FIELD EFFICACY OF BIOCAPSULES OF ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF VEGETABLE PESTS

PARVATHI MALOTH

(2018-11-122)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE VELLAYANI

THIRUVANANTHAPURAM-695522

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by

PARVATHI MALOTH

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2022

DECLARATION

I, hereby declare that the thesis entitled **"Field efficacy of biocapsules of entomopathogenic fungi for the management of vegetable pests"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani
Date: 21-01-2022

Parvathi Maloth
(2018-11-122)

CERTIFICATE

Certified that the thesis entitled **"Field efficacy of biocapsules of entomopathogenic fungi for the management of vegetable pests"** is a record of bonafide research work done independently by Ms. Parvathi Maloth under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

21/1/22

Dr. Reji Rani O. P.

Associate Professor
(Chairperson, Advisory Committee)
Department of Agrl. Entomology
College of Agriculture, Vellayani
Thiruvananthapuram 695 522

Vellayani

Date: 21.1.2022

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Parvathi Maloth (2018-11-122), a candidate for the degree of **Master of science in Agriculture** with major in Agricultural Entomology, agree that the thesis entitled "Field efficacy of biocapsules of entomopathogenic fungi for the management of vegetable pests" may be submitted by Ms. Parvathi Maloth, in partial fulfilment of the requirement for the degree.

Dr. Reji Rani O. P.
21/1/2022
(Chairperson, Advisory committee)
Associate Professor
Department of Agrl. Entomology
College of Agriculture, Vellayani

Dr. Anitha N.
(Member, Advisory committee)
Professor and Head
Department of Agrl. Entomology
College of Agriculture, Vellayani

Dr. Ambily Paul
(Member, Advisory committee)
Assistant Professor
Dept. of Agricultural Entomology
College of Agriculture, Vellayani

Dr. Sarada S.
(Member, Advisory committee)
Assistant Professor and Head
Department of Vegetable Science
College of Agriculture, Vellayani

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Parvathi Maloth

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Percentage
@	At the rate of
CD	Critical Difference
et al.	And others
Fig.	Figure
g	Gram
g^{-1}	Per gram
h	Hours
ha^{-1}	Hectare
HPMC	Hydroxy Propyl Methyl Cellulose
KAU	Kerala Agricultural University
kg	Kilogram
L	Litre
L^{-1}	Per litre
mL	Millilitre
mL^{-1}	Per milliliter
NS	Non-Significant
RBD	Randomised Block Design
sp. or spp	Species (singular or plural)
viz.,	Namely

Introduction

1. INTRODUCTION

Safe to eat vegetable production in the state demands use of ecofriendly tools for managing the pests. Agriculture has to face the destructive activities of numerous pests like fungi, weeds and insects which have serious effect on food production. Global crop yield is reduced by 20 to 40 per cent annually due to plant pest and diseases (FAO, 2012). With the advent of chemical pesticides, this crisis was resolved to a great extent. However, over dependence on chemical pesticides and their eventual uninhibited use has necessitated for alternatives mainly for environmental concerns.

Though biopesticides cover about one per cent of the total plant protection products globally, their number and the growth rate have been showing an increasing trend in the past two decades. Around 175 biopesticide's active ingredients and 700 products have been registered worldwide (Rao *et al*. 2007).

Entomopathogenic fungi (EPF), is a widely used organic tool especially in the case of vegetable production, which renders a cultivation practice that is free from pesticide residues. They constitute a group with over 750 species from 90 genera which are known to be entomopathogenic (Charnley, 1989). Widely studied entomopathogenic fungi belong to genera such as *Beauveria*, *Metarhizium*, *Lecanicillium*, *Hirsutella*, *Erynia* (Zoopthora), *Nomuraea*, *Aspergillus*, *Aschersonia*, *Paecilomyces*, *Tolypocladium*, *Leptolegnia*, *Culicinomyces*, *Coelomomyces*, and *Lagenidium* (Moore and Prior 1993) of which, *Beauveria* spp., *Metarhizium* spp., *Lecanicillium* spp. and *Isaria* spp. have been developed as successful mycoinsecticides for various groups of insect pests (Shahid *et al*., 2012)

The main advantages of EPF are their specificity to target pests, safety to the non-target organisms, high virulence, biopersistence, and safety to environment and human health. They can be made use of, where the pests develop resistance to the conventional insecticides, enabling them to be incorporated as an eco-friendly component in integrated pest management programs, especially in the case of vegetable crops.

Amaranthus, okra and cowpea are the common vegetables that are grown year round by the farmers of Kerala, to meet the ever time market demand. Often these crops are vulnerable to a wide variety of insect pests, tempting the farmers to use chemical insecticides indiscriminately, leading to pest resistance, resurgence and most importantly the residual problems in the harvested produce.

Market samples of amaranthus, the most commonly used leafy vegetable was reported to harbour pesticide residues (Srinivasan, 2012). It is attacked by a number of insect and non-insect pests of which its yield was reported to be hindered by major insect pests such as *Spoladea recurvalis* (F.) (beet webworm), *Spodoptera littoralis* (Boisduval) (cotton leafworm), *Hypolixus* sp. (F.) (amaranth stem weevils), *Liriomyza huidobrensis* (Blanchard) (pea leaf miner) and *Myzus persicae* (Sulzer) (green peach aphid) (Mureithi *et al*., 2017).

Around 72 species of insects and mites have been recorded in okra during different growth stages (Geroh, 2011). Among these insect pests, *Earias vittella* F. and *Earias insulana* Boisduval (Lepidoptera: Noctuidae) are the most important insects that cause damage on shoots and fruits (Aziz *et al*., 2011).

Cowpea *Vigna unguiculata* (L.) Walp, is grown throughout the tropics and subtropics as a vegetable, a pulse crop, a fodder crop and also as a cover crop. It is a nutritionally rich and highly priced vegetable and pulse in the domestic markets of Kerala. Though the crop is attacked by an array of pests, sucking pest predominantly the pea aphid *Aphis craccivora* Koch (Homoptera: Aphididae), is one of the most common pest species in the tropics with cosmopolitan and polyphagous occurrence leading to 20 to 40 per cent yield loss (Singh and Allen, 1980).

With the increasing awareness of eco-friendly approach of pest management, microbial control employing application of entomopathogens particularly fungi is found is to be promising. Formulations of microbial pesticides are largely talc based which are bulky and difficult to transport and use. Furthermore, the chances of contamination and loss of viability are more in these formulations.

Capsule is a stable formulation wherein the bioagent is encapsulated in coatings and thus protected from extreme environmental conditions such as UV radiation, rain and temperature. Possibility of getting contaminated is also meagre as the infective propagules are encapsulated in a protective covering. Capsules have more residual stability than spray formulations.

The present study entitled “Field efficacy of biocapsules of entomopathogenic fungi for the management of vegetable pests” was therefore envisaged to test the efficacy of the capsule formulations developed by Remya and Reji (2018) in managing defoliators in amaranthus, borers in okra and sucking pests in cowpea.

Review of Literature

2. REVIEW OF LITERATURE

The development of pest control measures using microorganisms, especially entomopathogens has received increasing attention in recent years (Enkerli *et al*., 2004). Biopesticides offer several advantages over the chemical pesticides, mainly on their safety to non target organisms and on their sustained mode of pest management.

Biological control using entomopathogenic fungi (EPF) is an exciting and rapidly advancing research area that plays an important role in safer pest management. EPF had been known as an important inhibitory factor of crop pests for more than a century and is continuously used in biological control of crop pests throughout the world (Jaber and Enkerli, 2017). They are unique in their mode of action unlike the other entomopathogens such as bacteria and viruses. Sustainability, comparatively broader host range, safety to other invertebrates and amenability to easy and cheaper mass production methods are some other attributes of these bioagents.

Majority of entomopathogenic fungi used for biological control of insects and mites are in the orders Hypocreales and Entomophthorales, of which the commercially produced species falls under Hypocreales. Fungi such as *Metarhizium* spp., *Beauveria bassiana*, *Lecanicillium* spp., and *Isaria fumosorosea* are being used against a large number of pests (Sharma *et al*., 2020).

2.1 EFFICACY OF ENTOMOPATHOGENIC FUNGI AGAINST PESTS OF VEGETABLES

2.1.1 Amaranthus

Amaranthus (*Amaranthus* spp.) one of the most popularly grown leafy vegetable in India is affected by various insect pests that feed on plant parts such as stems, leaves, flowers and seeds. A total of 92 insect pests belonging to 11 orders have been recorded from cultivated amaranthus (Rajeshkanna *et al*., 2017).

Akinlosotu (1977) documented the pests of amaranthus belonging to various insect orders. They included coleopterans such as amaranthus stem weevil *Hypolixus truncatulus* F., *H. nubilosus* (Curculionidae), tortoise beetle

Aspidomorpha exilis F. (Cassididae); lepiopterans such as, Hawaiian beet web worm *Spoladea recurvalis* F. (Pyralidae), leaf webber *Psara basalis* Walker (Crambidae), leaf roller *Haritalodes (Sylepta) derogata* F. (Crambidae). The polyphagous pests such as cotton bollworm *Helicoverpa armigera* Hubner (Noctuidae), *Spodoptera litura* F. (Noctuidae), grasshopper *Atractomorpha crenulata* (Acrididae) and aphids *Aphis craccivora* Koch (Aphididae) were also reported to infest amaranthus by Ebert *et al*. (2011).

2.1.1 Pathogenicity of *B. bassiana* to pests of amaranthus

B. bassiana infects and kills insects as they come in contact with the conidia for which it takes three to five days. In a study conducted by Long *et al*. (2000), it was revealed that the dead insect may serve as source of spores for secondary spread of the fungus and that even an infected adult male can transmit the fungus during mating.

2.1.1.1 Lepidoptera

2.1.1.1.1 Spoladea (Hymenia) recurvalis

In a field experiment conducted by James *et al*. (2006), it was observed that inoculation of *B. bassiana* (isolate Bba 5648) conidial suspension @ $1x10^8$ CFU mL^{-1} was more virulent to larvae of *S. recurvalis* than other strains, as it caused cent per cent mortality of larvae within five days after inoculation. They could observe mycosis in 83 per cent of the dead larvae.

Pooru (2015) reported cent per cent cessation of movement of *S. recurvalis* second instar larvae 72 h after treatment when *B. bassiana* was sprayed @ 10^8 CFU g^{-1} with both the doses, 10g and 20g L^{-1}.

In a laboratory study, Miller (2019) reported that *B. bassiana* (isolate ICIPE 725) conidial suspensions @ $1x10^8$ CFU mL^{-1} spraying on leaves of amaranthus caused 83 per cent mortality in second instar larvae of *S. recurvalis* after seven days of treatment.

2.1.1.1.2 Psara basalis

In a field experiment conducted by James *et al*. (2006), it was observed that inoculation of *B. bassiana* (isolate Bba 5644) conidial suspension @ $1x10^8$ mL^{-1} caused 100 per cent mortality of *P. basalis* larvae, where 83 per cent dead larvae showed fungal sporulation, while the isolates Bba5653 and Bba5654 caused 97 per cent mortality each and 33 per cent of the dead larvae manifested the fungal sporulation.

2.1.1.1.3 Spodoptera litura

While studying the pathogenecity of *B. bassiana* to *S. litura*, Gopalakrishnan and Narayanan (1989) observed that 2.4 x 10^7 spores mL^{-1} was the effective concentration that caused 43.33 per cent mortality in pupae seven days after treatment. They also found that at a lower concentration of 2.4 x 10^4 spores mL^{-1} there was 86.6 per cent malformation or mortality in pupae.

In a similar laboratory study, Malarvannam *et al*. (2010) observed that *S. litura* third instar larvae sprayed with *B. bassiana* @ $2.4x10^7$ and $2.4x10^4$ spores mL^{-1} failed to pupate. Rajanikanth *et al*. (2010) while evaluating the pathogenicity of isolates of *B. bassiana viz.* Bb-13, Bb-11, Bb-5A, one commercial isolate coded Bb-N and two local isoalates, Bb-L-1 and Bb- L-2 @ $2x10^8$ conidia mL^{-1} against third instar larvae of *S. litura*, observed that Bb-5A was superior among others where the population decreased from 1.27 to 1.17, 1.10 and 1.17 on the first, second third and fourth day respectively.

Kaur *et al*. (2011) tested the efficacy of *B. bassiana* against second, third and fourth instar larvae of *S. litura* using three varying concentrations 2.03×10^8, 4.03×10^6 and 1.47×10^5 spores mL^{-1} and found 100, 80 and 60 per cent mortality respectively in those treated with 2.03×10^8 spores mL^{-1}, while the mortality was reduced to 80, 60 and 50 per cent mortality respectively when treated with 4.03×10^6 spores mL^{-1} and 65, 50 and 40 per cent respectively, with 1.47×10^5 spores mL^{-1} within a week.

Karthikeyan and Selvanarayanan (2011) studied the bioefficacy of liquid formulation of *B. bassiana* under laboratory conditions against *S. litura* larvae at three different concentrations 0.15, 0.20 and 0.25 % to determine the effective concentration and reported that among the three concentrations, 0.25 % recorded 86.67 per cent mortality to *S. litura.*

In a laboratory study, Petlamul and Prasertsan (2012) evaluated 10 strains of the *B. bassiana* against third instar larvae of *S. litura* by dipping the larvae in conidial suspension for five seconds. The strain BNBCRC @ $1x10^8$ conidia mL^{-1} exhibited 100 per cent larval mortality on the fifth day with a highest germination percentage of 72. 22.

Baskar *et al*. (2012) evaluated the larvicidal and growth inhibitory activities of 10 different isolates of *B. bassiana* against the third instar larvae of *S. litura* and they reported that the isolate Bb10 @ $1x10^8$ spores mL^{-1} caused the highest larval mortality of 68.60 per cent, which was followed by the isolate Bb6 (50.27 per cent). They also observed abnormality of adults, although there was 100 per cent emergence on the fourth day.

In a field experiment, Pooru (2015) reported 100 per cent immobility of *S. litura* second instar larvae after 72 h treatment with *B. bassiana* @ 10^8 CFU g^{-1} sprayed at 20g L^{-1}. Asi *et al*. (2013) evaluated the efficacy of eight fungal isolates against third instar larvae of *S. litura*. Result showed that there was 63.26 per cent mortality by the isolate 25 @ $1x10^8$ conidia mL^{-1} ten day post treatment.

Similarly, Ummidhi and Vadlamani (2014), observed 83.3 to 94.3 per cent mortality of third instar larvae of *S. litura* treated with different oil formulations of *B. bassiana* isolates ARSEF,1725, 654, B55 and B51 @ 1×10^8 conidia mL^{-1}.

Manjushree *et al*. (2020) while assessing the efficacy of organic insecticides against *S. litura* on amaranthus, observed that *B. bassiana* @1×10^9 spores @ 1g L^{-1} recorded maximum reduction (64.92 per cent) in pest population 10 days after spraying while it was 53.04 in NSKE 5%, 47.68 in azadirachtin 10,000 ppm, 34.34 in

pongamia oil 5%, 39.85 in garlic extract 2%, 25.21 in *L. lecanii* @ 1×10^9 spore 1g L^{-1}. The chemical control with malathion 50 EC recorded 70.05 per cent, reduction.

2.1.1.2 Coleoptera

Perusal of literature revealed that the studies on the efficacy of entomopathogenic fungi on coleopteran pests are meagre. Villacarlos *et al*. (2004) reported the non efficacy of five isolates of *B. bassiana* conidial suspension @ $1x10^7$ conidia mL^{-1} in *Aspidomorpha* sp**.** and observed that neither the topical spray nor the contaminated food could bring about mortality or symptoms of mycosis.

2.1.1.3 Hemiptera

2.1.1.3.1 Aphis craccivora

In a laboratory study, Ekesi *et al*. (2000) reported that spraying the conidial suspension of *B. bassiana* isolate, CPD 11 @ 6.8×10^5 conidia mL^{-1} recorded 58 to 91 per cent mortality to the aphids within seven days of treatment.

Saranya *et al*. (2010) conducted a study with six different concentrations of *B. bassiana* ranging from 10^3 to 10^8 spores mL^{-1} on *A. craccivora*. The study revealed that at 10^8 spores mL^{-1} there was 96.66 per cent mortality after seven days of treatment, whereas with 10^3 spores mL^{-1} mortality was only 26.66 per cent.

As per the report of Abd *et al*. (2011) *B. bassiana* @ 5×10^6 spores mL^{-1} and $1x10^6$ spores mL^{-1} caused 100 per cent mortality to adults and nymphs of *A. craccivora* over four and five days, respectively.

Selvaraj and Kaushik (2014) reported that *B. bassiana* at higher spore concentration of $1x10^{10}$ spores mL^{-1} caused 43.50 per cent mortality of *A. craccivora* on the first day which gradually increased to 85.04 per cent on the seventh day after treatment. The study also revealed that even the spore suspension at a low concentration of $1x10^4$ spores mL^{-1} caused 20.85 per cent mortality on the first day itself while it reached up to 55.21 per cent on the seventh day.

Abdou *et al*. (2017) reported that spraying spore suspension of *B. bassiana* @ $1x10^8$ spores mL^{-1} directly on *A. craccivora* caused 84.98 per cent mortality after four

days of direct spraying on aphid. While the results of indirect treatment (plant treatment) showed 53.75 per cent mortality after six days of treatment. This study also revealed that overall infected aphids produced fewer offspring on days 4 and 6 when comparison with control.

Mweke *et al*. (2018) studied the pathogenicity of 23 fungal isolates of *B.bassiana* against adult *A. craccivora* in the laboratory and found that all the isolates were pathogenic to the aphids. Among these the isolate ICIPE 62 @ 1×10^8 conidia mL^{-1} caused 34.5 to 90 per cent mortality in adult apterous aphids after seven days of treatment. This study revealed the pathogenicity of all the fungal isolates to *A. craccivora.*

2.1.1.4 Arachinida

***2.1.1.4.1 Tetranychus* spp.**

In a laboratory study, Wekesa *et al*. (2005) reported that spraying the conidial suspension of *B. bassiana* (isolate GPK) @ 1.1 x 10^7 conidia mL^{-1} recorded 82.6 per cent mortality to *T. evansi* within 4.6 days. In another laboratory study, Wekesa *et al*. (2006) evaluated the susceptibility of various developmental stages of *T. evansi* (eggs, larvae, protonymphs, deutonymphs and adults) to *B. bassiana*. The study revealed that adults and deutonymphs were more susceptible to fungal infection than the larval and protonymphal stages at all the three concentrations tested 3.0×10^6, to 10^8 conidia mL^{-1}. Eggs were also found to be susceptible to fungal infection. They also reported that the treated female mites laid fewer eggs than the untreated ones.

Bugeme *et al*. (2009) reported that spraying conidial suspension of *B. bassiana* (isolates ICIPE279 ICIPE273 and ICIPE278) @ 1.0×10^7 conidia mL^{-1} caused 95.2, 95.5 and 99.0 per cent mortality in 4.1, 4.6 and 4.4 days respectively, in *T. urticae.*

In a greenhouse study carried out by Gatarayiha *et al*. (2010), on various crops using varying concentrations of *B. bassiana* (isolate R444) *viz*., 1.05×10^6, 2.1×10^6 and 4.2×10^6 conidia mL^{-1}, it was observed that the aqueous conidial suspension was more effective than its emulsifiable formulation on *T. urticae* population. With the highest rate of conidia (4.2×10^6 conidia mL^{-1}), mortality of adult mites ranged from 60 to 85.7

per cent in the former and 39.4 to 61.3 per cent in the latter, seven days after spraying. They also noticed that the leaf damage index was substantially reduced by 30 per cent in treated plots.

In a field experiment conducted by Tehri *et al*. (2015), it was observed that spraying the conidial suspension of *B. bassiana* @ 0.3×10^9, 0.3×10^8 and 0.3×10^7 conidia mL^{-1} showed significant reduction of *T. urticae* population. There was 59.64, 48.23 and 37.92 per cent reduction, respectively over control.

Wu *et al*. (2016) evaluated 12e *B. bassiana* isolates in *T. urticae,* which revealed that the strains SCWJ-2, SDDZ-9, LNSZ-26, GZGY-1-3 and WLMQ-32 were more potent, causing 37.6 to 49.5 per cent mortality in adult population, when sprayed @ 1×10^7 conidia mL^{-1}, four days post-treatment.

