) - A new host plant record for leaf roller *Haritalodes derogata* Fabricius (Lepidoptera: Crambidae)

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ABSTRACT: This study reports on the occurrence of the leaf roller moth, *Haritalodes derogata* (Fabricius, 1775) (Lepidoptera: Crambidae: Spilomelinae) on *Hibiscus arnottianus* (Family: Malvacea) in Palar Agriculture College, Melpatti, Pernambut Road, Kothamarikuppam, Tamil Nadu, India. The research delves into biological and morphometric data related to the developmental stages of the moth.

Keywords: *Hibiscus arnottianus, Haritalodes derogata,* Palar agricultural college, metamorphic data

Hibiscus arnottianus, commonly known as Koki'oke'oke'o or Koki'oke'oke'o'ula, is an ornamental plant of Malvaceae family. This is an evergreen, either a small tree or tall shrub, unique to Oahu and occasionally cultivated. Recognized for its sizable, aromatic, funnel-shaped flowers with five spreading elliptical white petals, the plant reaches a height of 10–30 ft (3–9 m), featuring multiple trunks and a dense, sometimes hairy, crown. Indigenous to Oahu and Wailau Valley, Molokai, the species flourishes in wet forests at elevations ranging from 1000 to 3000 ft (305–914 m). Previously abundant in mountainous areas near Honolulu, it serves as a prime example of localized endemism within the Hawaiian archipelago (Little, 1989). The study conducted by Manjula *et al.* (2020) in Karnataka sheds light on the complex insect interactions affecting *H. rosa-sinensis*. The plant contends with a total of 20 insect species across Hemiptera, Lepidoptera, and Coleoptera orders, featuring both defoliators and sap suckers. Notably, the sucking pests are dominated by coccids, with seven distinct species representing Pseudococcidae, Coccidae, Cerococcidae, and Monophlebidae families. Regarding the *Hibiscus* genus, the prevalence of sap-sucking pests, such as aphids, mealy bugs, and scales, surpasses that of defoliators. Notably, the current investigation serves as a novel contribution to this aspect of *Hibiscus* ecology. The study is founded on meticulous field collections and laboratory observations conducted by the primary author within the *H. arnottianus.*

In a recent survey conducted in November 2023 at Palar Agriculture College, Melpatti, Pernambut Road, Kothamarikuppam, Tamil Nadu an observation was made regarding the severe infestation of *H. arnottianus* (Fig. 1, 2, and 3), commonly known as *Vellaisembaruthi* in Tamil, within the Garden land area (GPS coordinates 12.8818° N, 78.7768° E, altitude). The affected plants exhibited visible leaf folding, indicative of infestation by lepidopteran larvae. Subsequently, these caterpillars were systematically collected and reared on their host plant within an insect rearing cage under controlled laboratory conditions, maintaining a temperature range of 25-31^{o}C and RH 60-75%. Larvae in various instars were gathered and provided with *H. arnottianus* leaves until the pupation stage. The emerged pupae were then allowed to develop into adults, and their identification was performed at the adult stage. Throughout this process, the duration and dimensions of each stage in the life cycle of the leaf folder were meticulously recorded, contributing to a comprehensive understanding of the species' developmental dynamics and impact on the host plant.

The findings indicate a notable infestation of *H. arnottianus* by the leaf folder known as *Haritalodes derogata* (Fabricius,1775) (Lepidoptera: Crambidae: Spilomelinae), with sporadic instances. This identification is consistent with the detailed description provided by Hosseini Tabesh *et al.* (2015). Regarding the genus *Haritalodes* Warren, 1890, which falls under the subfamily Spilomelinae and is one of the 322 known genera, it encompasses eleven identified species distributed in the Oriental, Palaearctic, Australian, and Afro-Tropical regions, as outlined in the work of Nuss *et al.* (2014). The pest occurs in India, Pakistan, Bangladesh, Burma, Australia, Africa, China, Japan, Sri Lanka and other parts of the world. The pest is active from the month of September to November (Mariselvi

and Manimegalai, 2016). Afrotropical (Africa, Sub-Saharan, Madagascar, Comoros, Seychelles, Reunion Island), Australian (Papua New Guinea, Australia, Samoa), Eastern Palaearctic (Japan, Philippines, Korea, Taiwan, China, Southeast Asia, Pakistan), Oriental (Nepal, Java, India, Burma, Bangladesh, Thailand, Malaysia, Indonesia, Guam, Singapore, Solomon Island, Andaman Island, Nicobar Island, Sri Lanka, Vietnam, Pakistan) (Leraut, 2005; Yamanaka, 2008; Robinson *et al.,* 2010).

