Microbial Consortium for the Management of Insect Pests of Bitter gourd (*Momordica charantia* L.)

by

NAVEEDA S.

(2013-21-107)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

Department of Agricultural Entomology

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2018

DECLARATION

I, hereby declare that this thesis entitled **"Microbial Consortium for the Management of Insect Pests of Bitter gourd (*Momordica charantia* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani
Date : 30.01.2019

Naveeda S
(2013-21-107)

CERTIFICATE

Certified that this thesis entitled **"Microbial Consortium for the Management of Insect Pests of Bitter gourd (*Momordica charantia* L.)"** is a record of bonafide research work done independently by Ms. Naveeda S (2013-21-107) 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.

Vellayani
Date: 30.01.2019

Dr. Anitha N.
(Major Advisor, Advisory committee)
Professor & Head (Agricultural Entomology)
College of Agriculture, Vellayani,
Thiruvananthapuram- 695 522

CERTIFICATE

4

We, the undersigned members of the advisory committee of **Ms. Naveeda S, (2013 - 21 - 107)** a candidate for the degree of **Doctorate of Philosophy in Entomology** agree that this thesis entitled **"Microbial Consortium for the Management of Insect Pests of Bitter gourd (*Momordica charantia* L.)"** may be submitted by **Ms. Naveeda S. (2013-21-107)** in partial fulfillment of the requirement for the degree.

Dr. Anitha N.
(Chairman, Advisory Committee)
Professor and Head
Department of Agricultural Entomology
College of Agriculture, Vellayani,

Dr. O.P. Reji Rani
(Member, Advisory Committee)
Assistant Professor
Department of Agricultural Entomology
College of Agriculture, Vellayani

30/1/19

Dr. Amritha . V. S.
(Member, Advisory Committee)
Assistant Professor
Department of Agricultural Entomology
College of Agriculture, Vellayani

Dr. K. B. Soni
(Member, Advisory Committee)
Professor
Department of Biotechnology
College of Agriculture, Vellayani,

Dr. K. N. Anith
(Member, Advisory Committee)
Professor
Department of Agricultural Microbiology
College of Agriculture, Vellayani

30-01-2019
Dr. Chandrika Mohan
Principal Scientist (Ag-Entomology)
ICAR-CPCRI, Reg. Station, Kayamkulam
EXTERNAL EXAMINER

ACKNOWLEDGEMENT

'Alhamdulillah' for giving me the courage, strength and confidence to complete my research work successfully

I am pleased to express my deepest gratitude to Dr. Anitha N., Professor and Head, Department of Agricultural Entomology, College of Agriculture, Vellayani and Chairman of the advisory committee. I am greatly indebted for her expert guidance and timely advice throughout the course of this work. It was her constant encouragement, valuable suggestions and constructive criticism which made this piece of work possible.

I am grateful to Dr. Reji Rani O.P., Assistant Professor, Department of Entomology and member of advisory committee for her valuable suggestions and critical evaluation during the course of this work. I am thankful to Dr. Amritha V.S., Assistant Professor, Department of Entomology and member of advisory committee for her benign help and critical suggestions during the course of investigation and for critical evaluation of manuscript.

I feel immense pleasure in expressing my thankfulness for Dr. Anith N, Professor, Department of Microbiology and member of advisory committee for the valuable suggestions, encouragement and support throughout the course of work.

I would like to place my heartfelt gratitude to Dr. K. B. Soni, Professor, Department of Biotechnology, for her valuable suggestions, encouragement and support throughout the course of work, as a member of advisory committee.

I am pleased to place my profound etiquette to Dr. Sheela. M.S, and Dr. K. Sudharma for the valuable guidance, suggestions, constant encouragement, constructive criticism and support during the course of my Ph.D. programme.

I take this opportunity to thank Dr. C. Nandakumar, and Dr. Hebsy Bai, Professor Department of Agricultural Entomology, College of Agricultural Vellayani,, for their support, priceless care and all the helpful advice rendered. Special thanks to Dr.Thomas Biju Mathew for allowing me to utilize the facilities at Pesticide Residue Lab. I am grateful to all my beloved teachers in Dept. of Agricultural Entomology. Dr. Faizal, Dr. Ambily Paul, Dr. T. Jiji, Dr. K. D. Prathapan, Dr Nazeema, Dr. Premila, Dr. Nisha M. S., Dr. Narayanan, and

Dr. Devaneshan for their support, empathy and guidance rendered to me during the course of my research work.

It must be mentioned here that without the support rendered to me at most crucial times by my beloved juniours Nithya, Divya, Lishma, Achu, and Milsha this work would have been a vain exercise. No words can express my gratitude towards you my dears. You were always there for me and supported me whenever I felt down. Alhamdulillah, I am blessed to have you all in my life. I will always cherish those wonderful moments we had together.

I was fortunate to have the best batch mates and juniors who supported me with their caring manner. Special thanks to Hari for your brotherly affection, care and support. Thanks to Akshay, Murali, Akhila, KP, Jasmy, Archana, Praveena, Gayathri, Varsha, Anju and Mithra for all the love and support. Thank you Vidya and Narmada for the invaluable support and selfless help.

I owe heartfelt gratitude to my seniors Sivakumar sir, Radhika madam, Asha chechi, Lekha chechi, Malini chechi, Anis chichi , Amritha chichi and Deepa chichi for their support and advice.

I render my profound thanks to Jiji chechi, Shifa chechi, Smitha chechi, Sindu chechi, Chinju , Sam, Santhosh, Babukuttan and Anjitha for their kindness and helpfulness for the successful completion of this work. I am obliged to all the labourers who helped in successful completion of my field work.

Special thanks to Animol chechi for all the help you offered during my thesis preparation.Thanks to Ambadi and my kutti makkalz (Appoos, Vichu, Chikuz, Anuja) for all the care and support.

No words are enough to express my feelings and affection towards my vaappa and umma for their constant support, meticulous care, sacrifices, encouragement and blessings which enabled me to pursue this endeavour successfully. Special thanks to amma and atha for their constant encouragement and timely help during my research work and thesis preparation With boundless affection, I would like to extend my hearty acknowledgement and indebtedness to my loving brother Nikku and sister Tanu for being so supportive and for keeping the spirit in me.

Words are scarce to express my love and gratitude to my dear husband Mr. Tariq Taj. Ikka, I love you for everything, for being so understanding and for putting up with me for the toughest times of my life. You are the most amazing person in my life. Thanks to my lovely children Zaira and Zayd for filling my life with colours and lifting all my worries with that cute smile.

This work is an outcome of the help and support from many people. Thanking you all,

Naveeda S

INDEX

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Sl. No.	Title	Appendix No.	Page No.
1	Media composition	I	

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Percentage
°C	Degree Celsius
mL^{-1}	Per millilitre
@	At the rate of
µm	micro meter
a.i.	Active ingredient
CD	Critical difference
Cm	Centimetre
Cfu	colony forming units
DAI	Days after inoculation
DAS	Days after storage
DAT	Days after treatment
EC	Emulsifiable Concentrate
et al.	And others
Fig.	Figure
g	Gram
Ha	Hectare

ha^{-1}	Per hectare
Hr	Hours
Kg	Kilogram
L^{-1}	Per litre
M	Meter
mg	Milligram
min.	Minute
mL	Millilitre
NS	Non Significant
SC	Soluble Concentrate
Spp.	Species
Viz.	Namely

Introduction

1. INTRODUCTION

Bitter gourd, *Momordica charantia* L. is an important annual vegetable belonging to the family Cucurbitaceae and is a good income generative crop for the farmers. A multitude of insect pests and diseases attack the crop during its various growth stages and affect both productivity and quality. The major pests are melon fly, *Bactrocera cucurbitae* (Coq.), epilachna beetle, *Henosepilachna septima* (Fab.) pumpkin caterpillar, *Diaphania indica* Saund. and pumpkin beetle *Aulacophora* spp.

Many chemical insecticides have been used for controlling these pests. The overdependence and uninhibited use of these pesticides has necessitated alternatives mainly for environmental concerns. Microbial pesticides offer an ecologically sound and effective solution to target pests. Microbial bio control agents like bacteria, virus, fungi and nematodes had been recognized as a valuable tool in ecofriendly pest management. Among various entomopathogenic microbes, entomopathogenic fungi play a stellar role and had considerable scope in pest management owing to their wide host range, specificity and safety to non-target organisms.

One of the prime limitations of fungi as microbial control agents is that, each species and strain within a species are usually efficacious in a narrow window of climatic conditions. In field condition, farmers must manage pest complexes under various weather conditions and therefore, prefer broad spectrum pesticides. This is one of the crucial reasons for less adaptation of biopesticides, regardless of all the advantages they possess. Theoretically, this issue could partly be addressed through the development of an appropriate co-formulation of two or more fungi or fungal strains with different host ranges and ecological tolerances or combining different microbes with wide host range (Wang *et al.*, 2002). Zhang *et al.*, (2006) reported the microbial mixtures offered better pest control than the microbial agents applied separately. In inundative applications of microbial control agents, combination treatment with two entomopathogens offers an attractive biorational strategy. If the

two entomopathogens complement each other, or act synergistically, a beneficial effect can be obtained.

In integrated pest management progammes (IPM), a pivotal role is given for chemical pesticides, despite the controversies of resistance development, residue problems and resurgence. The safe use of pesticide is one of the effective components of pest management programme. In field conditions, sometimes it demands the co-application of biocontrol agents along with chemical pesticides. In this context, it is important to have sufficient knowledge on the compatibility of biocontrol agents with pesticides for formulating an effective integrated pest management strategy.

Taking these into consideration, the present study focused on the following objectives:

- To isolate and identify the microbial pathogens of major pests of bitter gourd
- To develop a microbial consortium, that can effectively contain all the major pests of bitter gourd
- To test the compatibility of consortium with new pesticide molecules
- To evaluate the efficacy of microbial consortium against major pests of bitter gourd.

Review of literature

2. REVIEW OF LITERATURE

Bitter gourd is an important annual vegetable grown in India. The crop is attacked by a multitude of pests and diseases during its various growth stages. The literature pertaining to importance and nature of damage of major pests of bitter gourd and their management using different entomopathogenic fungi are reviewed below.

2.1. IMPORTANT PESTS OF BITTER GOURD

2.1.1. *Bactrocera cucurbitae*

Bitter gourd is infested by a number of pests of which melon fly, *B. cucurbitae* can be considered as the most prominent one and is the major limiting factor as it reduces the marketable yield as well as quality of fruits (Srinivasan, 1959; Lall and Singh, 1969). In bitter gourd, the damage by fruit flies account for 41 to 89 per cent (Kushwaha *et al.*, 1973; Gupta and Verma, 1978; Rabindranath and Pillai, 1986). Being polyphagous in nature, the pest causes damage to other cucurbits as well rendering the fruits unfit for human consumption. Depending on the environmental conditions and susceptibility of the crop species, the extent of losses varies between 30 to 100 per cent (Dhillon *et al.*, 2005; Shooker *et al.*, 2006)

2.1.2 *Diaphania indica*

D. indica, vernacularly known as pumpkin caterpillar is an important pest of cucurbitaceous vegetables. A number of cucurbits cultivated in India *viz.*, bitter gourd (*Momordica charantia* L.), cucumber (*Cucumis sativus* L.), melon (*C. melo* L.), gherkin (*C. sativus* L.), bottle gourd (*Lagenaria siceraria* Molina), snake gourd (*Trichosanthes anguina* L.), Luffa (*Luffa aegyptiaca* Mill.), little cucumber (*Melothria* spp.) serve as host species of the pest (Ayyar, 1923; Tripathi and Pandy, 1973; Clavijo *et al.*, 1995; Bacci *et al.*, 2006).

In bitter gourd, the pest causes economic loss by feeding on leaves and young flower buds. With a single larva per leaf, yield loss by *D. indica* was estimated to be 10 per cent (Schreiner, 1991). The larvae bore on young and mature fruits reducing its market value (Ravi, 1998). The pest accounts fruit damage to the range of 90 per cent (Jhala *et al.*, 2004) and leaf damage of the range 3 to 14 per cent (Singh and Naik, 2006) in bitter gourd. Mohammed *et al.* (2013) observed leaf damage and fruit damage by *D. hyalinata* in different cucurbits. Leaf damage of 24.92 per cent and 9.16 per cent was observed respectively in snake cucumber (*Cucumis melo* var. *flexuosus*) and pumpkin. Also fruit damage of 46.6 per cent in sweet melon and 20.13 per cent in snake cucumber was reported.

2.1.3 *Henosepilachna septima*

The genus *Henosepilachna* is an important defoliator and reported from a number of crops such as bitter gourd, brinjal, cucmber, pumpkin, melon, tobacco and tomato (Rath, 2005). Damage is caused by both adults and grubs of the beetle which are often found on the lower surfaces of the leaves, scrapping and feeding voraciously on the parenchyma and the lower epidermis between the veins and skeletonize it in a characteristic manner leaving intact the upper epidermis as well as the tougher tissues in the form of "window" (Tara and Sharma, 2017).

Ghule *et al.* (2014) reported 27 per cent leaf damage in pointed gourd by *H. septima*. When untreated, *Henosepilachna* resulted in 85 per cent leaf damage in bitter gourd leading to drying, defoliation, stunted growth and finally loss of yield in field (Borgia and Miranda, 2016).

2.1.4 *Aulacophora* spp.

The pumpkin beetle is a major polyphagous pest of cucurbits. Both the adults and the grubs cause severe damage to various cucurbit crops. Eggs of the insect are

usually seen in the collar region of the plants and the grubs cause damage by feeding on the roots (Maniruzzamman, 1981). The adult beetles are potent defoliators of almost all cucurbits. Adults also feed on the flowers and stem of cucurbits and causes damage (Butani and Jotwani, 1984). Prasad and Kumar, (2002) reported heavy yield loss in pumpkin and other cucurbits due to pumpkin beetles. The pest is known to cause a substantial yield loss of about 30 to100 per cent in field level (Dhillon *et al.*, 2005).

2.2. MANAGEMENT OF PESTS

2.2.1 Biological Control

2.2.1.1Entomopathogenic Fungi (EPF)

Fungi are omnipresent natural entomopathogens that causes natural epizootics in many host insects. More than 700 species of fungi from all the five subdivisions are known to be infectious to insects (Charnley, 1989). Insects from different orders such as Lepidoptera, Homoptera, Hymenoptera, Coleoptera, Diptera, Hemiptera, Orthoptera and Thysanoptera serve as natural hosts to various entomopathogenic fungi which render them uniqueness and suitability as promising biocontrol agents (Feng *et al.*, 1990; Ferron *et al.*, 1991; Negasi *et al.*, 1998; Castillo *et al.*, 2000; De La Rosa *et al.*, 2000; Lezama-Gutierrez *et al.*, 2000).

Approximately, 60 per cent of insect diseases are caused by fungi, the infective propagules of which could be isolated from the cadavers of infected insects. (Hajek and St. Leger, 1994; Meyling, 2007; Faria and Wraight, 2007). Exploiting this, many researchers have isolated entomopathogenic fungi and utilized them for various biocontrol studies.

Isolation of the fungi can be done directly by inoculating it in Potato Dextrose Agar (PDA) medium, provided external sporulation of the fungus is there. In the absence of external sporulation, the insect cadavers should be kept in a moist chamber prior to placement in PDA so that hyphal or conidial production occurs externally. (Goettel and Inglis, 1997).

Some of the entomopathogenic fungi isolated were *Metarhizium anisopliae* (Metschnikoff) Sorokin from *Heliothis armigera* Hubner (Gopalakrishnan and Narayanan, 1989), *Fusarium pallidoroseum* (Cooke) Sacc. from *Aphis craccivora* Koch (Hareendranath, 1989), *A. niger* and *Beauveria bassiana* (Balsamo) Vuillemin from *Dolycoris baccarum* Linnaeus (Assaf *et al.* 2011), *Lecanicillium* sp. from *Coccus viridis* Green (Ramanujam *et al.* 2011), *M. anisopliae* from *Chilo venosatus* Walker (Liu *et al.,* 2012), *Hirsutella* sp. from *Oliarus dimidiatus* Berg (Toledo *et al.*, 2013), *B. bassiana* and *Metarhizium* sp. from *Basilepta fulvicorne* Jacoby (Joseph, 2014), *Isaria javanica* (Friedrichs and Bally) Samson and Hywel-Jones from *Bemisia tabaci* Gennadius (Lokesh, 2014), *A. flavus* from *Leptocorisa acuta* (Thunb) (Nilamudeen, 2015), *B. bassiana* from *Metriona circumdata* Weise and *F. oxysporum* from *Cofana spectra* (Praveena, 2016).

2.2.1.1.1Metarhizium anisopliae

M. anisopliae is a soil borne mitosporic haploid fungus with a cosmopolitan distribution. It was first reported to cause disease on wheat cockchafer by Metschnikoff (1879). Epizootic by *M. anisopliae* was also reported from 200 insect species, many of which are agricultural pests which made it an effective biocontrol agent (Butt *et al.,* 2001; Nahar *et al.,* 2004).

The fungus, when treated at a concentration of 1 x 10^6 conidia mL^{-1} resulted in 100 per cent mortality of Mediterranean fruit fly, *Ceratitis capitata* (Wied.) (Castillo *et al.*, 2000). Mortality was also reported in the adults and immature stages

of fruit fly, *Anastrepha fraterculus* (Wied.) (Rodrigues-Destéfano *et al.*, 2005). Toledo *et al.* (2006) evaluated the efficacy of several strains of *M. anisopliae* to adults of Mexican fruit fly, *A. ludens* (Loew). The fungal strains were highly virulent against the fruit fly adults and caused mortality to the range of 75.2 to 89 per cent.

Topical application of different isolates of *M. anisopliae* at a concentration of $1x10^6$ conidia mL^{-1} caused mortality in *B. zonata* and *B. cucurbitae* (Sooker *et al.*, 2008). Thaochan and Sausa-Ard (2017) assessed the virulence of 64 indegenous isolates of *M. anisopliae* against adult melon flies, *Zeugodacus cucurbitae* (Coquillet), and reported mortality percentage varying from 4 to 100. *M. anisopliae* isolates PSUM02 and PSUM05 induced 100 per cent mortality within seven days, in both sexes of *Z. cucurbitae* while less than 50 per cent mortality of the treated insects was observed 7 days post treatment in other isolates. The LT_{50} and LT_{90} values ranged between 2.9 to 10.9 and 4.8 to 15.4 respectively.

The pathogenicity of green muscradine fungus against *D. indica*, one of the major defoliators of bitter gourd was proved by Praveena (2016). Five indigenous soil isolates of *Metarhizium* along with one NBAIR isolate (Ma4) when topically applied to second instar larvae of the pumpkin caterpillar resulted in mortality of the insects that ranged from 56.66 to 100 per cent with different strains.

M. anisopliae at two spore concentrations 10^8 and 10^{10} conidia mL^{-1} induced mortality in the grubs and adults of *H. vigintioctopunctata,* an important defoliator of solanaceous and cucurbitaceous crops (Rajendran, 2002). The mortality percentage was high at both the doses. The fungus was more effective to the younger instars as 100 per cent mortality was reported in the first and second instars, 7 days post treatment wherein more than 70 per cent mortality was observed in third and fourth instar grubs. The fungus was not that potent in controlling adult populations as only 17.4 per cent mortality was there in newly emerged adults.

Aqueous formulation of *M. anisopliae* @ 5×10^{12} conidia mL^{-1} caused mortality in the adults of *H. vigintioctopunctata* (Swaminathan *et al.*, 2010). Vishwakarma *et al.* (2011) reported 70.71 per cent reduction in the population of grubs of *H. vigintioctopunctata* in field with the application of *M. anisopliae* at 3 g L^{-1} water. Joseph (2014) observed that *H. vigintioctopunctata* adults and grubs treated with *M. anisopliae* @ 10^8 spores mL^{-1} showed a mortality of 17.66 per cent and 42.21 per cent for adults and grubs respectively. The virulane of green muscardine fungus, *M. anisopliae* against grubs of *H. vigintioctopunctata* was also observed by Kodandaram *et al.*, (2014), who observed 62 per cent mortality of the treated insects, 6 days post treatment .Viswanathan (2015) reported 66.58 per cent mortality of grubs of *H. vigintioctopunctata* seven days after treatment when treated with *M. anisopliae* at a spore concentration of 10^8 spores mL^{-1}. Only 0.16 per cent mortality was observed in adults stating the low potency of fungus in managing the adult population.

The pathogenicity of *M. anisopliae* to grubs and adults of *A. foveicollis* was proved by Joseph, (2014). A mean per cent mortality of 6.67 and 24.37 respectively for adults and grubs was visible after seven days of treatment with *M. anisopliae* @ 10^8 spores mL^{-1}. Bioassay studies resulted in LC_{50} value of 11.42 x 10^8 spores mL^{-1} and 4.91 x 10^8 spores mL^{-1}, 7 days post treatment for adults and grubs respectively.

The pathogenicity of two isolates of *M. anisopliae* to the bug *Leptoglossus occidentalis* (Heidemann) was proved by Barta (2010). The LC_{50} values obtained were 16.05 x 10^5 and 47.13 x 10^5 spores mL^{-1} for the different isolates.

2.2.1.1.2 Beauveria bassiana

B. bassiana, the white muscardine fungus is one of the most promising entomopathogen that has been widely used for pest management. The pathogenicity of this fungus to insects was first reported by Bassi (1836) and was first used for pest

management by Klochko (1969). *B. bassiana* has a cosmopolitan distribution and a wide host range making it one of the pioneer organisms for insect pathology studies. The fungus is known to infect 500 host species belonging to orders Coleoptera and Lepidoptera (Charnley and Collins, 2007; Zimmermann, 2008). The universal distribution, wide host range and non-toxic nature towards the non-target organisms made it an effective pathogen that can be successfully utilized for crop pest management (Prasad and Syed, 2010; Karthikeyan and Jacob, 2010)

Pathogenicity of *B. bassiana* to adults of fruit fly, *C. capitata* was studied by Muñoz (2000) and reported mortality levels ranging from 20 to 98.7 per cent with different strains. De La Rosa *et al.*, (2002) evaluated the pathogenecity of eight strains of the entomopathogenic fungus *B. bassiana* on larvae, pupae, and adult females of the Mexican fruit fly, *A. ludens*. The results stated that the adult fruit flies showed high susceptibility to the different strains and the mortality percentage ranged from 82 to 100 for the various strains tested. However all the strains tested showed low pathogenicity to the immature stages i.e. larva and pupa. Bioassays were also carried out to evaluate the effect of three different strains of *B. bassiana* (Bb16, Bb26, and Bb24) on adults of the fruit fly. The LT_{50} values obtained were 2.8, 3.7 and 4.2 days and LC_{50} values were 5.13 x 10^{5}, 3.12 x 10^{6} and 9.07 x 10^{6} conidia mL^{-1} for the strains Bb16, Bb26, and Bb24 respectively.

Cross infectivity of a new strain of *B. bassiana* (*B. bassiana* (ITCC No. 6063) isolated from okra leaf roller, *Sylepta derogata* (Fabricius) was done by Jiji *et al.*, (2006) against the fruit fly *B. dorsalis*. The fungus at a concentration of 10^{9} spores mL^{-1} caused 90 per cent mortality of the test insect at 6 DAT. Bioassay studies resulted in LC_{50} value of 7.0 x 10^{8} spores mL^{-1} at 3 DAT.

Aemprara (2007) studied the pathogenicity of 12 isolates of *Beauveria* spp. against fruit fly *B. dorsalis*. All the selected strains were pathogenic *to B. dorsalis*

and the mortality percentage ranged from 2 to 68. Bioassay of the most effective *Beauveria* sp. isolate 6241 on fruit fly resulted in mortality which increased with increase in spore concentration of the fungus. 50 per cent mortality of the test insect occurred at a concentration of 7.36 $x10^7$ spores mL^{-1} .

Amala (2010) reported 98.2 per cent pupal mortality of *B. cucurbitae* when treated with *B. bassiana* (ITCC No. 6063) at 2 x 10^7 spores mL^{-1}. The same dose caused 76.67 per cent mortality of adults 6 days post treatment. Bioassay of the fungus on adults of melon fly resulted in mortality ranged from 11.11 per cent to 76.67 per cent with an increase in the tested concentration from 2 x 10^3 spores mL^{-1} to 2 x 10^7 spores mL^{-1}. The concentration required to kill half of the population at 3 DAT was 1.8 x 10^8 spores mL^{-1}.

Marri *et al.,* (2016) studied the efficacy of a commercial formulation of *B. bassiana* (Botanigard® ES) for managing the various developmental stages of oriental fruit fly, *B. dorsalis*. The study stated that the effect of the fungi was dose dependent and the mortality increased with fungal spore concentration. A dose of 26.5 × 10^6 spores mL^{-1} killed 50 per cent of adult flies within 4 to 5 days and 99 per cent within 8 to 9 days. Adult emergence from infected pupae was also reduced from 97 per cent in the control to 46 per cent in the treated groups. Mortality also occurred in the adults emerged from the survived pupa and also in the adults treated directly.

B. bassiana was reported to infect *D. indica* and a mortality percentage of 86. 67 was recorded against the larvae (Jiji *et al.*, 2008). Lenin (2011) also reported 35 per cent reduction in the population of *D. indica* in field when commercial formulation of *B. bassiana* was applied at a dose of 2 g L^{-1}. Praveena (2016) observed 53.33 per cent mortality of second instar larvae of *D. indica* 7 days post treatment, when treated with *B. bassiana* at a spore concentration of 10^9 spores mL^{-1}

The effectiveness of *B. bassiana* and *B. tenella* in managing epilachna beetle, *Epilachna vigintioctopunctata* was studied by Klochko (1969). According to him 98 per cent of the test insects were killed by these fungi, 13 DAT. Padmaja and Kaur , (1998) reported *B. bassiana* to be effective against the second instar larvae of *E. vigintioctopunctata*. The larval stages were more susceptible to the fungi than pre-pupal and adult stages. Bioassays of four different isolates of *B. bassiana* on grubs and prepupal stages of hadda beetle resulted in LT_{50} of 1.33 to 4.86 days for the grubs and 2.75 to 5.67 days for the prepupal stage.

Rajendran and Gopalan, (1999) studied the effectiveness of direct spraying of white muscardine fungus, *B. bassiana* and reported that the fungus could kill 58.1 per cent first instar larvae and 35.2 per cent pre-pupal stage larvae. The adults were less susceptible to the fungus with the maximum mortality being 10.3 per cent in the case of newly emerged adults. The fungus caused 54.6 per cent hatchability of one-day old eggs of the spotted beetle.