2.1.1.2 *Pathogenicity of M. anisopliae*

2.1.1.2.1 Lepidoptera

2.1.1.2.1.1 Spoladea (*Hymenia) recurvalis*

Praveena (2016) reported that *M. anisopliae* isolates SP11 and Ma4 @ $28.01x10^7$ spores mL^{-1} caused mortality of 63.33 to 100 per cent, against second instar larvae of *S. recurvalis,* 14 days after inoculation. A pot culture experiment conducted to evaluate seven indigenous isolates *Fusarium solani* (Mart.) Sacc. (SP6), *M. anisopliae* (SP7, SP8, SP9, SP11 and SP13) and *Purpureocillium lilacinum* Thorn (Samson) S10 and two NBAIR isolates Bb5, Ma4 @ $1x10^8$ spores mL^{-1} against leaf webbers in amaranthus variety Arun, it revealed that the number of plants infested by the webbers, number of webbings $plant^{-1}$ and number of larvae web^{-1} was lowest in SP11 treatment, at 14 days after treatment. Yield was the highest (50.75 g $plant^{-1}$) in this treatment compared to others (32.25-46.75g $plant^{-1}$).

In a laboratory study, Miller (2019) reported that *M. anisopliae* (isolate ICIPE 30) caused 92 per cent larval mortality in second instar larvae of *S. recurvalis* after 4.8 days of inoculation.

2.1.1.2.1.2 Spodoptera litura

Asi *et al*. (2013) reported that *M. anisopliae* L6 @10^5 to 10^8 conidia mL^{-1} in insect immersion method caused 35.32, 49.20, 67.29 and 71.56 per cent mortality respectively in the eggs of *S. litura*. They also observed 41.16 and 43.03 percent mortality in the second and third larval instar 10 days after exposure to 10^7 and 10^8 mL^{-1} respectively and that there was less adult emergence of 88.75 per cent at the highest concentration.

Ummidhi *et al*. (2014) observed that spraying *M. anisopliae* M20 @ $1x10^8$ conidia mL^{-1} was pathogenic to second instar larvae of *S. litura*, the mortality being 40 to 88.33 per cent.

While studying the pathogenicity of *M. anisopliae* to *S. litura* infecting cabbage, Patait *et al*. (2008) observed that the isolate Ma2 from Tamil Nadu was found more effective against *S. litura* compared to other isolates Ma1 and Ma3.

In a laboratory study, Petlamul and Prasertsan (2012) evaluated 10 strains of the *M. anisopliae* against third instar larvae of *S. litura* and found that the isolates M33 and M36 caused 100 per cent mortality on the fifth day when the larvae were dipped in conidial suspension ($1x10^8$ conidia mL^{-1}) for 5 sec.

2.1.1.2.1.3 Haritalodes (*Sylepta*) *derogata*

Praveena (2016) reported that *M. anisopliae* isolates SP11 and Ma4 @ $28.01x10^7$ spores mL^{-1} caused 83.33 to 100 per cent a mortality of second instar larvae of *H. derogata* at 14 days after inoculation.

2.1.1.2.2 Hemiptera

2.1.1.1.2.2.1 Aphis craccivora

In a laboratory study, Ekesi *et al*. (2000) reported that spraying the conidial suspension of two isolates of *M. anisopliae* (CPD 4 and CPD 5) @ 3.1×10^5 and 2.7×10^5 conidia mL^{-1} caused 64 to 93 per cent and 66 to100 per cent mortality, respectively within seven days. In a bioassay conducted by Saranya *et al*. (2010) it was observed that *M. anisopliae* caused 80.76 per cent mortality in *A. craccivora* when sprayed @ $1x10^8$ spores mL^{-1}, seven days after treatment.

Mweke *et al*., (2018) conducted a laboratory evaluation with *M. anisopliae* isolate ICIPE 62 and reported that the fungus produced conidia on aphid cadavers (4.5×10^7 conidial mL^{-1}) six days post-treatment and caused 90 per cent mortality @ 1×10^8 conidial mL^{-1}.

2.1.1.2.3 Tetranychus spp.

A study conducted by Batta (2003) reported that *M. anisopliae* @ 5×10^6 conidia mL^{-1}, killed nymphs of *T. cinnabarinus*, within three to four days of treatment, with fungal outgrowth and sporulation on the cadavers within five days. They also observed that mortality of *T. cinnabarinus* ranged from 58.3 to 93.3 per cent under laboratory conditions and from 25.9 to 90.6 per cent under field conditions.

In another laboratory study, Wekesa *et al*. (2005) reported that spraying the conidial suspension of *M. anisopliae* (ICIPE 78) @ 1.0 x 10^7 caused 77.9 per cent mortality of *T. evansi* in 4.8 days. The same authors in 2006, evaluated the susceptibility of various stages of *T. evansi* to *M. anisopliae* and found that the adults and deutonymphs were more susceptible, with 93 and 78 per cent mortality respectively when compared to larval and protonymphal stages with 48 and 51 per cent mortality respectively. It was also observed that all the three tested concentrations 3.0×10^6, 10^7 and 10^8 conidia mL^{-1} were equally effective and that the eggs were also susceptible to fungal infection.

Bugeme *et al*. (2009) reported that spraying conidial suspension of *M. anisopliae* (isolate ICIPE25) @ $1.0x10^7$ conidia mL^{-1} caused 100 per cent mortality in *T. urticae* within six days.

2.1.1.3 Pathogenicity of other entomopathogenic fungi

In a laboratory bioassay, Saranya *et al*., (2010) observed that *L. lecanii* caused 100 per cent mortality @ $1x10^8$ conidia m L^{-1} in *A. craccivora* after seven days of treatment. They also observed that *Hirsutella thompsonii* Pat. @ $1x10^8$ conidia m L^{-1} caused 100 per cent mortality in *A. craccivora*, seven days after treatment. *Paecilomyces farinosus* Samson was reported to infect the larvae of *S. recurvalis* (Kuruvila and Jacob, 1980).

Revathi *et al*. (2011) observed that dipping third instar larvae of *H. armigera* in the conidial suspension of *N. rileyi* @1x10^7 conidia mL^{-1} resulted in 70 per cent mortality of *H. arimgera* within three days. Nithya and Rani (2019) reported that *L. lecanii* @ 1×10^8 spores mL^{-1} caused 100 per cent mortality in adult *Tetranychus* spp. in four days and also observed that @ 1×10^7 spores mL^{-1} caused 90, 100 per cent mortality in mites after four and five days of treatment respectively.

2.1.2 Okra

Insect pests of 72 species have been reported in okra *Abelmoschus esculentus* (L.) (Moench) (Rao and Rajendran, 2003) of which, the sucking pests comprising the leafhopper *Amrasca biguttula biguttula* Ishida (Hemiptera: Cicadellidae), aphids *Aphis gossypii* Glover (Hemiptera: Aphididae), whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and mite *Tetranychus cinnabarinus* Boisduval (Trombidiformes: Tetranychidae) causes more damage to the crop. At later stage, fruit borers like *Earias vittella* F. (Lepidoptera: Nolidae), *E. insulana* Boisduval (Lepidoptera: Nolidae) and *H. armigera* cause considerable losses to the crop to the tune of 91.6 per cent (Pareek and Bhargava, 2003).

The effectiveness of bio pesticides like *B. bassiana* and *M. anisopliae* against okra pests as reported by various workers is reviewed hereunder.

2.1.2.1 Pathogenicity of *B. bassiana* to pests of okra

2.1.2.1.1 Lepidoptera

2.1.2.1.1.1 Earias vittella

In a laboratory study, Manimekalai *et al*. (2010) reported that spraying the conidial suspension of *B. bassiana* @ 2.1 x 10^7 (4 gL^{-1}) caused maximum mortality in second instar larvae of *E. vittella*, after 72 h. Bioassay conducted in the third and fourth instars revealed that susceptibility decreased with increase in age of the larvae and required higher concentrations to kill them. Karthikeyan and Selvanarayanan (2011) studied the bioefficacy of liquid formulation of *B. bassiana* on *E. vittella* under laboratory conditions. Among the three different concentrations tested *viz.,* 0.15, 0.20 and 0.25 per cent, 0.25 per cent recorded the highest mortality of 73.33 per cent.

Field studies conducted by Nayak *et al*. (2012) on the efficacy of five bio-pesticides against *E. vittella* revealed that application of the formulation named Daman (*B. bassiana*) @ 1 kg ha^{-1} at 50, 60, 70, 80 and 90 days after sowing resulted in less fruit damage (number infested and weight loss basis).

Ali *et al*. (2015) conducted a laboratory experiment using *B. bassiana* and at three varying concentrations (1.5×10^{6} to1.5×10^{8} conidia mL^{-1}) and a commercial formulation of *Bacillus thuringiensis* Berliner (0.5 µg g^{-1}) against three field populations of *E. vittella* which were applied alone and in combination. It was concluded that highest larval mortality was observed in the population from Faisalabad which recorded the lowest pupation rate, adult emergence and egg eclosion and the lowest mortality was observed in population from Pakpattan. Overall results demonstrated the variation in virulence among the geographical isolates of the same species.

In a field experiment conducted by Devi *et al*. (2015), it was observed that *B. bassiana* @ 2000 g a. i ha^{-1} provided 68 per cent higher fruit yield in okra (87.54 q ha^{-1}) as compared to untreated control (52.10 q ha^{-1}).

Panbude *et al*. (2019) conducted a field experiment which concluded that spraying the conidial suspension of *B. bassiana* @ 1×10^{8} spores mL^{-1} showed less (23.68 per cent) infestation when compared to that in untreated plots (34.68 per cent infestation) 14 days after spraying.

2.1.2.1.1.2 Helicoverpa armigera

Revathi *et al*. (2011) observed that dipping third instar larvae of *H. armigera* in the conidial suspension *B. bassiana* @ $1x10^{7}$ conidia mL^{-1} resulted in 70 per cent mortality within three days. In a field experiment conducted by Sarkar *et al*. (2015), it was reported that spraying conidial suspsension of *B. bassiana*- CFU $1x10^{8}$ g^{-1} (5g L^{-1}) caused 34 per cent mortality of *H. armigera* in okra which was more or less equal to imidacloprid 17.8% SL 0.31 ml L^{-1} (43. 34 per cent) within 14 days of spraying. They also reported highest marketable fruit yield of okra (39.28 q ha^{-1}) in *B. bassiana* treated plots.

Tahir *et al*. (2019) reported that dipping second instar larvae of *H. armigera* in conidial suspension of *B. bassiana* isolates (WG-11 and WG-18) at 1×10^8 conidia ml^{-1} caused 91.89 and 100 per cent mortality respectively after 10 days.

B. bassiana isolates (APPRC-9604, APPRC-T5 and DLCO-EA-56) were reported to be effective to third instar larvae of *H. armigera* when sprayed at $1x10^8$ conidia mL^{-1} causing 91, 89 and 83 per cent mortality, respectively within 10 days of treatment, under laboratory conditions (Fite *et al*., 2020). They also reported that these isolates were effective to third instar larvae of *H. armigera* at $1x10^9$ conidia mL^{-1} as it resulted in 89, 78, 71 per cent mortality within 4.6, 5.5 and 6.2 days, respectively.

Finalo *et al*. (2021) observed that applying conidial suspension of *B. bassiana* isolates (Bb3 and Bb11) @ $1x10^9$ conidia mL^{-1} on third instar larvae of *H. armigera* resulted in 63.33 and 71.66 mortality, respectively after 15 days of application.

2.1.2.1.2 Hemiptera

2.1.2.1.2.1 *Bemisia tabaci*

Quesada-Moraga *et al*. (2006) evaluated the infectivity, thermal requirements, and toxicogenic activity of *B. bassiana* under laboratory conditions. Twenty-five native isolates and a commercially available mycoinsecticide (based on *B. bassiana*) were evaluated for virulence to fourth instar nymphs *B. tabaci*, at a concentration of 1×10^7 conidia mL^{-1}. They observed that all the isolates were pathogenic and the mortality rates varied from three to 85 per cent.

Rios *et al.* (2014) evaluated *B. bassiana* spore suspension (1.8×10^3 to 6.1×10^7 spores mL^{-1} against immature *B. tabaci*, on cherry tomato leaves dipped in spore suspension for 10 sec. They observed that $1x10^7$ spores mL^{-1} was very effective, killing more than 80 per cent population. They also observed mycelial growth on the cuticles of the immature insects, two to three days after death.

In an okra field experiment conducted by Janghel and Rajput (2016), it was observed that spraying the conidial suspension of *B. bassiana* @ 1×10^8 cfu g^{-1} reduced the population by 68.70 per cent after with a mean population of 2.43 and

2.21 whiteflies plant^{-1} at three and seven day after application. Patel *et al*. (2017) observed that spraying *B. bassiana* @ 2.5g L^{-1} in okra field, reduced the whitefly population to 4.30 per three leaves^{-1}, 14 days after treatment.

In a field experiment conducted by Raheem and Keridis (2017) it was reported that *B. bassiana* formulation Bio power @ 1x10^{8} and 1×10^{9} spores mL^{-1} exhibited 52 and 100 per cent reduction in population of *B. tabaci* by the sixth day.

2.1.2.1.2.2 Aphis gossypii

Herlinda *et al*. (2010) while evaluating 10 isolates of *B. bassiana*, isolate BPM @ 1 x 10^{6} conidia mL^{-1} resulted in 80.80 per cent mortality of third instar nymphs of *A. gossypii*, while the isolate BAgTb caused a death rate of 47.20 per cent, thus revealing the variation in virulence among the isolates, in infecting the same species of host insect.

In a laboratory study, Gurulingappa *et al*. (2011), observed that immersing the leaf discs in different conidial concentrations ranging from 10^{4} to 10^{7} conidia mL^{-1} for 5 sec., exhibited less reproductive rate and fecundity period of *A. gossypii*. Jandricic *et al*. (2014) reported that spraying conidial suspension of *B. bassiana* isolate 5493 at a dose of 1-3 mg conidia mL^{-1} (1-2 x10^{8} conidia mL^{-1}) resulted in 56 per cent mortality of *A. gossypi*, within three days.

Wakulima (2016) evaluated the virulence of three isolates of *B. bassiana* @ 1 × 10^{8} conidia mL^{-1} on *A. gossypii* and found that three isolates ICIPE10, ICIPE 273 and ICIPE 279 caused mortality of 57.4 per cent, 57.3 per cent, and 77.1 per cent respectively, seven days after inoculation.

2.1.2.2 Pathogenicity of *M. anisopliae*

2.1.2.1 Lepidoptera

2.1.2.1.1 Earias insulana

In a laboratory study, Hemat *et al*. (2019) reported that spraying the viable conidial suspension of *M. anisopliae* @ 1x10^{8}, 5x10^{7}, 2.5x10^{6}, 1.25x10^{5} and 0.625x10^{4} spores mL^{-1}, directly to the newly hatched larvae of *E. insulana* resulted in

99.48, 98.37, 95.71, 90.31, 81.13 per cent mortality respectively, after seven days. They also reported that under field conditions *M. anisopliae* @ 1×10^9 spores mL^{-1} was most effective than other concentrations exhibiting 100 per cent mortality.

Dar *et al*. (2020) observed that spraying *M. anisopliae* formulation Bioranza® WP 10% @ 1 x 10^8 spore mL^{-1} with Economy Micron ULVA (15 L Fed^{-1}) sprayer resulted in 92.8 per cent reduction in *E. insulana* infestation whereas with Hand-held Hydraulic sprayer (15 L Fed^{-1}), the mortality was 91 per cent, within seven days of treatment.

2.1.2.1.2 Helicoverpa armigera

Laboartory experiment carried out by Revathi *et al*. (2011) reported that dipping third instar larvae of *H. armigera* in the conidial suspension of *M. anisopliae* @ $1x10^7$ conidia mL^{-1} resulted in 70 per cent mortality within three days.

In a similar experiment under laboratory conditions, Tahir *et al*. (2019) reported that dipping second instar larvae of *H. armigera* in conidial suspension of *M. anisopliae* isolate (WG-07) @ 1×10^8 conidia mL^{-1} caused 83.86 per cent mortality by the 10^{th} day.

Fite *et al*. (2020) reported that spraying conidial suspension of *M. anisopliae* strain (DLCO-EA-40) @ $1x10^8$ conidia mL^{-1} caused 73 per cent mortality to third instar larvae of *H. armigera* within 10 days of treatment, under laboratory conditions.

2.1.2.2 Hemiptera

2.1.2.2.1 Amrasca biguttula biguttula

Maketon *et al*. (2008) reported that when leaves dipped in *M. anisopliae* (isolate CKM-048) suspension at 5 x 10^6 conidia mL^{-1} were fed to *A. biguttula biguttula* in the laboratory caused 73.33 per cent mortality. They also reported that the wettable powder formulation of the same isolate @ $1x10^9$ conidia g^{-1}, when sprayed consecutively thrice at seven day interval, yielded similar results.

2.1.2.2 .2 Aphis gossypii

Wakulima (2016) evaluated the efficacy of five isolates of *M. anisopliae* @ 1× 10^8 conidia mL^{-1} against *A. gossypii*. Results showed that the isolates ICIPE 30, ICIPE 62 and ICIPE 69 outperformed the other two, causing 77.0 per cent, and 74.1 per cent mortality in *A. gossypii*, seven days after inoculation. They also observed that ICIPE 62 produced more conidia on the cadavers than ICIPE 30 and ICIPE69.

Jandricic *et al*. (2014) reported that spraying conidial suspension of *M. anisopliae* isolate 5471 @ 1-3 mg conidia mL^{-1} ($1x10^8$ and $2x10^8$ conidia mL^{-1}) yielded 38 per cent mortality of *A. gossypii*.

2.1.2.2.3 Bemisia tabaci

Batta (2003) stated that *M. anisopliae* @ $5×10^6$ conidia mL^{-1}, killed *B. tabaci* nymphs within three days, with fungal outgrowth and sporulation observed on the cadavers in four to five days. The mortality ranged from 66.7 to 100 per cent under laboratory conditions and 30.0 to 92.2 per cent under field conditions.

In a laboratory study, Islam *et al*. (2014) reported that *M. anisopliae* (isolate GT_3) @ $7.2 × 10^6$ conidia mL^{-1} caused 84.3 per cent mortality to *B. tabaci* Raheem and Keridis (2017) reported that *M. anisopliae* formulation Bio magicat different concentrations @ $1×10^7$, $1×10^8$ and $1×10^9$ spores mL^{-1} caused death after three days. Hundred per cent mortality was noted on the sixth day when treated with $1×10^9$ spores mL^{-1}.

Rios *et al.* (2014) evaluated fungal virulence against immature *B. tabaci*, on cherry tomato leaves dipped in *M. anisopliae* spore suspension @ $2.8×10^3$ to $9.5×10^7$ spores mL^{-1} 10 sec. They observed that it was very virulent, killing more than 80 per cent of the population. Mycelia emerged from the cuticles of the immature insects two to three days after death, and most of the conidia were recorded on legs, wings, and thorax of cadavers.

2.1.2.3 Pathogenicity of other fungi to pests of okra

Raheem and Keridis (2017) reported that Bio catch (*L. lecanii*) @ $1×10^9$ spores mL^{-1} caused 100 per cent mortality of *B. tabaci* after six days of

treatment. In a field experiment was conducted by Devi *et al*. (2015) it was observed that *L. lecanii* at 1000 mL ha^{-1} resulted in 32.21 per cent reduction of shoot and fruit borer infestation, control.

Field experiment conducted by Janghel and Rajput (2016) reported that *L. lecanii* 1x 10^8 cfu g^{-1} resulted in 84.20 per cent reduction in population of *B. tabaci* within seven days after treatment at 40 days after germination.

Nithya (2015) reported that *L. lecanii* @ different concentrations $1x10^3$, 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 spores mL^{-1} caused 16.67, 23.33, 30, 53.33, 70 and 73.33 per cent mortality respectively in *B. tabaci* seven days after treatment.