Nature of infestation

The larva exhibits characteristic leaf-rolling behavior during its early stages, consuming green tissue initially and progressively devouring a substantial portion of the leaf as it matures (see Fig. 1 and 2). Remarkably, flowering is relatively unaffected by this pest, as illustrated in Fig. 3. Observable symptoms of larval infection include the formation of webs and leaf withering.The larval feeding process results in complete leaf consumption or the development of prominent holes. Subsequently, the affected leaves transition to a brown color before ultimately dropping from the plant. Severe infestations, denoted by a high number of leaf rolls, lead to stunted plant growth.Caterpillars employ silk spun by the spinnerets near their mouths to roll up leaves from the sides and secure the roll. Scrapping by larval instars is particularly intense during the first three stages (characterized by a transparent light green to yellow color). The presence of fecal pellets inside the leaf, which are subsequently dropped below the plant, indicates larval activity.In the fourth and fifth instars (displaying light pink to dark red coloration), feeding substantially diminishes. Larvae in these stages cease feeding altogether, constructing a new leaf folded completely with silken threads, in which they pupate without exposure to sunlight. This behavior signifies a crucial phase in the pest's life cycle.

Hosts

Several host plants of ten different families are known for *Haritalodes derogata* of which the most important ones belong to the family Malvaceae (*Abelmoschus esculentus, Abutilon sp., Alcea sp., Alcea rosea, Althaea sp., Althaea rosea, Gossypioideskirkii, Gossypium sp., G. barbadense, G. herbaceum, Hibiscus sp., H. cannabinus, H. mutabilis, H. parviformis, H. rosa-sinensis, H. sabdariffa, H. tiliaceus, Kydiacalycina, Sida sp., S. cordifolia, S. orientalis, Thespesia danis, T. lampas, T. populnea, Urena sp., U. lobata*) (Robinson *et al.,* 2010), *Hibiscus syriacus* L. and *Hibiscus mutabilis*L., (***Hosseini Tabesh et al., 2015).*** Our present report is a new addition of Host plant *H. arnottianus* for *H. derogata.*

Fig 1. Infestation of leaf roller

Fig.2. Larvae of leaf roller H. derogata

Fig. 3. Obtect pupa

Developmental stages

The antepenultimate larval stage ranges from 100-180 mm in length (mean ± SE: 12.3±0.68 mm) and 18-68 mg in weight (39±4.07 mg). Transitioning to the penultimate larval phase, lengths fluctuate between 20-24 mm (22.00±0.37 mm), and weights range from 90-124 mg (94.2±6.13 mg). In the ultimate larval stage, lengths extend to 25-30 mm (25.4±0.22 mm), with weights ranging from 130-220 mg (175.7±3.93 mg) (Fig. 4 and 5). The larva has been described detail by Atulukwu (2021). According to Anoike (1978) larvae of *Syleptaderogata* [*Sylleptederogata*], a pest of okra in Nigeria, underwent 5 or 6 instars when instars when reared singly in the laboratory; the life cycle was completed in 33.9+or-0.5 days.

Fig. 4. Female adult

The prepupal stage (Fig. 6) features lengths varying from 16-22 mm (18.5±0.69 mm) and weights spanning 60-90 mg (69.7±4.62 mg). The subsequent pupal stage presents lengths of 13-18 mm (14.40±0.60 mm) and weights ranging from 40-55 mg (46.1±1.27 mg). Pupae adopt an obtect form, exhibiting a distinctive chocolate brown hue and were found to exist 8-11 days. Mariselvi and Manimegalai (2016) reported that the period of pupation is about 6-12 days. Roychoudhury *et al.* (2017) have also reported of this species 7-10 days. Finally, the adult stage (Fig. 7) emerges with a length range of 25-32 mm (27.2±0.42 mm). These moths are light yellowish in color and have black and brown spots on their head, body, and abdomen. What makes them distinct is the presence of dark brown wavy lines on their wings, giving them a unique and eye-catching appearance. The diagnostic features of moth of this species have been described by Roychoudhury *et al.* (2017); Atulukwu (2021) and Mariselvi and Manimegalai (2016).