Thurkathipana and Mikunthan (2008) studied the effect of *B. bassiana* on grubs of *E. vigintioctopunctata* and stated that at a concentration of 10^8 spores mL^{-1}, mortality of grubs was observed 18 hr. after application in laboratory and 72 hr. post application in field conditions. A mean mortality of 63.33 per cent on epilachna beetle grubs due to *B. bassiana* infection in field was observed by Jiji *et al.* (2008). Studies conducted by Devi *et al.* (2008) revealed that various strains of *B. bassiana* were infective to epilachna beetle (*H. vigintioctopunctata*) causing a mortality that ranges from 70 to 100 per cent depending upon the strain.

Vishwakarma *et al.* (2011) reported significant reduction in the population of epilachna beetle (74.91per cent) when treated with *B. bassiana* @ 3.0 g L^{-1} of water. According to Ghosh and Chakraborty (2012), the microbial pesticide *B. bassiana* provided only 39.56 per cent suppression of the epilachna beetle population when compared to treatment with chemical insecticide (cartap hydrochloride 50 % SP @ 1

g L^{-1}). Joseph, (2014) reported that the adults and grubs of *H. vigintioctopunctata* recorded mean per cent mortality of 35.52 and 64.48 respectively after 7 days of treatment with *B. bassiana* @ 10^8 spores mL^{-1}. The LC_{50} values were 0.48 x 10^7 and 1.86 x 10^7 spores mL^{-1} respectively for grubs and adult at 10 DAT.

Misra and Sarangi, (2014) studied the effectiveness of *B. bassiana* in field in controlling the population of *H. septima* and reported that the fungus almost failed to prevent leaf damage in field with very negligible reduction in per cent leaf damage (1.57). Viswanathan (2015) observed 63.25 per cent mortality of grubs of *H. vigintioctopunctata* 7 DAT when treated with *B. bassiana* at a spore concentration of 10^8 spores mL^{-1}. However the fungus was less effective in reducing the population of adults, the mortality percentage being 0.16 at 7 DAT. Kodandaram *et al.*, (2014) observed 60.3 per cent mortality in the grubs of *H. vigintioctopunctata* treated with white muscardine fungus, *B. bassiana*.

Joseph, (2014) reported that the adults and grubs of *A. foveicollis* recorded a mean per cent mortality of 24.37 and 66.78 respectively after 7 days of treatment with *B. bassiana* @ 10^8 spores mL^{-1}. The corresponding LC_{50} values at 5 DAT were 5.27 x 10^8 and 2.15 x 10^8 spores mL^{-1} for adults and grubs respectively. According to Moorthi and Balasubramanian (2016), the fungus, *B. bassiana* is highly potent against red pumpkin beetle, *A. foveicollis* and the pest can be considered as a natural diet for the fungus. They also reported an LC_{50} value of 9 x 10^3 spores mL^{-1}, 12 days post treatment.

Two isolates of *B. bassiana* were reported to be pathogenic to the bug *L. occidentalis* by Barta (2010). The LC_{50} values obtained were 10.35 x 10^5 and 10.18 x 10^5 spores mL^{-1} for the two isolates.

2.2.1.1.3. Lecanicillium lecanii

Susceptibility of the larvae and pupae of *H. vigintioctopunctata* to fungal suspension of *Lecanicillium lecanii* at a concentration of 1.6×10^7 or 4.8×10^7 spores mL^{-1} was observed by Santharam *et al.*, (1978). Ghatak and Mondal (2008), recorded 60.99 per cent a reduction of epilachna beetle population when treated with *V. lecanii* at a concentration of 20 g L^{-1}. Kodandaraman (2014) reported *L. lecanii* to be pathogenic against grubs of *H. vigintioctopunctata* causing more than 40 per cent mortality of the treated insects, 6 days post treatment

2.2.1.1.4 Purpureocillium lilacinus

Jiji *et al.*, (2006) isolated an indigenous isolate of *P. lilacinus* from *B. cucurbitae*. The isolate, *P. lilacinus* (ITCC No. 6064) caused 100 per cent mortality of the treated melon flies, 3 days post treatment. Dead flies developed pinkish violet fluffy mycelial growth over them. The pupae when treated turned black in colour and failed to emerge. Bioassay on adult fruit fly revealed that 5.0×10^6 spores mL^{-1} was required to cause 50 per cent mortality of the treated insects, 2 days post treatment. At 3rd and 4th DAT, 8.0×10^5, 7.0×10^5 spores mL^{-1} were required to cause the same mortality percentage

Amala (2010) reported 100 per cent adult mortality and 92.5 per cent pupal mortality of melon fly *B. cucurbitae* when treated with *P. lilacinus* at 2×10^7 spores mL^{-1}. LC_{50} value of 1.3×10^8 spores mL^{-1} was obtained upon treatment of *P. lilacinus* (ITCC No. 6064) on melon fly *B. cucurbitae* 2 days post treatment Mortality of the treated insects increased with the tested concentration and 100 per cent mortality was obtained at 7 DAT when treated with a concentration of 1.5×10^7 spores mL^{-1}. She also reported mortality of larvae of *D. indica* on field application of *P. lilacinus* in bitter gourd.

2.2.1.1.5. Other Entomopathogenic Fungi

Paecilomyces farinosus Dicks ex Fr. as a pathogen of the larvae of *D. indica* was reported by Kuruvila and Jacob (1980). Visalakshy (2005) reported *Nomuraea rileyi* (Farlow) Samson as a pathogen of *D. indica.*

Beevi and Jacob (1982) reported *Fusarium moniliforme* var. *subglutinans* (Wollenw and Reinking) to be pathogenic to larvae of *H. vigintioctopunctata* causing 100 per cent mortality after 5 days in the third and fourth instar. An infection of 20 per cent was observed in the egg mass of *H. vigintioctopunctata* treated with *F. solani* (Mart.) Sacc (Bhagat and Munshi, 1999).

2.2.1.2 Entomopathogenic Bacteria

By the discovery of potential entomopathogenic bacteria, especially associated to species belonging to the genus *Bacillus* the paradigm of biocontrol changed upside down (Glare and O'Callaghan, 2000). Entomopathogenic bacteria and their toxins can be considered as the most commercially successful microbial insecticides. Entomopathogenic bacteria follows an oral mode of entry to the host body where they produce toxins and other pathogenic factors that disrupt the midgut epithelium rendering them access to the insect haemocoel. These organisms then multiply within the insect hemocoel causing septicemia and death of the host organism. The majority of bacterial pathogens of insects and related taxa occur in the family Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae, and Micrococcaceae.

2.2.1.2.1 Phylloplane Bacteria

Leaf surfaces are the natural habitat for a wide range of organisms including bacteria, fungi, algae and bryophytes. The term 'phylloplane' refers to the surface

area of a leaf which supports large and complex microbial communities comprising both pathogenic and non-pathogenic species. Andrews (1992) reported that microorganisms that stably colonize the surface of plant leaves act as potential biological agents to suppress foliar pathogens and insect defoliators. Extracellular chitinases are produced by some of the phylloplane bacteria which helps them to degrade the peritrophic membrane of leaf feeding insects rendering them potential biocontrol agents (Aggarwal *et al.*, 2015)

Occurrence of *Serratia entomophila*, highly potent entomopathogenic bacteria are repoted from phyllosphere region of tomato and banana (Akutsu *et al.*, 1993; Riveros *et al.*, 2002). Chattopadhyay and Sen (2013) treated the seeds of *Arachis hypogaea* L. with *S. entomophila* and the shoots and roots emerged when fed to *H. armigera* resulted in 86.6 and 90 per cent mortality respectively.

The chitinase secreted by *Alcaligenes paradoxus* strain KPM-012A isolated from tomato leaves and vitally entrapped in sodium alginate gel beads was potent enough to degrade the chitinous peritrophic membrane of phytophagous ladybird beetles *E. vigintioctopunctata* and thereby caused the death of insect (Otsu, *et al.*,2003). An entomopathogenic bacterium, *Pseudomonas fluorescens* KPM-018P isolated from tomato leaves caused death of the phytophagous ladybird beetles *E. vigintioctopuncta* on sprayed leaves. It was noted that the enzymatic activity of chitinase and protease in infected larvae increased considerably in parallel with rapid multiplication of KPM-018. Ingestion of bacterium by the larvae caused prompt death to suppression of their pupation (Otsu *et al.*, 2004).

Viswanathan (2015) isolated the bacteria *S. marcescens, P. aeruginosa*, *P. fluorescens*, *Microbacterium* sp., *Curtobacterium flaccumfaciens* and *Bacillus megaterium* strain Q3 from the phylloplane of brinjal. The isolates resulted in 86.60, 66.35, 43.21, 23.12, 33.18 and 25.93 per cent mortality of grubs of epilachna beetle, *H. vigintioctopunctata* 3 DAT.

Liz (2017) isolated the bacteria, *S. marcescens* from the phylloplane of bitter gourd. The bacterium resulted in 83.33 per cent mortality of grubs of epilachna beetle *H. vigintioctopunctata*, and 93.33 per cent mortality of larvae of pumpkin caterpillar *D. indica*.

2.2.1.3 Talc Formulation of Entomopathogenic Fungi and Shelf Life

Srikanth *et al.*, (2006) evaluated different dry formulations of *B. brongniartii* in terms of conidial viability and virulence against white grub, *Holotrichia serrata* (Fabricius) and reported longer shelf life for those formulated in talc and lignite

'Biopower', a talc based formulation of *B. bassiana*, was evaluated for its shelf life in three locations having different agro-climatic conditions *viz.*, Ooty, Coimbatore and Chennai. Greater conidial viability and better survivability upto 11 months were observed in Ooty followed by Coimbatore upto 9 months, where the average minimum and maximum temperature range between 5 to 25 °C and 19 to 35°C respectively. At Chennai, the viability of conidia was upto 9 months where an average minimum of 25 °C and maximum of 36 °C were recorded (Ramarethinam *et al.*, 2002).

Talc formulations of *B. bassiana* recorded 13 per cent reduced viability after the storage for 90 days (Chaudary *et al.*, 2001). Lokesh (2014) reported that the talc based formulation of *M. anisopliae* (Ma4), *L. lecanii* (Ll18), *B. bassiana* (Bb5a) upon viability tests produced a cfu of 129.19 x 10^6, 84.75 x 10^6 and 70.40 x 10^6 spores mL^{-1} respectively at 15 DAS which reduced to 26.46 x 10^6, 65.26 x10^6 and 66.24 x 10^6 spores mL^{-1} respectively at 90 DAS. Upon storage, the bioefficay against *Aphis gossypii*, reduced from 86.61, 97.80 and 93.28 per cent mortality at 15 DAS to 66.57, 79.82 and 75.56 per cent mortality at 90 DAS respectively for talc based formulations of Ma4, Ll18 and Bb5a.

Storage caused no significant reduction in the bioefficacy of talc based formulation of *B. bassiana* against grubs of *Cylas formicaricus*. However reduction in the bioefficacy was noted for the adults which reduced from 88.83 per cent mortality at 30 DAS to 85.16 per cent mortality at 90 DAS. (Joseph, 2014). Talc formulation of *M. anisopliae* displayed a reduction in bioefficacy against grubs and adults of sweet potato weevil *Cylas formicaricus* F. where the mortality percentages ranged from 66.59 to 55.46 for grubs and 77.71 to 68.49 for adults respectively at 30 and 90 DAS. The cfu values ranged from 1.86 x 10^9 to 0.29 x 10^9 spores mL^{-1} and 21.05 x 10^9 to 1.57 x 10^9 spores mL^{-1} respectively for talc formulation of *M. anisopliae* and *B. bassiana.*

2.2.1.3.1. Effect of Temperature on Storage of Formulation

Storage temperature plays a key role in determining the keeping quality of a formulation. Zhang *et al.* (1992) observed that wettable powder formulation of *B. bassiana* had more than 85 per cent germination, when stored for eight months at 10 to 20 °C. Gupta *et al.* (2000) noticed significant reduction in the sporulation of talc formulation of *M. anisopliae* with long term storage irrespective of the storage temperature. Low storage temperature maintained better condial viability in long term storage. Infectivity against white grub, *H. consanguinea* was not affected even at room temperature up to 20 days of storage. They concluded that for a bio pesticide to be effective in the field against its target pest, it must be stored under low temperature (4°C and 20°C for the retention of high viability and virulence).

Alves *et al.* (2002) tested the effect of different water-based and oil-based formulations on conidial viability of *M. anisopliae* stored at 10 °C and 27 °C and found that formulation stored at 10 °C was more viable (91 per cent germination) than that stored at 27 °C, 40 weeks after storage.

Rachappa (2003) reported that the strains of conidia of *M. anisopliae* under refrigeration provided longer life compared to ambient temperature during entire storage period. And he also reported that, among the different carrier materials evaluated, colony forming units (cfu) count of *M. anisopliae* was least affected by sorghum flour.

High storage temperature lowers the viability of *L. lecanii* conidia. Conidial viability declined rapidly when stored at 35 °C and was declined to nearly zero after three months while, spores retained viability when stored at 15 °C and 25 °C (Chen *et al.*, 2008).

Derakhshan *et al.* (2008) reported that the viability of *L. lecanii* stored in refrigerated condition was significantly higher than that stored in room temperature. Viability of talc and oil formulations of *L. lecanii* under refrigerated condition was found to be more than that stored at room temperature (Banu and Gopalakrishnan, 2012; Banu, 2013).

Das *et al.* (2013) studied the influence of temperature on viability of talc based wettable powder formulation of *B. bassiana.* Shelf life was evaluated at room temperature (24±1°C), refrigerated condition (4°C) and in deep freeze condition (–4°C to –6°C). The viability of conidia lasted upto 180 days with 20.22×10^{7}conidia g^{-1} under room temperature whereas under refrigeration the viability lasted for 210 days with the same conidial density. Under deep refrigeration, viability lasted for 300 days with same conidial load, but pathogenicity to rice hispa, *Dicladispa armigera* (Olivier) substantially reduced to 20 per cent which was 48 per cent under room temperature and 69.45 per cent under refrigeration.

Nithya (2015) reported that oil based and talc based formulation of *L. lecanii* stored in refrigeration recorded high cfu count and spore count than that stored in room temperature.

2.2.1.4 Mixtures of Pathogens

It is a general principle that complex associations in nature are more stable and there is a better chance of attaining successful biological control with a mixture of several antagonists than with a single one (Baker and Cook, 1982). Inglis *et al.* (1997) observed that the performance of entomopathogenic fungi, *B. bassiana* and *M. flavoviride* against the grass hopper, *Melanoplus sanguinipes* varied with fluctuating temperatures. They reported that a combination of both the fungi, however could produce a stable result in all the temperatures and produced an increase in mortality (100 per cent) than when used alone at varying temperaturs.

Geetha *et al.* (2012), assessed the interactions of entomopathogenic fungi *viz.*, *B. bassiana*, *B. brongniartii* and *M. anisopliae* among themselves and three other opportunistic soil fungi from the sugarcane ecosystem namely, *Fusarium saachari*, *Aspergillus* sp. and *Penecillium* sp. in vivo against *Galleria mellonella* larvae. The tested fungi, were co-applied on IV instar *G. mellonella* @ 1 x 10^7 mL^{-1}, in combinations of two, at the interval of 24 hr. either preceding or succeeding each other to assess their efficacy and sporulation rates. They observed that the efficacy of *B. bassiana* (90 per cent) and *B. brongniartii* (100 per cent) was not enhanced further but was negatively affected in most combinations with other fungi. In case of *M. anisopliae* compatibility was higher, resulting in higher mortality by application of *B. bassiana* before (100 per cent) or after (83.3 per cent) *M. anisopliae* than when it was applied alone (70 per cent).

An increased reduction in the attack of sweet potato weevil *C. formicaricus* was observed by Reddy *et al.* (2014) during co-application of *B. bassiana* and *M. brunneum* (20 mL $a.i.ha^{-1}$ + 45 m L $a.i.ha^{-1}$)

The interaction *in vivo* may not be an indication of *in vitro* interaction among different entomopathogens. Claro and Sillva, (2010) reported that the interaction

between the fungi, *B. bassiana, M. anisopliae* and *L. lecanii in vitro*, to be mutual inhibition at distance. However, the conidial mixtures did not change the conidial germination percentage with a percentage higher than 95 per cent for every species in co-culture. In dual cultures, the colony growth stopped by the third week and the margins of the colonies were about 4 to 6 mm away from each other.

2.2.2 Management with Chemicals

Dupo and Aganon, (2009) reported that Indoxacarb (Steward 30WDG) at the rate of 30 to 40 g a.i. ha^{-1} was able to effectively protect both leaves and fruits of bittergourd from the feeding damage of *D. indica*. During peak infestation, indoxacarb @ 30 g a.i. ha^{-1} (3 g product 16 L^{-1}) resulted in about 90 per cent larval control and indoxacarb @ 40 g a.i. ha^{-1} (4g product 16 L^{-1}) resulted in 93 per cent larval control. Indoxacarb @ 30 and 40 g a.i. ha^{-1} resulted in an yield of 1.5 to 6.5 t ha^{-1} and 1.7 to 6.4 t ha^{-1} respectively while the yield obtained in untreated control was 0.21 to 1.32 tons ha^{-1}

Bharathi *et al.*, (2011) evaluated the field efficacy of chlorantraniliprole 20 % SC (@ 10, 15, 20, 25 and 30 g a.i. ha $^{-1}$) against *B. cucurbitae*, *D. indica* and *A. foveicollis* in bitter gourd and reported that the the doses 20, 25 and 30 g a.i. ha $^{-1}$ effectively suppressed the population of the target insects compared to the untreated check

Viswanathan (2015) reported chlorantraniliprole 18.5 SC 0.006 % to be effective in reducing the population of epilachna beetle, *H. vigintioctopunctata* in brinjal plants causing 100 per cent mortality of the insects in the treated plants. Kodandaram *et al.*, (2014) reported that chlorantraniliprole 18.5 SC @ 150 g a.i. ha $^{-1}$ reduced the hatchability (70 per cent) of eggs of *H. vigintioctopunctata* and caused 86.7 and 10 per cent mortality of grubs and adults respectively under laboratory conditions.

Fipronil 5 % SC 0.01 %, flubendiamide 39.35% SC 0.1% and indoxacarb 14.5 % SC 0.001% resulted in 16.66, 93.33 and 96.66 per cent mortality of the grubs of *H. vigintioctopunctata*, 48 hr after treatment by leaf dip method in laboratory (Dash, 2016). For adults, the mortality percentage was 63.33 for both fipronil 5 % SC 0.01 % and flubendiamide 39.35% SC 0.1% 48 hr post treatment while 100 per cent mortality was observed indoxacarb 14.5 % SC 0.001%. During field studies, the lowest pest population was observed in indoxacarb 14.5 % SC 0.001% while fipronil 5 % SC 0.01 % recorded the maximum pest population after the untreated control

The efficacy of insecticides, chlorantraniliprole 18.5% SC @ 0.30 mL L^{-1}, flubendiamide 39.35% SC @ 0.10 mL L^{-1} and indoxacarb 14.5 % SC @ 1.00 mL L^{-1} in managing the population of fruit borer, *D. indica* was studied by Thamilarasi (2016). The treatments chlorantraniliprole 18.5% SC @ 0.30 mL L^{-1} and flubendiamide 39.35%SC @ 0.10 mL L^{-1} , were highly effective in managing the pest population as the plots sprayed with these insecticides were free of the pest after three days of spraying. In indoxacarb 14.5 % SC @ 1.00 mL L^{-1}, the per plant population of the borer reduced to zero after five days of spraying. However, the population increased to 0.8 larvae plant $^{-1}$, after fifteen days of spraying.

Balikai and Mallapur, (2017) conducted field experiments for two consecutive seasons to evaluate the efficacy of flubendiamide against pumpkin caterpillar, *D. indica*. They observed that flubendiamide 480 SC @ 60 g a.i. ha^{-1} and indoxacarb 14.5 SL @ 21.75 g a.i. ha^{-1} afforded the highest protection against fruit borer with 91.3 and 90.5 per cent reduction in infestation along with fruit yield of 10.45 and 10.24 t ha^{-1} during first season and 89.1 and 87.0 per cent reduction in infestation with 9.65 and 9.52 t ha^{-1} fruit yield during second season, respectively over the untreated check.

Flubendiamide 48% SC @ 80 mL a.i. ha^{-1} effectively managed *H. vigintioctopunctata* population in brinjal (Reshma, 2017). The reduction in

population of epilachna beetle over control was 60.62 and 62.43 per cent (1st and 2nd spray) during Kharif season and 86.35, 84.43 and 69.77 per cent (1st, 2nd and 3rd spray) during summer season

Nagaraju *et al.* (2018) tested the field efficacy of selective insecticides against cucumber moth, *D. indica* on bitter gourd and reported chlorantraniliprole 18.5% SC (0.2mL L^{-1}) as the best and effective treatment among flubendiamide 48% SC, spinosad 45 SC, emamectin benzoate 5% SG, dichlorvos 76% EC, azadirachtin 1500 ppm and *B. thuringiensis* in checking *D. indica* population and caused 86.85 per cent reduction in population.

2.2.3 Compatibility of EPF with Insecticides

One of the favourable aspects of an entomopathogen is its integration with other pest control measures, particularly the chemical method (Ramaraje Urs *et al.,* 1967). Effect of different pesticides had been reported to be varying from inhibitory to stimulatory on different entomopathogenic fungi.

2.2.3.1 B. bassiana

The compatibility of *B. bassiana* conidia with 10 widely used pesticides was assessed by Xu *et al.,* (2002). The results stated that inhibition in the conidial germination of *B. bassiana* increased with increasing pesticide concentrations and Fipronil 5 % FF, exhibited high compatibility with *B. bassiana* conidia with germination rates exceeding 90 per cent even at the highest concentration.

Rajanikanth *et al.*, 2010 studied the compatability of Indoxacarb (Avaunt 14.5SC 0.0145 per cent) with different strains of *B. bassiana*. At the tested dose, Indoxacarb caused no significant inhibition in the radial growth of *B. bassiana* strains. Significant inhibition of sporulation and spore viability in some strains to the range of 0.97 to 3.95 per cent reduction over control was noticed.

Flubendiamide 480 SC at different doses 36,48 and 60 ppm showed significant inhibition in the growth of *B. bassiana* at all the doses tested (Thilagam *et al.*, 2010). Slight toxicity of Thiodicarb to *B. bassiana* was observed by Amutha *et al.*, (2010). They also observed Indoxacarb to be highly toxic to *B. bassiana.* Rashid *et al.*,(2012) studied the effect of Fipronil 2.5 % EC at different concentrations on the germination, vegetative growth and sporulation of *B. bassiana* . At 400 ppm, Fipronil 2.5 % EC caused 46.04 per cent reduction in the germination of *B. bassiana*. At 1600 ppm, 76.64 per cent reduction in the vegetative growth and 88.14 per cent reduction in the sporulation of *B. bassiana* was observed. Nitin (2014) reported Flubendiamide 20 SC to be compatible with *B. bassiana*

2.2.3.2 M. anisopliae

Antonio *et al.*, (2001) reported Fipronil (Regent® 800 WG 15 to 250 g a.i ha^{-1}) to be incompatible with *M. anisopliae* as the insecticide reduces the conidial yield without affecting the vegetative growth.

Rachappa *et al.*, (2007) reported field dose of Indoxacarb to be less detrimental to *M. anisopliae* causing 39.7 per cent growth inhibition compared to the growth in control grown in unpoisoned media. Akbar *et al.*, (2012) also reported Indoxacarb (157 µL in 90 mL media) to be safe to conidial germination and growth of the different isolates of *M. anisopliae*

Slight inhibition in the conidial germination of *M. anisopliae* was noted with germination studies having Fipronil at a concentration of 50 and 100 ppm in the poisoned media. However complete inhibition of germination was there at a higher concentration of the insecticide (200 ppm). Also Fipronil at 10 ppm showed no reduction in the vegetative growth and sporulation of the fungi. At higher concentrations (40 ppm and 120 ppm), growth inhibition to the range of 60 to 76.4 per cent was noted (Rashid *et al.*, 2010) Amutha and Banu (2012) reported

Thiodicarb (75 WP) to be slightly toxic and Indoxacarb (14.5 % EC) to be highly toxic to *M. anisopliae*

Material and methods

3 MATERIALS AND METHODS

The study entitled 'Microbial consortium for the management of insect pests of bitter gourd (*Momordica charantia* L.)' was conducted at College of Agriculture, Vellayani during 2013-2017. The materials and methods adopted for the research are detailed below.

3.1. ISOLATION AND IDENTIFICATION OF MICROBIAL PATHOGENS ASSOCIATED WITH MAJOR PESTS OF BITTER GOURD

Frequent monitoring of 20 bitter gourd fields from Thiruvananthapuram and Kollam districts were carried out and the major pests of bitter gourd *viz., Bactrocera cucurbitae*, *Henosepilachna septima*, *Diaphania indica*, *Aulacophora foveicollis* and *Leptoglossus australis* were inspected. Cadavers of the mentioned insects as well as live and weak insects showing disease symptoms were collected and kept for isolation and was frequently observed for development of any fungal or bacterial infection.

3.1.1. Diagnosis of Microbial Infection

Based on the signs and symptoms present on the cadavers, a preliminary diagnosis was carried out in the laboratory for identifying the infectious agents.

3.1.2 Isolation of the Disease Causing Microorganisms

The disease causing agents were isolated from the cadavers adopting the methodology mentioned below.

3.1.2.1 Entomopathogenic Fungi

The insect cadavers suspected to be killed by fungal infection were kept in moist chambers consisting of petri plates lined with moist filter paper for hyphal

development. The cadavers were then surface sterilized with 0.1 % $HgCl_2$ for one min. followed by three washes in sterile water. The surface sterilized cadavers were then dried using sterile filter paper. The whole process was done under aseptic conditions in a laminar air flow chamber. The surface sterilized cadavers were placed in slants with Potato Dextrose Agar (PDA) and incubated at room temperature for development of mycelia. The fungal culture thus obtained was subcultured thrice and transferred to PDA slants. Fourteen day old slants were maintained at 4°C in a refrigerator.

3.1.2.2 Isolation and Maintenance of Microorganisms from Phylloplane of Bitter Gourd

The technique devised by Otsu *et al.* (2003) with slight modifications was employed for isolation of phylloplane microorganisms. Bitter gourd leaves were randomly collected and the upper surface of the detached leaves were pressed and left for two min. on Petri plates with sterile M9 minimal agar medium (12.8 g $Na_2HPO_4.7H_2O$, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 4 g glucose and 20 g agar in 1000 mL of water). The bacterial colonies obtained were brought to pure culture by streak purification and was refrigerated at 4°C in nutrient agar (NA) slants.

3.1.3. Screening of Isolated Entomopathogens for Pathogenicity

A preliminary screening of the isolated entomopathogens and phylloplane microorganism was done to confirm pathogenicity to the respective target insects. Fungal isolates were screened by spraying the spore suspensions on the test insects. A batch of ten insects was then transferred to trays with fresh food and was observed for disease development. For screening the bacterial isolates, the test insects were fed on leaves dipped in bacterial suspension with 10^8 cfu mL^{-1}. The test insects which ingested the treated food was then transferred to separate rearing jars with fresh food and observed for development of disease.