2.1.3 Cowpea

Chemical sprays for management of cowpea aphid were not cost effective and also eliminate the beneficial insects like parasites and predators from these cropping systems (Rabindra and Ramanujam 2007). The most common entomopathogenic fungi used for the management of sucking pests include those under the genus *Lecanicillium*. *L. lecanii* was reported to be effective with a wide host range among aphids, whiteflies, scales, mealy bugs, nematodes and powdery mildew disease (Goettel *et al*. 2008).

2.1.3.1 Pathogenicity of *Lecanicillium* spp. to sucking pests of cowpea

2.1.3.1.1 L. lecanii

Nirmala *et al*. (2006) reported that VI1 isolate of *L. lecanii* @ 1×10^7 spores mL^{-1} caused 2 to 80.8 per cent mortality in *A. craccivora* nymphs within two days. In a laboratory study, Saranya *et al*. (2010) reported that *L. lecanii* @ six different spore concentrations ($1x10^8$, $1x10^7$, $1x10^6$, $1x10^5$, $1x10^4$, $1x10^3$ spores mL^{-1}), resulted in 100, 100, 84, 60, 44, 28 per cent mortality respectively, in adult aphids within seven days of treatment.

Abd *et al*. (2011) reported that *L. lecanii* formulation Bio-Catch caused 100 per cent mortality to *A. craccivora* adults and nymphs @ 5.0 ml and 1.0 ml (5 x 10^6 spores mL^{-1}) respectively, over a period of three days. El-Salam *et al*. (2012) found

that *L. lecanii* (Bio-Catch) @ 1×10^8 spores mL^{-1} caused 80.7 per cent reduction within five days from the first spraying and then gradually decreased to 63.6 after the second spraying. Nithya (2015) reported that *L. lecanii* @ 1×10^8 spores mL^{-1} caused 100 per cent mortality of *A. craccivora* within five days and @ 1×10^7 spores mL^{-1} it caused 100 per cent mortality within six days of treatment.

Ramanujam *et al*. (2017) found that oil formulations of *L. lecanii* isolates Vl-8, Vl-12 and Vl-32 @ 1×10^8 spores mL^{-1} given as foliar spray suppressed *A. craccivora* population by 80.05, 65.88 and 66.83 per cent respectively. In a study conducted by Reddy and Sahotra (2020) using *L. lecanii* it was found that higher concentration of 10^9 spores mL^{-1} caused 43.33 - 93.33 per cent and the lowest concentration 10^5 spores mL^{-1} caused 10 - 53.33 per cent mortality of *A. craccivora* within eight days of treatment.

2.1.3.1.3 L. saksenae

The species, *L. saksenae* was not known for its entomopathogenicity until Reji *et al*. (2015) from Kerala, India, reported it to be infective to aphids *Aphis craccivora* (Koch), *A. gossypii* (Glover), whitefly *Bemisia tabaci* (Gennadius), jassid *Amrasca biguttula biguttula* (Ishida) and mealybug *Coccidohystrix insolita* (Green). Its infectivity to common sucking pests of vegetables was further demonstrated by Jasmy (2016) and the field efficacy in managing sucking pests of rice was proved by Sankar and Rani (2018). They reported paralysis and convulsions in rice bugs treated with the spore suspension at 10^7 spores mL^{-1} within 48 h of treatment. It was also reported to be safe to beneficial insects and predators of the rice ecosystem (Sankar and Rani, 2018).

In a bioassay study, (Sreeja and Rani 2019) observed that toxicity of secondary metabolities of *L. saksenae* @ 1000 ppm (crude toxin) to *C. insolita* revealed 95.98, 85.51 per cent mortality in the third instar nymphs and adults 48 h after treatment and 100 per cent mortality at 72 and 96 h after treatment on nymphs and adults brinjal mealybug, respectively.

2.1.3.1.2 L. muscarium

Under greenhouse condition Juliya (2019) screened various isolates of entomopathogenic fungi and reported that *L. muscarium* isolate GKVK 031 @ $1x10^6$ conidia mL^{-1} was more pathogenic to *A. craccivora*, causing 16.67 to 100 per cent

2.2 EFFICACY OF CAPSULE FORMULATIONS OF MICROBIAL AGENTS IN PLANT PROTECTION

Capsules ensure slow release of inoculam, compared to direct application by spraying or drenching. It is a stable formulation wherein the bioagent is encapsulated within coatings and thus protected from extreme environmental conditions like UV radiation, rain, temperature etc. and its residual stability is enhanced due to slow or controlled release (Burges and Jones, 1998).

Dureja and Palmar (2009) developed capsule formulation of *Trichoderma* sp. by mixing dried, and powdered hyphae with organic or inorganic carriers with or without adjuvants that can be stored for two years under ambient conditions.

Anandaraj (2016) developed hard gelatin coated bio-capsule of Plant Growth Promoting Rhizobacteria (PGPR) and claimed that the technology offers easiness in storage at normal temperature and delivery of the microbes under field conditions. Moreover, the technology was reported to be cost effective.

Hiltpold *et al.* (2012) reported the efficacy of alginate based capsules of entomopathogenic nematodes, *Steinernema* spp. and *Heterorhabditis bacteriophora* Poinar and claimed that the damage caused by corn root worm, *Diabrotica virgifera virgifera* Dejean was reduced, when compared to water spray.

Schoebitz *et al.* (2013) *Trichoderma viride* Pers. formulated as capsules and tablets were found to be superior to powder formulations using talc and charcoal powder in terms of viability of the organism upto 260 days after storage (Baghel *et al.* 2014).

Kim *et al.* (2015) developed calcium alginate capsules of entomopathogenic nematodes as a novel formulation for application in soil. It was observed that hardness of capsules was more in those which were pre-treated with Ca^{2+} at 4^0 C, whereas the population of nematodes was retained in those without pre-treatment.

Remya and Reji (2020b) developed capsules of *M. anisopliae* and *B. bassiana* using talc/chitosan as carrier material, which was 100 per cent organic and biodegradable as well. While evaluating these biocapsules against banana weevils in a pot culture study, they reported that *M. anisopliae* capsules were effective in reducing population of banana weevil *C. sordidus* when applied as a prophylactic treatment. The reduction in population was 55.56 and 47.22 per cent with talc based and chitosan based capsules. They also observed that in curative treatment, both the capsules were equally effective with 50 per cent reduction in chitosan based capsules, while it was 58.3 per cent in talc based capsules.

In a field experiment conducted by (Balakrishnan, 2020) prophylactic management by leaf sheath application of chitosan based *Beauveria* capsules @10^{10} spores mL^{-1} (4 $plant^{-1}$) at monthly intervals resulted in 90.9 per cent reduction in the population of grubs and adults of the pseudostem weevil *O. longicollis*. Curative management by leaf axil application and bore hole placement of capsules at weekly interval resulted in 51.51 to 60.8, 61.5 per cent reduction in population. In the same field experiment, bimonthly application of chitosan based *Metarhizium* capsules @ 10^{10} spores mL^{-1} (4 $plant^{-1}$) has shown 70 to 77.77 per cent reduction in population.

Remya and Reji (2021b) reported that capsules of *B. bassiana* @ 10^{10} spores mL^{-1} resulted in 91.67 per cent reduction in population of banana pseudostem weevil *O. longicollis*. They also observed that in curative treatment, plants treated with talc based capsules shown 100 per cent reduction in pest count. while that with spore suspension caused 83.33 per cent reduction in weevil population.

Materials and Methods

3. MATERIALS AND METHODS

The current research work on "Field efficacy of biocapsules of entomopathogenic fungi for the management of vegetable pests" was carried out at the Biocontrol Laboratory for Crop Pest Management, Department of Agricultural Entomology, College of Agriculture, Vellayani and Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram, during the year 2018-2021.

3.1 MAINTENANCE OF FUNGAL CULTURES

The entomofungal cultures maintained in the Biocontrol Laboratory, Department of Agricultural Entomology, College of Agriculture, Vellayani, were utilized for the study. *Beauveria bassiana* (Balsamo) Vuillemin isolate Bb5, *Metarhizium anisopliae* (Metsch.) Sorokin isolate Ma4 and *Lecanicillium lecanii* (Zimmermann) Zare and Gams isolate Vl8 were originally sourced from National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru. The indigenous isolate ITCC Lsvs1 -7714 of *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno, isolated and characterised by Reji *et al*. (2015) was the fourth candidate used in this study. Virulence of these entomopathogens was maintained by periodically passing them through their respective host insects. *B. bassiana* was periodically revived using the grubs of banana pseudostem weevil, *Odoiporus longicollis* Oliver and *M. anisopliae* using the grubs of rhizome weevil *Cosmopolites sordidus* Germer. *L. lecanii* was periodically passed through *Aphis craccivora* Koch and *L. saksenae* was passed through *Leptocorisa* sp. Pure and sub cultures of these fungi were maintained in Potato Dextrose Agar (PDA) slants.

3.2 PREPARATION OF BIOCAPSULES

Biocapsules of *B. bassiana* and *M. anisopliae* were prepared using 14 day old cultures while those of *L. lecanii* and *L. saksenae* were prepared from 21 day old cultures incubated at ambient conditions. The protocol developed by Remya and Reji (2018) was followed for capsule preparation.

3.2.1 Mass Culturing the Fungi

The fungi under study were mass multiplied by static liquid fermentation in Sabouraud Dextrose Broth (SDB) taken in 2L fermenter flask. Upon sporulation the conidia were harvested.

3.2.2 Preparation of Conidial Suspension

Sporulating cultures of *B. bassiana* and *M. anisopliae* (14 day old) and those of *L. lecanii* and *L. saksenae* (21 day old) were blended in a mixer-grinder by adding a drop of tween 20. The culture was then filtered through a double layered muslin cloth (Plate 1a). The filtrate served as the conidial suspension for further preparation of capsules.

3.2.3 Preparation of Primary Powder

Spore suspension after straining was taken in centrifuge bottles (Plate 1b) and centrifuged in a Remi R 23 centrifuge (Plate 1c, d) 20 min at 4000 rpm. The spore pellet collected at the bottom of the tube (Plate 2a, b) was washed gently with sterile distilled water, to remove the mycelial mat adhering to it. Primary powder was prepared by mixing the spore pellet and crude chitosan in the ratio 1:1 to obtain 10^{10} spores g^{-1} (Plate 2c, d).

3.2.4 Filling the Capsules

Filling material was prepared by mixing the primary powder with chitosan in the ratio 1:20. The empty Hydroxy Propyl Methyl Cellulose (HPMC) capsules of 0.8g were filled using a hand operated capsule filling device as illustrated in Plates 3 and 4, which yield 100 capsules in one set. The capsules were stored airtight under ambient conditions in plastic bottles for field evaluation studies.

3.3 FIELD EFFICACY OF BIOCAPSULES

Three separate field experiments were conducted to study the efficacy of biocapsules in managing the major pests of three different vegetable crops. The objective of first experiment was to evaluate the comparative efficacy of *B. bassiana* and *M. anisopliae* capsules and standardise its dose in managing defoliators, which

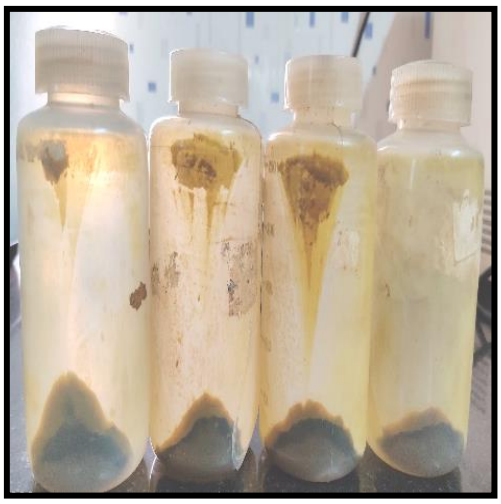
a. Straining the blended culture

b. Spore suspension in centrifuge bottles

c. Centrifugation in centrifuge

d. Centrifugation at 4000 rpm, 20 min

Plate 1. Preparation of capsule – centrifugation of spore suspension



a. Spore pellet at the bottom of the centrifuge bottles

b. *Metarhizium* spore pellet

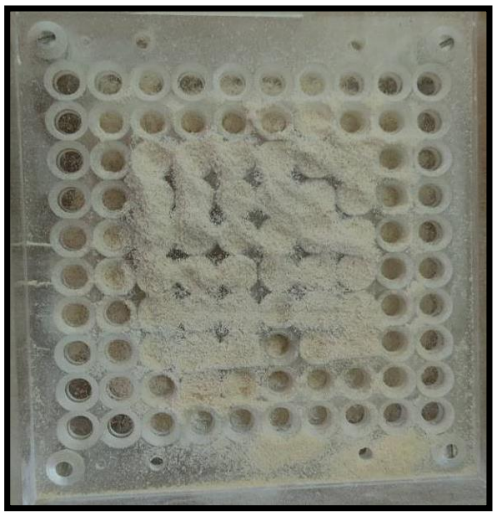
c. Spore pellet in chitosan

d. Primary powder

Plate 2. Preparation of primary powder

a. Cuboidal tray (lower portion)

b. Cuboidal tray (upper portion)



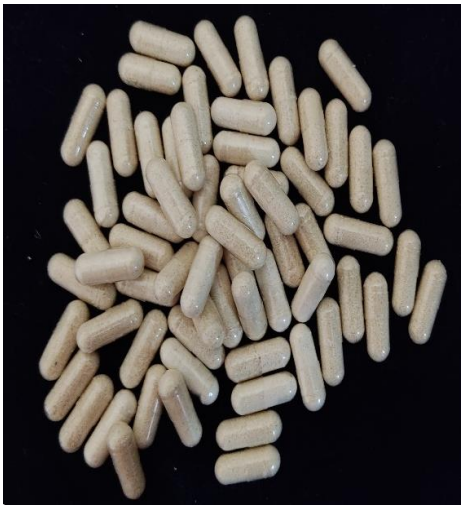
c. Placement of filler material

d. Locking the upper and lower

Plate 3. Capsule filling device

a. Assemblage of filling trays

b. Formation of capsules inside the tray



c. Capsules ready for packing

Plate 4. Filling the empty capsules

was carried out in amaranthus. The second experiment was to standardise the dose of these capsules for the management of okra fruit and shoot borer and the third experiment was to evaluate the efficacy and dose of *L. lecanii* and *L. saksenae* capsules against pea aphid in cowpea.

All the three experiments were carried out in the Instructional Farm College of Agriculture, Vellayani, during 2018-21, following the Package of Practices recommendations of Kerala Agricultural University (KAU, 2017), except for pest management. Periodic removal of weeds was followed as a cultural control measure in all the treatment as well as control plants.

3.3.1 Efficacy of Biocapsules in Managing Defoliators in Amaranthus

Seeds of KAU amaranthus variety Arun, procured from the Department of Vegetable Science, College of Agriculture, Vellayani was used for the experiment. The experimental plot was laid out in Randomized Block Design (RBD) consisting of 10 treatments replicated thrice with plot size of 1m x 1m (Plate 5a). The treatments were as follows.

T1 - 1 *Beauveria* capsule L^{-1}

T2 - 2 *Beauveria* capsule L^{-1}

T3 - 3 *Beauveria* capsule L^{-1}

T4 - 1 *Metarhizium* capsule L^{-1}

T5 - 2 *Metarhizium* capsule L^{-1}

T6 - 3 *Metarhizium* capsule L^{-1}

T7 - *Beauveria* spore suspension @ 10^8 mL^{-1} - 20 mL L^{-1}

T8 - *Metarhizium* spore suspension @ 10^8 mL^{-1} - 20g L^{-1}

T9 - Chemical check – flubendiamide 39.35 % SC (18.24 g a. i ha^{-1})

T10 - Untreated control

3.3.1.1 Method of Application of Capsules

Capsules of *B. bassiana* and *M. anisopliae* prepared as described in Para 3.2 were used in the experiment. Capsules at the respective doses were dispersed in water with 0.1% tween 80. Spraying was carried out using a knapsack sprayer. First spraying was given when 10 per cent of plants were infested and the second after one week of the first application.

3.3.1.2 Observations

Observations were made on pre and post count of larvae of the leaf roller *Spoladea (Hymenia*) *recurvalis* plant^{-1}, which was the dominant defoliator observed. For recording larval population, three plants were selected at random from each replication and the average was worked out. Incidence of other foliage and sap feeding insects observed throughout the crop period was also recorded. The number of natural enemies plot^{-1} was recorded by visual counting. Average yield plot^{-1} was noted for comparison of treatments.

3.3.2 Efficacy of biocapsules in managing borers in okra

Seeds of KAU okra variety Anjitha procured from Department of Vegetable Science, College of Agriculture, Vellayani was used for the experiment. The experimental plot was laid out in RBD of 10 treatments replicated thrice with plot size of 2m x 2m (Plate 5b). The treatments T1 to T8 and T10 were as same as those listed in Para 3.3.1. The chemical check (T9) was chlorantraniliprole 18.5 % SC (25 g a. i ha^{-1}).

3.3.2.1 Method of application

The method mentioned in 3.3.1.1 was followed

3.3.2.2 Observations

Pest population was assessed based on the number of shoots showing bore holes or wilting of the terminal shoot as well as fruits showing bore holes. For recording damaged shoots and fruits, three plants were selected randomly in each replication and average per plant was worked out. Incidence of other foliage and sap

feeding insects were observed throughout the crop period and recorded. The total number of natural enemies per plot was recorded by visual counting. Average yield per plot was noted for comparison of treatments.

3.3.3 Efficacy of biocapsules in managing sucking pests in cowpea

Seeds of KAU bush cowpea variety Bhagyalakshmi procured from Department of Vegetable Science, College of Agriculture, Vellayani were used for the experiment. The experimental plot was laid out in RBD with 10 treatments replicated thrice, with unit plot size of 2m x 2m (Plate 5c). The treatments were as follows

T1 - 1 *L. lecanii* capsule L^{-1}

T2 - 2 *L. lecanii* capsule L^{-1}

T3 - 3 *L. lecanii* capsule L^{-1}

T4 - 1 *L. saksenae* capsule L^{-1}

T5 - 2 *L. saksenae* capsule L^{-1}

T6 - 3 *L. saksenae* capsule L^{-1}

T7 - *L. lecanii* spore suspension @ 10^7 spores mL^{-1} 20 mL L^{-1}

T8 - *L. saksenae* spore suspension @ 10^7 spores mL^{-1} 20 g L^{-1}

T9 - Chemical check - Thiamethoxam 25 % WG (50 g a. i ha^{-1})

T10 - Untreated control

3.3.3.1 Method of application

The method mentioned in 3.3.1.1 was followed

3.3.3.1.2 Observations

Incidence of aphids on cowpea was recorded commencing from seventh week after planting. The population was recorded from three plants selected at random from each replication. Aphid population on 5cm long terminal twig were taken on 3rd and 7th day after treatment. Based on the intensity of infestation these twigs were

a. Layout of amaranthus field

b. Layout of okra field

c. Layout of cowpea field

Plate 5. Layout of the field experiments

classified into five classes following the method of Reji (2001). The classes were as follows.

Sl. No	Class	Description
1	zero (0)	No aphid
2	Very light (V)	From one aphid to a small colony confined to the very youngest leaves of the crown
3	Light (L)	Several aphid colonies present on the stem and not only confined to the uppermost leaves.
4	Medium (M)	Aphids present in large numbers, not in recognisable colonies but diffuse and infesting a large proportion of leaves and stem.
5	Heavy (H	Aphids present in large numbers very dense, infesting all the leaves and stem, the latter usually being black with aphids.

Collection of samples and estimation of aphid population were done following the method of Srikanth (1985). Five number of shoots in each class mentioned in the table above were collected from the experimental field. The sample shoots were cut with a sharp blade ensuring that the number of aphids falling from the shoots was reduced to a minimum. These were then put in plastic containers with provision of ample aeration and were brought to the laboratory. Each sample shoot was transferred to a white paper and were gently tapped to dislodge the aphids. The mean number of aphids (all stages) per twig in each class was recorded as follows

Class	Number of aphids sample^{-1}					Mean number of aphids class^{-1}
	1	2	3	4	5	
V	35	58	62	45	57	51.4
L	85	110	78	180	195	129.6
M	245	305	298	340	390	315.6
H	500	690	768	742	663	672.6

While taking observations from field aphid population was visually scored as 0, L, V, M and H and later substituted with the respective values for statistical analysis.

3.3.3.1.3 Incidence of other pests

Incidence of foliage and sap feeding insects were observed throughout the crop period and recorded.