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REFERENCES

Anioke, S. C. 1989. The biology of *Sylepta derogata* Fabricius (Pyralidae), a lepidopterous pest of okra in Eastern Nigeria. *International Journal of Pest Management,* **35**(1): 78-82.

Atuluku, S. I. 2021. Management of leaf roller *Sylepta derogata* Fab. (Lepidoptera: Pyralidae) on some

Table 1. Morphometrics of *H. derogate* developed on *H. arnottianus* in laboratory

Stage	Length(mm)		Weight (mg)	
	Range	Mean ± SE	Range	Mean ± SE
Larva				
Antepenultimate	10-18	12.3± 0.68	18-68	39± 4.07
Penultimate	20-24	22.00± 0.37	90-124	94.2± 6.13
Ultimate *	25-30	25.4± 0.22	130-220	175.7± 3.93
Prepupa	16-22	18.5±0.69	60-90	69.7±4.62
Pupa	13-18	14.40±0.60	40-55	46.1±1.27
Adult **	25-32	27.2±0.42	-	-

*Full grown ** Wing expanse of dry specimen. Data based on 10 observations

okra varieties in Samaru and Kadawa, Nigeria. *Postgraduate thesis, Ahmadu Bello University*, Zaria, Nigeria.

Hosseini Tabesh, B., Alipanah, H., &Sahragard, A. 2015. First record of *Haritalodes derogata* (Fabricius) (Lepidoptera: Crambidae: Spilomelinae) from Iran. *Journal of Crop Protection,* **4**(2): 167-171.

Leraut, P. 2005. Contribution à lettuce des generes *Pyralis* Linnaeus, *Pleuroptya* Meyrick *Haritalodes* Warren [Lepidoptera, Pyraloidea]. *Revue Française d'Entomologie (N. S.),* **27**(2): 77-94.

Little, E. L. 1989. *Common Forest Trees of Hawaii: Native and Introduced (Vol. 679)*. US Department of Agriculture, Forest Service.

Manjula, K. N., Renuka, S., Rishi, R. R. and Sundararaj, R. 2020. Insects infesting *Hibiscus rosa-sinensis* Linn. in Karnataka, India. *Entomon,* **45**(3): 231-236.

Mariselvi, S. and Manimegalai, K. 2016. Biochemical studies of cotton pest *Sylepta derogata* Fab. by Econeem, *Acorus calamus*, and *Piper longum* extracts. *International Journal of Scientific and Research Publications,* **6**(1): 388-402.

Nuss, M., Landry, B., Vegliante, F., Tränkner, A., Mally, R., Hayden, J., Bauer, F., Segerer, A., Li, H., Schouten, R., Solis, M. A., Trofimova, T., De Prins, J., and Speidel, W. 2014. Global Information System on Pyraloidea. Retrieved from www.pyraloidea.org

Robinson, G. S., Ackery, P. R., Kitching, I. J., Beccaloni G. W. and Hernandez, L. M. 2010. HOSTS-A Database of the World's Lepidopteran Hostplants. Natural History Museum, London. Retrieved from http://www.nhm.ac.uk/hosts

Roychoudhury, N., Meshram, P., Pandey, A. and Prajapati, N. 2017. *Hibiscus tiliaceus* Linn. (family Malvaceae)–a new host plant. *Indian Journal of Forest,* **40**: 151-154.

Yamanaka, H. 2008. Revisional study of some species of the genus *Haritalodes* Warren (Pyralidae, Pyraustinae) from Eastern Palaearctic and Oriental Regions. *Tinea,* **20**(4): 243-252.

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RESEARCH NOTE

Evaluation of valifenalate 6 % + mancozeb 60 % WG against downy mildew of cucumber

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ABSTRACT: Field studies were carried out to evaluate valifenalate 6 % + mancozeb 60 % WG against downy mildew of cucumber incited by *Pseudoperonospora cubensis*. The results revealed that, application of valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha had recorded lowest downy mildew severity (7.99%) with maximum yield of 12.23 t/ha which was significantly superior over standard check and untreated control. Therefore valifenalate 6 % + mancozeb 60 % WG can be an additional option for effective management of downy mildew of cucumber in order to break the fungicide resistance development by the pathogen against mancozeb. Subsequent testing on phytotoxicity revealed that there were no visual phytotoxic symptoms observed during the experimentation.