The insect cadavers obtained were surface sterilized and were then gently crushed in sterile distilled water. The suspension obtained was used as inoculum. After repeated streak purification by streak plate method in NA plates, the organism was brought to pure culture and transferred to NA slants. The isolate which could reproduce the symptoms and which could be re-isolated from the host cadavers were confirmed to be entomopathogenic and was selected for further studies.

3.1.4. Identification of Entomopathogens

3.1.4.1 Identification of Entomopathogenic Fungi

3.1.4.1.1 Morphological Identification

Morphological as well as cultural characteristics of the fungi were studied by growing the fungi in petri plates containing PDA. For this, fungal cultures were initially maintained in petri plates from which, mycelial discs of 5mm diameter were taken using sterile cork borers and were inoculated in the center of fresh PDA plates aseptically. Radial growth as well as other colony characters such as colour change of the mycelia was recorded daily. The conidial and conidiophore characters were studied using slide cultures prepared from 14 day old cultures of the respective fungi. The slides were viewed under a Motic BA 210 compound microscope under 40X magnification and measurements were taken.

3.1.4.1.2. Molecular Identification of fungus

DNA isolation using NucleoSpin® Plant II Kit (Macherey-Nagel)

For DNA isolation, a sample size of 100 mg of tissue/mycelium was taken and homogenized using liquid nitrogen. This homogenized tissue was transferred to a microcentrifuge tube and added four hundred microlitres of buffer PL1. After vortexing for 1 min., 10 µL of RNAase A solution was added, mixed and was

incubated at 65°C for 10 min. The lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for two min. After discarding the filter, the flow through liquid was collected. To this, 450 µL of buffer PC was added and mixed well. This solution was transferred to a Nucleospin Plant II column, centrifuged for one min. and the flow through liquid was discarded. To the column, 400 µL buffer PW1 was added and centrifuged at 11000 x g for one min. After discarding the flow through liquid, 700 µL PW2 was added. Centrifugation was done at 11000 x g and flow through liquid was discarded. Finally 200 µL of PW2 was added and centrifuged at 11000 x g for two minutes to dry the silica membrane. The column was transferred to a new 1.7 mL tube and 50 µL of buffer PE was added and incubated at 65^{0}C for five min. which is then centrifuged at 11000 x g for one min. to elute the DNA. The eluted DNA was stored at 4°C.

Agarose Gel Electrophoresis for DNA Quality check

The DNA isolated using NucleoSpin® Plant II Kit was quality checked using agarose gel electrophoresis. The buffers used for the study were 1µL of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) and TBE (Tris-Borate-EDTA) buffer containing 0.5 µg mL^{-1} ethidium bromide. 5µL of eluted DNA was taken and 1µL of 6X gel-loading buffer was added to it. 0.8% agarose gel was prepared in 0.5X TBE buffer containing 0.5 µg mL^{-1} ethidium bromide and the DNA samples were loaded to it. Electrophoresis was performed until bromophenol dye front has migrated to the bottom of the gel. With the help of a UV transilluminator (Genei), the gels were visualized and the images were captured under UV light using Gel documentation system (Bio-Rad).

PCR Analysis

PCR amplification reactions were carried out in a reaction volume containing 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2 mM each dNTPs (dATP, dGTP,

dCTP and dTTP), 1 µL DNA, 0.2 µL Phire Hotstart II DNA polymerase enzyme, 0.1 mg mL^{-1} BSA and 3 % DMSO, 0.5M Betaine, 5pM of forward and reverse primers . About 20 µL of reaction volume was required to conduct the experiment.

Primers used

Target	Primer Name	Direction	Sequence (5' → 3')
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTGCGG
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

PCR amplification profile

ITS & LSU

98 °C - 30 sec

98 °C - 5 sec
60 °C - 10 sec
72 °C - 15 sec
} 40 cycles

72 °C - 60 sec

4 °C - ∞

Agarose Gel electrophoresis of PCR products

PCR products were separated on 1.2 % agarose gels prepared in 0.5X TBE buffer containing 0.5 μg mL^{-1} ethidium bromide. 5 μL of PCR products were mixed with 1 μL of 6X loading dye and this mixture was loaded to the electrophoresis unit. The electrophoresis was performed at 75 V for 1 to 2 hr. The end point was marked with the migration of bromocresol blue front to the bottom of the gel. 0.5X TBE acted as the electrophoresis buffer and 2-log DNA ladder (NEB) was utilized as the molecular standard. For visualizing the gels, a UV transilluminator (Genei) was used and the obtained images were captured under UV light using Gel documentation system (Bio-Rad).

ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) was used for removing unwanted primers and dNTPs from a PCR product mixture. It consisted of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer. For the ExoSAP-IT treatment of PCR product mixture, 5 μL of the mixture was mixed with 2 μL of ExoSAP-IT and incubated at 37°C for 15 min. This was followed by enzyme inactivation at 80°C for 15 min.

Sequencing using BigDye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) was used for conducting the reaction.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated) - 10-20 ng

Primer	-	3.2 pM (either Forward or Reverse)
Sequencing Mix	-	0.28 µL
5x Reaction buffer	-	1.86 µL
Sterile distilled water	-	made up to 10µL

For all the primers used, the sequencing PCR temperature profile consisted of several cycles with the 1st cycle at 96°C for 2 min. followed by 30 cycles at 96°C for 30 sec. 50°C for 40 sec. and 60°C for 4 min.

Post Sequencing PCR Clean up

Post sequencing PCR clean up involved the use of two master mixes, master mix I and II. In the first step, master mix I was prepared with 10µL milli Q and 2 µL 125 mM EDTA per reaction. 12 µL of this was added to each reaction containing 10µL of reaction contents and was properly mixed. Master mix II was made of 2 µL of 3M sodium acetate pH 4.6 and 50 µL of ethanol per reaction. 52 µL of master mix II was added to each reaction. After mixing, the contents were incubated at room temperature for 30 min.followed by centrifuging at 14,000 rpm for 30 min. 100 µL of 70 % ethanol was added to the supernatant and again centrifuged at 14,000 rpm for 20 minutes followed by the ethanol wash. The pellet obtained was air dried and sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

Sequence Analysis

The sequence quality check was performed using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.

Nucleotide sequence analysis

BLAST (Basic Local Alignment Searching Tool) was used for analysing the nucleotide sequence.

3.1.4.2. Identification of entomopathogenic bacteria

3.1.4.2.1. Molecular Identification

Molecular characterization of bacterial isolates were done by 16S rRNA cataloging using universal primers.

Genomic DNA Isolation

Genomic DNA was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions.

Loop full bacterial culture was transferred to one mL sterile distilled water taken in a microcentrifuge tube. 180 µL of T1 buffer and 25 µL of proteinase K was added and incubated at 56 °C in a water bath until it was completely lysed. After lysis, 5 µL of RNase A (100 mg / mL) was added and incubated at room temperature for 5 minutes. 200 µL of B3 buffer was added and incubated at 70 °C for ten minutes. 210 µL of 100% ethanol was added and mixed thoroughly by vortexing. The mixture was pipetted into NucleoSpin® Tissue column placed in a 2 mL collection tube and centrifuged at 11000 × g for one minute. The NucleoSpin® Tissue column was transferred to a new 2 mL tube and washed with 500 µl of BW buffer. Wash step was repeated using 600 µL of b5 buffer. After washing the NucleoSpin® Tissue column was placed in a clean 1.5 mL tube and DNA was eluted out using 50 µL of BE buffer.

Agarose Gel Electrophoresis for DNA Quality and Quantity Check

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1 µL of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH – 8.0) was added to 5 µL of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µL / mL ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were observed in a UV Transilluminator GeNei®.

PCR Analysis

PCR amplification reactions were carried out in a 20 µL reaction volume which contained 1X PCR buffer (100 mM Tris HCl, pH – 8.3, 500 mM KCl), 0.2 mM each dNTP's (dATP, dGTP, dCTP and dTTP), 2.5 mM $MgCl_2$, 1 unit of AmpliTaq Gold DNA polymerase enzyme, 0.1 mg / mL BSA, 4% DMSO, 5 pM of forward and reverse primers and FTA disc as template.

Primers used :

Target	Primer Name	Direction	Sequence (5' → 3')
16S rRNA	16S-RS-F	Forward	CAGGCCTAACACATGCAAGTC
	16S-RS-R	Reverse	GGGCGGWGTGTACAAGGC

The amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied biosystems).

PCR amplification profile :

16S rRNA

95 °C	-	5.00 min	
95 °C	-	30 Sec	35 cycles
60 °C	-	40 Sec	
72 °C	-	60 Sec	
72 °C	-	7.00 min	
4 °C	-	∞	

Agarose Gel Electrophoresis of PCR Products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg / ml ethidium bromide. 1 µL of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75 V power supply with 0.5 TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder. The gels were visualized in a UV Transilluminator Genei®.

Sequence Analysis

The nucleotide sequence of 16S rRNA was compared with the sequence available in the database using the BLAST tool offered by National Centre for Biotechnology Information (NCBI). BLASTn provided by NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was carried for homology search.

3.2. PATHOGENICITY OF ENTOMOPATHOGENIC FUNGI

Pathogenicity studies of the fungi *viz., Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Lecanicillium* (*Verticillium*) *lecanii* (Zimmermann) Zare and Gams, *Purpureocillium lilacinus* (Thom), and isolated pathogens from the field were carried out on the following insect pests

1. Pumpkin caterpillar (*Diaphania indica* Saunders)
2. Melon fly (*Bactrocera cucurbitae* Coquillet)
3. Epilachna beetle (*Henosepilachna septima* Dieke)
4. Pumpkin beetle (*Aulacophora foveicollis* Lucas)
5. Leaf footed bug (*Leptoglossus australis* Fabricius)

3.2.1. Maintenance of Fungal Cultures

Different fungal isolates used for the study were *B. bassiana* (Bb5), *L. lecanii* (Ll8), and *M. anisopliae* (Ma4) (obtained from National Bureau of Agricultural Insect Resources (NBAIR, Bengaluru) and maintained in Biocontrol lab, Vellayani, Kerala Agricultural University, K.A.U isolates *P. lilacinus* (ITCC No.6064) and *B. bassiana* (ITCC No.6063) along with the field isolates. The fungi were subcultured and maintained in Potato Dextrose Agar (PDA) slants. Fourteen day old slants were stored under refrigeration for carrying out various experiments.

3.2.2. Maintenance of Stock Insects

3.2.2.1 B. cucurbitae

Bitter gourd fruits infested with fruit fly were collected from the field and kept in glass troughs containing sterile soil for pupation. Glass troughs of size 30 cm diameter were used for rearing the flies. Sterile soil to a depth of 4 cm was

maintained in each trough and the soil was kept moistened by sprinkling water. On pupation, the pupae were collected and transferred into another rearing jar 15× 20 cm for adult emergence. The jar was secured using a muslin cloth. The emerged flies were provided with honey and yeast solution (2 mL honey + 1g yeast in 10 mL water) soaked in cotton balls. The adult flies were supplied with fresh fruits for oviposition.

3.2.2.2 A. foveicollis

Field collected adults of *A. foveicollis* were released in glass jars containing fresh stem and leaves of salad cucumber for egg laying. To prevent withering of leaves, fresh end of the stem were provided with water soaked cotton pads. After oviposition, the adult beetles were transferred to separate jar. Stems with ovipositional punctures and leaves with eggs were transferred to trays and kept undisturbed for hatching. The grubs were reared in plastic trays with fresh roots and stems of salad cucumber. The trays were kept clean by timely removal of the decayed plant parts. Fifteen grubs were maintained per tray and reared up to adult emergence. The grubs on maturity were allowed to pupate on sterile soil maintained in trays. The emerged adults were then transferred into jars containing fresh stem and leaves for egg laying.

3.2.2.3 H. septima

Field collected adults as well as grubs of *H. septima* were reared on fresh bitter guard leaves kept in rearing jars of 15 cm x 20 cm size. A total of 10 adults/grubs were maintained per jar. Fresh bitter gourd leaves were provided at an interval of two days. Eggs were transferred to fresh jars and the emerged grubs were fed with fresh bitter gourd leaves. Adults upon emergence were transferred to separate rearing jars for maintaining the culture.

3.2.2.4 D. indica

Larvae of *D. indica* collected from field were reared on fresh bitter gourd leaves kept in rearing jars of 15 cm x 20 cm size. The leaves were kept fresh using moist cotton plugged to the stem. Mouths of the jars were tightly secured using fine muslin cloth and rubber band. Pupae were transferred to another rearing jar for adult emergence. On emergence, the adults were fed with 10 per cent honey solution soaked in cotton pads. A small drop of Vitamin E oil was also added to the honey solution to boost the egg laying. The first instar larvae were transferred to fresh jars to maintain the culture.

3.2.2.5 L. australis

Eggs of *L. australis* were collected from the field and surface sterilized with 0.1 % sodium hypochlorite solution for 2 min. followed by two to three washes in sterile water. The sterilized eggs were kept for emergence in rearing jars of 15 cm x 20 cm. Fresh stem as well as fruits of bitter gourd were provided as food for the emerging nymphs. Feed were changed regularly and the jars were covered using muslin cloth and rubber band. The emerged nymphs were used for conducting the study.

3.2.3. Pathogenicity Tests

The fungal isolates mentioned in 3.2.1 were tested for its pathogenicity against the target insects mentioned in 3.2.2. Pathogenicity tests were conducted against second instar larvae of *D. indica*, second instar grubs and adults of *H. septima*, adults of *A. foveicollis*, adults and pupae of *B. cucurbitae* and third instar nymphs and adults of *L. australis*. Pathogenicity tests were conducted using spore suspensions of various fungi. The fungi were cultured in Potato Dextrose Broth (PDB) and 14 day old cultures were used for the study. The cultures were blended for 2 min. using a mixer, strained and the spore suspensions were used for treating

insects. Spore count of the suspensions was estimated using a Neubaeur's haemocytometer.

The spore suspensions were applied directly on to the test insect using an atomizer. Daily observations on mortality of the test insect, feeding behavior and disease symptoms were noted up to 12 days after treatment. Dead insects from the experiment were kept separately in moist chambers for symptom development and pathogenicity was confirmed by establishing Koch's Postulates.

3.3 BIOASSAY

Bioassay of the pathogens mentioned in 3.2.1 was conducted to find their effective concentration against the insects which were found susceptible as per 3.2.3. The spore suspension obtained as in 3.2.3 was then filtered through a double layered muslin cloth. The filtrate was then centrifuged at 5000 rpm for 25 min in Hermile labortechnic Z323K centrifuge. The pellet obtained was washed with sterile distilled water and again suspended in 10 mL sterile distilled water. Spore count of the suspension was enumerated using Neubeur's haemocytometer and was adjusted to 10^9 spores mL^{-1} after making necessary dilutions. The suspension was then serially diluted to obtain various lower concentrations (10^8, 10^7, 10^6 and 10^5 spores mL^{-1}) and were sprayed directly over the test insects using an atomizer. Insects sprayed with sterile distilled water served as control. Four replications were maintained for each test insect and each replication contained 15 insects. Fresh feed was provided daily and mortality were recorded at 24 h interval.

The observations obtained were subjected to probit analysis after necessary correction using Abbott's formula (Abbott, 1925). SPSS Statistics Version 21 was used for the calculation of parameters like LC_{50}, LC_{90}, LT_{50} values and fiducial limits.

3.4. DEVELOPMENT OF MICROBIAL CONSORTIUM

3.4.1. Compatibility of Entomopathogens

3.4.1.1 Dual Culture Studies

In vitro compatibility between various fungi was tested using dual culture plate technique (Dennis and Webster, 1971) using PDA medium in Petri plates of diameter 9 cm. The test organisms between which compatibility has to be assessed were grown separately in PDA plates for 5 days from which mycelial discs of 5mm diameter was excised using a sterile cork borer.

Mycelial discs of both the fungi were inoculated in the same Petri plate 2 cm away from the edge. Five replications were maintained for each treatment and the plates were incubated at room temperature. Plates with only one fungus served as control. The plates were incubated for 10 days and daily observations on mycelial growth were taken in the dual culture plates as well as in the control plates. The percentage inhibition on growth of one organism by other is calculated by the formula;

$$L = \frac{(C - T)}{C} \times 100$$

Where L = Percentage inhibition of radial growth of the selected fungus

C = Radial growth of the selected fungus (mm) in control

T = Radial growth of the selected fungus (mm) in treatment

Based on the ability of one organism to overgrow and inhibit the growth of other, a score has been given as per modified Bell's scale (Bell *et al.*, 1982). Where

R1 = 100 % over growth

R2 = 75 % over growth

R3 = 50 % over growth

R4 = locked at the point of contact

Fungal combinations used for studying in vitro compatibility are:

- *M. anisopliae* + *B. bassiana*
- *M. anisopliae* + *P. lilacinus*
- *M. anisopliae* + *L. lecanii*
- *B. bassiana* + *P. lilacinus*
- *B. bassiana* + *L. lecanii*
- *P. lilacinus* + *L. lecanii*

3.4.2. Laboratory Evaluation of the Consortium

3.4.2.1 Against Individual Species

The fungal combinations which were found compatible as per experiment 3.4.1 were tested for pathogenicity against major pests of bitter gourd listed in 3.2. The treatments tested include the microbial consortiums developed based on in vitro compatibility studies, along with its component fungi. For preparing the fungal combinations, spore pellets of the component fungi obtained as in experiment 3.3 was suspended in sterile water and was mixed till the desired spore count of 10^{8} spores mL^{-1} was reached. Each treatment was replicated thrice and ten insects were maintained per replication. Insects sprayed with sterile water served as control.

3.4.2.2. Against Mixed Population

The fungal combinations which were found compatible as per experiment 3.4.1 were tested for pathogenicity along with its component fungi on a mixed population where a single experimental unit was a representative of all the test insects mentioned in 3.2. Each replication contained six representatives from each of the test insects mentioned in experiment 3.2. Three replications were maintained per treatment and observations on mortality of insects were noticed.

3.5. DEVELOPMENT OF TALC FORMULATION OF THE CONSORTIUM

The microbial combination which offered the best result against all the test insects in experiment 3.4.2 was formulated in talc and used for further studies.

3.5.1. Preparation of Spore Suspensions

Components of the selected consortium were cultured in PDB at room temperature. Spore suspension was prepared as in 3.3 and the spore count of the suspension was adjusted to 10^9 spores mL^{-1} after making necessary dilutions. This spore suspension was used for making the formulation.

3.5.2. Development of Talc Formulation

The carrier material for the formulation, talc was sterilized prior to formulating by autoclaving at 1.06 kg cm^{-2} and 121°C for 40 min. Fifteen mL spore suspension (10^9spores mL^{-1}) of each of the component fungus (prepared as per 3.4.3.1) of the selected consortium was added to 90 g of sterile talc and mixed thoroughly. This was shade dried till the recommended moisture limit of 10 -12 %

was reached. The formulated material was stored at room temperature in sterile polythene bags.

3.5.3 Assessment of Shelf Life

The shelf life studies of the talc formulation developed in experiment 3.4.3.2. were done by storing them in polyethylene bags at two different temperatures, at room temperature and under refrigeration. Spore count and cfu were estimated at fortnightly intervals for a period of three months. Bioefficacy of the formulation was also assessed at monthly intervals on the target insects mentioned in 3.2.

3.5.3.1.Estimation of Colony Forming Units (cfu)

Dilution plate method (Aneja, 1996; 2003) was used to estimate the colony forming units (cfu). Ten gram of the talc based formulation of the consortium was mixed with 90 mL of sterile water to get a dilution of 10^{-1}. Serial dilutions were made to obtain concentrations of 10^{-3} and 10^{-5} and 10^{-7}. One mL of this diluted formulation was poured to each Petri plate over which 15 mL of molten PDA was added and gently rotated for uniform spreading of the suspension. The plates were then incubated at room temperature for seven days. Four replications were maintained for each treatment. Cfu was estimated using the formula,

Cfu = number of colonies x dilution factor / volume of sample

3.5.3.2. Enumeration of Spore Count

In order to find the spore count of the developed formulation, one gram of the talc formulation was weighed and mixed in 10 mL sterile water. This was filtered using a double layered muslin cloth. A drop from this filtrate was used to assess the spore count using a Neubaeur's haemocytometer. Number of spores present in 1 mL of the filtrate was assessed using the formula,

$$\text{Number of spores } mL^{-1} = \frac{Dx}{nk}$$

Where, D - dilution factor

x - total number of spores counted from 30 to 50 squares

n - number of small squares counted

k - volume of one small square in cm^3

3.6. DETERMINATION OF EFFECTIVE DOSE OF THE FORMULATED CONSORTIUM

To determine the effective dose of the formulated consortium, different doses of the formulated consortium *viz.,* 15, 20, 25, 30, 35 and 40 g L^{-1} were tested on the second instar larvae of *D. indica* and were assessed based on mortality of the test insect. Three replications of 10 insects each were maintained for all the treatments. Insects sprayed with talc alone and insects sprayed with sterile water were kept as control.

3.7. COMPATIBILITY OF THE CONSORTIUM WITH INSECTICIDES

The components of the developed consortium, were tested for compatibility with six different insecticides using Poison food technique (Zentmeyer, 1955). The growth and sporulation of the fungi grown on PDA poisoned with various insecticides was assessed. The insecticides selected were the ones which are generally used for pest management in bitter gourd. The experiment was conducted in CRD with four replications. The various insecticides used for the study are listed below:

1. Flubendiamide 20% WG @ 0.01%

2. Fipronil 5 % SC @ 0.01%

3. Thiodicarb 75 % WP @ 0.1 %

4. Chlorantraniliprole 18.5 SC @ 0.006%

5. Indoxacarb 14.5% SC @ 0.015%

For preparation of the poisoned media, appropriate amount of insecticides were measured and added separately to conical flasks containing sterile molten PDA and mixed thoroughly. This was poured to 9 mm petri plates and allowed to solidify. Fungal discs of 0.5 cm diameter was cut from ten days old culture plates of the respective fungi using a sterile cork borer and was placed centrally in petri plates containing poisoned media. The plates were incubated at room temperature. The whole procedure was conducted aseptically in a laminar air flow chamber. Fungal discs inoculated into plates containing sterile PDA without insecticide served as control.

3.7.1 Estimation of Growth of the Fungi

Observations on the colony diameter of the fungi were made daily, until the fungi fully covered the control plates.

3.7.2 Estimation of Spore Count

To assess the sporulation of the fungi, at 14 days after inoculation, 10 mL sterile water was poured into each petri plate with sporulating fungi and the surface was gently scrubbed using a sterile spatula and the spore suspension was collected. Spore count was estimated using an improved Neubauer's haemocytometer after making necessary dilutions.

3.8. FIELD EVALUATION OF THE CONSORTIUM

Based on lab study conducted on various pests of bitter gourd (experiment 3.5), promising dose of the formulated consortium was identified and tested under field conditions along with the insecticide found compatible as per experiment 3.6, Malathion as chemical check and an untreated control. Bitter gourd variety Preethi was raised under pandal system in the instructional farm, College of Agriculture, Vellayani. The experiment was laid out in Randomized Block Design with nine treatments replicated thrice.

Plots of size 2×2 m^2 were maintained and five plants were kept per plot. The recommended POP of KAU (KAU, 2016) was followed except for the plant protection measures which were given as per the treatments fixed in the study Spraying was done during evening hours.

Pretreatment counts on *B. cucurbitae*, *D. indica, H. septima* and *A. foveicollis* were taken and post treatment counts were taken at first, third, fifth, seventh and fourteenth days after treatment (DAT).

Table 1: Treatments for field evaluation of the consortium

Treatment	Method of application
Talc based formulation of consortium I	Two spraying
Compatible insecticide from 3.7	Two spraying
T1 followed by T2	Spraying

T2 followed by T1	Spraying
Malathion 0.1%	Two spraying
Untreated check	

3.8.1. Assessment of population of the target insects

For assessing the population and damage by various target insects, counts were taken from three plants per replication. Random counts were taken from selected plants for different pests, the details of which are given below

- *D. indica* - Number of larvae present in 10 leaves plant^{-1}
- *H. septima*- Number of adults/grubs present in 10 leaves plant^{-1}
- *A. foveicollis*- Number of adults present in 10 leaves plant^{-1}
- *B. cucurbitae* Number of damaged fruits per plot
- Number of natural enemies plant^{-1}

3.8.2. Estimation of Yield

Yield was recorded separately from each treatment at the time of harvest and the data was subjected to analysis of variance.

Results

4 RESULTS

4.1 ISOLATION AND IDENTIFICATION OF MICROBIAL PATHOGENS ASSOCIATED WITH MAJOR PESTS OF BITTER GOURD

The details of the entomopathogenic isolates obtained are given below. One fungus was isolated from cadavers of *H. septima* grub and *D. indica* larvae collected from the farmer's field in Thiruvananthapuram district. One bacterium was isolated from the bitter gourd phylloplane which was found to be pathogenic to *D.indica* larvae and *H.septima* grub. Identification of the pathogen was done on the basis of morphological and molecular characters and the details are given below.

4.1.1 Identification of Fungus

4.1.1.1 Morphological Characters

The mycelium of the fungus was white in PDA and attained slight violet tinge on aging. Lower side had violet to dark violet pigmentation (Plate 1A). The mycelium had a thin mat like appearance. The radial growth of the fungus reached 8.5 to 9 cm at 10 days after inoculation at a temperature of 28 ± 2°C. Both macro and micro conidia were present. Macro conidia were 3 to 5 septate, slightly crescent shaped with tapering ends and the micro conidia were oval shaped and aseptate (Plate 1B).

4.1.1.2 Molecular Characters

The sequence obtained through the 16S rRNA sequencing is given below and was subjected to nucleotide BLAST

TGTGACATACCAATTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAAC
GGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCT
GAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTC

TGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTAT
TCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCCCGGG
TTTGGTGTTGGGGATCGGCGAGCCCTCGCGGCAAGCCGGCCCCGAAATCT
AGTGGCGGTCTCGCTGCAGCTTCCATTGCGTAGTAGTAAAACCCTCGCAA
CTGGTACGCGGCGCGGCCAAGCCGTTAAACCCCCAACTTCTGAATGTTGA
CCTCGGATCAGGTAGGAATACCCGCTGAACTTAA

The BLAST result showed that the fungus had 100 per cent similarity to the *Fusarium verticilloides* isolate ATLOY2 having the accession number KX957964.1 The isolate also showed 100 per cent similarity with *F. proliferatum* isolate LrLF8 having accession number MG543710.1 and *Fusarium* sp. ZL-2014 strain EM-1-17 having accession number KJ629549.1.