3.3.3.1.4 Natural enemy population

The number of natural enemies was recorded by visual counting.

3.4 STATISTICAL ANALYSIS

The data obtained from field experiments were subjected to analysis of variance (ANOVA) using WASP 1 (Web).

Results

4. RESULTS

Results of the research work entitled "Field efficacy of biocapsules of entomopathogenic fungi for the management of vegetable pests" carried out in the Biocontrol Laboratory for Crop Pest Management, Department of Agricultural Entomology, College of Agriculture, Vellayani, Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram, during the year 2018-2021, is depicted below

4.1 FIELD EFFICACY OF BIOCAPSULES

Efficacy of biocpasules prepared from entomopathogenic fungi such as *B.bassiana*, *M.anisopliae*, *L. lecanii* and *L.saksenae* in managing the major pests of three different vegetable crops, amaranthus, okra and vegetable cowpea is detailed below.

4.1.1 Against Defoliators in Amaranthus

Efficacy of biocapsules of *Metarhizium anisopliae* (Ma4) and *Beauveria bassiana* (Bb5) was tested against the major defoliator observed in the field, the leaf webber *Spoladea* (*Hymenia) recurvalis* (Plate 6).

4.1.1.1 *Spoladea* (*Hymenia) recurvalis*

4.1.1.1.1 First spraying

Analysis of data on mean larval count (Table1) noted on third DAS (Days After Spraying) revealed that, spraying 3 capsules L^{-1} of *B. bassiana* and *M. anisopliae* was equally good in reducing the population, compared to their dosages 2 and 1 capsules L^{-1}. The mean population was 2.89 and 2.56 larvae plant $^{-1}$ in the first two treatments respectively, while the count was 3.22 and 3.0 when sprayed @ 2 capsules L^{-1} of *Beauveria* and *Metarhizium* respectively. The mean larval count was 3.56 and 3.45 $plant^{-1}$, when the dosage was reduced to 1 capsule L^{-1} of each of them respectively and their effect was on par with each other.

Among the biocontrol treatments it was the plots treated with spore suspension of both the fungi that exhibited maximum reduction in population. The mean larval

count was 2.33 and 2.00 plant^{-1} in the case of *B. bassiana* and *M. anisopliae* sprayed @ 10^8 spores mL^{-1}.

Highest reduction in population was noted in plots treated with flubendiamide 39.35 % SC@ 0.1 mL $^{-1}$, where the mean population was 1.67 larvae plant^{-1}.

After one week, there was a narrow decline in population. Among the capsule treated plots, the lowest population was recorded in plots treated with 3 capsules L^{-1} of *M. anisopliae* (2.11 larvae plant^{-1}), which was on par with the effect of the same dose of *B. bassiana* (2.39). With the lower dose of 2 capsules L^{-1}, the population was 2.56 and 2.78 respectively with *Metarhizum* and *Beauveria,* which did not differ significantly. Single capsule L^{-1} was the inferior treatment where the mean larval count recorded was 3.0 and 3.11 respectively with *Metarhizum* and *Beauveria.* Lowest larval count was recorded in spore suspension of *Metarhizium* @ 10^8 spores mL^{-1}which was closely followed by *Beauveria* @ 10^8 spores mL^{-1}. Population in flubendiamide 39.35 % SC @ 0.1 mL^{-1} treated plot was the lowest among treatments (1.11 larvae plant^{-1}).

4.1.1.1.2 Second spraying

After three days of second spraying, among the capsule treatments, the dosage 3 capsules L^{-1} of *M. anisopliae* was the most effective treatment which was statistically on par with followed by *B. bassiana* treatment @ 3 capsules L^{-1}. The mean population noted was 1.44 and 1.67 respectively. With the lower dose of 2 capsules L^{-1} the population recorded with both the fungi was on par (1.89 and 2.0 respectively). Single capsule treatment with *Metarhizium* recorded 2.5 larvae plant^{-1} which differed significantly from the corresponding dose of *Beauveria* (2.78 larvae plant^{-1}).

The mean larval count noted after 7 days was 1.34 and 1.67 plant^{-1} respectively with the dosages of 2 capsules L^{-1} of *Metarhizium* and *Beauveria* capsules respectively, which were on par with other. The corresponding larval count noted in plots treated @ 1 capsule L^{-1} was 2.22 and 2.0 plant^{-1} respectively with *Metarhizium* and *Beauveria* which were in parity with each other. Spore suspensions were found to be superior to capsules in bringing down the larval population.

Table 1. Efficacy of biocapsules in managing *Spoladea* (*Hymenia*) *recurvalis* in amaranthus

Treatments (L^{-1})	*Mean larval count $plant^{-1}$				
	First spraying			Second spraying	
	Precount	3 DAT	7 DAT	3 DAT	7 DAT
1 *Beauveria* capsule	4.22 (2.04)	3.56 ^{ab} (1.87)	3.11 ^{ab} (1.74)	2.78 ^{ab} (1.64$^{)}$	2.00 ^{ab} (1.56)
2 *Beauveria* capsules	4.11 (2.01)	3.22 ^{ab} (1.76)	2.78 ^{abc} (1.64)	2.00 ^{bc} (1.38)	1.67 ^{bc} (1.45)
3 *Beauveria* capsules	3.33 (1.76)	2.89 ^{abcd} (1.63)	2.39 ^{bcd} (1.47)	1.67 ^{cd} (1.22)	1.00 ^{de} (1.19)
1 *Metarhizium* capsule	4.22 (2.05)	3.45 ^{ab} (1.82)	3.00 ^{ab} (1.69)	2.50 ^{abc} (1.53)	2.22 ^{ab} (1.61)
2 *Metarhizium* capsules	4.00 (1.99)	3.00 ^{abc} (1.71)	2.56 ^{abc} (1.57)	1.89 ^{bc} (1.34)	1.34 ^{bcd} (1.32)
3 *Metarhizium* capsule	4.11 (2.01)	2.56 ^{abcd} (1.59)	2.11 ^{bcd} (1.44)	1.44 ^{cd} (1.19)	0.67 ^{de} (1.07)
Beauveria spore suspension @ 10^8 mL^{-1} (biocontrol check 1) - 20 mL L^{-1}	3.78 (1.93)	2.33 ^{bcd} (1.51)	1.78 ^{cde} (1.30)	0.94 ^{def} (0.93)	0.33 ^{ef} (0.90)
Metarhizium spore suspension @ 10^8 mL^{-1} (biocontrol check 2) - 20 g L^{-1}	3.89 (1.94)	2.00 ^{cd} (1.38)	1.50 ^{de} (1.17)	0.39 ^{f} (0.62)	0.00 ^{f} (0.71)
Flubendiamide 39.35 % SC (18.24 g a.i ha^{-1}) (chemical check)	4.33 (2.08)	1.67 ^{d} (1.29)	1.11 ^{e} (1.04)	0.67 ^{ef} (0.80)	0.44 ^{def} (0.97)
Untreated control	4.11 (2.02)	3.89 ^{a} (1.96)	3.67 ^{a} (1.90)	3.67 ^{a} (1.88)	3.06 ^{a} (1.87)
CD (0.05)	NS	(0.38)	(0.37)	(0.40)	(0.35)

NS - Not Significant. Values in the parentheses are square root transformed,

* Mean of three replications, DAT - Days after treatment

There was no larval population at all in the *Metarhizium* treated plots while it was negligible in *Beauveria* treated plots (0.33 larva plant^{-1}). The corresponding count in flubendiamide 39.35 % SC treated plot was 0.44 larva plant^{-1}.

4.1.1.2 Effect on natural enemy population

Table 2 reveals the mean natural enemy population in the experimental plot. The natural enemies comprised of spiders such as *Tetragnatha* sp. and *Mantis* sp. (Plate 6). Analysis of data on total count of natural enemies per plant revealed that there was no significant variation in their count before and after treatment. Their population did not vary significantly among the treatments even after two sprayings. It varied from 1.89, 2.33 to 3.89, 3.45 plot $^{-1}$ in biocontrol treatments, while it was 1.56 in plots treated with flubendiamide 39.35% SC, three DAT. At the end of the experimental period, the biocontrol plots recorded a population of 2.33 to 3.67 plot^{-1} while that in flubendiamide 39.35% SC treatment it was 1.44 plot^{-1}. The natural enemy population noted in untreated plot varied from 2.56 to 4.56 plot^{-1} during the experimental period.

4.1.1.3 Effect of biocapsules treatments on the yield of amaranthus

Analysis of data on yield recorded from 1x1 m^2 plot revealed that there was significant variation among treatments. Among the biocapsules highest yield of 2.67 kg was obtained from plots treated with *Metarhium* capsules @ 3 L^{-1}, which was significantly lower than the yield obtained from plots treated with its spore suspension (3.23 kg) @ 10^8 spores mL^{-1} as well as from plots treated with flubendiamide 39.35% SC (2.9 kg). The yield recorded from plots treated with 2 *Metarhium* capsules L^{-1} was 2.10 kg which was significantly higher than its single capsule treatment (1.20 kg) as well as from yield recorded from plots treated with 2 capsules L^{-1} of *Beauveria* (1.87 kg) and 1 capsule of *Beauveria* (1.3 kg). The yield from untreated plot was significantly lower (0.8 kg) Table 3.

Table 2. Effect of biocapsules on natural enemy population in amaranthus

Treatments (L^{-1})	No. of natural enemies $plot^{-1}$*				
	First spraying			Second spraying	
	Precount	3 DAT	7 DAT	3 DAT	7 DAT
1 *Beauveria* capsule	2.44 (1.56)	2.67 (1.78)	2.45 (1.54)	2.67 (1.62)	2.67 (1.61)
2 *Beauveria* capsules	3.11 (1.76)	2.50 (1.72)	3.05 (1.74)	2.78 (1.66)	3.67 (1.91)
3 *Beauveria* capsules	3.44 (1.85)	3.45 (1.98)	3.11 (1.75)	1.89 (1.28)	2.44 (1.52)
1 *Metarhizium* capsule	3.67 (1.91)	3.17 (1.90)	2.33 (1.52)	2.33 (1.49)	2.78 (1.64)
2 *Metarhizium* capsules	2.89 (1.68)	2.00 (1.47)	3.89 (1.96)	3.22 (1.79)	3.39 (1.83)
3 *Metarhizium* capsule	3.00 (1.73)	3.22 (1.93)	3.33 (1.81)	3.28 (1.79)	2.33 (1.52)
Beauveria spore suspension @ 10^8 mL^{-1} (biocontrol check 1) - 20 mL L^{-1}	2.39 (1.51)	2.89 (1.82)	3.22 (1.79)	3.11 (1.76)	3.61 (1.90)
Metarhizium spore suspension @ 10^8 mL^{-1} (biocontrol check 2) - 20 g L^{-1}	2.89 (1.64)	2.33 (1.66)	2.78 (1.66)	2.67 (1.63)	2.67 (1.63)
Flubendiamide 39.35 % SC (18.24 g a.i ha^{-1}) (chemical check)	1.89 (1.37)	1.56 (1.43)	2.67 (1.61)	2.17 (1.46)	1.44 (1.18)
Untreated control	2.78 (1.61)	2.56 (1.73)	4.56 (2.11)	3.89 (1.96)	3.67 (1.91)
CD (0.05)	NS	NS	NS	NS	NS

*Plot size 1m x 1 m. Mean of three replications. Figures in parentheses are square root transformed values. DAT - Days after treatment. NS - Non significant

Table 3. Effect of biocapsules on yield of amaranthus

Treatments (L^{-1})	Yield (kg $plot^{-1}$) *
1 *Beauveria* capsule	1.30^{g}
2 *Beauveria* capsules	1.87^{f}
3 *Beauveria* capsules	2.30^{d}
1 *Metarhizium* capsule	1.20^{g}
2 *Metarhizium* capsules	2.10^{e}
3 *Metarhizium* capsules	2.67^{c}
Beauveria spore suspension @ 10^{8} mL^{1} (biocontrolcheck 1) - 20 mL L^{-1}	3.10^{a}
Metarhizium spore suspension @ 10^{8} mL^{-1} (biocontrol check 2) - 20 g L^{-1}	3.23^{a}
Flubendiamide 39.35 % SC (18.24 g a.i ha^{-1}) (chemical check)	2.90^{b}
Untreated control	0.80^{h}
CD (0.05)	0.20

*Plot size 1m x 1m. Mean of three replications. Values sharing same alphabets in superscript are statistically on par based on ANOVA

a. *Spoladea* (*Hymenia*) *recurvalis* caterpillars

c. *Tetragnatha* sp.

b. *Mantis* sp.

Plate 6. Pest and natural enemies recorded in amaranthus

4.1.2 Against Borers and Defoliators in Okra

Efficacy of biocapsules of *Metarhizium* and *Beauveria* in managing the borer pests in okra was assessed based on the damage caused by the fruit and shoot borer, *Earias vitella* (Plate 7) and leaf roller *Sylpeta derogata.*

4.1.2.1 *Earias vittella*

Treatment with biocapsules in okra was evaluated based on the symptom expression by the fruit and shoot borer *Earias vittella*.

4.1.2.1.1 Based on number of infested shoots

Table 4 indicates the infestation level in terms of shoot damage.

4.1.2.1.1.1 First spraying

The mean of infested shoots plant^{-1} recorded on the day prior to treatment did not vary significantly. It was 1 to 1.73 infested shoots plant $^{-1}$.

Three days after spraying, the mean damage noted in plots with 2 and 3 capsules L^{-1} of *Beauveria,* was on par with those observed with 2 and 3 capsules L^{-1} of *Metarhizium.* It was 1.27, 1.13, 1.2 and 1.13 plant $^{-1}$ respectively. The plots treated with spore suspension of *Beauveria* @10^{8} spores mL^{-1} recorded least number of damaged shoots plant^{-1} (0.60 plant $^{-1}$), while that in *Metarhizium* spore suspension @10^{8} spores mL^{-1} was 1.0. plant$^{-1.}$ Highest shoot damage was noted in plots treated with one *Beauveria* capsules L $^{-1}$ (1.53) which was on par with the damage observed in untreated plots (1.60). The corresponding damage in plots treated with single capsule L $^{-1}$ was 1.33 plant $^{-1}$, which was significantly lower than single capsule L^{-1} of *Beauveria*.

Seven days after treatment, shoot damage recorded in plots treated with 3 capsules of *Beauveria* and 2 and 3 capsules of *Metarhizium* was on par with each other (0.4, 0.53 and 0.46 plant^{-1}respectively). Single capsule treatment with *Beauveria* and *Metarhizium* recorded a higher shoot damage of 0.8 and 0.93 plant^{-1}respectively. Spore suspension of *Metarhizium* and *Beauveria* @ 10 ^{8} spores mL^{-1} was the best treatment recording 0.1 no. of infested shoot plant^{-1}, followed by spore suspension of

a. *Earias vittella* infesting shoot

b. *Earias vittella* infesting fruit

Sylepta derogata

Plate 7. Major pests observed in okra

Beauveria 10^8 spores mL^{-1} (0.23 infested shoot $plot^{-1}$). Chlorantraniprole 18.5 % SC was inferior to *Metarhizium* spore @ 10^8 spores mL^{-1} (0.33 infested shoot $plant^{-1}$), but superior to all other treatments.

4.1.2.1.1.2 Second spraying

After the second spraying, 3 DAT minimum infestation was noted in plots treated with *Beauveria* @ 3 capsules L^{-1}, which was on par with that observed with chlorantraniprole 18.5 % SC treatment (0.27 no. of infested shoots $plant^{-1}$ in both). This was closely followed by the treatment with *Metarhizium* @ 3 capsules L^{-1} (0.33 no. of infested shoots $plant^{-1}$). In spore suspension treatment with *Beauveria* @ 10^8 spores mL^{-1} the shoot damage was 0.13, while in *Metarhizium* spore suspension @ 10^8 spores mL^{-1}, it was negligible (0.1), both being on par with each other. Treatment with 1 and 2 capsules L^{-1} of both the fungi was inferior causing 1.07 and 0. 6 number of infested shoots $plant^{-1}$ in the case of *Beauveria* and 0.73 and 0.4 infested shoots $plant^{-1}$ in the case of *Metarhizium.* The mean number of infested shoots in the untreated plot was 1.27 infested shoots $plant^{-1}$, which was the highest damage recorded at this point of time.

At the end of the experimental period seven DAT the infestation noted in plots treated with *Beauveria* @ 3 capsules was much lower (0.17 infested shoots $plant^{-1}$) and on par with that noted in plots treated with *Metarhizium* @ 3 capsules L^{-1} and 2 capsules L^{-1} (0.23 no. of infested shoots $plant^{-1}$ in both). In plots treated with 2 capsules of *Beauveria*, infestation observed was 0.4 which was superior to single capsule treatment, where the mean number of infested shoots was 0.47 in the case of *Metarhizium* capsule and 0.6 in the case of *Beauveria* capsule. In spore suspension treatment with *Metarhizium* @ 10^8 spores mL^{-1} there was 0.1 damaged shoots, while in *Beauveria* spore suspension @ 10^8 spores mL^{-1}, the mean number of damage shoots was negligible (0.07), both being on par with each other. It was 0.13 no. of infested shoots $plant^{-1}$ in chemical control and 1.0 no. of infested shoots $plant^{-1}$ in untreated control.

Table 4. Efficacy of biocapsules on shoot damage in okra by *Earias vittella*

Treatments (L^{-1})	* Mean number of infested shoots $plant^{-1}$				
	First spraying			Second spraying	
	Precount	3 DAT	7 DAT	3 DAT	7 DAT
1 *Beauveria* capsule	1.73 (1.31)	1.53 ^{a} (1.24$^{)}$	0.80 ^{bcd} (1.10)	1.07 ^{ab} (1.02)	0.60 ^{b} (1.05)
2 *Beauveria* capsules	1.53 (1.24)	1.27 ^{ab} (1.12)	0.87 ^{abc} (1.16)	0.60 ^{cd} (0.76)	0.40 ^{bcd} (0.95)
3 *Beauveria* capsules	1.13 (1.06)	1.13 ^{ab} (1.06)	0.40 ^{bcde} (0.95)	0.27 ^{efg} (0.51)	0.17 ^{de} (0.82)
1 *Metarhizium* capsule	1.53 (1.24)	1.33 ^{ab} (1.16)	0.93 ^{ab} (1.18)	0.73 ^{bc} (0.83)	0.47 ^{bc} (0.97)
2 *Metarhizium* capsules	1.47 (1.21)	1.20 ^{ab} (1.10)	0.53 ^{bcde} (1.01)	0.40 ^{cde} (0.61)	0.23 ^{de} (0.85)
3 *Metarhizium* capsule	1.13 (1.05)	1.13 ^{ab} (1.05)	0.46^{bcde} (0.97)	0.33 ^{def} (0.55)	0.23 ^{de} (0.85)
Beauveria spore suspension @ 10^{8} mL^{-1} (biocontrol check 1) - 20 mL L^{-1}	1.40 (1.17)	0.60 ^{c} (0.78)	0.23 ^{de} (0.85)	0.13 ^{fg} (0.36)	0.07 ^{e} (0.75)
Metarhizium spore suspension @ 10^{8} mL^{-1} (biocontrol check 2) - 20 g L^{-1}	1.00 (1.00)	1.00 ^{bc} (1.00)	0.10 ^{e} (0.78)	0.10 ^{g} (0.31)	0.10 ^{e} (0.78)
Chlorantraniliprole 18.5 % SC (25 g a. i ha^{-1}) (chemical check)	1.40 (1.18)	1.27 ^{ab} (1.10)	0.33 ^{cde} (0.90)	0.27 ^{efg} (0.51)	0.13 ^{e} (0.79)
Untreated control	1.73 (1.31)	1.60 ^{a} (1.27)	1.40 ^{a} (1.37)	1.27 ^{a} (1.13)	1.00 ^{a} (1.23)
CD (0.05)	NS	0.24	0.27	0.23	0.14

NS - Not Significant.Values in the parentheses are square root transformed, * Mean of three replications, DAT - Days after treatment

4.1.2.1.2 Based on number of infested fruits

Efficacy of the capsules assessed based on the number of infested fruits (bore holes) is presented in Table 5.