Keywords: Cucumber, valifenalate, disease severity, downy mildew, fungicide, phytotoxicity

Cucumber (*Cucumis sativus* L.) is an annual vegetable crop, grown during warm season across the world. In India, the crop is extensively cultivated under natural condition as it is grown in tropical, subtropical and milder temperate zones during *Kharif* and summer. On the onset of *rabi*, it is taken up in protected controlled conditions (Rai *et al*., 2008). Cucumber is attacked by various fungal, bacterial, phytoplasmal and viral diseases. Among them, fungal diseases such as *Alternaria* leaf blight, *Cercospora* leaf spot, *Septoria* leaf spot, target leaf spot, anthracnose, downy mildew, powdery mildew, *Fusarium* wilt, *Verticillium* wilt and gummy stem blight have a prominent role in reducing cucumber yield.

Downy mildew of cucumber incited by *Pseudoperonospora cubensis* is a common threatening disease which appears as angular, yellow lesions on the top surface of the infected leaf and corresponding underside of the leaf shows gray to black fuzz (sporangia) which gives dirty or velvety appearance. It causes abundant reduction in yield in terms of quality and quantity (Lebeda and Cohen, 2011). *P. cubensis* is an obligate parasite and overwinters on live cucurbit plants in areas with mild winter climates or plants growing in greenhouse/ protected environment (Keinath, 2015). *P. cubensis* attacks at least 50 cucurbit species which includes cucumber, cantaloupe, muskmelon, pumpkin, squash and watermelon (Lebeda and Widrlechner, 2003).

Traditionally, cucumber downy mildew was managed by an integrated approach that combined planting of resistant cultivars, cultural practices that reduce leaf wetness and a timely but limited application of broad-spectrum protectant or oomycete-specific fungicides (Holmes *et al*., 2006). Fungicide acts as an important tool for management of downy mildew in case of highly susceptible varieties during humid weather. The Fungicide Resistance Action Committee (FRAC) considers *P. cubensis* a pathogen with a high risk for development of resistance. Systemic fungicides should be used in combination with protectant fungicides to reduce the chance of developing fungicide resistant strains of the pathogen (Urban and Lebeda, 2006). Keeping all above in view, the present investigation was carried out to evaluate new combi-fungicide valifenalate 6 % + mancozeb 60 % WG against severity of downy mildew of cucumber and yield parameters.

The field experiment was laid out during 2020-21 and 2021-22 in a randomized complete block design with nine treatments and replicated thrice using local cucumber variety with a spacing of 125 × 90 cm. The first foliar spray of recommended fungicides was given as per the respective treatments after the onset of diseases, the second spray was given 10 days after the imposition of first spray and the last spray was given at an interval of 10 days. The disease severity on each treatment was recorded prior to the imposition of the

treatment and 10th day after first, second and third spray during the experimentation and the per cent disease severity was analyzed statistically. The final scoring of the disease severity was recorded as per disease index by following 0-5 scale as described by Jamadar and Desai (1997) given below (Table 1). Fifteen leaves randomly selected on five cucumber plants/plot were assessed for scoring the severity of diseases. The data were computed to percent disease index (PDI) using following formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Number of leaves observed}} \times \frac{100}{\text{Maximum disease rating value}}$$

The PDIs were suitably transformed into arcsine values and analyzed. The weight of cucumber fruits harvested/plucked were summed up for calculating total yield/plot and converted into t/ha and statistically analyzed.

Table 1. Disease rating scale used to assess the severity of downy mildew on cucumber leaves

Grade	Description (% leaf area infected)
0	No infection of downy mildew
1	0-10
2	10.1-15
3	15.1-25
4	25.1-50
5	> 50

To know the phytotoxic effect, the fungicide was sprayed at recommend dose (x, 1.5 x and 2.0 x), cucumber plants were observed at 1, 3, 5, 7 and 10 days after each application for phytotoxic symptoms like leaf injury, wilting, vein clearing, necrosis, yellowing, stunting, epinasty and hyponasty.