4.1.2 Identification of Bacterium

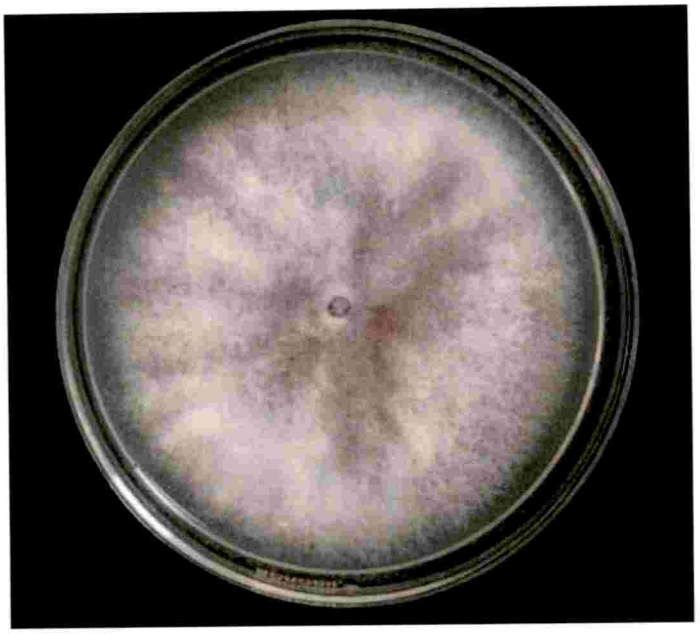
4.1.2.1 Morphological Characters

The bacteria produced crimson red colonies when cultured on nutrient agar (Plate 2). Rapid growth was noticed and the colonies were smooth and had a shiny appearance.

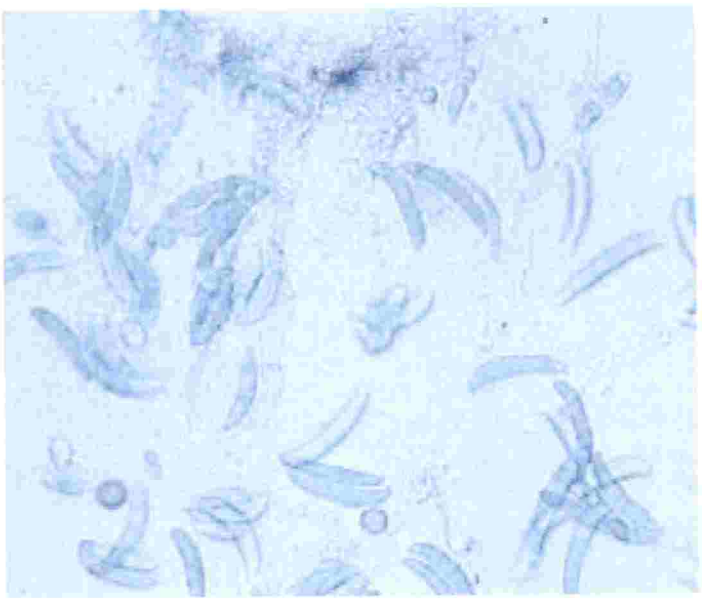
4.1.2.2 Molecular Characters

The sequence obtained through the ITS sequencing is given below and was subjected to nucleotide BLAST.

GGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAA
CTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGG
GGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAG
TAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAG



(A) Growth of *Fusarium verticilloides* in PDA



(B) Conidia

Plate1: Colony morphology and conidia of *Fusarium verticilloides*

GATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG
CCGCGTGTGTGAAGAAGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGG
AAGGTGGTGAACTTAATACGTTCATCAATTGACGTTACTCGCAGAAGAAG
CACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGC
GTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTC
AGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGAAACTGGCAA
GCTAGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATG
CGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAA
GACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAG
GCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGC
CGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGG
AGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGAC
ATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTGAGAC
AGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGT
CCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCGGCCGGGA
ACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGT
CAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCG
TATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTAC
GTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCG
CTAGTAATCGTAGATCA

The BLAST result showed that the bacterium had 99 per cent similarity to the *Serratia marcescens* strain RSC-14 having the accession number CP012639.1 and *S. marcescens* strain CAV1492 with accession number CP011642.1. The isolate also showed 98 per cent similarity with *Enterobacter cloacae* strain coIR/S. The bacterium was identified as *Serratia marcescens*.

(A) Crimson red coloured colonies of *S. marscecens*

(B) *Henosepilachna septima* grub

(C) *Diapania indica* larvae

Plate 2: Colony morphology (A) and symptoms of *Serratia marcescens* infection in (B) *H. septima* grub (C) *D. indica* larvae

4.2. PATHOGENECITY OF ENTOMOPATHOGENS TO VARIOUS TEST INSECTS

Pathogenicity of different entomopathogenic fungi such as *B. bassiana, M. anisopliae, L. lecanii, F. verticilloides* and bacterium *S. marcescens* were tested against the major pests of bitter gourd. Spore suspensions of the respective fungi used for conducting tests were prepared from 14 day old cultures in PDB. 48 hr old culture of the bacteria grown in nutrient agar medium was used for conducting pathogenicity trials. Infection in insects was confirmed through Koch's postulate. The results on pathogenicity of fungi and bacterium are presented in Table 2

4.2.1. *B. cucurbitae*

4.2.1.1. *Adults*

The adults of *B. cucurbitae* infected with various fungi became less active and had reduced food intake. Mortality was noticed from second day onwards in *M. anisopliae* and *P. lilacinus* treated insects and three days post treatment in *B. bassiana* treated insects. In all the cases, white mycelial growth was seen covering the cadaver leaving the wings intact for *M. anisopliae* and *B. bassiana* (Bb5, ITCC 6063). The mycelial growth turned green for *M. anisopliae* and slightly vinaceous brown in case of *P. lilacinus.* (plate 3). White fluffy mycelial mat is seen covering the body of the insect in case of *B. bassiana* (Bb5, ITCC 6063), two days post death.

On treatment with four different fungal isolates at a concentration of 10^8 spores mL^{-1}, mortality was observed in the adults of *B .cucurbitae* three days post treatment. The highest mean mortality percentage was observed in *M. anisopliae* and *P. lilacinus* treated insects having mortality percentage of 11.67 each and was significantly higher compared to the rest of the treatments (Table 3).

Table 2: Pathogenicity of various entomopathogens to the major pests of bitter gourd

Pest	Stage of pest	*B. bassiana*	*M. anisopliae*	*L. lecanii*	*P. lilacinus*	*F. verticilloides*	*S. marscescens*
B. cucurbitae	A	+	+	-	+	-	-
	P	+	+	-	+	-	-
H. septima	A	+	+	-	-	-	-
	G	+	+	-	-	-	+
D. indica	L	+	+	-	+	+	+
L. australis	N	+	+	-	-	-	-
	A	+	+	-	-	-	-
A. foveicollis	A	+	+	-	-	-	-
A. gossipii	A	+	+	+	-	+	-

A-adult, P-pupa, G-grub, L-larvae, N-nymph

+ Pathogenic

- Non-pathogenic

Table 3. Mortaliy of *Bactrocera cucurbitae* adults treated with various entomopathogenic fungi

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)				
	3DAT**	4 DAT *	5 DAT *	6 DAT *	7 DAT *
B. bassiana (Bb5)	5.00 $(2.19)^b$	11.67 $(19.80)^c$	51.67 $(45.96)^c$	81.67 $(64.72)^b$	83.54 $(66.07)^b$
M. anisopliae (Ma4)	11.67 $(3.46)^a$	45.00 $(42.12)^a$	68.34 $(55.78)^a$	88.34 $(70.20)^a$	93.54 $(76.11)^a$
B. bassiana (ITCC6063)	5.00 $(2.19)^b$	8.34 $(16.58)^c$	41.67 $(40.19)^d$	75.00 $(60.04)^c$	80.00 $(63.51)^b$
P. lilacinus (ITCC6064)	11.67 $(3.46)^a$	25.00 $(29.72)^b$	58.33 $(49.81)^b$	83.34 $(66.02)^b$	93.54 $(76.24)^a$
Control	0.00 $(0.74)^c$	0.00 $(0.74)^d$	0.00 $(0.74)^e$	0.00 $(0.74)^d$	3.34 $(7.85)^c$
C D (0.05)	(1.060)	(5.170)	(2.647)	(3.756)	(8.460)

Figures in parenthesis are ** square root transformed values

*angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

(A) *M. anisopliae*

(B) *B. bassiana*

(C) *P. lilacinus*

Plate 3: *Bactrocera cucurbitae* adults and pupae infected by different entomopathogens

At four days after treatment, a mean mortality percentage of 45 was observed in insects treated with *M. anisopliae* and was statistically superior to all other treatments. This was followed by *P. lilacinus* having a mean mortality percentage of 25. Least mortality was observed in *B. bassiana* isolates Bb5 and ITCC 6063 having a mean mortality percentage of 11.67 and 8.34 respectively. A similar trend was observed five days after treatment with *M. anisopliae* having the highest mortality percentage of 68.34.

At six days after treatment, the mortality was high in all the treatments. Maximum mortality of 88.34 per cent was observed in *M. anisopliae*. This was significantly high compared to all the other treatments. The next superior treatments were *P. lilacinus* and *B. bassiana* (Bb5) which were statistically on par and had a mean mortality percentage of 83.34 and 81.67 respectively. 75 per cent mortality was observed in insects treated with *B. bassiana* (ITCC 6063) which was the least compared to all the other treatments.

P. lilacinus and *M. anisopliae* recorded the highest mean mortality percentage of 93.54 at 7 days after treatment and was statistically superior to all the treatments. The next superior treatments were the *B. bassiana* isolates Bb5 and ITCC 6063 having a mortality percentage of 83.54 and 80 respectively. In control, 3.34 per cent mortality was observed seven days after treatment.

4.2.1.2 *Pupa*

The infected pupae became blackish initially and later white patches of mycelia were seen for *B. bassiana* and *M. anisopliae* (plate 3). For *M. anisopliae,* the white patches turned slighltly yellowish. *P. lilacinus* infected pupae showed white mycelial growth which later turned slightly brown.

The data on the cumulative mortality of *B. cucurbitae* pupa treated with four different fungal isolates at a concentration of 10^8 spores mL^{-1} are given in Table 4. At 7 DAT, the highest cumulative mortaliy of 100 per cent was obtained in *P. lilacinus*.

Table 4. Mortaliy of *Bactrocera cucurbitae* pupae treated with different entomopathogenic fungi

Treatments @ 10^8 spores mL^{-1}	Pupal mortality at 7DAT	Adult emergence at 7DAT
B. bassiana (Bb5)	90.00 (71.86)[b]	10.00 (18.14)[b]
M. anisopliae (Ma4)	98.75 (86.28)[a]	1.25 (3.72)[c]
B. bassiana (ITCC6063)	86.25 (68.45)[b]	13.75 (21.55)[b]
P. lilacinus (ITCC6064)	100.00 (89.35)[a]	0.00 (0.65)[c]
Control	0.00 (0.65)[c]	100 (89.35)[a]
CD (0.05)	(5.618)	(5.618)

Figures in parenthesis are angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

No adult emergence was observed in *P. lilacinus* treated pupae. Mortality obtained in *M. anisopliae* was on par with that of *P. lilacinus*, the mortality percentage being 98.75 with 1.25 per cent adult emergence. This was followed by *B. bassiana* isolates Bb5 and ITCC 6063 with mortality percentages of 90 and 86.25 respectively and was on par. 13.75 per cent adults emerged in pupa treated with *B. bassiana* (ITCC 6063) and 10 per cent adult emergence was noted in pupa treated with *B. bassiana* (Bb5). All the pupae emerged in control.

4.2.2. *D. indica*

Of the various fungi tested, *M. anisopliae*, *B. bassiana*, *P. lilacinus* and *F. verticilloides* were found pathogenic to *D. indica.* Reduced feeding and movement were the first noticeable symptoms of mycosis. In both *M. anisopliae* (Ma4) and *B. bassiana* (Bb5, ITCC 6063) treated insects, death commenced on three DAT while death was observed four DAT in *P.lilacinus* and *F. verticilloides*. The infected larvae were mummified. Spiracular regions of the *M. anisopliae* (Ma4) treated larvae developed black coloured spots at first and later white coloured mycelial growth was seen through the intersegmental regions. The white mycelia covered the entire body of the insect in three days and turned green after two days. Slight pinkish colouration was observed in *B. bassiana* infected cadaver and was completely covered with white mycelial growth four days after death (plate 4). White fluffy mycelia covered the insect cadaver three days after death in *P. lilacinus* treated insects while in *F. verticilloides* treated insects, the cadaver showed white mycelial growth which later turned slightly pinkish.

The second instar larvae of *D. indica* on treatment with four different fungal isolates at a concentration of 10^8 spores mL^{-1} three days after treatment, the highest mean mortality percentage of 15 was observed with *M. anisopliae* which was significantly higher than all other treatments (Table 5). At four days after treatment, a mean mortality percentage of 30 was observed on insects treated with *M. anisopliae*

Table 5. Mortaliy of *Diaphania indica* larvae treated with various entomopathogenic fungi

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)				
	3 DAT **	4 DAT *	5 DAT *	6 DAT *	7 DAT *
B. bassiana (Bb5)	2.50 (1.34)^{b}	7.50 (2.61)^{b}	17.50 (24.52)^{b}	37.50 (37.65)^{b}	55.00 (47.87)^{b}
M. anisopliae (Ma4)	15.00 (3.88)^{a}	30.00 (5.48)^{a}	42.50 (40.66)^{a}	60.00 (50.81)^{a}	82.50 (65.44)^{a}
B. bassiana (ITCC6063)	2.50 (1.34)^{b}	5.00 (1.97)^{bc}	10.00 (15.35)^{bc}	32.50 (34.70)^{b}	50.00 (44.98)^{bc}
P. lilacinus (ITCC6064)	0.00 (0.71)^{b}	5.00 (1.97)^{bc}	15.00 (22.49)^{bc}	20.00 (26.18)^{c}	42.50 (40.66)^{c}
F. verticilloides	0.00 (0.71)^{b}	2.50 (1.34)^{bc}	7.50 (13.82)^{c}	20.00 (26.18)^{c}	30.00 (33.04)^{d}
Control	0.00 (0.71)^{b}	0.00 (0.71)^{c}	0.00 (0.91)^{d}	0.00 (0.91)^{d}	0.00 (0.91)^{e}
C D (0.05)	(1.176)	(1.721)	(9.819)	(7.160)	(5.629)

Figures in parenthesis are ** square root transformed values

*angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

(A) *B. bassiana*

(B) M. *anisopliae*

(C) *P. lilacinus*

(D) *F. verticilloides*

Plate 4: *Diaphania indica* larvae infected with different entomopathogens

and was statistically superior to all other treatments. This was followed by *B. bassiana* (Bb5) having a mean mortality percentage of 7.5. *P. lilacinus*, *B. bassiana* (ITCC 6063) and *F. verticilloides* recorded a mean mortality percentage of 5, 5 and 2.5 respectively and were on par. A similar trend was observed five days after treatment with *M. anisopliae* having the highest mortality percentage of 42.5 followed by *B. bassiana* Bb5 (17.5 per cent). *P. lilacinus*, *B. bassiana* (ITCC 6063) and *F. verticilloides* resulted in mortality of 15, 10 and 7.5 per cent and was on par. At six days after treatment, the mortality was high in all the treatments except *F. verticilloides* and *P. lilacinus* with 20 per cent cumulative mortality each. *M. anisopliae* proved highly pathogenic to *D. indica* and had a mean mortality percentage of 60. This was significantly high compared to all the other treatments. The next superior treatments were *B. bassiana* (Bb5) and *B. bassiana* (ITCC 6063) which depicted a mean mortality percentage of 37.5 and 32.5 respectively.

At seven days after treatment, the highest mean mortality percentage (82.5 per cent) was observed in insects treated with *M. anisopliae* and was statistically superior to all the treatments. The next superior treatment was *B. bassiana* Bb5 (55 per cent) and was followed *B. bassiana* ITCC 6063 which depicted a mean mortality percentage of 50 and on par with *P. lilacinus* (42.5 per cent). Among the treatments, *F. verticilloides* was least pathogenic to *D. indica* and had a mortality percentage of 30. No mortality was observed in control.

4.2.3. *H. septima*

4.2.3.1 Grubs

The grubs of *H. septima* treated with various entomopathogenic fungi showed reduced feeding and activity two days post treatment. Mortality was initiated three days post treatment in *M. anisopliae* and five days post treatment in *B. bassiana*. Low mortality was observed in *F. verticilloides* treated insects and mortality was seen six days post treatment. In all the cases, white mycelial growth covered the cadaver first

which later turned green in *M. anisopliae* (plate 5) and slightly greyish in *F. verticilloides*.

Mortality was observed in *H. septima* grubs treated with *M. anisopliae* three days post treatment while no mortality was observed in *B. bassiana* (Bb5, ITCC 6063) and *F. verticilloides* upto five days after treatment. At five days after treatment, mortality was observed in all the treatments except control, the highest mortality percentage being 25 observed for *M. anisopliae* treated insects (Table 6). This was significantly high compared to all the other treatments. *B. bassiana* (Bb5) reported a mean mortality percentage of 17.5 and was followed by *B. bassiana* ITCC 6063 (7.50 per cent). *F. verticilloides* treated insects showed a mortality percentage of 5, five days after treatment. At seven days after treatment, highest mean mortality percentage was observed in *M. anisopliae* (45) and was on par with *B. bassiana* Bb5 (37.5 per cent). This was followed by *B. bassiana* ITCC 6063 and with *F. verticilloides* having mean mortality percentages of 22.5 and 17.5 and were on par. A similar trend was observed nine days after treatment with *M. anisopliae* having 52.5 per cent mortality followed by *B. bassiana* Bb5 having a mean mortality percentage of 47.5. *F. verticilloides* treated insects depicted a low mortality percentage of 30.

4.2.3.2. Adults

The treated adults behaved normally for the first five days post treatment. Clustering of the adults was noticed six days post treatment and mortality was initiated ten days post treatment in *B. bassiana* and nine days post treatment in *M. anisopliae*. Reduced egg laying was also noticed in the case of treated insects and scattered egg laying was shown by the treated females (plate 5).

The mortality of adults when treated with various fungi was comparatively low compared to the grubs. Upon treatment with three fungal isolates, *M. anisopliae* and *B. bassiana* (Bb5 and ITCC 6063) at a concentration of 10^8 spores mL^{-1}, no significant difference in mortality was observed between the treatments at six days

Table 6. Mortaliy of *Henosepilachna septima* grubs treated with various entomopathogenic fungi

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)		
	5 DAT **	7 DAT*	9 DAT*
B. bassiana (Bb5)	17.50 (4.21)[a]	37.50 (37.66)[a]	47.50 (43.56)[ab]
M. anisopliae (Ma4)	25.00 (5.03)[a]	45.00 (42.12)[a]	52.50 (46.44)[a]
B. bassiana (ITCC 6063)	7.50 (2.61)[b]	22.50 (28.23)[b]	42.50 (40.68)[b]
F. verticilloides	5.00 (1.67)[bc]	17.50 (24.54)[b]	30.00 (33.06)[c]
Control	0.00 (0.91)[c]	0.00 (0.91)[c]	0.00 (0.91)[d]
C D (0.05)	(1.428)	(5.666)	(4.847)

Figures in parenthesis are ** square root transformed values

*angular transformed values

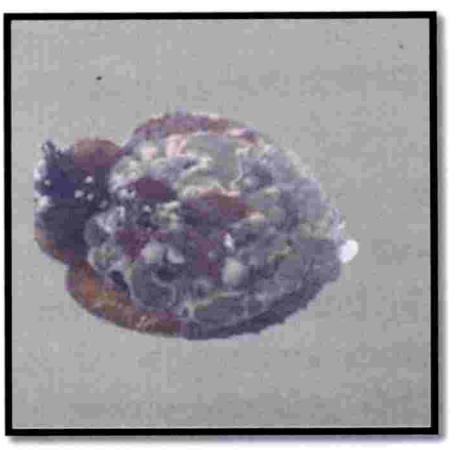
DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

(A) B. bassiana

(B) M. anisopliae

(C) *F. verticilloides*

(A) *B. bassiana*



(B) *M. anisopliae*

Plate 5: *Henosepilachna septima* grubs and adults infected with different entomopathogens

after treatment. At eight days after treatment, the treatments differed significantly for its virulence against adults of epilachna beetle, the highest mortality being 12.5 per cent observed for *M .anisopliae* treated insects (Table 7). The two *Beauveria* isolates, Bb5 and ITCC 6063 resulted in mean mortality percentage of 5 and 2.5 respectively and were on par. At 10 days after treatment, 20 per cent mortality was noticed in insects treated with *M. anisopliae* and was statistically superior compared to *B. bassiana* isolates Bb5 and ITCC 6063 having 10 and 7.5 per cent mortality respectively.

4.2.4. *A. foveicollis*

A. foveicollis adults treated with *B. bassiana* (Bb5, ITCC 6063) and *M. anisopliae* showed symptoms like reduced feeding and sluggish movement five days after treatment. The beetles tend to hide behind the leaves upon treatment. Mortality of the adults was seen from the fifth day onwards in *M. anisopliae* and *B. bassiana*. Initially, white mycelial growth was observed in the cadavers from the intersegmental regions and around the eyes and antennae for both the fungi three days after death. In *M. anisopliae* treated insects, the mycelial growth turned green after three days whereas white spores emerged over the cadavers of *B. bassiana* (Bb5, ITCC 6063) treated insects within three days.

A. foveicollis adults on treatment with three different fungal isolates at a concentration of 10^8 spores mL^{-1} showed mortality five days post treatment (Table 8). Maximum mortality was observed in the treatment *B. bassiana* (Bb5) and had a mean mortality percentage of 12.5. This was on par with *M. anisopliae* which resulted in 10 per cent mortality. At five days after treatment, *B. bassiana* ITCC 6063 resulted in 5 per cent mortality. At seven days after treatment, the highest mortality percentage of 27.5 was observed in insects treated with *B. bassiana* Bb5 and was statistically on par with *M. anisopliae* (25 per cent). A mortality percentage of 12.5 per cent was observed in insects treated with *B. bassiana* (ITCC 6063). Significant differences in

Table 7. Mortaliy of *Henosepilachna septima* adults treated with different entomopathogenic fungi

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)		
	6 DAT	8 DAT	10 DAT
B. bassiana (Bb5)	2.50 (1.34)	5.00 (1.98)[b]	10.00 (3.24)[b]
M. anisopliae (Ma4)	5.00 (1.98)	12.50 (3.56)[a]	20.00 (4.56)[a]
B. bassiana (ITCC 6063)	0.00 (0.74)	2.50 (1.34)[b]	7.50 (2.60)[b]
Control	0.00 (0.74)	0.00 (0.70)[b]	0.00 (0.70)[c]
C D (0.05)	(N S)	(1.578)	(1.211)

Figures in parenthesis are square root transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

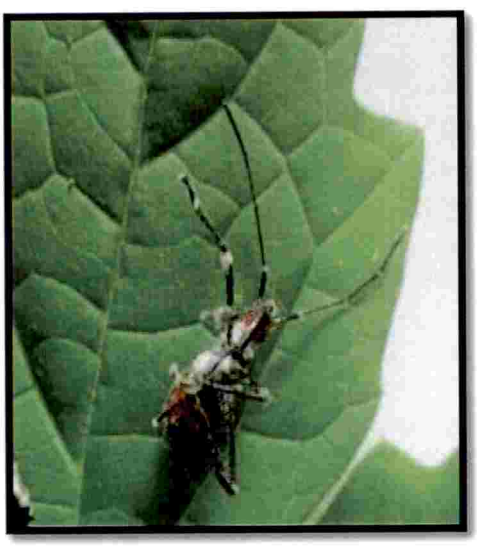
Table 8. Mortaliy of *Aulacophora foveicollis* adults treated with various entomopathogenic fungi

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)		
	5 DAT**	7DAT **	10DAT*
B. bassiana (Bb5)	12.50 (3.88)[a]	27.50 (5.24)[a]	45.00 (42.12)[a]
M. anisopliae (Ma4)	10.00 (3.56)[a]	25.00 (5.03)[a]	37.50 (37.66)[a]
B. bassiana (ITCC6063)	5.00 (1.98)[b]	12.50 (3.56)[b]	22.50 (28.23)[b]
Control	0.00 (0.70)[b]	0.00 (0.71)[c]	0.00 (0.91)[c]
C D (0.05)	(1.356)	(0.953)	(5.660)

Figures in parenthesis are ** square root transformed values *angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

(A) *M. anisopliae*



(B) B. bassiana

Plate 6: *Leptoglossus autralis* nymphs infected with different entomopathogens

mortality were observed among the treatments 10 days post treatment. Highest mortality was observed in *B. bassiana* (Bb5) treated insects (45 per cent) which was statistically on par with the treatment *M. anisopliae* which resulted in 37.5 per cent mortality. This was followed by *B. bassiana* (ITCC 6063) with 22.5 per cent mortality.

4.2.5. *L. australis*

The numphs of *L. australis* when treated with *B. bassiana* and *M. anisopliae* showed reduced activity two days post treatment. Mortality was noticed from three days post treatment for both the fungi. Upon incubation, fungal hyphae were seen radiating out from the joints of leg, antennae and around compound eyes plate. Later the white mycelia covered the entire body of the insect. In *M. anisopliae* infected nymphs, green coloured spores were observed two days post incubation (plate 6).

Mortality was noticed in the nymphs of *L. australis* three days post treatment when treated with three different fungal isolates at a concentration of 10^8 spores mL^{-1} (Table 9). The highest mortality of 18.33 per cent and was observed in *M. anisopliae* treated insects. This was followed by *B. bassiana* (Bb5) (6.67 per cent) and *B. bassiana* (ITCC 6063) (5.00 per cent) and were statistically on par. At five days post treatment, 33.33 per cent mortality was observed in *M. anisopliae* treated insects which was statistically superior compared to the rest of the treatments. The next superior treatment was *B. bassiana* Bb5 having a mean mortality percentage of 23.34. It took 7 days to cause more than 50 per cent mortality, highest being 61.05 per cent with *M. anisopliae* and was on par with *B. bassiana* (Bb5) the per cent mortality being 49.38 per cent. *B. bassiana* (ITCC 6063) recorded a mortality of 26.05 per cent. 5 per cent mortality was observed in control. Significant differences were observed among the isolates at eight DAT. The highest mortality (78.12 per cent) was observed in *M. anisopliae* and was statistically superior to the rest of the treatments. The

Table 9. Mortaliy of *Leptoglossus australis* nymphs treated with various entomopathogenic fungi

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)			
	3DAT**	5DAT*	7 DAT*	8 DAT*
B. bassiana (Bb5)	6.67 (2.45)^b	23.34 (28.82)^b	49.38 (44.65)^a	60.77 (51.27)^b
M. anisopliae (Ma4)	18.33 (4.33)^a	33.33 (35.21)^a	61.05 (51.50)^a	78.12 (62.29)^a
B. bassiana (ITCC6063)	5.00 (2.19)^b	15.00 (22.71)^c	26.05 (30.60)^b	44.67 (41.94)^b
Control	0.00 (0.71)^c	0.00 (0.74)^d	5.00 (9.46)^c	5.00 (9.46)^c
C D (0.05)	(1.270)	(3.810)	(10.664)	(9.432)

Figures in parenthesis are ** square root transformed values *angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

treatments *B. bassiana* (Bb5) and *B. bassiana* (ITCC6063) resulted in mortality of 60.77 and 44.67 per cent and were statistically on par.