4.1.2.1.2.1 First Spraying

Three DAT, *Metarhizium* capsules @ 3 L^{-1} was found to be superior over all other treatments. The mean number of bore hole observed was 0.89 $plot^{-1}$ which was closely followed by the effect of *Beauveria* capsules @ 3 L^{-1} (1.44 bore holes $plant^{-1}$). Effect of 2 *Metarhizium* capsules was superior (1.99 bore holes $plant^{-1}$) to the corresponding dose of *Beauveria* capsules (2.44 bore holes $plant^{-1}$). Single capsule treatment of both the fungi was ineffective, as the number of bore holes recorded was significantly higher (3.33 and 3.0 $plant^{-1}$). Among the biocontrol check, *Metarhizium* spore suspension @ 10^8 spores mL^{-1}, was superior (1.11 bore holes $plant^{-1}$) to *Beauveria* spore suspension @ 10^8 mL^{-1} (1.55 bore holes). The number of infested fruits in chlorantraniprole 18.5 % SC (25g a.i ha^{-1}) treated plot was 1.78 $plant^{-1}$, which was significantly superior over single and double capsule treatments as well as the untreated plot (3.55 bore holes $plant^{-1}$).

The trend observed was almost similar at seven DAT where minimum number of infested fruits was noted in plots treated with *Metarhizium* capsules @ 3 L^{-1} and was found to be superior over all other treatments (0.55 bore holes $plant^{-1}$). The mean number of infested fruits noted with three capsules L^{-1} of *Beauveria* was 1.44 which was significantly lower in effect. The mean number of bore holes noted in plots treated with 2 capsules L^{-1} of *Metarhizium* and *Beauveria* was 1.33 and 2.11 shoots $plant^{-1}$respectively, which was significantly lower than the biocontrol checks with their respective spore suspensions @ 10^8 spores mL^{-1} (0.66 and 0.89 infested shoots $plant^{-1}$ respectively with *Metarhizium* and *Beauveria*). The corresponding damage noted in plots treated with chlorantraniprole 18.5% SC was 1.00 number of bore holes $plot^{-1}$. All the treatments were superior compared to the untreated control which recorded maximum number of damaged fruits (3.0 bore holes $plant^{-1}$).

4.1.2.1.2.1 Second Spraying

At three DAT also, *Metarhizium* capsules @ 3 L^{-1} was found to be superior, recording the 0.22 damaged fruit plant $^{-1}$ and was equivalent to chemical treatment with chlorantraniprole 18.5% SC, where the mean number of damaged fruit was 0.33 $plant^{-1}$. The corresponding damage noted with *Beauveria* capsules @ 3 L^{-1} was 0.66 fruit $plant^{-1}$. *Metarhizium* capsules @ 2 $L^{-1}$and *Beauveria* capsules @ 2 L^{-1} were inferior, resulting in 0.88 and 1.22 number of damaged fruits plant $^{-1}$, respectively. The spore suspension of *Metarhizium* @ 10^8 mL^{-1}, plot $^{-1}$, resulted in least fruit damage (0.11 fruit $plant^{-1}$), while that noted in the plots treated with *Beauveria* spore suspension @ 10^8 mL^{-1} was 0.55 fruit $plant^{-1}$. Maximum fruit damage was noted in the untreated plots (2.33 $plant^{-1}$).

At the end of the experimental period (seven DAT), there was no infestation at all in the plots treated with *Metarhizium* capsules @ 3 L^{-1}, which was on par with *Metarhizium* spore suspension @ 10^8 mL^{-1}. The next superior treatment was *Beauveria* capsules @ 3 L^{-1} (0.33), which was on par with its spore suspension @ 10^8 mL^{-1} (0.22 fruit $plant^{-1}$). All other treatments had significantly lower effect, where *Metarhizium* capsules @ 2 L^{-1} recorded 0.44 damage fruits. The number of damaged fruit in plots treated with *Beauveria* capsules @ 2 L^{-1} was 0.66 $plant^{-1}$, which was superior to *Metarhizium* capsules @ 1 capsule L^{-1} and *Beauveria* capsules @ 1 capsule L^{-1} (0.89 and 1.11 $plant^{-1}$, respectively). The corresponding damage noted in chlorantraniprole 18.5% SC treated plot was 0.11 fruit $plant^{-1}$. Highest fruit damage was recorded in untreated plots (1.33 fruit $plant^{-1}$).

4.1.2.2 *Sylepta derogata*

Infestation of okra leaf roller *H. derogata* was assessed based on the mean larval count (Table 6). The mean precount recorded from field was 1.22-2.11 larvae $plant^{-1}$.

4.1.2.2 .1 First spraying

Three days after first spraying no significant variations in larval count was noted among the treatments. it ranged from 0.89 to 2 caterpillars $plant^{-1}$.

Table 5. Efficacy of biocapsules on fruit damage in okra by *Earias vittella*

Treatments (L^{-1})	* Mean number of infested fruits				
	First spraying			Second spraying	
	Pre count	3 DAT	7 DAT	3 DAT	7 DAT
1 *Beauveria* capsule	3.45 (1.85)	3.33 ^{ab} (1.82)	2.33 ^{ab} (1.52)	1.77 ^{ab} (1.49)	1.11 ^{ab} (1.26)
2 *Beauveria* capsules	2.33 (1.62)	2.44 ^{bc} (1.56)	2.11 ^{bc} (1.45)	1.22 ^{bcd} (1.29)	0.66 ^{bcd} (1.07)
3 *Beauveria* capsules	3.22 (1.75)	1.44 ^{f} (1.19)	1.44 ^{cde} (1.19)	0.66 ^{cdef} (1.08)	0.33 ^{de} (0.91)
1 *Metarhizium* capsule	3.22 (1.75)	3.00 ^{ab} (1.70)	2.00 ^{bcd} (1.38)	1.44 ^{abc} (1.39)	0.89 ^{abc} (1.18)
2 *Metarhizium* capsules	2.56 (1.59)	1.99 ^{cd} (1.40)	1.33 ^{de} (1.14)	0.88 ^{cde} (1.15)	0.44 ^{cde} (0.95)
3 *Metarhizium* capsules	2.67 (1.60)	0.89 ^{f} (0.94)	0.55 ^{f} (0.73)	0.22 ^{ef} (0.84)	0.00 ^{e} (0.71)
Beauveria spore suspension @ 10^8 mL^{-1} (biocontrol check 1) - 20 mL L^{-1}	3.22 (1.75)	1.55 ^{de} (1.23)	0.89 ^{ef} (0.94)	0.55 ^{def} (1.02)	0.22 ^{de} (0.84)
Metarhizium spore suspension @ 10^8 mL^{-1} (biocontrol check 2) - 20 g L^{-1}	3.56 (1.88)	1.11 ^{ef} (1.05)	0.66 ^{f} (0.81)	0.11 ^{f} (0.78)	0.00 ^{e} (0.71)
Chlorantraniliprole 18.5 % SC (25 g a. i ha^{-1}) (chemical check)	2.56 (1.59)	1.78 ^{cde} (1.33)	1.00 ^{ef} (1.00)	0.33 ^{ef} (0.90)	0.11 ^{e} (0.78)
Untreated control	3.78 (1.95)	3.55 ^{a} (1.88)	3.00 ^{a} (1.73)	2.33 ^{a} (1.68)	1.33 ^{a} (1.34)
CD (0.05)	NS	(0.29)	(0.27)	(0.33)	(0.25)

NS - Not Significant.Values in the parentheses are square root transformed, * Mean of three replications, DAT - Days after treatment

After one week, among the biocapsule treatments *Metarhizium* capsules sprayed @ 3 capsules L^{-1} recorded the least population of 0.89 larva $plant^{-1}$, followed by the population recorded in plots treated with *Beauveria* capsules @ 3 capsules L^{-1} (1.17 larvae $plant^{-1}$). The population recorded in plots treated with 2 capsules *Metarhizium* was significantly lower (1.44 larvae $plant^{-1}$) than that recorded in plots treated with *Beauveria* @ 2 capsules L^{-1} (1.56 larvae $plant^{-1}$). Single capsule treatment was less effective with *Metarhizium* and not effective with *Beauveria* capsule (1.83 larvae $plant^{-1}$) as it was on par with the population recorded in untreated plots (2.0 larvae $plant^{-1}$). The corresponding population recorded in chlorantraniprole 18.5 % SC treated plot was 0.61 larva $plant^{-1}$.

4.1.2.2 .1 Second spraying

The population recorded after three days of second spraying varied significantly among treatments with a mean larvae population of 0.44 $plant^{-1}$ recorded with *Beauveria* capsules @ 3 L^{-1} which was on par with the chemical. This was closely followed by the larval count noted in *Metarhizium* @ 3 L^{-1} (0.66 larva $plant^{-1}$). In plots treated with 2 capsules $L^{-1,}$ *Metarhizium* was superior (0.78 larva $plant^{-1}$) to *Beauveria* (1.00 larvae $plant^{-1}$) and with 1 capsule it was 0.78 and 1.0 larvae plant-1 respectively. The larval count absolutely nil when spore suspension of *Metarhizium* @ 10^8 spores mL^{-1} was sprayed and was negligible (0.06 larva $plant^{-1}$) in plots treated with *Beauveria* spore suspension 10^8 spores mL^{-1}. In the untreated plot the population was 2.11 larvae $plant^{-1}$.

At the end of the experimental period, that is one week after second spraying, among the capsule treatments *Beauveria* @ 3 L^{-1} recorded the least larval count (0.22 $plant^{-1}$, while it was 0.33 $plant^{-1}$ in *Metarhizium* @ 3 capsule L^{-1}. The mean number of larvae recorded in plots treated with 2 capsules was significantly higher than the former (0.66 $plant^{-1}$, with *Beauveria* and 0.06 with *Metarhizium*). Single capsule treatment recorded 0.78 $plant^{-1}$ with *Metarhizium* and 1.0 $plant^{-1}$ with *Beauveria*.

There were no larvae at all in plots treated with spore suspensions of both the fungi, while it was 0.11 $plant^{-1}$ in chlorantraniprole 18.5 SC @ 2 ml L^{-1} and 1.83 $plant^{-1}$ in untreated plot.

Table 6. Efficacy of biocapsules in managing *Sylepta derogata* in okra

Treatments (L^{-1})	* Mean larval count $plant^{-1}$				
	First spraying			Second spraying	
	Pre count	3 DAT	7 DAT	3 DAT	7 DAT
1 *Beauveria* capsule	1.78 (1.30)	1.95 (1.39)	1.83 [a] (1.52)	1.56 [ab] (1.43)	1.00 [b] (1.23)
2 *Beauveria* capsules	2.11 (1.62)	1.83 (1.33)	1.56 [ab] (1.43)	1.00 [cd] (1.22)	0.66 [bcd] (1.07)
3 *Beauveria* capsules	1.55 (1.42)	1.39 (1.17)	1.17 [abcd] (1.29)	0.44 [ef] (0.97)	0.22 [de] (0.84)
1 *Metarhizium* capsule	1.33 (1.14)	1.72 (1.31)	1.61 [ab] (1.45)	1.44 [bc] (1.39)	0.78 [bc] (1.12)
2 *Metarhizium* capsules	1.66 (1.47)	1.44 (1.19)	1.44 [abc] (1.39)	0.78 [def] (1.13)	0.06 [cde] (1.13)
3 *Metarhizium* capsules	1.67 (1.22)	0.89 (0.94)	0.89 [bcd] (1.18)	0.66 [cde] (1.07)	0.33 [cde] (0.90)
Beauveria spore suspension @ 10^8 mL^{-1} (biocontrol check 1) - 20 mL L^{-1}	2.00 (1.38)	0.89 (0.94)	0.78 [cd] (1.13)	0.06 [fg] (1.23)	0.00 [e] (0.71)
Metarhizium spore suspension @ 10^8 mL^{-1} (biocontrol check 2) - 20 g L^{-1}	1.22 (1.10)	1.06 (1.00)	1.06 [bcd] (1.23)	0.00 [g] (0.71)	0.00 [e] (0.71)
Chlorantraniliprole 18.5 % SC (25 g a. i ha^{-1}) (chemical check)	1.67 (1.22)	1.00 (1.00)	0.61 [d] (1.05)	0.44 [ef] (0.97)	0.11 [e] (0.78)
Untreated control	2.00 (1.38)	2.00 (1.38)	2.00 [a] (1.56)	2.11 [a] (1.62)	1.83 [a] (1.52)
CD (0.05)	NS	NS	(0.28)	(0.25)	(0.23)

NS - Not Significant.Values in the parentheses are square root transformed, * Mean of three replications, DAT - Days after treatment

Table 7. Effect of biocapsules on natural enemy population in okra

Treatments (L^{-1})	No. of natural enemies $plot^{-1}$*				
	First spraying			Second spraying	
	Pre count	3 DAT	7 DAT	3 DAT	7 DAT
1 *Beauveria* capsule	1.56 (1.23)	1.45 (1.20)	1.22 (1.10)	1.00 (1.00)	1.33 (1.14)
2 *Beauveria* capsules	1.56 (1.23)	1.22 (1.10)	1.56 (1.24)	1.78 (1.33)	1.78 (1.33)
3 *Beauveria* capsules	1.78 (1.34)	1.78 (1.33)	1.56 (1.24)	2.22 (1.48)	2.00 (1.38)
1 *Metarhizium* capsule	1.33 (1.13)	1.33 (1.14)	1.89 (1.34)	1.56 (1.24)	1.67 (1.29)
2 *Metarhizium* capsules	0.89 (0.94)	0.89 (0.94)	1.33 (1.14)	1.56 (1.24)	1.89 (1.37)
3 *Metarhizium* capsules	1.89 (1.34)	1.56 (1.21)	2.00 (1.38)	1.78 (1.29)	1.67 (1.28)
Beauveria spore suspension @ 10^8 mL^{-1} (biocontrol check 1) - 20 mL L^{-1}	2.45 (1.57)	2.22 (1.49)	2.33 (1.52)	2.56 (1.59)	2.56 (1.60)
Metarhizium spore suspension @ 10^8 mL^{-1} (biocontrol check 2) - 20 g L^{-1}	1.56 (1.23)	1.45 (1.20)	2.11 (1.45)	2.22 (1.49)	2.11 (1.45)
Chlorantraniliprole 18.5 % SC (25 g a. i ha^{-1}) (chemical check)	2.11 (1.45)	1.56 (1.21)	1.22 (1.10)	1.22 (1.10)	1.00 (1.00)
Untreated control	1.22 (1.08)	1.11 (1.04)	1.11 (1.04)	1.67 (1.28)	2.33 (1.52)
CD (0.05)	NS	NS	NS	NS	NS

*Plot size 2 x 2 m. Mean of three replications. Figures in parentheses are square root transformed values. DAT - Days after treatment. NS - Non significant

a. *Mantis* sp.

b. *Thomius projectus*

c. *Plexippus* sp.

d. *Olios* sp.

Plate 8. Natural enemies recorded in okra field

4.1.2.3 Effect on natural enemy population

Table 7 represents the mean count of natural enemies encountered in the okra plots treated with biocapsules. The natural enemies comprised spiders such as *Plexippus* sp. and *Olios* sp. *and Thomius projectus* Tikader and *Mantis* sp. (Plate 8). Analysis of data revealed that there is no significant difference in population among the treated and untreated plots before and after treatment. It varied from 0.89 to 2.56 $plot^{-1}$ throughout the experimental period the end of the experimental period, the mean count was 1.33.to 2.56 $plot^{-1}$ in biocontrol plots, while it was 1.00 $plot^{-1}$ in chlorantraniprole 18.5 SC treated plots.

4.1.2.4 Effect of biocapsules on the yield of okra

Analysis of yield data recorded from the experimental plots revealed the average yield per plot varied from 1.92 to 2.37 kg $plot^{-1}$. There was no significant variation in yield among the treatments (Table 8).

4.1.3 Cowpea

The major sucking pests encountered was the pea aphid *Aphis craccivora* Koch (Plate 9). Other sucking pests noted were *Riptortus pedestris* F. and *Nezara virudula* L. which were less in number

4.1.3.1 *Aphis craccivora*

Table 9 depicts the aphid population in cowpea before and after spraying the biocapsules. There was no significant variation in the population among the plots before the treatment as well as after three days of treatment.

4.1.3.1.1 First Spraying

One week after spraying, the lowest population was noted in those plots treated with *L. saksenae* at 3 capsules L^{-1} (195.33) which was on par with that observed in *L. lecanii* at 3 capsules L^{-1} (215.33) as well as the chemical treatment with thiaimethoxam 25WG (180.0). The mean population noted in plots treated with one and two capsules of *L. saksenae* and *L. lecanii* was significantly higher. It was 325 and 361.67 in the case of *L. saksenae* and *L. lecanii* sprayed at 2 capsules L^{-1},

Table 8. Effect of biocapsules on the yield of okra

Sl. No	Treatments (L^{-1})	Yield (kg $plot^{-1}$) *
1	1 *Beauveria* capsule	1.94
2	2 *Beauveria* capsules	2.00
3	3 *Beauveria* capsules	2.12
4	1 *Metarhizium* capsule	1.92
5	2 *Metarhizium* capsules	2.15
6	3 *Metarhizium* capsules	2.25
7	*Beauveria* spore suspension @ 10^8 mL^{-1} (biocontrol check 1) - 20 mL L^{-1}	2.37
8	*Metarhizium* spore suspension @ 10^8 mL^{-1} (biocontrol check 2) - 20 g L^{-1}	2.22
9	Chlorantraniliprole 18.5 % SC (25 g a. i ha^{-1}) (chemical check)	2.15
10	Untreated control	1.93
	CD (0.05)	NS

*Plot size 2 x 2 m. Mean of three replications. NS – Non significant

respectively, while it was 331.33 and 382 respectively, when sprayed with single capsules of *L. saksenae* and *L. lecanii*. Population noted in plots treated with spore suspension of these fungi, were on par with each other, the values being 248.33 for *L. saksenae* and 247.33 for *L. lecanii.* Highest population was recorded in untreated plots (564.67).

4.1.3.1.2 Second Spraying

The population reduced significantly after the second spraying. Observations taken on the third day revealed that, the plots treated with 3 capsules of *L. saksenae* and *L. lecanii* was on par with each other (83.33 and 72.67 respectively). Aphid population recorded from plots treated with 2 capsules L^{-1} of *L. saksenae* was 185.67 which were significantly lower than its corresponding dose of *L. lecanii* (225.67). When single capsule L^{-1} was sprayed the population noted was higher. It was 317 with *L. saksenae* and 315 with *L. lecanii* which did not vary significantly. Population noted in plots treated with spore suspension was higher than the dose 3 capsules L^{-1}, but lower than those observed with 2 and 1 capsule L^{-1}.

At the end of the experimental period the population reduced drastically to 20.01 in *L. saksenae* 3 capsules L^{-1} and 27.33 in *L. lecanii* @ 3 capsules L^{-1}, which were on par with each other. Lower doses were not effective in reducing the population to very low levels. It was 157.00 in *L. saksenae* and 177.67 in *L. lecanii* when 2 capsules were used and the population was much higher i. e. 234.33 and 248.33 when single capsule L^{-1} was sprayed. *L. lecanii* spore suspension was better than *L. saksenae* spore suspension at 10^7 spores mL^{-1} (63.33 and 90.67). thiamethoxam 20 WG treated plots recorded lowest population (15.33).