The pooled data of 2020-21 and 2021-22 revealed that downy mildew intensity in the trial ranged between 8.54 and 9.74 before implementation of treatments and was non-significant with each other. All the spray treatments were significant in managing disease in comparison to untreated control (Table 2). The fungicides were tested against downy mildew of cucumber and the results revealed that, the treatment plots sprayed with three applications of valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha - with 10 days interval has recorded minimum severity of downy mildew (7.99 %) which was followed by valifenalate 6 + mancozeb 60 % WG at 2500 g/ha (T_4) and at 2000 g/ha (T_3) with 8.33 and 9.20 per cent downy mildew, respectively which are on par with each other and are significantly superior over remaining treatments including standard check (T_7-mancozeb 75 % WP at 2000 g/ha with 24.09 %) and untreated control check (47.78 %). Among all the tested chemicals, the maximum percentage of reduction over control of downy mildew in cucumber was recorded with valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha (T_5) (83.29 %) which was followed by valifenalate 6 % + mancozeb 60 % WG at 2500 g/ha (T_4) (82.56) and at 2000 g/ha (T_3) (80.79) (Table 2).

Yield data recorded in response to different treatments are presented in table 2. Maximum yield of 12.23 t/ha was recorded with valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha (T_5) treatment followed by valifenalate 6 % + mancozeb 60 % WG at 2500 g/ha (T_4) and at 2000 g/ha (T_3) with 11.99 and 11.60 t/ha, respectively. These treatments are on par with each other and significantly superior over the rest of the treatments including standard (9.41 t/ha). The economics of valifenalate 6 % + mancozeb 60 % WG in managing the downy mildew of cucumber revealed that, the maximum BC ratio was recorded by valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha (T_5) with 1.31 followed by valifenalate 6 % + mancozeb 60 % WG at 2500 g/ha (T_4) and at 2000 g/ha (T_3) with 1.28 and 1.21, respectively against 0.68 by the standard check (Table 2). Further the phytotoxicity of valifenalate 6 % + mancozeb 60 % WG was tested, the observations revealed that, no visual phytotoxic symptoms such as leaf injury, leaf vein clearing, yellowing, stunting, wilting, necrosis, hyponasty and epinasty were observed on cucumber.

Table 2. Effect of formulation of valifenalate 6 % + mancozeb 60 % WG on downy mildew and yield of cucumber (Pooled data of 2020-21 and 2021-22

Tr. No	Treatment detail	Dose (g or ml/ ha)	Downy mildew (Severity (PDI))				ROC after 3rd spray (%)	Yield (t/ ha)	Benefit cost ratio
			Before spray	After I spray	After II spray	After III spray			
T1	Control	-	9.52 (17.95)*	27.08 (31.35)	38.06 (38.06)	47.78 (43.72)	-	7.14	-
T2	Valifenalate 6 % + mancozeb 60 % WG	1500	9.74 (18.16)	13.53 (21.57)	15.06 (22.82)	17.11 (24.42)	64.20	10.65	0.98
T3	Valifenalate 6 % + mancozeb 60 % WG	2000	9.29 (17.72)	8.26 (16.33)	8.94 (16.94)	9.20 (17.49)	80.79	11.60	1.21
T4	Valifenalate 6 % + mancozeb 60 % WG	2500	9.51 (17.95)	7.22 (15.57)	7.90 (16.32)	8.33 (16.92)	82.56	11.99	1.28
T5	Valifenalate 6 % + mancozeb 60 % WG	3000	8.54 (16.97)	6.74 (15.01)	7.67 (16.06)	7.99 (16.67)	83.29	12.23	1.31
T6	Valifenalate 10 % WG	1500	8.76 (17.15)	13.41 (21.47)	16.05 (23.60)	18.36 (25.34)	61.67	9.78	0.79
T7	Mancozeb 75 %WP	2000	8.62 (17.06)	17.91 (25.01)	19.23 (25.98)	24.09 (29.37)	49.66	9.41	0.68
T8	Ametoctradin 27 % + dimethomorph 20.27 %	800-1000	9.69 (18.12)	15.83 (23.44)	17.67 (24.85)	20.40 (26.84)	57.29	9.75	0.69
T9	Cymoxanil 8 % + mancozeb 64 % WP	1500	9.51 (17.96)	16.28 (23.77)	17.23 (24.51)	20.40 (26.84)	57.35.	9.66	0.73
	S. Em ±		**0.42**	**0.41**	**0.39**	**0.39**	**-**	**0.19**	
	CD at 5%		**NS**	**1.22**	**1.16**	**1.17**	**-**	**0.57**	