4.2.6. Symptoms of Bacterial Infection

Mortality was observed in the grubs of *H. septima* released on leaves treated with *S. marscesens* one day post treatment. The grubs were active on the first day and later showed reduction in feeding. Change in colour from orange red to brown was observed in the dead insects along with a putrefied smell (plate 2). Slightest pressure on the body of dead insects resulted in rupture of the body wall and orange red coloured body fluid exuded out. 32.5 per cent mortality of the treated insects was noticed one DAT. The mortality percentage was increased to 72.5 and 92.5 per cent respectively at three DAT and five DAT (Fig.1).

D. indica released on leaves treated with *S. marcescens* showed mortality I DAT. The insects displayed reduced feeding one day post treatment. The dead insects exhibited a slight orange tinge initially and later changed to brown colour (plate 2) Foul odour and change in consistency was observed in the cadaver. One day post treatment, 42.5 per cent mortality was observed in the treated insects. The mortality percentage was increased to 82.5 and 100 per cent at 3 DAT and 5 DAT respectively.

4.3. BIOASSAY

Bioassay of the fungi *B. bassiana, M. anisopliae* and *P. lilacinus* against various stages of the test insects were carried out and LC_{50}, LC_{90} and LT_{50} values were calculated. The results are given in Tables 10 to 20.

4.3.1. *M. anisopliae*

4.3.1.1. B. cucurbitae

The mortality of adults of *B. cucurbitae* was noticed from the second day after treatment (Table 10). The mortality percentage ranged from 2.22 to 33.33, 6.67 to 40,

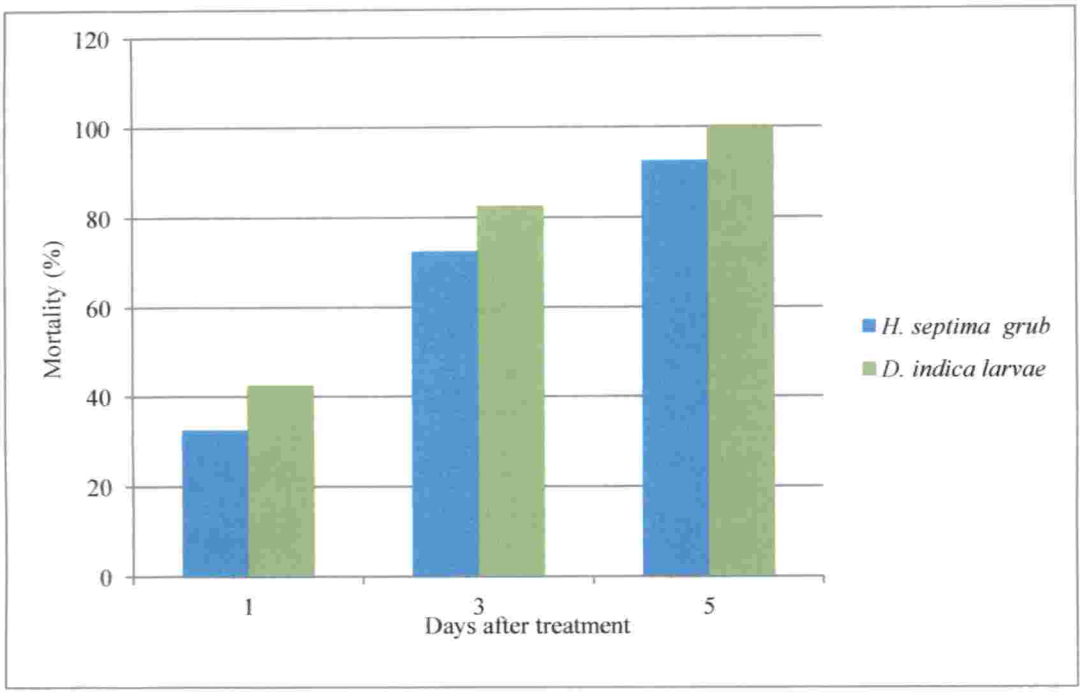


Fig.1. Effect of *S. marcescens* on the grubs of *H. septima* and larvae of *D. indica*

Table 10 .Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *Bactrocera cucurbitae* treated with *M .anisopliae*

Concentration (spores mL^{-1})	Cumulative mortality (%)			LT_{50} Days
	3 DAT	5 DAT	7 DAT	
2.3×10^9	31.11	51.11	100.00	4.25
2.3×10^8	15.56	38.89	82.22	5.51
2.3×10^7	11.11	24.44	55.56	6.72
2.3×10^6	6.67	17.78	40.00	7.84
2.3×10^5	2.22	11.11	33.33	8.10
Control	0.00	0.00	0.00	

Probit analysis					
Days after treatment	Spores $mL^{-1} \times 10^9$				chi-square
	LC_{50}	Fiducial limit for LC_{50}	LC_{90}	Fiducial limit for LC_{90}	
5	1.87	1.28 – 3.44	4.95	3.01 – 8.52	4.126
7	1.55	1.19 – 2.02	3.72	3.04 – 4.82	3.914

DAT- Days After Treatment

11.11 to 55.56, 15.56 to 82.22 and 31.11 to 100 per cent, at 10^{5}, 10^{6}, 10^{7}, 10^{8}, and 10^{9} spores mL^{-1} on three, five and seven days after treatment (DAT).

LT_{50} recorded was 4.25 days at the concentration of 10^{9} spores mL^{-1}. The LT_{50} values increased to 5.51, 6.72, 7.84 and 8.08 days as concentration reduced to 10^{8}, 10^{7}, 10^{6}, 10^{5} spores mL^{-1}, respectively. The LC_{50} values obtained by the probit analysis of dose-mortality responses of the insect on the fifth and seventh DAT were 1.87×10^{9} and 1.55×10^{9} spores mL^{-1}, respectively. The corresponding LC_{90} values were 4.95×10^{9} and 3.72×10^{9} spores mL^{-1} at third, fifth and seventh DAT

4.3.1.2. D. indica

The second instar larvae *D. indica*, upon treatment with various spore concentrations of *M. anisopliae* showed mortality 3 DAT (Table 11). The mortality percentage ranged from 2.22 to 15.56, 4.44 to 26.67, 8.89 to 40, 13.33 to 75.56 and 31.11 to 100 per cent, at 10^{5}, 10^{6}, 10^{7}, 10^{8} and 10^{9} spores mL^{-1} on three, five and seven DAT.

At the concentration of 10^{9} spores mL^{-1}, it took 3.76 days to cause 50 per cent mortality. The LT_{50} values increased to 5.69, 7.95, 9.27 and 11.23 days as concentration reduced to 10^{8}, 10^{7}, 10^{6}, 10^{5} spores mL^{-1}, respectively. The LC_{50} values obtained by the probit analysis of dose-mortality responses of the insect on the fifth and seventh DAT were 2.04×10^{9} and 0.18×10^{9} spores mL^{-1}, respectively. The corresponding LC_{90} values were 4.88×10^{9} and 0.53×10^{9} spores mL^{-1} at fifth and seventh DAT

4.3.1.3. H. septima

4.3.1.3.1. Grub

The mortality percentage ranged from 8.89 to 20, 13.33 to 33.33, 17.79 to 37.78, 28.89 to 44.44 and 55.56 to 80 per cent, at 10^{5}, 10^{6}, 10^{7}, 10^{8} and 10^{9} spores

Table 11 .Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of *Diaphania indica* larvae treated with *M. anisopliae*

Concentration (spores mL^{-1})	Cumulative mortality (%)			LT_{50} Days
	3 DAT	5 DAT	7 DAT	
2.7×10^9	31.11	77.78	100	3.76
2.7×10^8	13.33	31.11	75.56	5.70
2.7×10^7	8.89	20.00	40.00	7.96
2.7×10^6	4.44	17.78	26.67	9.28
2.7×10^5	2.22	8.89	15.56	11.24
Control	0.00	0.00	0.00	

Probit analysis					
Days after treatment	Spores $mL^{-1} \times 10^9$				chi-square
	LC_{50}	Fiducial limit for LC_{50}	LC_{90}	Fiducial limit for LC_{90}	
5	2.04	1.58 – 2.69	4.88	3.94 - 6.48	4.403
7	0.18	0.13 – 0.26	0.53	0.41- 0.77	4.104

DAT- Days After Treatment

mL^{-1} on five, seven and nine DAT (Table 12). LT_{50} recorded was 4.30 days at the concentration of 10^9 spores mL^{-1}. The LT_{50} values increased to 9.74, 11.04, 11.69 and 15.66 days as concentration reduced to 10^8, 10^7, 10^6, 10^5 spores mL^{-1}, respectively.

The LC_{50} values obtained by the probit analysis of dose-mortality responses of the insect on the fifth, seventh and ninth DAT were 3.92×10^9, 2.82×10^9 and 1.56×10^9 spores mL^{-1} respectively. The corresponding LC_{90} values were 8.70×10^9, 7.50×10^9 and 5.90×10^9 spores mL^{-1} at fifth, seventh and ninth DAT

4.3.1.3.2. Adults

The mortality of adults of *H. septima* treated with various spore concentrations of *M. anisopliae* (Table 13) increased from 2.22 to 31.11, 4.44 to 40 and 6.67 to 53.33 per cent at eighth, tenth and twelfth DAT. The higher concentration of 2.3×10^9 spores mL^{-1} recorded the minimum time of 11.25 days to kill 50 per cent of the population. The LT_{50} values increased to 26.28, 29.14, 29.94 and 33.76 days as concentration reduced to 10^8, 10^7, 10^6, 10^5 spores mL^{-1}, respectively.

The LC_{50} values obtained by the probit analysis of dose-mortality responses of the insect on the twelth DAT was 2.12×10^9 spores mL^{-1} and the corresponding LC_{90} value was 4.45×10^9 spores mL^{-1}.

4.3.1.4. A. foveicollis

The mortality percentage ranged from 2.22 to 11.11, 4.44 to 15.56, 6.67 to 20, 11.11 to 33.33 and 42.22 to 86.67 per cent, at 10^5, 10^6, 10^7, 10^8 and 10^9 spores mL^{-1} on fifth, seventh and tenth DAT (Table 14). LT_{50} recorded was 5.56 days at the concentration of 10^9 spores mL^{-1}. The LT_{50} values increased to 13.28, 21.46, 22.71 and 23.51 days as concentration reduced to 10^8, 10^7, 10^6, 10^5 spores mL^{-1}, respectively.

The LC_{50} values obtained by the probit analysis of dose-mortality responses of

Table 12 .Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *Henosepilachna septima* treated with *M. anisopliae*

Concentration (spores mL^{-1})	Cumulative mortality (%)			LT_{50} Days
	5 DAT	7 DAT	9 DAT	
4.5×10^9	55.56	66.67	80.00	4.30
4.5×10^8	28.89	35.56	44.44	9.74
4.5×10^7	17.78	26.67	37.78	11.04
4.5×10^6	13.33	17.78	33.33	11.69
4.5×10^5	8.89	13.33	20.00	15.66
Control	0.00	0.00	0.00	

Probit analysis					
Days after treatment	Spores $mL^{-1} \times 10^9$				chi-square
	LC_{50}	Fiducial limit for LC_{50}	LC_{90}	Fiducial limit for LC_{90}	
5	3.93	2.99 – 5.68	8.70	6.63 – 13.05	2.226
7	2.82	2.08 – 4.07	7.50	5.72 – 11.17	5.180
9	1.56	0.97 – 2.34	5.91	4.49 - 8.78	4.672

DAT- Days After Treatment

Table 13. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of *Henosepilachna septima* adults treated with *M. anisopliae*

Concentration (spores mL^{-1})	Cumulative mortality (%)			LT_{50} Days
	8DAT	10 DAT	12 DAT	
2.3×10^9	31.11	40.00	53.33	11.25
2.3×10^8	13.33	17.78	22.22	26.23
2.3×10^7	6.67	11.11	13.33	29.14
2.3×10^6	4.44	8.89	11.11	29.94
2.3×10^5	2.22	4.44	6.67	33.76
Control	0.00	0.00	0.00	

Probit analysis					
Days after treatment	Spores $mL^{-1} \times 10^9$				chi-square
	LC_{50}	Fiducial limit for LC_{50}	LC_{90}	Fiducial limit for LC_{90}	
12	2.12	3.42- 6.54	4.45	3.42-6.54	3.217

DAT- Days After Treatment

Table 14. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *Aulacophora foveicollis* treated with *M. anisopliae*

Concentration (spores mL^{-1})	Cumulative mortality (%)			LT_{50} Days
	5DAT	7 DAT	10 DAT	
3.5×10^9	42.22	66.67	86.67	5.56
3.5×10^8	11.11	17.78	33.33	13.28
3.5×10^7	6.67	8.89	20.00	21.46
3.5×10^6	4.44	6.67	15.56	22.71
3.5×10^5	2.22	4.44	11.11	23.51
Control	0.00	0.00	0.00	

Probit analysis					
Days after treatment	Spores $mL^{-1} \times 10^9$				chi-square
	LC_{50}	Fiducial limit for LC_{50}	LC_{90}	Fiducial limit for LC_{90}	
7	2.64	2.16 – 3.33	5.07	4.21 – 6.52	2.806
10	1.55	1.19 – 2.02	3.72	3.04 – 4.82	3.914

DAT- Days After Treatment

the insect on the seventh and tenth DAT were 2.63×10^9 and 1.55×10^9 spores mL^{-1}, respectively. The corresponding LC_{90} values were 5.08×10^9 and 3.72×10^9 spores mL^{-1}.

4.3.2. *B. bassiana*

4.3.2.1. B. cucurbitae

Mortality was observed in the adults of *B. cucurbitae* treated with various spore concentrations of *B.bassiana* from the third day after treatment (Table 15). The mortality percentage ranged from 2.22 to 24.44, 6.67 to 34.44, 11.11 to 55.56, 11.11 to 82.22 and 24.44 to 100 per cent, at 10^5, 10^6, 10^7, 10^8, and 10^9 spores mL^{-1} on three, five and seven DAT.

LT_{50} recorded was 4.85 days at the concentration of 10^9 spores mL^{-1}. The LT_{50} values increased to 5.51, 6.72, 9.84 and 14.89 days as concentration reduced to 10^8, 10^7, 10^6, 10^5 spores mL^{-1}, respectively. The LC_{50} values obtained by the probit analysis of dose-mortality responses of the insect on the fifth and seventh DAT were 2.87×10^9 and 1.65×10^9 spores mL^{-1}, respectively. The corresponding LC_{90} values were 4.95×10^9 and 4.72×10^9 spores mL^{-1} at fifth and seventh DAT

4.3.2.2. D. indica

The mortality of second instar larvae of *D. indica* treated with various concentrations of *B. bassiana* was noticed from the fourth day after treatment. The mortality percentage ranged from 7.5 to 27.5, 10 to 32.5, 17.5 to 42.5, 20 to 57.5 and 37.5 to 65 per cent, at 10^5, 10^6, 10^7, 10^8 and 10^9 spores mL^{-1} on five, six and seven DAT (Table 16). LT_{50} recorded was 5.81 days at the concentration of 10^9 spores mL^{-1}. The LT_{50} values increased to 6.83, 7.48, 8.01 and 8.40 days as concentration reduced to 10^8, 10^7, 10^6, 10^5 spores mL^{-1}, respectively. The LC_{50} values obtained by the probit analysis of dose-mortality responses of the insect on the sixth and seventh DAT were 2.13×10^9 and 1.20×10^9 spores mL^{-1}, respectively. The corresponding LC_{90}

Table 15. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *Bactrocera cucurbitae* treated with *B. bassiana*

Concentration (spores mL^{-1})	Cumulative mortality (%)			LT_{50} Days
	3DAT	5DAT	7DAT	
$2.7x10^{9}$	24.44	61.11	100.00	4.85
$2.7x\ 10^{8}$	11.11	55.56	82.22	5.51
$2.7x\ 10^{7}$	11.11	34.44	55.56	6.72
$2.7x\ 10^{6}$	6.67	17.78	34.44	9.84
$2.7x\ 10^{5}$	2.22	6.67	24.44	14.89
Control	0.00	0.00	0.00	

Probit analysis					
Days after treatment	Spores mL^{-1} x 10^{9}				chi-square
	LC_{50}	Fiducial limit for LC_{50}	LC_{90}	Fiducial limit for LC_{90}	
5	2.87	1.68 – 3.54	4.95	3.01 – 8.52	4.126
7	1.65	1.19 – 2.02	4.72	3.04 – 5.82	4.954

DAT- Days After Treatment

Table 16. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of *Diaphania indica* larvae treated with *B. bassiana*

Concentration (spores mL^{-1})	Cumulative mortality (%)			LT_{50} Days
	5 DAT	6 DAT	7 DAT	
2.7×10^9	37.50	50.00	65.00	5.82
2.7×10^8	20.00	42.50	57.50	6.84
2.7×10^7	17.50	30.00	42.50	7.48
2.7×10^6	10.00	25.00	32.50	8.01
2.7×10^5	7.50	17.50	27.50	8.40
Control	0.00	0.00	0.00	

Probit analysis					
Days after treatment	Spores $mL^{-1} \times 10^9$				chi-square
	LC_{50}	Fiducial limit for LC_{50}	LC_{90}	Fiducial limit for LC_{90}	
6	2.13	1.35 - 4.60	6.74	4.39-16.07	5.174
7	1.20	0.56 - 2.58	5.85	3.79-14.30	4.104

DAT- Days After Treatment

values were 5.17×10^9 and 4.10×10^9 spores mL^{-1} respectively at sixth and seventh DAT

4.3.2.3. H. septima

4.3.2.3.1. Grubs

The mortality of second instar grubs of *H. septima* treated with various spore concentrations of *B. bassiana* was noticed from the fourth day after treatment and the mortality percentage ranged from 2.22 to 15.56, 4.44 to 22.22, 8.89 to 26.67, 15.56 to 48.89 and 40 to 75.56 per cent, at 10^5, 10^6, 10^7, 10^8 and 10^9 spores mL^{-1} on five , seven and nine DAT (Table 17).

The minimum time required to kill 50 per cent of the population was 6.11 days at the higher spore concentration of 2.7×10^9. The maximum time of 13.29 days was taken for the lowest dose of 2.7×10^5.

The spore concentration required to kill 50 percent of the population seven days after treatment was 1.82×10^9. Lower concentrations of 1.05×10^9 resulted in 50 per cent mortality at nine DAT. The corresponding LC_{90} values were 4.57×10^9 and 3.20×10^9 spores mL^{-1} at seven and nine DAT.

4.3.2.3.2. Adults

Adults of epilachna beetle, *H. septima* showed mortality from the fifth day after treatment. The mortality percentage ranged from 0.00, 0.00 to 2.22, 2.22 to 6.67, 4.44 to17.78 and 17.78 to 37.78 at 10^5, 10^6, 10^7, 10^8 and 10^9 spores mL^{-1} on tenth, twelfth and fourteen DAT (Table 18).

None of the tested concentration was sufficient enough to cause 50 per cent mortality of the adults at the observed duration.

Table 17. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *Henosepilachna septima* treated with *B. bassiana*

Concentration (spores mL^{-1})	Cumulative mortality (%)			LT_{50} Days
	5 DAT	7 DAT	9 DAT	
2.7×10^9	40.00	57.78	75.56	6.11
2.7×10^8	15.56	33.33	48.89	9.01
2.7×10^7	8.89	22.22	26.67	12.28
2.7×10^6	4.44	17.78	22.22	12.29
2.7×10^5	2.22	11.11	15.56	13.29
Control	0.00	0.00	0.00	

Probit analysis					
Days after treatment	Spores $mL^{-1} \times 10^9$				chi-square
	LC_{50}	Fiducial limit for LC_{50}	LC_{90}	Fiducial limit for LC_{90}	
7	1.82	1.33 - 2.79	4.57	3.38 - 7.35	5.144
9	1.05	0.10 - 3.78	3.20	1.72 -17.86	9.842

DAT- Days After Treatment

Table 18. Cumulative per cent mortality of adults of *Henosepilachna septima* treated with *B. bassiana*

Concentration (spores mL^{-1})	Cumulative mortality (%)		
	10 DAT	12 DAT	14 DAT
3.2×10^9	17.78	31.11	37.78
3.2×10^8	4.44	8.89	17.78
3.2×10^7	2.22	4.44	6.67
3.2×10^6	0.00	2.22	2.22
3.2×10^5	0.00	0.00	0.00
Control	0.00	0.00	0.00

DAT- Days After Treatment

4.3.2.4. A. foveicollis

The results on the mortality of adults of *A. foveicollis* treated with various spore concentrations of *B. bassiana* are presented in Table 19. The mortality ranged from 0 to 4.44, 2.22 to 11.11, 4.44 to 15.56, 8.89 to 38.44 and 33.33 to 68.89 per cent, at 10^5, 10^6, 10^7, 10^8 and 10^9 spores mL^{-1} on eight, nine and ten DAT. LT_{50} recorded was 8.83 days at the concentration of 10^9 spores mL^{-1}. The LT_{50} values increased to 19.81, 24.09, 24.75 and 25.58 days as concentration reduced to 10^8, 10^7, 10^6, 10^5 spores mL^{-1}, respectively.

The LC_{50} values obtained by the probit analysis of dose-mortality responses of the insect on the nineth and tenth DAT were 2.93×10^9 and 2.34×10^9 spores mL^{-1}, respectively. The corresponding LC_{90} values were 5.67×10^9 and 5.67×10^9 spores mL^{-1} at 9 and 10 DAT

4.3.3. *P. lilacinus*

4.3.3.1. B. cucurbitae

Mortality was observed in the adults of *B. cucurbitae* treated with various spore concentrations of *P. lilacinus* from the second day after treatment (Table 20). The mortality percentage ranged from 2.22 to 28.44, 6.67 to 45.44, 11.11 to 68.54, 17.78 to 96.88 and 25.56 to 100 per cent, at 10^5, 10^6, 10^7, 10^8, and 10^9 spores mL^{-1} on three, five and seven DAT.

LT_{50} recorded was 3.85 days at the concentration of 10^9 spores mL^{-1}. The LT_{50} values increased to 4.06, 6.12, 8.14 and 11.89 days as concentration reduced to 10^8, 10^7, 10^6, 10^5 spores mL^{-1}, respectively. The LC_{50} values obtained by the probit analysis of dose-mortality responses of the insect on the fifth and seventh DAT were 1.87×10^9 and 1.05×10^9 spores mL^{-1}, respectively. The corresponding LC_{90} values were 3.45×10^9 and 2.72×10^9 spores mL^{-1} respectively at fifth and seventh DAT.

Table 19. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *Aulacophora foveicollis* treated with *B. bassiana*

Concentration (spores mL^{-1})	Cumulative mortality (%)			LT_{50} Days
	8DAT	9 DAT	10 DAT	
3.4×10^9	33.33	57.78	68.89	8.83
3.4×10^8	8.89	17.78	38.44	19.81
3.4×10^7	4.44	11.11	15.56	24.09
3.4×10^6	2.22	6.67	11.11	24.75
3.4×10^5	0.00	2.22	4.44	25.58
Control	0.00	0.00	0.00	

Probit analysis					
Days after treatment	LC_{50} (spores $mL^{-1} \times 10^9$)	Fiducial limit for LC_{50} (spores $mL^{-1} \times 10^9$)	LC_{90} (spores $mL^{-1} \times 10^9$)	Fiducial limit for LC_{90} (spores $mL^{-1} \times 10^9$)	chi-square
9	2.94	2.37 – 3.83	5.67	4.59 – 7.59	4.749
10	2.34	1.87 – 3.04	5.67	4.59 – 7.59	5.254

DAT- Days After Treatment

Table 20. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *Bactocera cucurbitae* treated with *P. lilacinus*

Concentration (spores mL^{-1})	Cumulative mortality (%)			LT_{50} Days
	3 DAT	5 DAT	7 DAT	
2.4×10^{9}	25.56	73.34	100.00	3.85
2.4×10^{8}	17.78	58.56	96.88	4.06
2.4×10^{7}	11.11	32.44	68.54	6.12
2.4×10^{6}	6.67	25.56	45.44	8.14
2.4×10^{5}	2.22	11.11	28.44	11.89
Control	0.00	0.00	0.00	

Probit analysis					
Days after treatment	(spores $mL^{-1} \times 10^{9}$)				chi-square
	LC_{50}	Fiducial limit for LC_{50}	LC_{90}	Fiducial limit for LC_{90}	
5	1.87	1.68 – 2.54	3.45	3.01 – 6.52	4.362
7	1.05	0.89 – 2.02	2.72	2.04 – 5.82	6.954

DAT- Days After Treatment

4.4 COMPATIBILITY OF ENTOMOPATHOGENIC FUNGI

4.4.1 Compatibility of *M. anisopliae* with *B. bassiana*

The results on the dual culture studies conducted to determine the compatibility of *M. anisopliae* with *B.bassiana* are presented in Table 21. At 7 days after inoculation (DAI), the mean diameter of *M. anisopliae* was 3.13 cm in dual culture plates and in the control plates, the diameter was 3.18 cm. Same pattern was observed for *B. bassiana* as well. A similar trend was followed in 10 DAI (plate 7). At 14 DAI, the growth of both the fungi locked each other at the point of contact. No overlapping was observed and the margins of the colonies were about 3 to 5 mm apart from each other.

The percentage inhibition in the growth of *B. bassiana* by *M. anisopliae* worked out at 14 DAI showed no inhibition at all as both control and dual culture plates showed the same growth rate

4.4.2 Compatibility of *M. anisopliae* with *P. lilacinus*

A reduction in the growth of *P. lilacinus* in dual culture in comparison with the control was observed at 14 DAI with a percentage inhibition of 57.89 (Table.22). The mean growth diameter of *P. lilacinus* was 1.18 cm, 2.33 cm and 2.4 cm in dual culture compared to 1.45 cm, 3.43 cm and 5.7 cm in control at 7, 10 and 14 DAI respectively (plate 7). *M. anisopliae* showed a mean growth of 2.4 cm, 4.23 cm and 5.60 cm in dual culture plates compared to 2.7 cm, 6.08 cm and 6.53 cm radial diameter in control plates. *P. lilacinus* reduced the growth of *M. anisopliae* by 14.18 per cent at 14 DAI. In dual cultures, the colony growth stopped by the second week and the margins of the colonies were about 1 to 1.5 cm away from each other.