4.1.3.2 Effect of capsules on natural enemy population in cowpea

The predatory spiders noted in cowpea ecosystem were *Hermippus* sp., *Plexippus* sp. and *Xanthogramma* sp. and the coccinellid predators were *Coccinella septumpunctata* L. (Plate 10). The total population encountered in the cowpea field is furnished in Table 10. Analysis of data revealed that, all through the

a. Aphid population infesting pod

b. Aphid population infesting shoot

Plate 9. *Aphis craccivora* infestation in cowpea field

Table 9. Efficacy of biocapsules in managing *Aphis craccivora* in cowpea

Treatments (L^{-1})	*Number of aphids 5cm $shoot^{-1}$				
	First spraying			Second spraying	
	Pre count	3 DAT	7 DAT	3 DAT	7 DAT
1 *L. lecanii* capsule	449.33 (21.19)	391.33 (19.77)	382.00 ^{b} (19.53)	315.00 ^{b} (17.70)	248.33 ^{b} (15.72)
2 *L. lecanii* capsules	493.00 (22.18)	469.67 (21.64)	361.67 ^{b} (18.97)	225.67 ^{c} (15.01)	177.67 ^{c} (13.32)
3 *L. lecanii* capsules	354.33 (18.78)	337.00 (18.31)	215.33 ^{d} (14.64)	72.67 ^{g} (8.50)	27.33 ^{f} (5.20)
1 *L. saksenae* capsule	375.00 (19.38)	345.67 (18.59)	331.33 ^{bc} (18.20)	317.00 ^{b} (17.81)	234.33 ^{b} (15.28)
2 *L. saksenae* capsules	369.33 (19.18)	355.67 (18.80)	325.00 ^{bc} (17.95)	185.67 ^{cd} (13.57)	157.00 ^{c} (12.50)
3 *L. saksenae* capsules	368.33 (19.10)	347.33 (18.56)	195.33 ^{d} (13.94)	83.33 ^{g} (9.12)	20.01 ^{f} (4.16)
L. lecanii spore suspension @ 10^{7} spores mL^{-1} - 20 mL L^{-1}	384.67 (19.44)	359.67 (18.73)	247.33 ^{cd} (15.60)	137.33 ^{e} (11.70)	63.33 ^{e} (7.87)
L. saksenae spore suspension @ 10^{7} spores mL^{-1}- 20 g L^{-1}	426.33 (20.64)	397.00 (19.90)	248.33 ^{cd} (15.65)	153.67 ^{de} (12.36)	90.67 ^{d} (9.52)
Thiamethoxam 25 % WG (50 g a. i ha^{-1})	364.33 (19.02)	342.67 (18.43)	180.00 ^{d} (13.40)	55.00 ^{g} (7.40)	15.33 ^{g} (3.88)
Untreated control	500.67 (22.38)	484.67 (22.01)	564.67 ^{a} (23.74)	549.00 ^{a} (23.43)	555.67 ^{a} (23.57)
CD (0.05)	NS	NS	2.71	1.61	1.04

NS - Not Significant. Values in the parentheses are square root transformed
DAT - Days after treatment, * Mean of three replications

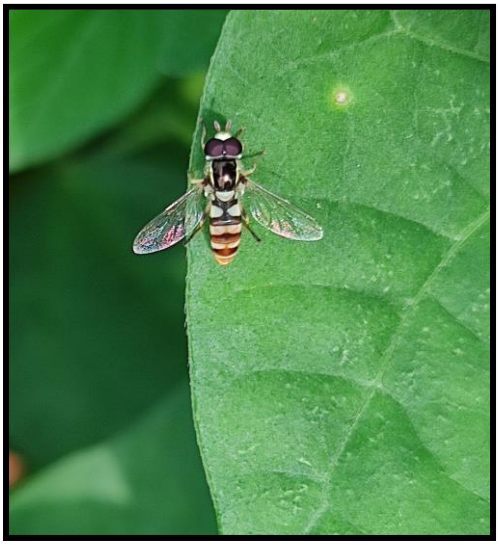
Table 10. Effect of biocapsules on natural enemy population in cowpea

Treatments (L^{-1})	No. of natural enemies $plot^{-1}$*				
	First spraying			Second spraying	
	Pre count	3 DAT	7 DAT	3 DAT	7 DAT
1 *L. lecanii* capsule	4.17 (2.27)	5.00 (2.23)	3.67 (1.88)	3.67 (1.88)	4.00 (1.96)
2 *L. lecanii* capsules	3.67 (1.88)	3.33 (1.81)	3.33 (1.72)	3.33 (1.72)	3.00 (1.65)
3 *L. lecanii* capsules	4.00 (1.96)	3.00 (1.65)	4.33 (2.08)	3.67 (1.88)	4.00 (1.96)
1 *L. saksenae* capsule	4.00 (1.98)	4.00 (1.96)	2.67 (1.55)	3.00 (1.67)	2.67 (1.55)
2 *L. saksenae* capsules	3.33 (1.74)	3.00 (1.65)	4.33 (2.07)	2.67 (1.55)	4.33 (2.07)
3 *L. saksenae* capsules	3.67 (1.90)	3.33 (1.74)	2.67 (1.55)	3.00 (1.71)	2.67 (1.55)
L. lecanii spore suspension @ 10^7 spores mL^{-1} - 20 mL L^{-1}	5.00 (2.23)	3.00 (1.62)	4.67 (2.13)	3.67 (1.81)	3.67 (1.88)
L. saksenae spore suspension @ 10^7 spores mL^{-1} - 20 g L^{-1}	3.33 (1.79)	3.33 (1.82)	4.33 (2.07)	3.33 (1.79)	3.00 (1.71)
Thiamethoxam 25 % WG (50 g a.i ha^{-1})	4.33 (2.08)	3.50 (1.87)	2.00 (1.41)	1.00 (1.00)	0.83 (0.93)
Untreated control	2.67 (1.55)	3.00 (1.65)	3.67 (1.81)	3.67 (1.88)	4.67 (2.13)
CD (0.05)	NS	NS	NS	NS	NS

*Plot size 2 x 2m. Mean of three replications. Figures in parentheses are square root transformed values. DAT - Days after treatment. NS - Non significant

a. *Hermippus* sp.

b. *Plexippus* sp.



c. *Xanthogramma* sp.

d. Coccinella septempunctata

Plate 10. Natural enemies observed in cowpea

field trial, population did not vary much among the treatments. The count varied from 0.83 to5.0 plot^{-1}.

The population deviation noted between pre count and last count revealed that, thiamethoxam 25 % WG reduced the natural enemy population by 80.83 per cent while it was unaffected in *L. lecanii*.

4.1.3.3 Effect of biocapsules on the yield of cowpea

Comparision of yield obtained from 2 m^2 of cowpea plot revealed that there was significant variation in yield from plots sprayed with various treatments. Highest yield of 1.85 kg was recorded in plots treated with *L. saksenae* @ 2 capsules L^{-1} followed by that in thiamethoxam treated plots (1.65 kg). The yield obtained from plots with *L. saksenae* @ 3 capsules was 1.56 kg which was higher than its corresponding dose of *L. lecanii* (1.29 kg) but lower than the yield obtained from plots treated with its spore suspension @10^7 spores mL^{-1} (1.59 kg). Single dose application of *L. saksenae* was superior (1.45 kg) to that of *L. lecanii* (1.0 kg). In the untreated plot the average yield recorded was significantly low (1.1kg).

The increase in yield varied from 30.63 to 66.66 per cent in *L. saksenae* treatments, while it was 16.21 to 30.63 in *L. lecanii* treatments and 48.64 per cent in chemical treatment.

Table 11. Effect of biocapsules on yield of cowpea

Treatments (L^{-1})	Yield ($kg\ plot^{-1}$) *
1 *L. lecanii* capsule	1.00^{e}
2 *L. lecanii* capsules	1.29^{cde}
3 *L. lecanii* capsules	1.45^{bcd}
1 *L. saksenae* capsule	1.45^{bcd}
2 *L. saksenae* capsules	1.85^{a}
3 *L. saksenae* capsules	1.56^{abc}
L. lecanii spore suspension @ 10^7 spores mL^{-1} - 20 mL L^{-1}	1.41^{bcd}
L. saksenae spore suspension @ 10^7 spores mL^{-1} - 20 g L^{-1}	1.59^{abc}
Thiamethoxam 25 % WG (50 g a.i ha^{-1})	1.65^{ab}
Untreated control	1.11^{e}
CD (0.05)	0.37

*Plot size 2m x 2m. Mean of three replications. Values sharing same alphabets in superscript are statistically on par based on ANOVA

Discussion

5. DISCUSSION

Microbial pesticides based on entomopathogenic fungi have become the topmost commercially exploited biopesticides in the generation of chemical free farming and sustainable development. Utilization of entomopathogenic fungi (EPF) is gaining popularity in biological control, because of greater food safety concerns and environmental awareness. Their products are particularly target-specific, advantageous and recommendable, but very much challenging to develop a formulation that can retain viability during long time storage. Furthermore, the conventional microbial formulations fail to deliver its expected vigour at the target site.

Capsule is a stable formulation wherein the bioagent is encapsulated and thus protected from extreme environmental conditions such as UV radiation, rain and temperature. Possibility of getting contaminated is also meager as the infective propagules are encapsulated. It is a smart formulation with slow release and reduced bulkiness in storage. Easiness in transport and less hazardous nature to the user are some other added advantages of capsules. Their validation for management of vegetable pests is therefore an impetus in IPM programmes. The results of the research work entitled “Field efficacy of biocapsules of entomopathogenic fungi for the management of vegetable pests” are discussed below.

The fungi used were *Beauveria bassiana* (Balsamo) Vuillemin (Bb5), *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ma4), and *Lecanicillium lecanii* (Zimmermann) Zare and Gams (Vl8) which were originally sourced from National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru and the indigenous isolate ITCC Lsvs1 -7714 of *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno, isolated and characterised by (Reji *et al*. 2015). The spore load maintained in the capsules was 10^{10} spores mL^{-1}, which is 100 times the normal infective dose worked out by previous researchers. The protocol developed by Remya and Reji (2020a) for preparation of fungal based biocapsules was followed for preparing capsules. These were 100 per cent organic and biodegradable as the coating material

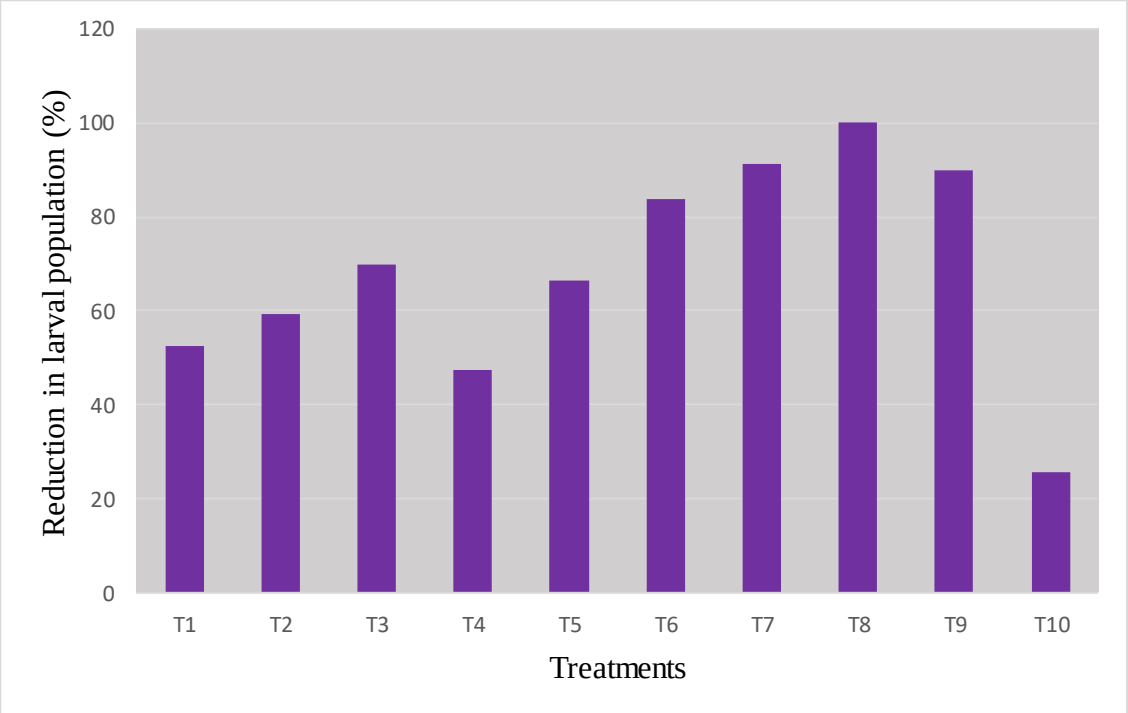
standardised by them was cellulose (HPMC-Hydroxy Propyl Methyl Cellulose) and the carrier material selected for slow release was chitosan, the natural biopolymer obtained from shell of crustaceans.

The existing formulations available in the market have a spore load of 10^8. Capsule being a smart formulation which is concise and precise, the spore load was increased several times with a view to reduce bulkiness as well as to ensure sufficient quantity of inoculam in the targeted area. With this back ground, three sets of experiments were laid out in the field to test its efficacy in managing the major pests of vegetables. They were (1) Efficacy of biocapsules in managing defoliators in amaranthus (2) Efficacy of biocapsules in managing borers in okra and (3) Efficacy of biocapsules in managing sucking pests in cowpea.

In the first experiment, the major pest observed was the leaf webber *Spoladea* (*Hymenia) recurvalis* F. the destructive defoliator pest of amaranthus. Results of this experiment revealed that spraying of *Metarhizium* capsules @ 3 L^{-1} was more effective, causing 83.69 per cent reduction in the population of larvae (Fig.1) than *Beauveria* capsules @ 3 L^{-1} (69.97 per cent). Two and one capsules of *Metarhizium* and *Beauveria* caused 66.5, 47.39, 59.36 and 52.60 per cent reduction in larval population, respectively.

Although evaluation of capsule formulations of entomopathogenic fungi for vegetable pests is the first of its kind, various researchers have proved by now, the efficacy of *B. bassiana* and *M. anisopliae* in managing *S. recurvalis* using spore suspensions and talc based formulations.

In a field experiment conducted by James *et al*. (2006) it was observed that *B. bassiana* (isolate Bba 5644) conidial suspension @ $1x10^8$ mL^{-1} caused 100 per cent mortality of the leaf webber *S. recurvalis*, where 83 per cent dead larvae showed fungal sporulation, while the isolates Bba5653 and Bba5654 caused 97 per cent mortality each and 33 per cent of the dead larvae manifested the sporulation. They also reported that, Bba 5644 was virulent to larvae of *P. basalis* larvae resulting in 100 per cent mortality of larvae within five days. In a similar study conducted by Pooru (2015) there was 100 per cent cessation of movement of *S. recurvalis* larvae



T1 - *Beauveria* capsule @ 1 L^{-1}

T2 - *Beauveria* capsule @ 2 L^{-1}

T3 - Beauveria capsule @ 3 L^{-1}

T4 - *Metarhizium* capsule @ 1 L^{-1}

T5 - *Metarhizium* capsule@ 2 L^{-1}

T6 - *Metarhizium* capsule @ 3 L^{-1}

T7 - *Beauveria* spore suspension @ 10^8 mL^{-1}

T8 - *Metarhizium* spore suspension @ 10^8 mL^{-1}

T9 - Flubendiamide 39.35 SC (18.24 g a.i ha^{-1})

T10 - Untreated control

Fig .1 Effect of biocapsules in managing *Spoladea recurvalis* in amaranthus

72h after treatment when *B. bassiana* was sprayed @ 10^8 CFU g^{-1} with both the doses, 10g and 20g L^{-1}.

Praveena (2016) while studying the efficacy of the same isolates used in the present study *viz.* Bb5 and Ma4, reported that the efficacy of the indigenous isolate SP 11 of *M. anisopliae* (from Vellayani, Kerala, India) was superior as it reported the lowest number of plants infested by the webbers, number of webbings $plant^{-1}$ and number of larvae web^{-1}, 14 days after treatment, The mortality reported by her on *S. recurvalis* was 63.33 to 100 per cent under laboratory conditions. In her study, Bb 5 and Ma 4 @ 1 x 10^9 spores mL^{-1} caused 46.66 and 100 per cent mortality to *S. recurvalis* larvae respectively, seven days after treatment.

In concurrence to the present study, Miller (2019) reported that *M. anisopliae* (isolate ICIPE 30) @ $1x10^8$ mL^{-1} caused 92 per cent larval mortality in the second instar larvae of *S. recurvalis* after 4.8 days of spraying which was more effective than *B. bassiana* (isolate ICIPE 725) that caused 83 per cent mortality, seven days after treatment.

Efficacy of flubendiamide 39.5% SC the chemical check used in this study is a proven insecticide for the management of defoliators of leafy vegetables such as amaranth and cabbage. Muralikrishna *et al*. (2019) observed 100 per cent mortality of second instar larvae *of S. recurvalis* 36 h after treatment in amaranthus. So also, Sambathkumar (2020) reported that flubendiamide 39.5% SC (0.1 mL L^{-1}) caused 100 per cent mortality in cabbage leaf webber, *Crocidolomia binotalis* Zeller 96 h after treatment.

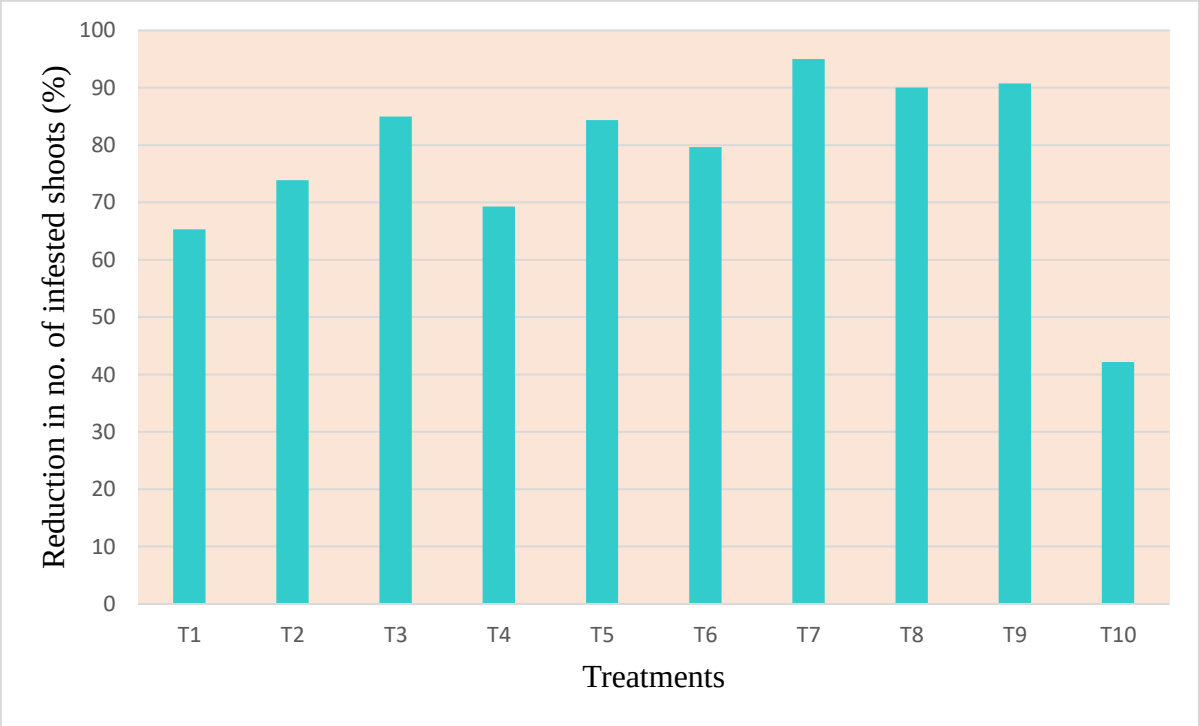
Even though precisely targeted formulations of flubendiamide are expected to be safe for non-target organisms, several recent studies have shown its toxic potential on many non-target organisms. In a study by Sarkar *et al*. (2014) it was observed that treatment concentrations (0.5, 1, 2 5, 10, and 20 $\mu g ml^{-1}$) of flubendiamide, inhibited acetylcholinesterase activity in third instar larvae of *Drosophila melanogaster* Meigen indicating its neurotoxic potential. In addition, larvae exposed to flubendiamide also manifested increased amounts of stress protein hsp 70. The larvae expressing such stress response when allowed to emerge as adults displayed severe eye structure

deformities as per scanning electron microscopic images.

Another study by Yan *et al*. (2014), reported that extensive application of flubendiamide has led to increasingly prominent resistance in diamondback moth, *Plutella xylostella* (L.), where they detected a point mutation (G4946E) that caused flubendiamide resistance in it. Acute and joint toxicity of flubendiamide was reported by Wei *et al*. (2014) on Chinese tiger frog *Hoplobatrachus chinensis* Wiegmann tadpoles. Furthermore, alterations in protein metabolism of fresh water fish *Labeorohita* F. Hamilton have been reported by Nirmalakallagadda and Rathnamma (2014). Disruption of enzyme activity in tropical soil after flubendiamide application was reported by (Shrinivas and David, 2015). Consistently, Liu *et al*., 2017 reported that exposure to flubendiamide could cause oxidative stress and DNA damage in earthworms *Eisenia fetida* Savigny. Chronic flubendiamide exposure could induce oxidative stress in water buffalo *Bubalus bubalis* L. calves (Ranjan *et al*. 2018).