*Figures in the parenthesis are Arcsine transformed values; NS: Non significant; ROC: Reduction over control

The outcomes of present investigation are in agreement with Kagadi *et al*. (2002) who reported that prophylactic spray with mancozeb serve as protective layer on foliage and destroy the sporangia landed on the foliage thereby delaying in onset of the disease. Alavi and Dehpour (2010) explained that application of mancozeb at 2000 ppm reduced the severity of downy mildew in cucumber with 24.4 per cent. Valifenalate is a new active ingredient discovered by Isagro Ricerca, belonging to group of CAA (Carboxilic Acid Amides), subgroup valinamide carbamates. The oomycetes fungi *P. cubensis* is the exclusive target of valifenalate, as it mainly inhibits the cell wall synthesis (Bermano *et al*., 2010). Noorulla *et al*. (2019) and Srividhya *et al*. (2019) also reported that curative sprays with metalaxyl + mancozeb at 0.2 % and dimethomorph at 0.1 % + mancozeb at 0.2 % at weekly interval at onset of the disease were found most effective against the disease in field conditions.

Based on the results of the present study, it can be concluded that valifenalate 6 % + mancozeb 60 % WG at 3000 g/ha (T_5) is effective in reducing downy mildew

disease of cucumber with high economic returns and could be an affective component of integrated disease management in cucumber.

REFERENCES

Alavi, S. V. and Dehpour, A. A. 2010. Evaluation of the nano-silver colloidal solution in comparison with the registered fungicide to control greenhouse cucumber downy mildew disease in the north of Iran. *Acta Horticulturae*, **877**: 1643-1646.

Anonymous .2020. Food and Agricultural Organization (FAO). *www.fao.org.faostat.in.*

Bermano, A., Giambelli, A. and Burri, L. 2010. Valifenalate (Valis): a new active ingredient for control of downy mildew on grapevine and vegetables. *Phytopathological days*, Cervia (RA), Italy, **2**: 103-110.

Holmes, G., Wehner, T. and Thornton, A. 2006. An old enemy re-emerges. *American Vegetable Grower*, **2**: 14-15.

Jamadar, M. M. and Desai, S. A. 1997. Bioefficacy of dimethomorph against downy mildew of grapevine. *Advances of Agriculture Research in India*, **4**: 81-85.

Kagadi, S. R., Deadman, M. L., Pawar, D. R. and Gadre, U. A. 2002. Effects of fungicide control of downy mildew (*Pseudoperonospora cubensis*) on yield and disease management of ridge gourd (*Luffa acutangula*). *The Plant Pathology Journal*, **18**(3): 147-151.

Keinath, A. P. 2015. Cucurbit downy mildew management. Clemson cooperative extension, Horticulture: *Plant Pathology*, 90.

Lebeda, A. and Cohen, Y. 2011. Cucurbit downy mildew (*Pseudoperonospora cubensis*) biology, ecology, epidemiology, host-pathogen interaction and control. *European Journal of Plant Pathology*, **129**: 157-192.

Lebeda, A. and Widrlechner, M. P. 2003. A set of cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *Journal of Plant Diseases and Protection*, **110**: 337-349.

Noorulla, H., Thulasiram, K. and Shashidhar, K. R. 2019. Integrated approach for management of downy mildew of cucumber caused by *Pseudoperonospora cubensis*. *Journal of Pharmacognosy and Phytochemistry*, **8**(4): 510-513.

Rai, M., Pandey, S. and Kumar, S. 2008. Cucurbit research in India: a retrospect. Proceedings of the IX eucarpia meeting on genetics and breeding of cucurbitaceae. 285-293.

Srividya, K. B., Pawar, K. B., Narute, T. K., Lohate, S. R. and Chavan, S. K. 2019. Bio-efficacy of botanicals against downy mildew (*Pseudoperonospora cubensis*) of cucumber under protected condition. *International Journal of Recent Scientific Research,* **10**(9): 34553-34556.

Urban, J. and Lebeda, A. 2006. Fungicide resistance in cucurbit downy mildew - methodological, biological and population aspects. *Annals of Applied Biology*, **149**: 63-75.

Wheeler, B. E. J. 1969. An introduction to plant diseases. John Wiley Publications, London, 301.

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