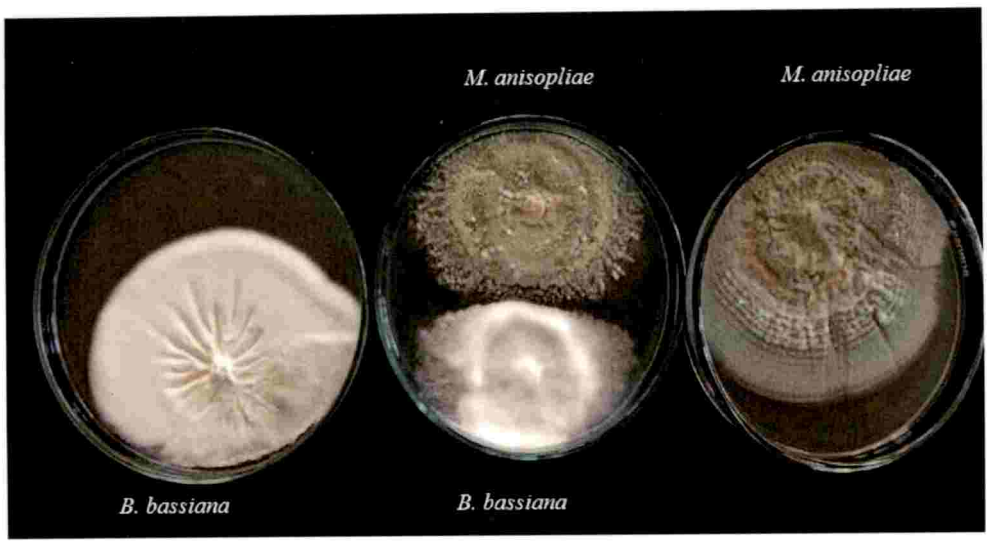
4.4.3 Compatibility of *M. anisopliae* with *L. lecanii*

Table 21: Colony diameter and percentage inhibition of *M. anisopliae* and *B. bassiana* in dual culture

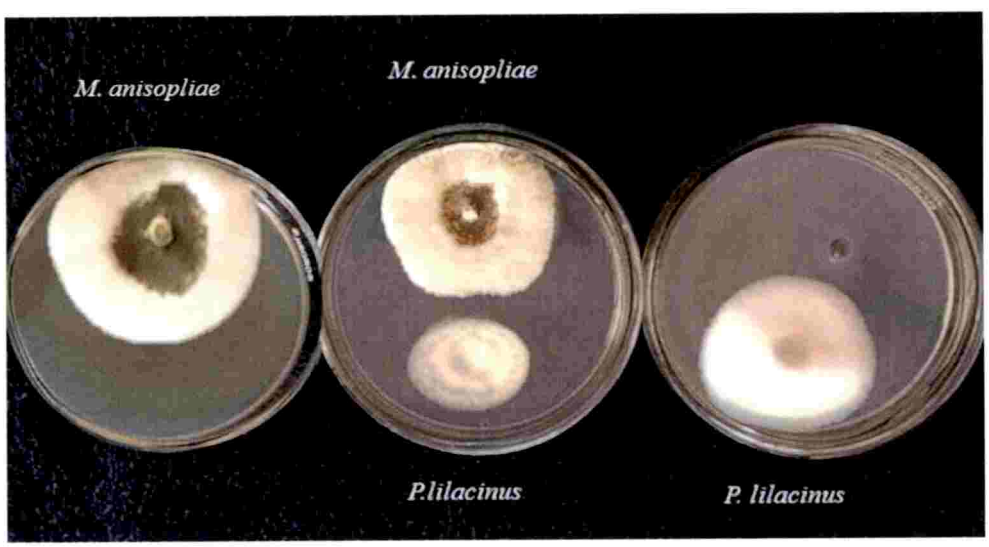
Days after inoculation	Mean colony diameter (cm)				Percentage inhibition	
	Dual culture		Control			
	M. anisopliae	*B. bassiana*	*M. anisopliae*	*B. bassiana*	*M. anisopliae*	*B. bassiana*
7	3.13	2.28	3.18	2.35	1.57	3.19
10	4.23	3.38	4.28	3.40	1.17	0.74
14	5.23	4.93	5.28	4.93	0.94	0.00

Table 22: Colony diameter and percentage inhibition of *M. anisopliae* and *P. lilacinus* in dual culture

Days after inoculation	Mean colony diameter (cm)				Percentage inhibition	
	Dual culture		Control		*M. anisopliae*	*P. lilacinus*
	M. anisopliae	*P. lilacinus*	*M. anisopliae*	*P. lilacinus*		
5	1.20	0.65	1.30	0.80	7.69	18.75
7	2.40	1.18	2.70	1.45	11.11	18.97
10	4.23	2.33	6.08	3.43	30.45	32.12
14	5.60	2.40	6.53	5.70	14.18	57.89



(A) Interaction of *M. anisopliae* and *B. bassiana* in dual cultures at 14 DAI



(B) Interaction of *M. anisopliae* and *P. lilacinus* in dual cultures at 14 DAI

Plate 7: *In vitro* compatibility of *M. anisopliae* with *B. bassiana* and M. anisopliae with *P. lilacinus* in dual cultures

A reduction in the colony diameter of *L. lecanii* was observed when co-inoculated with *M. anisopliae* in comparison with control at 7, 10 and 14 DAI (Table. 23). In dual cultures, *L. lecanii* displayed mean growth of 1.08 cm, 2.20 cm and 2.33 cm while in control, the growth were 1.70 cm, 3.40 cm and 3.73 cm respectively at 7, 10 and 14 DAI. A mean growth of 2.28 cm, 4.10 cm and 5.38cm in dual culture plates and 2.70 cm, 6.08 cm and 6.53 cm in control plates was observed for *M. anisopliae* (plate 8). Growth in dual cultures stopped two weeks after inoculation and a margin of 1 cm was observed between the two fungi. At 14 DAI, *M. anisopliae* caused 37.58 per cent inhibition for the growth of *L. lecanii*

4.4.4 Compatibility of *B. bassiana* with *P. lilacinus*

P. lilacinus when co-cultured with *B. bassiana* caused reduction in the colony diameter of the latter with 16.3 per cent inhibition, 14 DAI (Table.24). The mean growth of *B. bassiana* was 1.95 cm, 2.60 cm and 2.83 cm in dual culture compared to 2.10 cm, 2.88 cm and 3.38 cm in control at 7, 10 and 14 DAI respectively (plate 8). The colony diameter of *P. lilacinus* showed not much variation in dual cultures and control with the diameter being 2.05 cm, 4.40 cm and 5.08 cm in dual culture plates and 2.13 cm, 5.18 cm and 5.15 cm in control plates. In dual cultures, the colony growth stopped by the second week and the margins of the colonies were about 1.00 to 1.5 cm away from each other.

4.4.5 Compatibility of *B. bassiana* with *L. lecanii*

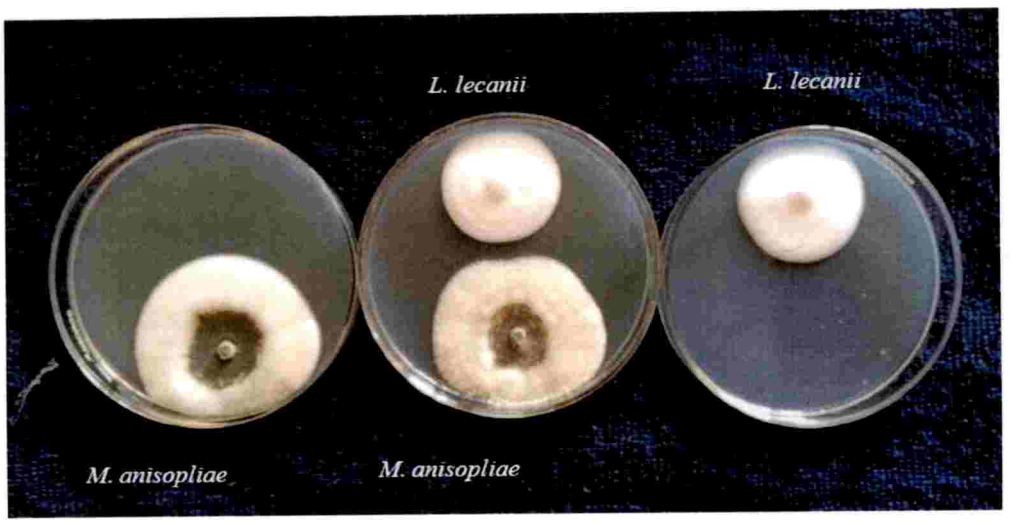
Mean growth diameter of *L. lecanii* in dual cultures and control showed not much variation when co- inoculated with *B. bassiana* at 7, 10 and 14 DAI (Table.25). In dual cultures, *L. lecanii* displayed mean growth of 1.43 cm, 1.85 cm and 3.15 cm while in control, the growth were 1.45 cm, 1.88 cm and 3.40 cm respectively at 7, 10 and 14 DAI (plate 9). A mean growth of 2.00 cm, 2.93 cm and 4.9 cm in dual culture plates and 2.28 cm, 2.93 cm and 4.98 cm in control plates was observed for

Table 23: Colony diameter and percentage inhibition of *M. anisopliae* and *L. lecanii* in dual culture

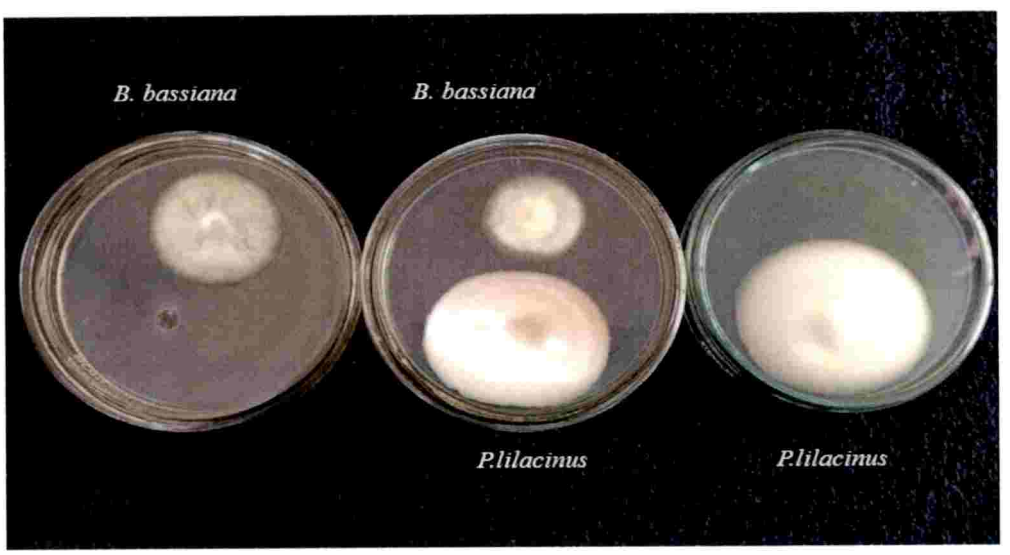
Days after inoculation	Mean colony diameter (cm)				Percentage inhibition	
	Dual culture		Control		*M. anisopliae*	*L. lecanii*
	M. anisopliae	*L. lecanii*	*M. anisopliae*	*L. lecanii*		
5	1.20	0.65	1.30	0.93	7.69	29.73
7	2.28	1.08	2.70	1.70	15.74	36.76
10	4.10	2.20	6.08	3.40	32.51	35.29
14	5.38	2.33	6.53	3.73	17.62	37.58

Table 24: Colony diameter and percentage inhibition of *B. bassiana* and *P.lilacinus* in dual culture

Days after inoculation	Mean colony diameter (cm)				Percentage inhibition	
	Dual culture		Control		*B. bassiana*	*P. lilacinus*
	B. bassiana	*P. lilacinus*	*B. bassiana*	*P. lilacinus*		
5	1.45	1.80	1.60	1.85	9.37	2.70
7	1.95	2.05	2.10	2.13	7.14	3.53
10	2.60	4.40	2.88	5.18	9.57	14.98
14	2.83	5.08	3.38	5.15	16.30	1.46



(A) Interaction of *M. anisopliae* and *L. lecanii* in dual cultures at 14 DAI

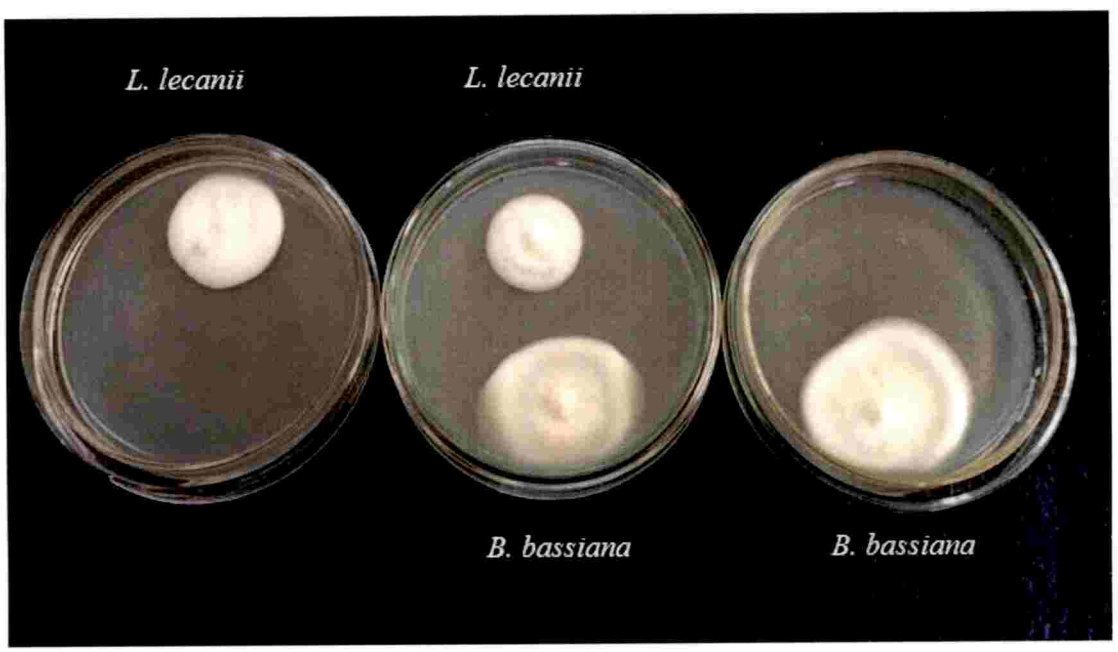


(B) Interaction of *B. bassiana* and *P. lilacinus* in dual cultures at 14 DAI

Plate 8: *In vitro* compatibility of *M. anisopliae* with *L. lecanii* and *B. bassiana* with *P. lilacinus* in dual cultures

Table 25: Colony diameter and percentage inhibition of *B. bassiana* and *L. lecanii* in dual culture

Days after inoculation	Mean colony diameter (cm)				Percentage inhibition	
	Dual culture		Control		*B. bassiana*	*L. lecanii*
	B. bassiana	*L. lecanii*	*B. bassiana*	*L. lecanii*		
5	1.70	1.35	1.73	1.40	1.45	2.90
7	2.00	1.43	2.28	1.45	12.09	1.10
10	2.93	1.85	2.93	1.88	0.00	0.85
14	4.90	3.15	4.98	3.40	1.51	5.03



Interaction of *B. bassiana* and *L.lecanii* in dual cultures at 14 DAI

Plate 9: *In vitro* compatibility of *B. bassiana* with *L.lecanii* in dual cultures

B.bassiana. Growth in dual cultures stopped two weeks after inoculation and a margin of 1.8 to 2 cm was observed between the two fungi.

In vitro studies on the compatibility of different entomopathogenic fungi showed that in dual cultures, growth of different fungi locked at the point of contact. No over growth was observed in any of the combinations. The relationship between different entomopathogenic fungi can be mutual inhibition.

4.5 LABORATORY EVALUATION OF CONSORTIUM

The interaction between different entomopathogenic fungi *in vitro* is not necessarily an indicator of their interaction *in vivo*. It is necessary to study the effect of different fungal combinations *in vivo* to know their actual effect of target pests when used together. The fungi which performed well in the pathogenicity trials against the various test insects were selected for studying their combined effect on various test insects. In pathogenicity trials, the fungi *M. anisopliae* and *B. bassiana* caused more than 50 per cent mortality to the test insects *viz., B. cucurbitae, H. septima, D. indica, A. foveicollis* and *L. australis*. The fungus *P. lilacinus* also induced mortality in fruit flies as well as in *D. indica*. Combinations of these three fungi, were tested for efficacy against the test insects in laboratory. The combinations tried were named as consortium 1, consortium 2 and consortium 3 which were combinations of the fungi *M. anisopliae* with *B. bassiana, M. anisopliae* with *P. lilacinus* and *B. bassiana* with *P. lilacinus* respectively. The consortium and the component fungi were tested at a spore concentration of 10^8 spores mL^{-1}. For the consortium, same volume of each fungal spore suspension was mixed to the desired concentration of 10^8 spores mL^{-1}.

4.5.1. *B. cucurbitae*

4.5.1.1. Adult

B. cucurbitae adults upon treatment with three entomopathogenic fungi *viz., B. bassiana, M. anisopliae* and *P. lilacius* and their combinations at a spore count of 10^8 spores mL^{-1} showed mortality two days after treatment (Table 26). The mixtures of various fungi proved to be more pathogenic to the adults in comparison with the fungi using individually. At three days after treatment, the most effective treatment was consortium 2 and had a mortality percentage of 41.67. This was on par with consortium 1 which depicted a mortality percentage of 35. The next superior treatment was consortium 3 which resulted in 25 per cent mortality. Among the individual components tested, *M. anisopliae* and *P. lilacinus* proved to be equally pathogenic to fruit flies and resulted in 16.67 and 15 per cent mortality respectively.

It took five days for fungal mixtures to reach 90 per cent mortality while the fungi when treated individually took seven days to reach the same. At five days after treatment, the highest mortality (96.67 per cent) was observed in consortium 1 which was on par with consortium 2 having 95 per cent mortality. Among the fungal mixtures tried, consortium 3 was least effective to the adults of fruit flies and had a mortality percentage of 75. This was followed by the treatments *M. anisopliae* and *P. lilacinus* which had 66.67 and 61.67 per cent mortality and were statistically on par with *B. bassiana*. 55 per cent mortality was observed in *B. bassiana* treated insects. No mortality was observed in control.

Cent per cent mortality was observed in insects treated with consortium 1 and consortium 2 at six days after treatment. At seven days after treatment, *P. lilacinus* (93.33) was the superior treatment after consortium 1 and consortium 2 which had 100 per cent mortality. The treatment, *M. anisopliae* was equally effective as *P. lilacinus* and resulted in 93.33 per cent mortality, seven days after treatment. The lowest mortality rate was observed in Consortium 3 which was on par with the treatment *B. bassiana* and had 81.67 and 83.33 per cent mortality respectively.

4.5.1.2 Pupae

Table 26 . Cumulative per cent mortality of *Bactrocera cucurbitae* adults treated with microbial combinations and its component micro-organisms in laboratory

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)		
	3DAT	5 DAT	7 DAT
Consortium 1 (*M. anisopliae* + *B. bassiana*)	35.00 (36.26)[a]	96.67 (82.15)[a]	100.00 (89.26)[a]
Consortium 2 (*M. anisopliae* + *P. lilacinus*)	41.67 (40.17)[a]	95.00 (80.54)[a]	100.00 (89.26)[a]
Consortium 3 (*B. bassiana* + *P. lilacinus*)	25.00 (29.96)[b]	75.00 (60.13)[b]	81.67 (64.73)[c]
M. anisopliae	16.67 (23.98)[c]	66.67 (54.78)[bc]	91.67 (73.42)[b]
B. bassiana	5.00 (11.41)[d]	55.00 (47.88)[c]	83.33 (66.02)[c]
P. lilacinus	15.00 (22.71)[c]	61.67 (51.78)[bc]	93.33 (76.97)[b]
Control	0.00 (0.74)[e]	0.00 (0.74)[d]	0.00 (0.74)[d]
C D (0.05)	(5.249)	(8.467)	(5.614)

Figures in parenthesis are angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

The data on the mortality of *B. cucurbitae* pupae and percentage adult emergencce on treatment with different fungal consortium and its component fungi are presented in Table 27. Zero per cent adults emerged from pupae treated with consortium 2 at seven days after treatment, with 1.67 per cent adult emergence and 98.33 per cent pupal mortality. The next superior treatment was *P. lilacinus* with 5 per cent adult emergence and 95 per cent pupal mortality. The treatments *M. anisopliae*, *B. bassiana* and consortium 3 were on par and showed 88.33, 85 and 81.67 per cent pupal mortality and 11.67, 15 and 18.33 per cent adult emergence respectively. Cent per cent adult emergence was noticed in control.

4.5.2. D. indica

Mortality was noticed in the second instar larvae of *D. indica,* when treated with the entomopathogenic fungi *B. bassiana*, *M. anisopliae* and *P. lilacinus* and their combinations at a concentration of 10^8 spores mL^{-1} at three days after treatment (Table 28). While only meager mortality was there in other treatments, consortium 1 resulted in 24.44 per cent mortality and was statistically superior to all other treatments. This was followed by *M. anisopliae* and consortium 2 which resulted in 8.89 and 4.44 per cent mortality respectively. No mortality was observed in the rest of the treatments.

At four days after treatment, mortality was observed in all the treatments except control. The fungal combination, consortium 1 proved to be highly effective and resulted in 44.44 per cent mortality, four DAT. This was followed by *M. anisopliae* and consortium 2 which were on par and had 17.78 and 15.56 per cent mortality respectively.

More than 50 per cent mortality (57.78) was observed in insects treated with consortium 1, five days after treatment. This was statistically superior to the rest of the treatments. The next superior treatment was *M. anisopliae* which resulted in 31.11

Table 27 . Cumulative per cent mortality of *Bactrocera cucurbitae* pupae treated with microbial combinations and its component micro-organisms in laboratory

Treatments @ 10^8 spores mL^{-1}	Adult emergence at 7 DAT*	Pupal mortality at 7 DAT**
Consortium 1 (*M. anisopliae* + *B. bassiana*)	1.67 (4.30)[d]	98.33 (85.78)[a]
Consortium 2 (*M. anisopliae* + *P. lilacinus*)	0.00 (0.74)[d]	100.00 (89.35)[a]
Consortium 3 (*B. bassiana* + *P. lilacinus*)	18.33 (25.58)[b]	81.67 (64.73)[c]
M. anisopliae	11.67 (19.48)[b]	88.33 (70.53)[c]
B. bassiana	15.00 (22.70)[b]	85.00 (67.29)[c]
P. lilacinus	5.00 (11.41)[c]	95.00 (78.62)[b]
Control	100.00 (89.26)[a]	0.00 (0.65)[d]
C D (0.05)	(6.718)	(6.752)

Figures in parenthesis are ** square root transformed values *angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

Table 28. Cumulative per cent mortality of *Diaphania indica* larvae treated with microbial combinations and its component micro-organisms in laboratory

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)			
	3 DAT**	4 DAT *	5 DAT *	6 DAT *
Consortium 1 (*M. anisopliae* + *B. bassiana*)	24.44 (4.98)[a]	44.44 (41.80)[a]	57.78 (49.48)[a]	100.00 (89.26)[a]
Consortium 2 (*M. anisopliae* + *P. lilacinus*)	4.44 (2.02)[c]	15.56 (23.14)[b]	22.22 (28.07)[c]	53.33 (46.93)[bc]
Consortium 3 (*B. bassiana* + *P. lilacinus*)	0.00 (0.71)[d]	4.44 (10.23)[c]	8.89 (17.12)[d]	37.78 (37.88)[d]
M. anisopliae	8.89 (3.03)[b]	17.78 (24.84)[b]	31.11 (33.88)[b]	60.00 (50.86)[b]
B. bassiana	0.00 (0.71)[d]	4.44 (10.23)[c]	11.11 (19.27)[d]	55.56 (48.20)[bc]
P. lilacinus	0.00 (0.71)[d]	2.22 (5.48)[c]	8.89 (17.12)[d]	48.89 (44.37)[c]
Control	0.00 (0.71)[d]	0.00 (0.74)[c]	0.00 (0.74)[e]	0.00 (0.74)[e]
C D (0.05)	(0.891)	(9.939)	(5.092)	(5.764)

Figures in parenthesis are ** square root transformed values *angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

per cent mortality five days after treatment and was followed by consortium 2 (22.22 per cent). The treatments *B. bassiana* (Bb5), consortium 3 and *P. lilacinus* were on par and resulted in a mean mortality of 11.11, 8.89 and 8.89 per cent respectively. The insects in the control where water spray was applied showed no mortality at all.

At six DAT, consortium 1 continued to be the best treatment and resulted in 100 per cent mortality of the test insects. A mortality percentage of 60 was observed in insects treated with *M. anisopliae* and was the next superior treatment after consortium 1. The treatments, *B. bassiana* and consortium 2 resulted in 55.56 and 53.33 per cent mortality and were on par with *P. lilacinus* which reported 48.89 per cent mortality.

4.5.3 *H. septima*

4.5.3.1Grub

The second instar grubs of *H. septima* on treatment with three fungal consortiums and its individual components at a concentration of 10^8 spores mL^{-1} showed mortality 3 DAT in all the treatments except in control (Table 29). Insects treated with consortium 1 showed the highest mortality percentage of 36.67 and was statistically superior to the rest of the treatments. The next superior treatments were *M. anisopliae* , *B. bassiana* and consortium 2 which resulted in mortality percentages of 15, 10 and 10 per cent respectively and were statistically on par. At 5 days after treatment, consortium 1 caused more than 50 per cent mortality (58.33 per cent) of the treated insects while its components took seven days to cause the same. *M. anisopliae* caused 30 per cent mortality which was followed by 21.67 per cent mortality caused by consortium 2. *B. bassiana* resulted in 18.33 per cent mortality and was on par with consortium 3 which showed 15 per cent mortality.

Table 29. Cumulative per cent mortality of *Henosepilachna septima* grubs treated with microbial combinations and its component micro-organisms in laboratory

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)			
	3 DAT	5 DAT	7 DAT	9 DAT
Consortium 1 (*M. anisopliae* + *B. bassiana*)	36.67 (37.25)[a]	58.33 (49.81)[a]	88.33 (70.53)[a]	100.00 (89.26)[a]
Consortium 2 (*M. anisopliae* + *P. lilacinus*)	10.00 (18.19)[b]	21.67 (27.69)[c]	30.00 (33.18)[d]	36.67 (37.25)[d]
Consortium 3 (*B. bassiana* + *P. lilacinus*)	5.00 (11.41)[c]	15.00 (22.71)[d]	25.00 (29.87)[d]	31.67 (34.22)[e]
M. anisopliae	15.00 (22.38)[b]	30.00 (33.18)[b]	50.00 (45.00)[b]	51.67 (45.96)[b]
B. bassiana	10.00 (18.19)[b]	18.33 (25.27)[cd]	38.33 (38.23)[c]	43.33 (41.16)[c]
Control	0.00 (0.74)[d]	0.00 (0.74)[e]	0.00 (0.74)[e]	0.00 (0.74)[f]
C D (0.05)	(6.479)	(3.201)	(4.837)	(2.589)

Figures in parenthesis are angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

The fungal consortium, consortium 1 proved to be highly pathogenic to the grubs of *H. septima* at seven days after treatment and resulted in a mortality percentage of 88.33 which was statistically superior to all other treatments and was higher than those of its components (*M. anisopliae* - 50 per cent, *B. bassiana*- 38.33 per cent) when treated individually. The other two fungal combinations tried, consortium 2 and consortium 3 resulted in the lowest mortality percentage of 30 and 25 per cent respectively and were on par.