These findings not only provide enough scientific evidences for flubendiamide toxicity to non-target organisms, but also its undesirable attributes with an ecological perspective and furthermore, its fate as serious environmental pollutant. Hence, safer alternatives such as biopesticides should be the prime IPM tool, though the diamide insecticides could cause 100 per cent kill of lepidopteran pests of vegetable crops.

In the experiment to test the efficacy of biocapsules in managing borers in okra, it was revealed that spraying of *Beauveria* capsule @ 3 L^{-1} and *Metarhizium* capsule @ 2 L^{-1} were equally effective, causing 84.96 and 84.35 per cent reduction in the shoot damage by *Earias vittella* (F.) respectively, (Fig. 2). On comparison of fruit damage caused in different treatments. spraying of *Metarhizium* capsule @ 3 L^{-1} was more effective causing 100 per cent reduction (Fig. 3), which was on par with *Beauveria* capsule @ 3 L^{-1} (89.75). The lower dose of two capsules of *Metarhizium* and *Beauveria* resulted in 82.81 and 71.67 per cent reduction respectively. While with low doses of capsule, it was 72.36 and 67.82 per cent respectively, revealing that the quantity of inoculam plays an important role in the viruelnce of these fungi.



T1- *Beauveria* capsule @ 1 L^{-1}`

T2 - *Beauveria* capsule @ 2 L^{-1}

T3 - Beauveria capsule @ 3 L^{-1}

T4 - *Metarhizium* capsule @ 1 L^{-1}

T5 - *Metarhizium* capsule@ 2 L^{-1}

T6 - *Metarhizium* capsule @ 3 L^{-1}

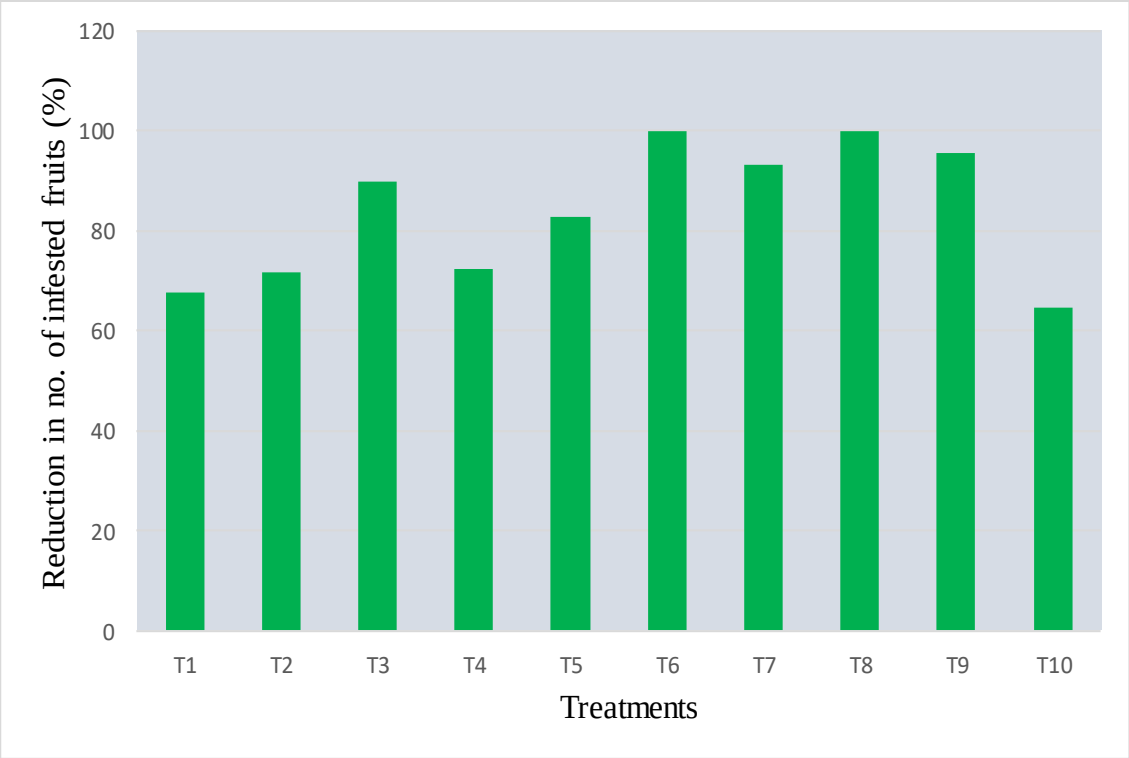
T7 - *Beauveria* spore suspension @ 10^8 mL^{-1}

T8 – *Metarhizium* spore suspension @ 10^8 mL^{-1}

T9 - Chlorantraniliprole 18.5 SC (25 g a. i ha^{-1})

T10 - Untreated control

Fig. 2 Effect of biocapsules on shoot damage by *Earias vittella* in okra



T1 - *Beauveria* capsule @ 1 L^{-1}

T2 - *Beauveria* capsule @ 2 L^{-1}

T3 - Beauveria capsule @ 3 L^{-1}

T4 - *Metarhizium* capsule @ 1 L^{-1}

T5 - *Metarhizium* capsule@ 2 L^{-1}

T6 - *Metarhizium* capsule @ 3 L^{-1}

T7 - *Beauveria* spore suspension @ 10^8 mL^{-1}

T8 - *Metarhizium* spore suspension @ 10^8 mL^{-1}

T9 - Chlorantraniliprole 18.5 SC (25 g a. i ha^{-1})

T10 - Untreated control

Fig. 3 Effect of biocapsules on fruit damage by *Earias vittella* in okra

Unformulated spore suspension of *Metarhizium* caused 90.0 per cent reduction in damage, while that with *Beauveria* was 95 per cent. So also, the reduction in fruit damage was 100 and 93.16 respectively. Formulation of a living entity usually tends to reduce the viability of conidia, but here, in the encapsulation technique where the conidia is protected from desiccation and contamination has very much reduced the deterioration was very much reduced.

Efficacy of *B. bassiana* in managing *E. vittella* was earlier reported by numerous researchers. Manimekalai *et al*. (2010) could obtain 72.36 per cent mortality of second instar larvae, 72 h after treatment in a bioassay conducted using the conidial suspension @ 2.1 x 10^7 (4 gL^{-1}). Susceptibility decreased with increase in age of the larvae and required higher concentrations to kill them. Karthikeyan and Selvanarayanan (2011) studied the bioefficacy of liquid formulation of *B. bassiana* on *E. vittella* under laboratory conditions and found that 0.25 per cent was more effective (73.33 per cent mortality) than 0.15 and 0.20 per cent. In a field study conducted by Nayak *et al*. (2012) the formulation named Daman of *B. bassiana* when applied @ 1 kg ha^{-1} at 50, 60, 70, 80 and 90 days after sowing resulted in less fruit damage. A field study by Rajput and Tayde (2017) reported that *B. bassiana* 8.08% @ 2000 g ha^{-1} recorded lowest infestation (18.58) of shoot and fruit borer after 14 days of treatment. Similary, Panbude *et al*. (2019) conducted a field experiment which concluded that spraying the conidial suspension of *B. bassiana* @ 1×10^8 spores mL^{-1} showed less (23.68 per cent) infestation when compared to that in untreated plots (34.68 per cent infestation) 14 days after spraying.

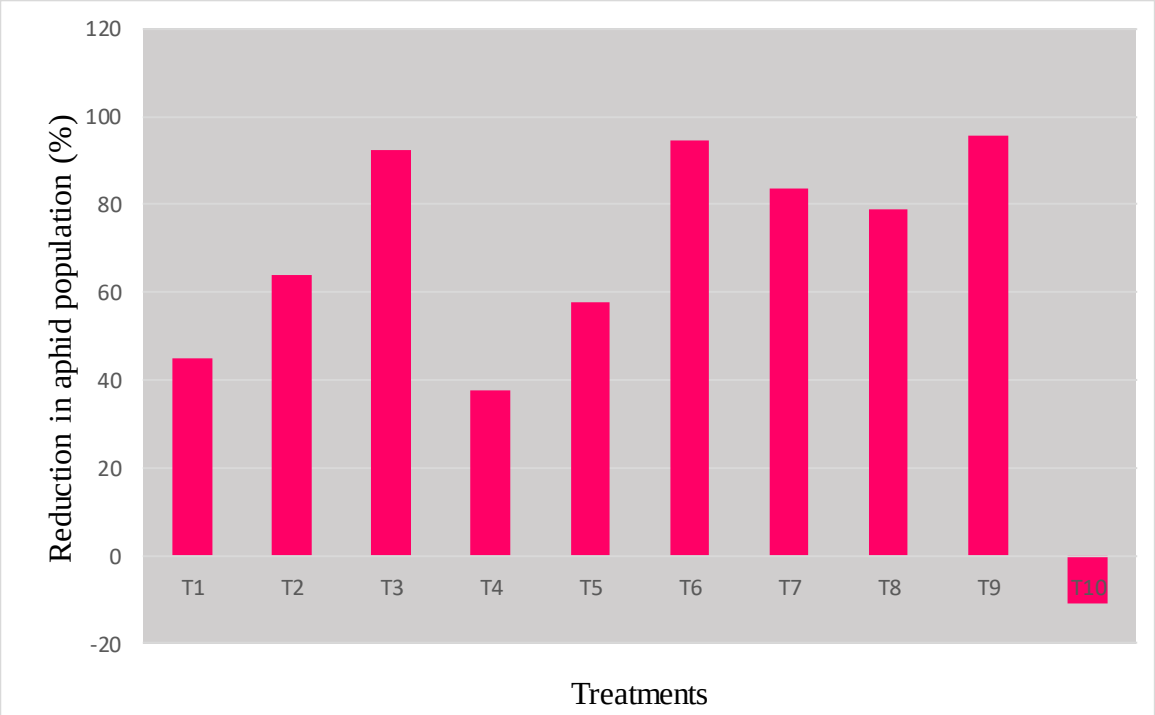
The efficacy of *M. anisopliae* in managing *E. vittella*, observed in this study is supported by the report of Nalini and Kumar (2016), which states that application of its *M. anisopliae* formulation @ 4 g L^{-1} resulted in 12.41 per cent fruit infestation when compared to that in untreated plots (33.72 per cent), 10 days after spraying. Panbude *et al*. (2019) conducted a field experiment which concluded that spraying the conidial suspension of *M. anisopliae* @ 1×10^8 spores mL^{-1} showed less (22.05) infestation when compared to that in untreated plots (34.68 per cent), 14 days after spraying. Likewise, Kaveri and Kumar (2020) observed that *M. anisopliae* @ 5gm L^{-1} resulted in only 17.34 per cent fruit infestation by *E. vittella* in okra 14 DAS, while it

was 24.60 in untreated plots. A field study by Mulani *et al*. (2021) observed that *M. anisopliae* Bio-magic and *B. bassiana* (Biosoft) 4 g L^{-1} resulted in 8.34, 2.89 per cent shoot infestation when compared to that in untreated plots (13.58), 14 days after spraying.

In this study it was observed that spraying chlorantraniprole 18.5 SC @ 2mL^{-1} (ai ha $^{-1}$) resulted in 90.71 per cent reduction in shoot damage and 95.70 per cent in fruit damage, seven days after spraying. It is notable that this green labelled insecticide, which is widely recommended for the management of borer pests in vegetable crops has adverse effects on non target organisms such as predators, parasites and pollinators. Vijayasree *et al*. (2015) reported its residue while spraying single and double doses (30 and 60 a. i. ha^{-1}) as 0.48 and 0.91 mg kg^{-1} with a halflife of 1.70 days.

Hussain *et al*. (2012) found that, chlorantraniliprole was intermediately toxic to the predator *Chrysoperla zastrowi sillemi* Esben-Petersen larvae, while Sabry *et al*. (2014) observed that chlorantraniliprole @ 36.5, 4.7 and 43.7 ppm caused 40, 86.7 and 36.7 per cent mortality to the second instar larvae of *C. carnea*, *Coccinella septempunctata* (L.), and the larvae of the egg parasitoid, *Trichogramma evanescens* Westwood. Moreover, Smagghe *et al*. (2013) reported that pollen exposure to relevant environmental concentrations of chlorantraniliprole 7 mgL^{-1}**,** affected bumble bee behaviour *Bombus terrestris* (L.). Chlorantranilprole being a new generation green labelled insecticide widely recommended for borer pests in vegetables and other crops pose increased chances of developing resistance in insect pests. Impact of indiscriminate use of insecticides, eventhough green labelled, could be harmful to the environment as well as to the non target organisms.

Third experiment which tested the efficacy of capsules of *L. lecanii* (Vl8) and *L. saksenae* (ITCC 7714) revealed that foliar application of *L. saksenae* capsule @ 3 L^{-1} and *L. lecanii* capsule @ 3 L^{-1} were equally effective causing 94.38 and 92.28 per cent reduction in the population of *A. craccivora* in cowpea (Fig 4). Lower efficacy recorded with 1 capsule L^{-1} (37.51 and 44.73 per cent reduction) of these fungi



T1 - *L. lecanii* capsule @ 1 L^{-1}

T2 - *L. lecanii* capsule @ 2 L^{-1}

T3 - *L. lecanii* capsule @ 3 L^{-1}

T4 - *L. saksenae* capsule @ 1 L^{-1}

T5 - *L. saksenae* capsule @ 2 L^{-1}

T6 - *L. saksenae* capsule @ 3 L^{-1}

T7 - *L. lecanii* spore suspension @ 10^7 mL^{-1}

T8 - *L. saksenae* spore suspension @ 10^7 mL^{-1}

T9 - Thiamethoxam 25 WG (50 g a.i ha^{-1})

T10 - Untreated control

Fig 4. Effect of biocapsules in managing *Aphis craccivora* in cowpea

compared to its higher doses, is attributed to the reduced probability of aphids acquiring a lethal dose of conidia from foliar spray or from the treated leaf surfaces.

It is notable that there was an increase in yield over all other treatments, which may be attributed to the endophytic nature of the isolate as reported by Divyasree (2019). The insecticidal, nematicidal and antimicrobial metabolites produced by this fungus as reported by Sreeja and Reji (2019) might have contributed to the yield.

This geographical isolate of *L. saksenae*, ITCC 7714 from soils of Vellayani was reported to be pathogenic to various sucking pests of vegetables (Reji *et al*. 2015) and rice (Sankar and Reji, 2018). It was proved to be an ideal candidate in IPM as it was compatible with many of the new generation insecticides (Keerthana, 2019). The same isolate has been reported to be effective to *A. craccivora* causing seven per cent increase in yield of cowpea (AICRP- BCCP Annual report, 2020).

Pathogenicity of *L. lecanii* is a well known fact across the world. It is the most widely used species of *Lecanicillium* reported to be effective to sucking pests of various crop pests. In a laboratory study by Nirmala *et al*. (2006) *L. lecanii* VI-l, reported high mortality of 80.80 per cent in *A. craccivora* @ $1x10^7$ spores mL^{-1}. Vu *et al*. (2007) found that *L. lecanii* 41185 @ $1x10^7$ conidial mL^{-1} showed the highest pathogenicity for *M. persicae* and *Aphis gossypii* Glover, with 100 per cent, reduction five and two days after treatment, respectively. Suresh *et al*. (2012) reported 71.62 per cent suppression of *A.craccivora* under field conditions, when the isolate VL-3 isolate @ $1x10^9$ spores mL^{-1} was given as foliar spray.

Ramanujam *et al*. (2017) reported that Vl 8 was more effective to Vl 12 and Vl32, as their oil formulations @ 1×10^8 spores mL^{-1} given as foliar spray suppressed *A. craccivora* population by 80.05, 65.88 and 66.83 per cent respectively. Nithya and Reji (2019) while testing the oil formulation of *L. lecanni* @ 1 x 10^8 spores mL^{-1} reported that spraying chitin based oil formulation of Vl8 could result in 99 per cent reduction in population of *A. craccivora* in cowpea field, after two sprayings carried out at fortnight intervals. In a laboratory study by Reddy and Sahotra (2020) *L. lecanii*

@ higher concentration of 10^9 spores mL^{-1} caused 93.33 per cent mortality of *A. craccivora* within eight days of treatment.

Thiamethoxam is a neonicotinoid insecticide belonging to thianicotinyl compounds and is recommended for sucking pests with moderate level of toxicity (blue labelled). It is effective to sucking pest such as aphids, rice hoppers, rice bugs and mealy bugs under laboratory as well as field trials Senn *et al*. (1998).

In the present study the percentage reduction noticed in aphid population was 95.79, when sprayed with thiamethoxam 25 WG (50 a. i ha $^{-1}$) @ 2g L^{-1}. Its efficacy in managing aphids were reported by several workers (Jyothsna *et al*., 2012; Aioub *et al*. 2015; Choudhary *et al*., 2017 and Patil *et al*., 2018) worldwide, causing 60 to 100 per cent mortality. Due to its high efficacy, it has been widely used by the growers and therefore various studies have indicated the developed resistance in a wide range of insect pests.

Residual toxicity of 356.03 and 302.10 was reported by Patil *et al*. (2018) at 24, and 48 h after treatment in *A. craccivora* with toxicity period of 11 and 12 days respectively. The mechanism of the resistance of insects to neonicotinoids is currently thought to be caused by the increased activity of MFOs (Rauch and Nauen, 2003).

Nasreen *et al*. (2007) found that thiamethoxam was moderately harmful to *C. carnea* larvae at lower concentration, whereas it was toxic at recommended and higher concentrations. Lundgren (2009) reported that thiamethoxam can harm beneficial predators *Orius insidiosus* Say, *Nabis americoferus* Carayon, by reducing survival, decreasing development rates, and reducing fecundity. Seagraves and Lundgren (2012) reported that the generalist predator community in the soybean foliage was reduced approximately by 25 per cent by thiamethoxam treatment. He *et al*. (2012) proved that systemic application of the field recommended concentration of thiamethoxam resulted in 100 per cent death of *Serangium japonicum* Chapin, predator of *B. tabaci*, within 24 h after treatment. Yao *et al*. (2015) also found that thiamethoxam is highly toxic to *S. japonicum* regardless of exposure route.

The safety of entomopathogenic fungi to the beneficial insects and natural enemies was earlier elucidated by several workers, worldwide. Lacey *et al*. (1997) opined that, the entomopathogenic fungi considered for pest management are unlikely to constitute a risk to beneficial insects and other non-target organisms, unless those organisms are very closely related to the target pest.

A commercially formulated isolate of *B. bassiana* (Naturalis) was reported to be extremely efficient in controlling a number of greenhouse pests, such as white flies, thrips and mites, with no impact on beneficial insects (Wright & Kennedy, 1996). Likewise, Butt *et al*. (1998) reported that there was no detrimental effect to the honey bees when treated by *M. anisopliae*. Askary and Brodeur (1999) demonstrated that *L. lecanii* is pathogenic to the aphid parasitoid *Aphidius nigripes* Ashmead only when aphid populations are heavily infected by the fungus.

Thungrabeab and Tongma (2007) reported that *B. bassiana* 1×10^8 conidia mL^{-1} isolate (Bb.5335) was non-pathogenic to natural enemies *C. zastrowi sillemi* and *Dicyphus tamaninii* Wagner. In a field study by Kpindou *et al*. (2013) it was revealed that *M. anisopliae* isolate (Met 31) and *B. bassiana* (isolate Bb 11) were least harmful biopesticides to natural enemies.

Singh *et al*. (2021) reported that the population of *C. undecimpunctata*, *Cheilomenes sexmaculatua* F. and *Scymnus* sp. was high in fields treated by myco-formulations of *B. bassiana* Biopower and *M. anisopliae viz*., Bio-Protector @10^8 CFU g^{-1} when compared to imidachloprid treated plot.

Therefore, it is concluded that biocapsules of *Metarhizium* and *Beauveria,* can effectively manage defoliators in amaranthus and borers in okra and those of *L. lecanii* and *L. saksenae* can be recommended for pea aphids, without affecting the natural enemies and yield significantly.

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Summary

6. SUMMARY

The present study entitled “Field efficacy of biocapsules of entomopathogenic fungi for the management of vegetable pests” was carried out at the Biocontrol Laboratory for Crop Pest Management, Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, during the year 2018-2021. The investigation was focused on validating the efficacy of biocapsules of entomopathogenic fungi and standardizing their dosage for the management of defoliators, borers and sucking pests of vegetables.

The entomopathogenic fungi selected for biocapsules preparation were *Beauveria bassiana* (Balsamo) Vuillemin isolate Bb5, *Metarhizium anisopliae* (Metschnikoff) Sorokin isolate Ma4, and *Lecanicillium lecanii* (Zimmermann) Zare and Gams isolate Vl8 and the indigenous isolate ITCC Lsvs-1 7714 of *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno. The capsules were prepared following the protocol developed by Remya and Reji (2018). They were prepared with HPMC as the coating material and chitosan as the carrier with a spore load of 10^{10} spores mL^{-1}. Three separate field experiments were carried out for testing the efficacy of biocapsules Efficacy of biocapsules in managing defoliators in amaranthus (2) Efficacy of biocapsules in managing borers in okra and (3) Efficacy of biocapsules in managing sucking pests in cowpea.

In each experiment three doses of capsules were tested *viz*. 1 capsule L^{-1}, 2 capsules L^{-1} and 3 capsules L^{-1}, which were given as foliar spray by dispersing the contents in water and spraying them at five per cent level of infestation. Second spraying was given after a fortnight.

In the first field experiment to evaluate the efficacy of biocapsules in managing defoliators in amaranthus, the observations were recorded on the mean larval count before and after treatment at three and seven days after treatment, The natural enemy population and total yield per plot was also noted treatment wise.

The results revealed that, *Metarhizium* and *Beauveria* capsules @ 3 L^{-1} sprayed twice (at weekly intervals) was effective causing 83.69 and 69.97 per cent reduction in population of *Spoladea recurvalis* F. respectively. Lower doses of 2 and 1 capsules L^{-1} were less effective causing, 47.39 to 66.5 per cent reduction. Spraying spore suspensions of these fungi @ 10^8 mL^{-1} were very much effective, causing 91.27 to 100 per cent reduction, while in flubendiamide 39.35 SC, it was 89.84 per cent. Treatment with *Metarhizium* and *Beauveria* capsules did not affect the natural enemy population significantly, the mean population being, 2.33 to 3.67 $plant^{-1}$. The corresponding population was 1.44 in flubendiamide 39.5 SC and 3.67 in untreated control. The yield recorded in the plots treated with *Metarhizium* and *Beauveria* capsules @ 3 L^{-1} was high (2.67 and 2.30 kg $plot^{-1}$) when compared to that in untreated plot (0.80 kg $plot^{-1}$).

In the second experiment to evaluate the efficacy of biocapsules to okra fruit and shoot borer *Earias vitella*. F. the mean number of infested shoots and fruits were assessed at three and seven days after each of the sprayings. Total count of natural enemies and the cumulative yield per plot were also recorded.

Results of the experiment concluded that, *Beauveria* capsule @ 3 L^{-1} and *Metarhizium* capsule @ 3 L^{-1} were equally effective when sprayed at weekly intervals leading to 84.96 and 79.64 per cent reduction in the shoot damage, respectively. The percentage reduction in shoot damage was only 65.32 to 73.86 per cent in lower doses of capsules. In plots treated with spore suspensions, the mean shoot damage recorded was 90 to 95 per cent. Percentage reduction in chlorantraniliprole 18.5 SC was 90.71. Considering the fruit damage, *Metarhizium* capsule @ 3 L^{-1} was found to be the best treatment causing 100 per cent reduction in damage caused by *E. vittella*, while it was 89.75 per cent with *Beauveria* capsule @ 3 L^{-1}. Reduction in fruit damage ranged from 67.82 to 82.81 per cent in the lower doses of capsules. Highest reduction in the fruit damage observed with spore suspensions of *Beauveria* and *Metarhizium* @ 10^8 mL^{-1} (93.16 and 100 per cent, respectively).

Similar results were obtained in the case of okra leaf roller, where *Metarhizium* and *Beauveria* capsule @ 3 L^{-1} were found to cause 80.23 to 96.38 per

cent reduction in population, while with lower doses it ranged from 41.35 to 68.72 per cent. Hundred per cent reduction was noted with spore suspensions of *Beauveria* and *Metarhizium* @ 10^8 mL^{-1}, while in chlorantraniliprole 18.5 % SC it was 93.41 per cent. Treatment with biocapsules capsules did not cause any adverse affect on natural enemy population, in okra field. The yield obtained from different treatments did not vary significantly.

In order to test the efficacy of biocpsules in managing sucking pests in cowpea, *A. craccivora*, the major pest was selected as the test insect. The population of aphids in the 5 cm terminal twig was assessed by scoring them under different classes such as 0, V, L, M and H, and later substituting the corresponding values which were prefixed by counting 10 number of shoots and calculating their mean.

Analysis of data revealed that, foliar application of *L. saksenae* capsule @ 3 L^{-1} and *L. lecanii* capsule @ 3 L^{-1} were equally effective to *A. craccivora* sprayed twice (at weekly intervals) causing 94.38 and 92.28 per cent reduction in the population respectively. Reduction in population noted was 57.54 to 63.96 per cent with lower dose @ 2 capsules L^{-1}, while it was least with single capsule treatment (37.51 to 44.73 per cent). The spore suspensions were more effective than the lower doses (78.73 - 83.53 per cent reduction). The chemical check thiamethoxam 25 WG recorded 95.79 per cent reduction in population. Biocapsule treatment did not affect natural enemy population significantly. The yield recorded in the plots with *L. saksenae* capsules @ 2 and 3 L^{-1} was high (1.85 and 1.56 kg $plot^{-1}$) when compared to other treated plots and untreated plots (1 - 1.45 kg $plot^{-1}$).

The salient findings of the investigation are

- Biocapsules of *Metarhizium* and *Beauveria* @ 3 capsules L^{-1}, were effective in managing the defoliators *S. recurvalis* in amaranthus and *S. derogata* in okra @ 3 capsules L^{-1} causing (69.97 to 85.80) per cent reduction in damage caused by these pests.
- Both the capsules were equally effective @ 3 capsules L^{-1} to fruit and shoot borer, *E. vittella* in okra.

- Biocapsules of *L. lecanii* and *L. saksenae* @ 3 capsules L^{-1} were effective in managing cowpea aphid *A. craccivora*.
- None of the biocaspule treatments had any adverse effect on natural enemy population.
- *L. saksenae* treatment increased the yield in cowpea significantly.

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FIELD EFFICACY OF BIOCAPSULES OF ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF VEGETABLE PESTS

by

PARVATHI MALOTH

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ABSTRACT

The study entitled "Field efficacy of biocapsules of entomopathogenic fungi for the management of vegetable pests" was conducted at the Biocontrol Laboratory for Crop Pest Management, Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, during the year 2018-2021. The objective of the study was to evaluate the efficacy of biocapsules of *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Lecanicillium lecanii* (Zimmermann) Zare and Gams and *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno for the management of major groups of vegetable pests. The study also intended to standardize the dose of biocapsules in managing amaranthus leaf webber *Spoladea* (*Hymenia*) *recurvalis* F., okra shoot and fruit borer *Earias vittella* F. and cowpea aphid *Aphis craccivora* Koch. The biocapsules of fungi were formulated at a higher spore load of 10^{10} with HPMC coating and chitosan as carrier, following the protocol developed by Remya and Reji (2019).

In the first field experiment to evaluate the efficacy of biocapsules in managing defoliators in amaranthus, it was revealed that, *Metarhizium* and *Beauveria* capsules @ 3 L^{-1} sprayed twice (at weekly intervals) was effective causing 83.69 and 69.97 per cent reduction in population of *S. recurvalis* respectively. Lower doses of 2 and 1 capsules L^{-1} were less effective causing, 47.39 to 66.5 per cent reduction in larval population. Spraying spore suspensions of these fungi @ 10^8 mL^{-1} resulted in 91.27 to 100 per cent reduction, while in flubendiamide 39.35 SC, it was 89.84 per cent. Treatment with *Metarhizium* and *Beauveria* capsules did not affect the natural enemy population significantly, the mean population being 2.33 to 3.67 $plant^{-1}$. The corresponding population was 1.44 in flubendiamide 39.5 SC and 3.67 in untreated control. The yield recorded in the plots treated with *Metarhizium* and *Beauveria* capsules @ 3 L^{-1} was high (2.67 and 2.30 kg $plot^{-1}$) when compared to that in untreated plot 0.80 kg $plot^{-1}$.

Results of the second experiment to evaluate the efficacy of biocapsules in managing fruit and shoot borer *E. vittella* in okra concluded that, *Beauveria* capsule

@ 3 L^{-1} and *Metarhizium* capsule @ 3 L^{-1} were equally effective when sprayed at weekly intervals leading to 84.96 and 79.64 per cent reduction in the shoot damage respectively. The percentage reduction in shoot damage was only 65.32 to 73.86 per cent reduction in lower doses of capsules. In plots treated with spore suspensions, the mean shoot damage recorded was 90 to 95 per cent. Percentage reduction in chlorantraniliprole 18.5 SC was 90.71. Considering the fruit damage, *Metarhizium* capsule @ 3 L^{-1} was found to be the best treatment causing 100 per cent reduction in damage caused by *E. vittella*, while it was 89.75 per cent with *Beauveria* capsule@ 3 L^{-1}. Reduction in fruit damage ranged from 67.82 to 82.81 per cent in the lower doses of capsules. Highest reduction in the fruit damage observed with spore suspensions of *Beauveria* and *Metarhizium* @ 10^8 mL^{-1} (93.16 and 100 per cent, respectively). Similar results were obtained in the case of okra leaf roller, *Metarhizium* @ 2 L^{-1} and *Beauveria* capsule @ 3 L^{-1} were found to be the best treatment causing 96.38 and 85.80 per cent reduction in population of *Sylepta derogata* F., while it was 80.23 per cent with *Metarhizium* capsule @ 3 L^{-1}. Reduction in population ranged from 41.35 - 68.72 per cent in the lower doses of capsules. Highest reduction in the population of *S. derogata* observed with spore suspensions of *Beauveria* and *Metarhizium* @ 10^8 mL^{-1} (100 per cent). Percentage reduction in chlorantraniliprole 18.5 % SC was 93.41. Treatment with biocapsules capsules did not cause any adverse affect on natural enemy population, in okra field. The yield obtained from different treatments did not vary significantly.

Third experiment in cowpea field revealed that, foliar application of *L. saksenae* capsule @ 3 L^{-1} and *L. lecanii* capsule @ 3 L^{-1} were equally effective to *A. craccivora* when sprayed twice (at weekly intervals) causing 94.38 and 92.28 per cent reduction in the population respectively. Reduction in population noted was 57.54 to 63.96 per cent with lower dose @ 2 capsules L^{-1} while it was least with single capsule treatment (37.51 to 44.73 per cent). The spore suspensions were more effective resulted than the lower doses (78.73 - 83.53 per cent reduction). The chemical check thiamethoxam 25 WG recorded 95.79 per cent reduction in population. Biocapsule treatment did not affect natural enemy population significantly. The yield recorded in the plots with *L. saksenae* capsules @ 2 and 3 L^{-1}

was high (1.85 and 1.56 kg plot^{-1}) when compared to other treated plots and untreated plot (1- 1.45 kg plot^{-1}).

Therefore, it is concluded that biocapsules of *Metarhizium* and *Beauveria,* can effectively manage defoliators in amaranthus and borers in okra and those of *L. lecanii* and *L. saksenae* can be recommended for pea aphids, without affecting the natural enemies and yield significantly.

സംഗ്രഹം

വെള്ളായണി കാർഷിക കോളേജിലെ എന്റമോളജി വിഭാഗത്തിൽ 2018-21 കാലയളവിൽ നടത്തിയ ബയോ ക്യാപ്സ്യൂളുകളുടെ കൃഷിയിട പ്രവർത്തനശേഷി വിലയിരുത്തുക " എന്ന ഗവേഷണ പദ്ധതിയുടെ പ്രധാന ഉദ്ദേശങ്ങളും കണ്ടെത്തലുകളും മാണ് ഇവിടെ പ്രതിപാദിച്ചിരിക്കുന്നത് .

ഈ പദ്ധതിയു ടെ പ്രധാന ഉദ്ദേ ശം പച്ചക്കറി വിളകളെ ബാധിക്കുന്ന ഇലതീനി പുഴുക്കൾ , തുരപ്പൻ പുഴുക്കൾ ,നീരൂറ്റിക്കുടിക്കുന്ന കീടങ്ങൾ എന്നിവയ്ക്കെതിരെ മിത്ര കുമിളുകളായ മെറ്റാറൈസിയം ,ബിവേറിയ, ലെക്കാ നിസിലിയം ,എന്നിവയാൽ നിർമിച്ച ബയോ കാപ്സ്യൂളുകൾ ഫലപ്രദമാണോ എന്ന് കണ്ടെത്തുക എന്നതായിരുന്നു. ഇതോടൊപ്പം കൃഷിയിടങ്ങളിൽ പ്രയോഗിക്കേണ്ട അളവ് തിട്ടപ്പെടുത്തുക, ഇവ മിത്ര പ്രാണികളെ പ്രതികൂലമായി ബാധിക്കുന്നില്ല എന്ന് ഉറപ്പു വരുത്തുക എന്നീ ഉദ്യമങ്ങളും നിർവഹിച്ചിരുന്നു .

ഒന്നാമത്തെ കൃഷിയിട പരീക്ഷണം ഇലക്കറി വിളയായ ചീരയിലായിരുന്നു ചീരയുടെ പ്രധാന കീടമായ ഇല ചുരുട്ടി പുഴുവിനെതിരെ വിവിധ ഡോസുകൾ പരിക്ഷിക്കുകയുണ്ടായി. ഒരു ലിറ്റർ വെള്ളത്തിൽ 1 , 2 ,3 , എന്നീ അളവിൽ ക്യാപ്സ്യൂളുകൾ ലയിപ്പിച്ചു സ്പ്രേ ചെയ്യുന്നതിനോടൊപ്പം മിത്ര കുമിളുകളും ,നൂതന രാസ കീടനാശിനിയായ ഫ്ലുബെന്റിയാമൈഡും ഉപയോഗിച്ചു ഇവയുടെ പ്രവർത്തനം താരതമ്യ പെടുത്തിയിരുന്നു. മൂന്ന് ക്യാപ്സ്യൂൾ ഒരു ലിറ്റർ വെള്ളത്തിൽ എന്ന നിരക്കിൽ രണ്ടു തവണകളായി ആഴ്ചയിൽ ഒരിക്കൽ സ്പ്രേ ചെയ്യുന്നതു ചീരയിലെ പുഴുക്കളെ 83.69 %വരെ നിയന്ത്രിക്കുന്നതിന് സഹായകം ആകുമെന്നാണ് കണ്ടെത്തൽ .എന്നാൽ 2 ,1 എന്നീ അളവുകൾക് പ്രവർത്തന ശേഷി കുറവാണെന്നും കണ്ടെത്തി. ഫ്ലുബെന്റിയാമൈഡും തളിച്ച ചീര തോട്ടങ്ങളും 89.84 % വും മിത്ര കുമിൾ ലായിനി നേരിട്ട് പ്രേയോഗിച്ച തോട്ടങ്ങളിൽ 91 - 100 % നിയന്ത്രണവും രേഖപെടുത്തിയിരുന്നു. മിത്രകുമിളുകൾ ഉപയോഗിച്ച കൃഷിയിടങ്ങളിൽ യാതൊരു നിയന്ത്രണ മാർഗങ്ങളും സ്വീകരിക്കാത്തവയിൽ ഉള്ളതിനേക്കാൾ നേരിയ വിള വർധനവും കണ്ടിരുന്നു.

രണ്ടാമത്തെ കൃഷിയിട പരീക്ഷണം വെണ്ടയിലെ കായും തണ്ടും തുരക്കുന്ന പുഴുക്കൾക്കെതിരെയായിരുന്നു രണ്ടാഴ്ചയിൽ ഒരിക്കൽ മിത്രകുമിൾ ക്യാപ്സ്യൂൾ മൂന്നെണ്ണം വീതം ഒരുലിറ്റർ വെള്ളത്തിൽ ലയിപ്പിച്ചു വൈകുന്നേരങ്ങളിൽ തളിക്കുന്നതുമൂലം 79.64 - 84.96 % വരെ കുറവ് കീടാക്രമണത്തിൽ രേഖപെടുത്തിരിക്കുന്നു ഇവിടെ മെറ്റാറൈസിയം ക്യാപ്സ്യൂളുകൾ ബിവെറിയെക്കാൾ മികച്ചതാണെന്നും രാസകീടനാശനിയായ ക്ലോറാൻട്രാൻലിപ്രോലിനോളം പ്രവർത്തന ശേഷി ഇല്ലെന്നും കണ്ടെത്തി. എന്നാൽ വിളവ് താരതമ്യപ്പെടുത്തിയപ്പോൾ ജൈവകീടനിയന്ത്രണ മാർഗവും രാസകീടനിയന്ത്രണമാർഗവും തുല്യനിലവാരത്തിലായിരുന്നു.

മൂന്നാമത്തെ കൃഷിയിട പരീക്ഷണം പയറിൻറ്റെ പ്രധാന കീടമായ മുഞ്ഞക്കെതിരെ ആയിരുന്നു. ഇവിടെ ലേക്കനിസിലിയം ലെക്കനി, ലെക്കനിസിലിയം സാക്സനെ എന്നീ മിത്രകുമിളുകൾ ഉപയോഗിച്ചുള്ള ക്യാപ്സ്യൂളുകൾ ആണ് പരീക്ഷിച്ചത് .രണ്ടുതരം ക്യാപ്സ്യൂളുകളും ഒരു പോലെ യുള്ളപ്രവർത്തനശേഷിയാണ് പ്രകടിപ്പിച്ചത്. ഒരു ലിറ്റർ വെള്ളത്തിൽ മൂന്നെണ്ണം ഉപയോഗിച്ചുള്ള ഡോസാണ് മികച്ചതെന്നും, രാസകീടനാശനിയായ തയാമൊത്തോക്സാമിന് തുല്യമായപ്രവർത്തനമാണ് രേഖപ്പെടുത്തിയത്.92 .79% വരെ കുറവ് മുഞ്ഞ ആക്രമണത്തിൽ ഉണ്ടായി.ഈ മിത്രകുമിളുകളോ അവയുടെ ക്യാപ്സ്യൂളുകളോ മിത്രപ്രാണികളെ പ്രതികൂലമായി ബാധിച്ചില്ല. എന്ന് മാത്രമല്ല, കാർഷിക സർവകലാശാല വികസിപ്പിച്ചെടുത്ത വെള്ളായണി ഇനമായ ലെക്കാനിസില്യം സാക്സനെ വിളവിൽ വർദ്ധനവ് ഉണ്ടാക്കാനും കഴിവുണ്ടെന്ന് തെളിയിക്കപ്പെട്ടു.

ആയതിനാൽ മിത്ര കുമിളുകളായ മെറ്റാ റൈസിയം, ബവേറിയ, എന്നിവയാൽ നിർമിക്കപ്പെട്ട ബയോ ക്യാപ്സ്യൂളുകൾ പച്ചക്കറി വിളകളിലെ ഇലതീനി പുഴുക്കൾക്കും, തണ്ടു തുരപ്പൻ പുഴുക്കൾക്കെതിരെയും ലെക്കാ നിസില്യം എന്ന മിത്രകുമിളുകൾ ഉപയോഗിച്ചുള്ള ബയോ ക്യാപ്സ്യൂളുകൾ മുഞ്ഞ പോലുള്ള നീരൂറ്റി കുടിക്കുന്ന കീടങ്ങൾക്കെതിരെയും ഫലപ്രദമായി ഉപയോഗിക്കാം.ഇത്തരം ജൈവ ക്യാപ്സ്യൂളുകൾ മിത്രകീടങ്ങൾക്കോ പ്രകൃതിക്കോ ദോഷകരമല്ല .