When treated with consortium 1, the grubs of *H. septima* showed cent per cent mortality at nine days after treatment. The next superior treatment was *M. anisopliae* which had 51.67 per cent mortality followed by *B. bassiana* (43.33 per cent), consortium 2 (36.67 per cent) and consortium 3 (31.67 per cent). No mortality was observed in control.

4.5.3.2 Adult

Adults of *H. septima* inoculated with different fungal consortium and its component fungi showed differences in mortality at seven days after treatment (Table 30). Mortality was first noticed at five days after treatment after inoculation with Consortium 1 at a concentration of 10^8 spores mL^{-1} while it was seen only at seven days after treatment for other treatments. At seven days after treatment, consortium 1 resulted in 35 per cent mortality of the treated insects which was superior compared to the rest of the treatments. *M. anisopliae* and consortium 2 resulted in 7.5 per cent mortality each and were on par. This was followed by *B. bassiana* and consortium 3, the mortality percentage being 2.5 and 5.00 per cent respectively.

At nine days after treatment, 55 per cent mortality was observed in the insects treated with consortium 1 while *M. anisopliae* and *B. bassiana* when treated individually resulted in 25 and 12.5 per cent mortality respectively. The mortality percentage in the treatments consortium 2, *B. bassiana* and consortium 3 were on par.

Table 30. Cumulative per cent mortality of *Henosepilachna septima* adults treated with microbial combinations and its component micro-organisms in laboratory

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)		
	7 DAT	9 DAT	11 DAT
Consortium 1 (*M. anisopliae* + *B. bassiana*)	35.00 (36.22)[a]	55.00 (47.88)[a]	55.00 (47.93)[a]
Consortium 2 (*M. anisopliae* + *P. lilacinus*)	7.50 (14.05)[b]	10.00 (18.44)[c]	22.50 (28.18)[b]
Consortium 3 (*B. bassiana* + *P. lilacinus*)	5.00 (9.67)[bc]	10.00 (18.44)[c]	22.50 (28.18)[b]
M. anisopliae	7.50 (14.05)[b]	25.00 (29.89)[b]	22.50 (28.18)[b]
B. bassiana	2.50 (5.29)[bc]	12.50 (20.47)[c]	15.00 (22.94)[b]
Control	0.00 (0.91)[c]	0.00 (0.91)[d]	2.50 (5.28)[e]
C D (0.05)	(11.265)	(3.946)	(8.001)

Figures in parenthesis are angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

At 11 DAT, the mortality of the treated insects reached 55 per cent in consortium 1. Results obtained in the treatments *M. anisopliae*, consortium 2, consortium 3 and *B. bassiana* were on par. The lowest mortality percentage (15) was observed in insects treated with consortium 3

4.5.4. *A. foveicollis*

The data on the evaluation of different fungal consortium and its component fungi on *A. foveicollis* adults are presented in Table 31. Excluding control, mortality was observed in all the treatments and the highest being 33.33 per cent in consortium 1 at five days after treatment. This was statistically superior compared to the rest of the treatments. *M. anisopliae* with a mortality percentage of 13.33 was the next superior treatment followed by consortium 2 and *B. bassiana* which had 10 per cent mortality each and were on par. Consortium 3 resulted in the lowest mortality, the mortality percentage being 6.67 and was on par with consortium 2 and *B. bassiana*.

A similar trend was followed at seven days after treatment with consortium 1 being the most effective treatment with a mortality percentage 56.67 followed by *B. bassiana* (36.67 per cent), consortium 2 (28.33 per cent) and *M. anisopliae* (25 per cent). Mortality percentages obtained in consortium 2 and *M. anisopliae* were on par. The lowest mortality percentage (16.67) was observed in consortium 3. At 10 days after treatment, consortium 1 resulted in 64.91 per cent mortality followed by *B. bassiana* (43.86 per cent). The treatments *M. anisopliae,* consortium 2 and consortium 3 were on par, the mortality percentages being 36.81, 28.07 and 21.05 respectively. Five per cent mortality was observed in control, 10 days after treatment.

4.5.5 *L. australis*

The third instar nymphs of *L. australis* when treated with different fungal combinations and its component micro-organisms showed mortality three days after treatment (Table 32). The highest mortality of 38.33 per cent was observed with the

Table 31. Cumulative per cent mortality of *Aulacophora foveicollis* adults treated with microbial combinations and its component micro-organisms in laboratory

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)		
	5 DAT	7 DAT	10 DAT
Consortium 1 (*M. anisopliae* + *B. bassiana*)	33.33 (35.21)^{a}	56.67 (48.84)^{a}	64.91 (53.72)^{a}
Consortium 2 (*M. anisopliae* + *P. lilacinus*)	10.00 (18.19)^{bc}	28.33 (32.05)^{c}	28.07 (31.96)^{cd}
Consortium 3 (*B. bassiana* + *P. lilacinus*)	6.67 (13.03)^{c}	16.67 (23.98)^{d}	21.05 (27.25)^{d}
M. anisopliae	13.33 (21.09)^{b}	25.00 (29.87)^{c}	36.84 (41.51)^{b}
B. bassiana	10.00 (18.19)^{bc}	36.67 (37.25)^{b}	43.86 (37.32)^{bc}
Control	0.00 (0.74)^{d}	0.00 (0.74)^{e}	5.00 (9.46)^{e}
C D (0.05)	(7.112)	(4.444)	(7.548)

Figures in parenthesis are angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

combination of *M. anisopliae* and *B. bassiana*, consortium 1 which was statistically superior to the rest of the treatments, especially *M. anisopliae* and *B. bassiana* the components of consortium 1 which resulted in 21.67 per cent and 8.33 per cent mortality respectively. At five days after treatment, consortium 1 resulted in a mortality percentage of 60. The next superior treatment was *M. anisopliae* which had 36.67 per cent mortality. The mortality in consortium 2 was on par with that of *B. bassiana* and showed 31.67 and 30 per cent mortality respectively. Lowest mortality at five days after treatment was observed in consortium 3, the mortality percentage being 23.33.

More than 50 per cent mortality of the treated insects was noticed in all the treatments, seven days after treatment. Cent per cent mortality was observed in the nymphs treated with consortium 1 followed by *M. anisopliae* (72.73 per cent mortality). The treatments consortium 2, *B. bassiana* and consortium 3 were on par and showed 56.37, 50.91 and 49.09 per cent mortality respectively. A mortality percentage of 8.33 was observed in control.

4.5.6. Effect of Consortium in a Mixed Population

The data on the evaluation of different fungal consortium and its component fungi on a mixed population comprising representatives of all the test insects are presented in Table 33. Excluding control, mortality was observed in all the treatments and the highest being 23.33 per cent in consortium 1 at 3 days after treatment. This was followed by consortium 2 having a mortality percentage of 11.11.

At five DAT, the highest mortality was observed in consortium 1 (41.11 per cent) followed by consortium 2 (30.00 per cent). The mortality obtained in *M. anisopliae, B. bassiana* and consortium 3 was on par, the mortality percentages being 25.56, 22.22 and 17.78 respectively.

Table 32. Cumulative per cent mortality of *Leptoglossus australis* nymphs treated with microbial combinations and its component micro-organisms in laboratory

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)		
	3 DAT	5 DAT	7 DAT
Consortium 1 (*M. anisopliae* + *B. bassiana*)	38.33 (38.23)[a]	60.00 (50.80)[a]	100 (89.26)[a]
Consortium 2 (*M. anisopliae* + *P. lilacinus*)	18.33 (25.27)[b]	31.67 (34.22)[bc]	56.37 (48.67)[c]
Consortium 3 (*B. bassiana* + *P. lilacinus*)	5.00 (11.41)[c]	23.33 (28.82)[d]	49.09 (44.49)[c]
M. anisopliae	21.67 (27.69)[b]	36.67 (37.25)[b]	72.73 (58.64)[b]
B. bassiana	8.33 (16.58)[c]	30.00 (33.18)[c]	50.91 (45.52)[c]
Control	0.00 (0.74)[d]	0.00 (0.74)[e]	8.33 (12.36)[d]
C D (0.05)	(4.444)	(3.454)	(9.928)

Figures in parenthesis are angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

Table 33. Cumulative per cent mortality of insects in a mixed population treated with microbial combinations and its component micro-organisms in laboratory

Treatments @ 10^8 spores mL^{-1}	Cumulative mortality (%)		
	3 DAT*	5 DAT**	7 DAT**
Consortium 1 (*M. anisopliae* + *B. bassiana*)	23.33 (4.88)^{a}	41.11 (39.87)^{a}	78.89 (62.67)^{a}
Consortium 2 (*M. anisopliae* + *P. lilacinus*)	11.11 (3.40)^{b}	30.00 (33.19)^{b}	43.33 (41.16)^{c}
Consortium 3 (*B. bassiana* + *P. lilacinus*)	5.56 (2.43)^{cd}	17.78 (24.92)^{d}	33.33 (35.24)^{d}
M. anisopliae	7.78 (2.86)^{bc}	25.56 (30.35)^{bc}	50.00 (45.00)^{b}
B. bassiana	3.33 (1.79)^{de}	22.22 (28.07)^{cd}	44.44 (41.80)^{c}
P. lilacinus	1.11 (1.13)^{ef}	12.22 (20.43)^{e}	25.56 (30.35)^{e}
Control	0.00 (0.70)^{f}	0.00 (0.74)^{f}	0.00 (0.74)^{f}
C D (0.05)	(0.931)	(3.411)	(2.652)

Figures in parenthesis are * square root transformed values

**angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

At seven DAT, consortium 1 continued to be the most effective treatment with a mortality percentage of 78.89 followed by *M. anisopliae* (50.11 per cent), *B. bassiana* (44.44 per cent) and consortium 2 (28.33 per cent).

4.6 EFFECTIVE DOSE OF CONSORTIUM I

The effective dose of consortium 1 for field study was fixed based on the mortality of second instar larvae of *D. indica.* The data on the mortality of *D. indica* larvae tested with different doses of talc based formulation of consortium 1 is presented in Table 34.

The talc based formulation of consortium I at different doses induced mortality in the second instar larvae of *D. indica*, three days post treatment. An increasing trend in mortality was noticed with the increase in dose of the consortium from 15 g L^{-1} to 40 g L^{-1}. At three days after treatment, the dose 40 g L^{-1} recorded the highest mortality of 31.11 per cent. This was followed by 30 g L^{-1} and 25 g L^{-1} with 26.67 and 17.78 per cent mortality respectively. The lowest mortality of 4.44 per cent was observed in the dose 15 g L^{-1}.

At five days after treatment, mortality observed with the doses, 40 g L^{-1}, 35 and 30 gL^{-1} were on par the mortality percentages being 77.78, 71.11 and 71.11 respectively. Also, the mortality observed in the lower doses 25, 20 and 15 g L^{-1} were on par with a mortality of 40.00, 33.33 and 31.11 per cent respectively. .

At seven days after treatment, 100 per cent mortality was observed with the doses 40 g L^{-1} and 35 g L^{-1} followed by 30 and 25 g L^{-1} which recorded 88.89 per cent and 73.33 per cent mortality respectively.

The dose 35 g L^{-1} was selected as the field dose as it offered the same level of mortality as the higher dose.

4.7. SHELF LIFE OF TALC FORMULATION OF THE CONSORTIUM

Table 34. Percentage mortality of larvae of *Diaphania indica* treated with talc based formulation of the consortium I in laboratory

Treatments ($g\ L^{-1}$)	Cumulative mortality (%)		
	3 DAT	5DAT	7DAT
15	4.44 $(10.23)^{d}$	31.11 $(33.87)^{c}$	55.56 $(48.20)^{d}$
20	11.11 $(19.27)^{c}$	33.33 $(35.26)^{bc}$	57.78 $(49.48)^{d}$
25	13.33 $(21.42)^{c}$	40.00 $(39.20)^{b}$	73.33 $(59.02)^{c}$
30	17.78 $(24.84)^{bc}$	71.11 $(57.64)^{a}$	88.89 $(70.73)^{b}$
35	26.67 $(30.98)^{ab}$	71.11 $(57.51)^{a}$	100.00 $(89.26)^{a}$
40	31.11 $(33.88)^{a}$	77.78 $(61.93)^{a}$	100.00 $(89.26)^{a}$
Talc	0.00 $(0.74)^{e}$	0.00 $(0.74)^{d}$	0.00 $(0.74)^{e}$
Control (water spray)	0.00 $(0.74)^{e}$	0.00 $(0.74)^{d}$	0.00 $(0.74)^{e}$
CD (0.05)	(6.556)	(4.691)	(4.631)

Figures in parenthesis are angular transformed values

DAT- Days After Treatment, Figures in a column when followed by the same letter do not differ significantly

The data on colony forming units, mean spore count and bioefficacy of the talc based product of the fungal consortium, consortium I against the test insects are presented in Tables 35 and 36.

4.7.1 Viability

The viability of the talc based formulation of consortium I stored at room temperature and refrigeration (Table 35) was assessed in terms of cfu at fortnightly intervals.

The quantity of viable spores in the talc formulation during 15 and 30 days after storage (DAS) under room temperature was significantly higher and recorded 9.8×10^7 and 9.63×10^7 cfu g^{-1} respectively. The cfu at 45, 60 and 75 DAS, the viable spore load in the talc formulation obtained were 9.23×10^7, 8.87×10^7 and 8.23×10 cfu g^{-1} and were on par. The lowest cfu count was obtained at 90 DAS with a count of 6.83×10^7 cfu g^{-1}.

Under refrigeration, the count of viable spores was high compared to that stored in room temperature and recorded a high cfu of 11.13×10^7, 10.19×10^7, 11.07×10^7 and 11.43×10^7 cfu g^{-1} at 15, 30, 45 and 60 DAS and were on par. Significantly lower cfu of 9.20×10^7 cfu g^{-1} was obtained 75 DAS which was statistically superior to the cfu count obtained 90 DAS (7.23×10^7 cfu g^{-1}).

4.7.2 Spore count

The talc based formulation of consortium I stored at room temperature recorded the highest spore count of 5.77×10^9 and 5.81×10^9 spores g^{-1} at 15 and 45 DAS and were on par (Table 35). This was followed by 4.85×10^9, 4.46×10^9, 3.15×10^9 and 2.59×10^9 spores g^{-1} respectively after 45, 60, 75 and 90 DAS. Under refrigeration, the spore counts at 15, 30 and 45 DAS were on par and recorded 6.10×10^9, 6.09×10^9 and 6.03×10^9 spores g^{-1} respectively. At 60 DAS, the spore count

Table 35. Mean spore count and cfu of the talc based formulation of consortium I at different intervals after storage under refrigeration and room temperature

Days After Storage	Mean number of cfu (n x 10^7 spores g^{-1})		Spore count (n x10^9 spores g^{-1})	
	Room temperature	Refrigeration	Room temperature	Refrigeration
15	9.80 (3.13)[a]	11.13 (3.33)[a]	5.77 (2.40)[a]	6.10 (2.46)[a]
30	9.63 (3.11)[a]	10.97 (3.31)[a]	5.81 (2.41)[a]	6.09 (2.47)[a]
45	9.23 (3.03)[ab]	11.07 (3.32)[a]	4.85 (2.21)[b]	6.03 (2.45)[a]
60	8.87 (2.97)[bc]	11.43 (3.39)[a]	4.46 (2.12)[c]	5.86 (2.42)[b]
75	8.23 (2.87)[c]	9.20 (3.04)[b]	3.15 (1.78)[d]	5.59 (2.36)[c]
90	6.83 (2.61)[d]	7.23 (2.68)[c]	2.59 (1.61)[e]	5.17 (2.27)[d]
CD(0.05)	(0.147)	(0.128)	(0.035)	(0.030)

* Figures in parenthesis are square root transformed values, Figures in a column when followed by the same letter do not differ significantly

137

obtained was 5.86×10^9 spores g^{-1} and was significantly higher compared to the spore counts at 75 (5.59×10^9 spores g^{-1}) and 90 DAS (5.17×10^9 spores g^{-1}).

4.7.3. Bioefficacy

The adults and pupae of *B. cucurbitae* showed no significant difference in mortality when applied with talc based formulation of consortium I @ 35 g L^{-1} upto 90 DAS (Table 36). For adults of epilachna beetle, *H. septima*, the highest mortality recorded was at 15 (64.44 per cent) and 30 DAS (64.44 per cent) and were on par. The mortality obtained was 60 and 57.78 per cent at 45 and 60 DAS and was on par with the mortality obtained at 75 and 90 DAS (55.56 per cent each)

When the grubs of *H. septima* were subjected to treatment with the talc based formulation, 100 per cent mortality was observed during 15 and 30 DAS and showed statistical parity. The mortality obtained at 45, 60, 75 and 90 DAS were also on par and recorded 91.11, 88.89, 86.67 and 86.67 per cent respectively.

Cent per cent mortality was observed for the larvae of *D. indica* treated with the talc based formulation of consortium I at 15 and 30 DAS. Significantly lower mortality (95, 92.5, 92.5 and 87.5 per cent) was observed at 45, 60, 75 and 90 DAS and was on par. For adults of *A. foveicollis*, the highest mortality (71.11 and 73.33 per cent) was obtained at 15 and 30 DAS of the talc based formulation and showed statistical parity. This was followed by 60 per cent mortality obtained 45 DAS. The mortality percentages obtained at 60, 75 and 90 DAS was on par and recorded 57.78, 51.11 and 48.89 per cent mortality respectively.

4.8 COMPATIBILITY OF ENTOMOPATHOGENIC FUNGI WITH INSECTICIDES

4.8.1 Compatibility of *M. anisopliae*

Table 36. Effect of storage of the consortium I on mortality of the target insects

Days After Storage	Mean percentage mortality					
	B. cucurbitae		*H. septima*		*D. indica* (larvae)	*A. foveicollis*
	Adult	Pupa	Adult	Grub		
15	100.00 (89.35)	100.00 (89.35)	64.44 (53.42)^{a}	100.00 (89.26)^{a}	100.00 (89.09)^{a}	71.11 (57.51)^{a}
30	100.00 (89.354)	100.00 (89.35)	64.44 (53.42)^{a}	100.00 (89.26)^{a}	100.00 (89.09)^{a}	73.33 (59.02)^{a}
45	100.00 (89.35)	98.33 (85.27)	60.00 (50.80)^{ab}	91.11 (72.88)^{b}	95.00 (80.33)^{ab}	60.00 (50.80)^{b}
60	100.00 (89.35)	98.33 (85.27)	57.78 (49.48)^{ab}	88.89 (70.74)^{b}	92.50 (75.94)^{bc}	57.78 (49.48)^{bc}
75	96.67 (83.43)	98.33 (85.27)	55.56 (45.65)^{b}	86.67 (69.01)^{b}	92.50 (75.94)^{bc}	51.11 (45.65)^{bc}
90	96.67 (81.17)	96.67 (81.17)	55.56 (48.64)^{b}	86.67 (68.59)^{b}	87.50 (69.54)^{c}	48.89 (44.37)^{c}
CD (0.05)	N S	N S	(5.402)	(5.693)	(10.014)	(6.057)

Figures in parenthesis are angular transformed values, Figures in a column when followed by the same letter do not differ significantly

The mean colony diameter of *M. anisopliae* at different intervals grown on Petri plates with poisoned media are presented in Table 37. The longest colony diameter (2.97 cm) at seven days after inoculation (DAI) was observed in Flubendiamide 20 WG 0.01 % which was on par with the untreated control (3.10 cm). This was found to be on par with Fipronil 5% SC 0.01% (2.70 cm) and Indoxacarb 14.5% SC 0.015% (2.77 cm). The next best treatment was Chlorantraniliprole 18.5 SC 0.006 % and Thiodicarb 75% WP 0.1 % having a mean diameter of 2.47cm each.

At 14 DAI, the longest colony diameter among the treated plates was observed in Chlorantraniliprole 18.5 SC 0.006 % (4.53 cm) (plate 10). The next best treatment was Thiodicarb 75% WP 0.1 % having a mean diameter of 4.00 cm and was found to be on par with Flubendiamide 20 WG 0.01 % (4.3 cm). The plates treated with Fipronil 5% SC 0.01 % had depicted the shortest radial growth.

A mean colony diameter of 6.00 cm was observed at 21 days after treatment in plates treated with Chlorantraniliprole 18.5 SC 0.006 %. This was followed by Thiodicarb 75% WP 0.1 % having a mean diameter of 5.37 cm and was found to be on par with Flubendiamide 20 WG 0.01 % (5.23 cm) and Fipronil 5% SC 0.01 % (5.27 cm). The lowest radial growth (5.03 cm) was observed in plates treated with Indoxacarb 14.5% SC 0.015 % at 21 days after treatment. Longest mean diameter of *M. anisopliae* has been observed against the control having a mean diameter of 8.33 cm at 21 days after inoculation.

The spore count of fungus was the highest in the untreated control (6.29×10^8 spores mL^{-1}) and all other treatments were significantly lower. At 14 days after inoculation, the treatment with Chlorantraniliprole 18.5 SC 0.006 % was found to be less inhibitive to sporulation of *M. anisopliae* with a mean spore count 4.89×10^8 spores mL^{-1}. Spore counts of all other treatments were significantly lower. Indoxacarb 14.5% SC 0.015% supported a spore count of 3.73×10^8 spores mL^{-1} and

Table 37. Colony diameter and spore count of *M. anisopliae* on PDA media treated with different insecticides

Treatments	Dosage	Mean colony diameter (cm)			Spore count at 14DAI (n x 10^8 spores mL^{-1})
		7 DAI	14 DAI	21 DAI	
Flubendiamide 20% WG	0.01%	2.97 $(1.73)^{a}$	3.83 $(1.96)^{cd}$	5.23 $(2.28)^{cd}$	3.45 $(1.86)^{cd}$
Fipronil 5%SC	0.01%	2.70 $(1.65)^{ab}$	3.37 $(1.84)^{e}$	5.27 $(2.30)^{cd}$	2.67 $(1.64)^{e}$
Thiodicarb 75% WP	0.1%	2.47 $(1.57)^{b}$	4.00 $(2.00)^{c}$	5.37 $(2.31)^{c}$	3.33 $(1.84)^{d}$
Chlorantraniliprole 18.5 SC	0.006%	2.47 $(1.57)^{b}$	4.53 $(2.12)^{b}$	6.00 $(2.44)^{b}$	4.89 $(2.21)^{b}$
Indoxacarb 14.5%SC	0.015%	2.77 $(1.66)^{ab}$	3.67 $(1.92)^{d}$	5.03 $(2.24)^{d}$	3.73 $(1.94)^{c}$
Control		3.10 $(1.77)^{a}$	6.30 $(2.51)^{a}$	8.33 $(2.89)^{a}$	6.29 $(2.51)^{a}$
C D (0.05)		(0.119)	(0.056)	(0.053)	(0.095)

Figures in parenthesis are square root transformed values

*DAI- Days After Inoculation, Figures in a column when followed by the same letter do not differ significantly

Plate 10: *In vitro* compatibility of *M. anisopliae* with different insecticides at 14 DAI

was on par with Flubendiamide 20% WG 0.01 % (3.45×10^8 spores mL^{-1}). This was followed by Thiodicarb 75% WP 0.1 % with a mean spore count of 3.73×10^8 spores mL^{-1}. The lowest spore count was observed in Fipronil 5% SC 0.01 % which showed a mean spore count of 2.67×10^8 spores mL^{-1}.

4.8.2 Compatibility of *B. bassiana* (Bb5)

B. bassiana when cultured in poisoned food media, 7DAI, control plates having *B.bassiana* grown in PDA without chemicals supported a mean colony diameter of 3.7 cm which was on par with the mean diameter in Chlorantraniliprole 18.5 SC 0.006 % (3.43 cm) (Table 38). Fipronil 5% SC 0.01 % reported a mean growth of 3.00 cm seven DAI and was statistically on par with Chlorantraniliprole 18.5 SC 0.006 %. Thiodicarb 5% WP 0.1 % and Flubendiamide 20% WG 0.01% depicted a mean growth of 2.47 cm and 2.57 cm respectively and were statistically on par. Least growth was observed in Indoxacarb 14.5% SC 0.015% which depicted a mean diameter of 2.03 cm.

A similar trend was observed 14 DAI where maximum growth was supported by the control plates having a mean diameter of 5.50 cm and was on par with Chlorantraniliprole 18.5 SC 0.006 % having a mean diameter of 5.03 cm (plate 11). Media poisoned with Flubendiamide 20% WG 0.01%, Fipronil 5% SC 0.01 % and Indoxacarb 14.5% SC 0.015% showed a mean growth rate of 4.33 cm, 4.40 cm and 4.07 cm and were statistically on par. Maximum reduction in growth of *B. bassiana* was noticed in Thiodicarb 5% WP 0.1 % having a mean diameter of 3.50 cm.

Out of five pesticides, Chlorantraniliprole 18.5 SC 0.006 % was found to be least inhibitory having the longest diameter of 5.40 cm at 21 DAI. This was on par with Flubendiamide 20% WG 0.01% and Fipronil 5% SC 0.01 % having a mean diameter of 5.27 cm and 4.93 cm respectively. Thiodicarb 5% WP 0.1 % was inhibitory to the growth of *B. bassiana* and showed a mean diameter of 3.93 cm and

Table 38 . Colony diameter and spore count of *B. bassiana* on PDA media treated with different insecticides

Treatments	Dosage	Mean colony diameter (cm)			Spore count at 14DAI (n x 10^8 spores mL^{-1})
		7 DAI	14 DAI	21 DAI	
Flubendiamide 20% WG	0.01%	2.57 $(1.60)^{c}$	4.33 $(2.09)^{b}$	5.27 $(2.30)^{b}$	4.56 $(2.13)^{c}$
Fipronil 5%SC	0.01%	3.00 $(1.74)^{b}$	4.40 $(2.09)^{b}$	4.93 $(2.22)^{bc}$	3.45 $(1.85)^{e}$
Thiodicarb 75% WP	0.1%	2.47 $(1.57)^{c}$	3.50 $(1.87)^{c}$	3.93 $(1.99)^{d}$	3.12 $(1.77)^{f}$
Chlorantraniliprole 18.5 SC	0.006%	3.43 $(1.86)^{ab}$	5.03 $(2.24)^{a}$	5.40 $(2.33)^{b}$	5.61 $(2.36)^{b}$
Indoxacarb 14.5%SC	0.015%	2.03 $(1.42)^{d}$	4.07 $(2.01)^{b}$	4.80 $(2.19)^{c}$	4.12 $(2.03)^{d}$
Control		3.70 $(1.92)^{a}$	5.50 $(2.34)^{a}$	6.23 $(2.49)^{a}$	6.95 $(2.63)^{a}$
C D (0.05)		(0.126)	(0.137)	(0.104)	(0.050)

Figures in parenthesis are square root transformed values

*DAI- Days After Inoculation, Figures in a column when followed by the same letter do not differ significantly

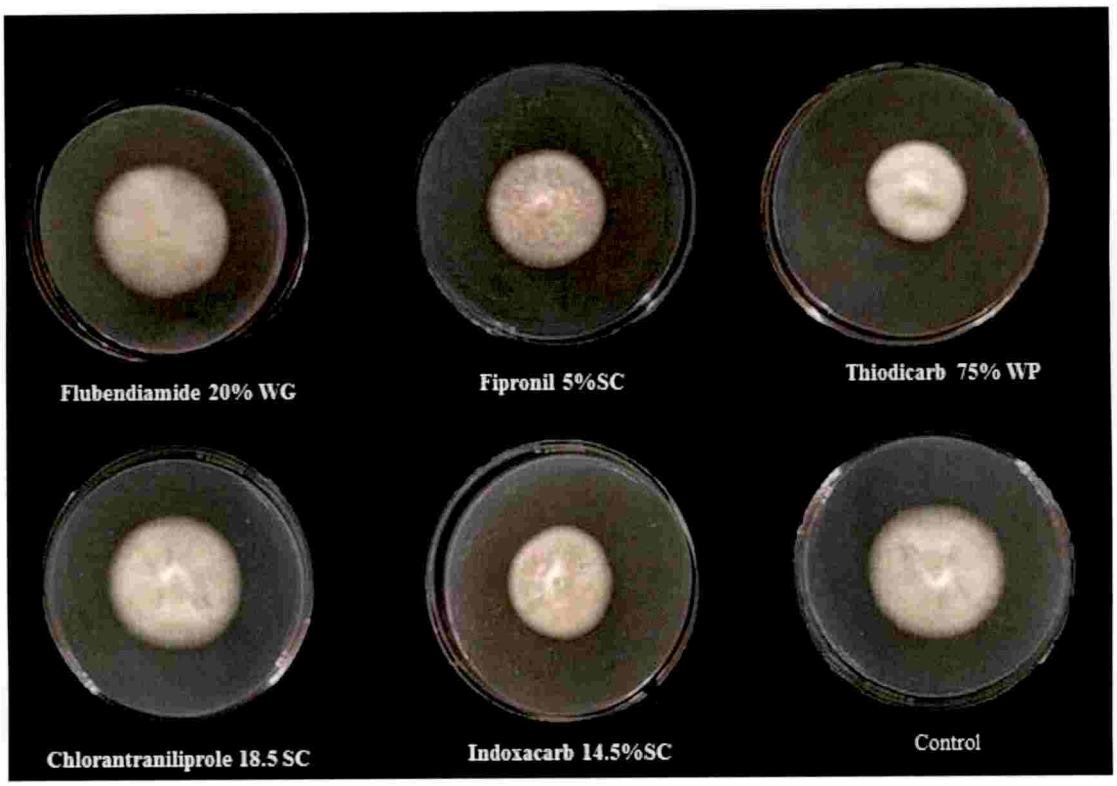


Plate 11: In vitro compatibility of B. bassiana with different insecticides at 14 DAI

was the lowest among the tested insecticides. In control, the mean diameter observed was of 6.23 cm.

The spore counts of the fungus grown on media poisoned with various pesticides were significantly lower compared to the untreated control. At 21 days after inoculation the spore count in untreated media was 6.95×10^{8} spores mL^{-1}. The treatment with Chlorantraniliprole 18.5 SC 0.006 % was found to be less inhibitive to sporulation of *B.bassiana* with a mean spore count 5.61×10^{8} spores mL^{-1}. Significantly lower spore count was observed in other treatments. Flubendiamide 20% WG supported a spore count of 4.56×10^{8} spores mL^{-1} and was followed by Indoxacarb 14.5% SC 0.015% (4.14×10^{8} spores mL^{-1}), Fipronil 5% SC 0.01 % (3.45×10^{8} spores mL^{-1}) and Thiodicarb 5% WP 0.1 % (3.12×10^{8} spores mL^{-1})

4.9. FIELD EVALUATION OF THE MICROBIAL CONSORTIUM

The results of the field studies conducted to test the efficacy of the standardized dose of the microbial consortium developed on various pests of bitter gourd viz; *B. cucurbitae, H. septima, D. indica, A. foveicollis* are presented in Tables 39 to 43

4.9.1Population of pests

4.9.1.1H.septima

The population of *H. septima* recorded at different intervals after application of the treatments was presented in Table 39. One day post treatment, significant reduction in the population was observed in the treatments consortium I followed by chlorantraniliprole 18.5 SC 0.006% (15.33 insects ten $leaves^{-1}$), chlorantraniliprole 18.5 SC 0.006% (14.22 insects ten $leaves^{-1}$) and malathion (14.33 insects in ten leaves) and were on par.

After three days of treatment, the lowest population of 4.33 insects in ten leaves was observed in plots treated with consortium I followed by chlorantraniliprole 18.5

Table 39. Effect of treatments on the population of *Henosepilachna septima* in field

Treatments	Mean population *				
	1 DAS	3 DAS	5 DAS	7 DAS	14 DAS
T1- Consortium I @ 35 g L^{-1}	23.22 (4.81)^{a}	12.89 (3.58)^{b}	3.78 (2.06)^{b}	2.11 (1.51)^{b}	2.22 (1.50)^{bc}
T2- Chlorantraniliprole 18.5 SC 0.006%	15.33 (3.91)^{b}	5.22 (2.27)^{c}	0.67 (1.08)^{e}	0.56 (0.76)^{d}	2.22 (1.46)^{bc}
T3- T1 followed by T2	16.00 (3.99)^{b}	4.33 (2.09)^{c}	0.11 (0.80)^{f}	0.56 (0.76)^{d}	1.22 (1.18)^{c}
T4- T2 followed by T1	23.11 (4.80)^{a}	14.67 (3.82)^{b}	3.11 (1.87)^{c}	1.89 (1.18)^{c}	2.00 (1.41)^{bc}
T5- Malathion 0.1 %	14.33 (3.79)^{b}	5.44 (2.32)^{c}	2.22 (1.62)^{d}	2.44 (1.55)^{b}	2.89 (1.66)^{b}
T6- Untreated check	21.11 (4.59)^{a}	17.33 (4.16)^{a}	16.44 (4.11)^{a}	11.22 (3.34)^{a}	6.33 (2.48)^{a}
CD (0.05)	(0.354)	(0.252)	(0.166)	(0.312)	(0.316)

* Population in 10 leaves per plant, figures in parenthesis are square root transformed values

Figures in a column when followed by the same letter do not differ significantly

SC 0.006% and was on par with the population observed in plots treated with chlorantraniliprole 18.5 SC 0.006% (5.22 insects ten leaves^{-1}) and malathion (5.44 insects in ten leaves). Next best treatment was consortium I @ 40 gL^{-1} and chlorantraniliprole 18.5 SC 0.006% followed by consortium I with a mean population of 12.89 insects ten leaves^{-1} and 14.67 insects in ten leaves. The population level in the untreated control plot was significantly higher than other treatments.

On fifth day after treatment, the lowest *H. septima* population was observed in consortium followed by chlorantraniliprole 18.5 SC 0.006% treated plot with a mean population of 0.11 insects in ten leaves and it was significantly lower compared to other treatments. A significantly higher population of 0.67 insects in ten leaves was observed in chlorantraniliprole 18.5 SC 0.006% and was the next best treatment. In Malathion, the mean population of 2.22 insects in ten leaves was observed and was significantly better than the treatments chlorantraniliprole 18.5 SC 0.006% followed by consortium I and consortium I where the population recorded were 3.11 and 3.78 insects in ten leaves. However the populations in these treatments were significantly lower than those in the untreated control.

On seventh day after treatment the lowest epilachna population was observed in chlorantraniliprole 18.5 SC 0.006% and consortium I followed by chlorantraniliprole 18.5 SC 0.006% treated plots with a mean population of 0.56 insects in ten leaves. In chlorantraniliprole 18.5 SC 0.006% followed by consortium I, the mean population of 1.89 insects ten leaves^{-1} was observed and was significantly better than the untreated control which recorded 11.22 insects in ten leaves. A population of 2.11 insects in ten leaves was observed in consortium I treatment and it was on par with Malathion (2.44 insects in ten leaves).

On 14th day the lowest pest population of 1.22 insects ten leaves^{-1} was recorded in consortium I followed by chlorantraniliprole 18.5 SC 0.006% treated plots. Epilachna populations of 2.00, 2.22 and 2.22 insects in ten leaves was recorded in chlorantraniliprole 18.5 SC 0.006% followed by consortium I, consortium I and

chlorantraniliprole 18.5 SC 0.006% and these were on par. In malathion treated plots, 2.89 insects in ten leaves was observed and was significantly lower compared to untreated control (6.33 insects in ten leaves).

Overall observations on population of *H. septima* revealed that the treatments consortium I followed by chlorantraniliprole 18.5 SC 0.006%, chlorantraniliprole 18.5 SC 0.006%, consortium I and chlorantraniliprole 18.5 SC 0.006 followed by consortium I were effective in controlling the epilachna population within a shorter period of seven days but the population of *H. septima* was in an increasing phase seven days after treatment.

4.9.1.2. D. indica

The populations of *D .indica* recorded in the treated plots at different intervals of time are presented in Table 40. One day after treatment, consortium I followed by chlorantraniliprole 18.5 SC 0.006% treated plots recorded the lowest diaphania population of 0.08 larvae in ten leaves and was significantly superior to others. Next best treatment was chlorantraniliprole 18.5 SC 0.006% with a mean population of 0.42 larvae in ten leaves followed by chlorantraniliprole 18.5 SC 0.006% followed by consortium I and malathion with the mean populations of 1.33 and 1.08 larvae in ten leaves respectively. Consortium I recorded a population of 1.75 larvae in ten leaves and was significantly lower compared to the untreated control plots (2.67 larvae in ten leaves)

Significantly lower reduction of insects was recorded in the treatments with chlorantraniliprole 18.5 SC 0.006% and consortium I followed by chlorantraniliprole 18.5 SC 0.006% with the mean population of 0.17 larvae in ten leaves each at 3 days post treatment. This was significantly lower than other treatments. This was followed by malathion, consortium I and chlorantraniliprole 18.5 SC 0.006% followed by consortium I with mean population of 1.08, 1.92 and 1.58 larvae in ten leaves respectively.

The lowest pumpkin caterpillar population at five days after treatment was observed in chlorantraniliprole 18.5 SC 0.006% and consortium I followed by chlorantraniliprole 18.5 SC 0.006% treated plot with mean population of 0.08 larvae in ten leaves and were on par. The treatments consortium I and chlorantraniliprole 18.5 SC 0.006% followed by consortium I recorded a mean population of 0.5 and 0.75 larvae in ten leaves and was significantly better than the treatment malathion (1.17 larvae in ten leaves).

On seventh day, the lowest pest population of 0.08 larvae in ten leaves was observed in the treatment consortium I. Significantly higher populations (0.17, 0.17 and 0.42 larvae in ten leaves) was recorded in consortium I followed by chlorantraniliprole 18.5 SC 0.006%, chlorantraniliprole 18.5 SC 0.006% followed by consortium I and chlorantraniliprole 18.5 SC 0.006% treated plots and these were on par. All the treatments reduced the population of the pest compared to the chemical check malathion (1.17 larvae in ten leaves) and the untreated control (2 larvae in ten leaves).

On 14th day, the lowest pumpkin caterpillar population (0.42 and 0.5 larvae in ten leaves) was observed in chlorantraniliprole 18.5 SC 0.006% and chlorantraniliprole 18.5 SC 0.006% followed by consortium I treated plots. This was followed by consortium I and consortium I followed by chlorantraniliprole 18.5 SC 0.006% with mean population of 0.83 larvae in ten leaves. Significantly higher population of 1.5 larvae in ten leaves was observed in the treatment malathion compared to the untreated control (3.08 larvae in ten leaves).

Overall observations on population of *D. indica* revealed that the insecticide chlorantraniliprole 18.5 SC 0.006% was effective in controlling the insect population upto fourteen days after treatment. The population of the *D. indica* was in a decreasing trend in consortium I treated plots at a long run period.

4.9.1.3. A. foveicollis

Table 40. Effect of treatments on the population of *Diaphania indica* in field

Treatments	Mean population *				
	1 DAS	3 DAS	5 DAS	7 DAS	14 DAS
T1- Consortium I @ 35 g L^{-1}	1.75 $(1.50)^{b}$	1.92 $(1.55)^{b}$	0.75 $(1.11)^{c}$	0.08 $(0.75)^{d}$	0.83 $(1.15)^{c}$
T2- Chlorantraniliprole 18.5 SC 0.006%	0.42 $(0.96)^{d}$	0.17 $(0.80)^{d}$	0.08 $(0.75)^{d}$	0.42 $(0.96)^{c}$	0.42 $(0.96)^{d}$
T3- T1 followed by T2	0.08 $(0.75)^{e}$	0.17 $(0.80)^{d}$	0.08 $(0.75)^{d}$	0.17 $(0.80)^{cd}$	0.83 $(1.15)^{c}$
T4- T2 followed by T1	1.33 $(1.36)^{c}$	1.58 $(1.44)^{b}$	0.50 $(0.99)^{c}$	0.17 $(0.80)^{cd}$	0.50 $(0.98)^{d}$
T5- Malathion 0.1 %	1.08 $(1.26)^{c}$	1.08 $(1.26)^{c}$	1.17 $(1.29)^{b}$	1.17 $(1.29)^{b}$	1.50 $(1.42)^{b}$
T6- Untreated check	2.67 $(1.78)^{a}$	2.67 $(1.77)^{a}$	2.58 $(1.76)^{a}$	2.00 $(1.58)^{a}$	3.08 $(1.89)^{a}$
CD (0.05)	(0.135)	(0.165)	(0.141)	(0.189)	(0.141)

* Population in 10 leaves per plant, figures in parenthesis are square root transformed values

Figures in a column when followed by the same letter do not differ significantly

The data on the population of *A. foveicollis* is presented in Table 41. One day after treatment, the lowest mean population of 0.92 and 1.17 adults in ten leaves was observed in plots treated with chlorantraniliprole 18.5 SC 0.006% and in consortium I followed by chlorantraniliprole 18.5 SC 0.006% and were on par. The next superior treatment was the chemical check malathion with populations of 1.75 adults in ten leaves. This was followed by chlorantraniliprole 18.5 SC 0.006% followed by consortium I and consortium I with mean populations of 2.75 and 2.25 adults in ten leaves respectively and were on par.

At three days after treatment, the lowest pumpkin beetle population (0.5 adults in ten leaves) was observed in chlorantraniliprole 18.5 SC 0.006%. The treatments malathion and consortium I followed by chlorantraniliprole 18.5 SC 0.006% were on par with a mean population of 0.83 and 1.08 adults in ten leaves and was significantly lower compared to the treatments consortium I (1.67 adults in ten leaves) and chlorantraniliprole 18.5 SC 0.006% followed by consortium I (1.75 adults in ten leaves) .

At five days after treatment, all the treatments significantly reduced the per plant population of pumpkin beetle adults compared to the untreated control which recorded a mean population of 3.67 adults in ten leaves. The lowest pumpkin beetle population (0.83 adults in ten leaves) was observed in chlorantraniliprole 18.5 SC 0.006%. The treatments malathion, chlorantraniliprole 18.5 SC 0.006% followed by consortium I , consortium I followed by chlorantraniliprole 18.5 SC 0.006% and consortium I were on par with mean populations of 1.33,1.42, 1.5 and 1.5 adults in ten leaves.

On 7th day, the lowest pest population of 1.75 adults in ten leaves was observed in the treatment chlorantraniliprole 18.5 SC 0.006%. Significantly higher populations (1.83, 2.17, 2.42 and 2.83 adults in ten leaves) was recorded in consortium I followed by chlorantraniliprole 18.5 SC 0.006%, malathion, consortium I and chlorantraniliprole 18.5 SC 0.006% followed by consortium I treated plots and these

Table 41. Effect of treatments on the population of *Aulacophora foveicollis* in field

Treatments	Mean population *				
	1 DAS	3 DAS	5 DAS	7 DAS	14 DAS
T1- Consortium I @ 35 g L^{-1}	2.75 $(1.66)^{b}$	1.67 $(1.28)^{b}$	1.50 $(1.22)^{b}$	2.42 $(1.55)^{bc}$	2.17 $(1.46)^{c}$
T2- Chlorantraniliprole 18.5 SC 0.006%	0.92 $(0.96)^{d}$	0.50 $(0.69)^{d}$	0.83 $(0.90)^{c}$	1.75 $(1.33)^{e}$	2.17 $(1.47)^{c}$
T3- T1 followed by T2	1.17 $(1.08)^{d}$	0.83 $(0.90)^{cd}$	1.50 $(1.23)^{b}$	1.83 $(1.35)^{de}$	2.33 $(1.51)^{c}$
T4- T2 followed by T1	2.25 $(1.49)^{bc}$	1.75 $(1.31)^{b}$	1.42 $(1.18)^{b}$	2.83 $(1.67)^{b}$	2.83 $(1.67)^{b}$
T5- Malathion 0.1 %	1.75 $(1.31)^{c}$	1.08 $(1.03)^{c}$	1.33 $(1.15)^{b}$	2.17 $(1.46)^{cd}$	2.33 $(1.52)^{bc}$
T6- Untreated check	5.25 $(42.29)^{a}$	4.33 $(2.07)^{a}$	3.67 $(1.91)^{a}$	3.50 $(1.86)^{a}$	3.75 $(1.93)^{a}$
CD (0.05)	(0.184)	(0.211)	(0.216)	(0.146)	(0.155)

* Population in 10 leaves per plant; figures in parenthesis are square root transformed values

Figures in a column when followed by the same letter do not differ significantly

were on par. All the treatments reduced the population of the pest compared to the untreated control (3.5 adults in ten leaves).

On 14th day, the lowest pumpkin beetle population (2.17, 2.17, 2.33 and 2.33 adults in ten leaves) was observed in consortium I, chlorantraniliprole 18.5 SC 0.006% consortium I followed by chlorantraniliprole 18.5 SC 0.006% and malathion treated plots. This was followed by chlorantraniliprole 18.5 SC 0.006% followed by consortium I with mean population of 2.83 adults in ten leaves. Significantly higher population of 3.75 adults in ten leaves was observed in the untreated control.

4.9.1.4. B. cucurbitae

The data on the percentage of fruit fly infested fruits in different treatments is presented in Table 42. One week after application of the treatments, the lowest infestation was recorded in plots treated with consortium I followed by chlorantraniliprole 18.5 SC 0.006% with a mean percentage infestation of 8.05. Significantly higher infestation (9.13, 12.86 and 16.36 per cent) was recorded in the treatments consortium I, chlorantraniliprole 18.5 SC 0.006% and chlorantraniliprole 18.5 SC 0.006% followed by consortium I and were on par. The chemical check malathion recorded a per cent infestation of 22.72 which was significantly higher compared to other treatments and was significantly lower compared to the untreated check (68.97 per cent infestation).

At two week post treatment, infestation was the lowest in plots treated with chlorantraniliprole 18.5 SC 0.006% (12.42 per cent) Significantly higher infestation(13.68, 20.65 and 21.28 per cent) was recorded in the treatments consortium I followed by chlorantraniliprole 18.5 SC 0.006% , consortium I and chlorantraniliprole 18.5 SC 0.006% followed by consortium I. The chemical check and the untreated control recorded a per cent infestation of 31.55 and 61 per cent respectively.

4.9.2 Yield of bitter gourd

Table 42. Effect of treatments on *Bactrocera cucurbitae* infestation in field

Treatments	Percentage of fruit fly infestation		
	Precount	One week after application	Two weeks after application
T1- Consortium I @ 35g L^{-1}	50.95 (45.55)	8.03 $(16.32)^{e}$	20.65 $(26.92)^{cd}$
T2- Chlorantraniliprole 18.5 SC 0.006%	50.83 (45.48)	12.86 $(20.97)^{cd}$	12.42 $(20.49)^{e}$
T3- T1 followed by T2	52.18 (46.29)	9.13 $(17.57)^{de}$	13.68 $(21.54)^{de}$
T4- T2 followed by T1	61.58 (51.86)	16.36 $(23.84)^{c}$	21.28 $(27.48)^{c}$
T5- Malathion 0.1 %	60.10 (50.88)	22.72 $(28.21)^{b}$	31.55 $(34.15)^{b}$
T6- Untreated check	65.54 (54.23)	68.97 $(56.17)^{a}$	61.00 $(51.50)^{a}$
CD (0.05)	NS	(3.811)	(5.733)

*Figures in parenthesis are angular transformed values, figures in a column when followed by the same letter do not differ significantly

The data on mean yield of bitter gourd harvested at different intervals after treatment are given in the Table 43. Significant differences in yield was observed between the treatments and the maximum yield of 14.53 t ha^{-1} was obtained from the treatment consortium I followed by chlorantraniliprole 18.5 SC 0.006% and was statistically superior compared to other treatments. Chlorantraniliprole 18.5 SC 0.006% and consortium I recorded an yield of 13.76 and 13.24 t ha^{-1} and was statistically on par with yield obtained from the treatments Malathion 0.1 % (11.90 t ha^{-1}) and chlorantraniliprole 18.5 SC 0.006% followed by consortium I (11.97 t ha^{-1}). The lowest yield was obtained from the untreated control and was 4.61 t ha^{-1}.

The data on benefit cost ratio (Table 43) revealed that consortium I followed by chlorantraniliprole 18.5 SC 0.006% gave the maximum returns with a benefit cost ratio 2.41 folowed by Chlorantraniliprole 18.5 SC 0.006% (2.27) and Consortium I @ 35g L^{-1} (2.21).

Table 43. Effect of treatments on the yield of bitter gourd

Treatments	Yield (t ha^{-1})	B:C ratio
T1- Consortium I @ 35g L^{-1}	13.24 $(3.63)^{ab}$	2.21
T2- Chlorantraniliprole 18.5 SC 0.006%	13.76 $(3.70)^{ab}$	2.27
T3- T1 followed by T2	14.53 $(3.82)^{a}$	2.41
T4- T2 followed by T1	11.97 $(3.46)^{b}$	2.00
T5- Malathion	11.90 $(3.45)^{b}$	1.99
T6- Untreated check	4.61 $(2.14)^{c}$	0.78
CD (0.05)	(0.344)	

* Figures in parenthesis are square root transformed values

Discussion

5. DISCUSSION

Insect pests and the damage they cause is an important factor that hinders crop production. Chemical management, though an important option for pest management, the overdependence and uninhibited use necessitated alternatives mainly for environmental concerns. Entomopathogenic microbial organisms have already established their role as potential biocontrol agents and can be utilized as an ecologically sound and effective solution to target pests. Among the microbial insect pathogens, entomopathogenic fungi play a key role in pest management and are widely demanded because of their efficacy, safety to non-target organisms and easiness in mass production. Microbial pathogens being host specific demands the application of different pathogens for management of different pests in the same eco-system. This is costly and time consuming.

Bitter gourd (*Momordica charantia L.*) is an important annual vegetable grown in Kerala for domestic consumption and for export. Cultivation of the crop is often hampered by the infestation of a number of pests of which the major pests are melon fly, *Bactrocera cucurbitae*. epilachna beetle, *Henosepilachna septima*, pumpkin caterpillar, *Diaphania indica* and pumpkin beetle, *Aulacophora* spp.

The important pests of bitter gourd was monitored from twenty bitter gourd fields in Thiruvananthapuram and Kollam districts as a part of identifying natural epizootics in these organisms and isolating disease causing organisms. One entomopathogenic fungi was isolated from the infected cadavers of *H. septima* and *D. indica* and one entomopathogenic phyllopane bacterium was also isolated. The fungus isolated from both epilachna beetle and pumpkin caterpillar was identified as *Fusarium verticilloides*. To date, no reports are available on the pathogenicity of *F. verticilloides* against *H. septima* grub and *D. indica* larvae. However, the production of beauveriacin, a secondary metabolite having insecticidal action by the fungus was reported by Ghiasian *et al.* (2005). Pelizza *et al.* (2011) observed

F. verticilloides causing natural epizootics in *Tropidacris collaris* (Stoll), a harmful grasshopper species. In laboratory studies, the isolate also caused 58 ± 6.53 per cent mortality against another grasshopper *Ronderosia bergi* (Stål), 10 days post treatment. In the present study, the field isolate of *F. verticilloides* at a spore concentration of 1.6 x 10^{8} spores mL^{-1}could impart only 30 per cent mortality for *D. indica* larvae and *H. septima* grub. Also, cross inoculation to other test insects viz. *B. cucurbitae*, *A. foveicollis* and *L. australis* for pathogenicity produced negative results.

A phylloplane bacterium was also isolated from bitter gourd leaf surface by stamping the surface of leaves on minimal media with chitin as the sole carbon source. The bacteria upon screening for pathogenicity against the test insects, developed disease in epilachna beetle grub and *D. indica* larvae and caused mortality. Based on 16 S rRNA homology, the organism was identified as *Serratia marcescens*

The insects infested with *S. marcescens* developed mortality, one day post treatment. Change in colour and consistency of the body along with a putrefied smell was observed for both the insects and the dead insects turned slighty orange red to brown in colour. Exudation of orange red coloured body fluid from the dead insects was observed in both the insects. The symptoms observed were typical of a bacterial infection as described by Tanada and Kaya, (1993). They observed rapid break down of internal tissues and organs to viscid consistency in insects infested with bacteria. The cadaver become very soft and darkens in colour accompanied by a putrid odour. They also noted development of amber colouration in case of infection by *Serratia sp.*

In the present study, *H. septima* grubs and *D. indica* larvae fed with S. *marcescens* treated leaves produced 92.5 and 100 per cent mortality respectively at 5 DAT. The results were in line with the findings of Viswanathan (2015) who reported 93.28 per cent mortality of epilachna beetle grub, *H. vigintioctopunctata*, fed with

S. marcescens treated leaves 5 DAT. The bacterium is known to cause mortality in apple maggot fly, *Rhagoletis pomonella* (Lauzon *et al.*, 2003), *Spodoptera litura* (Aggarwal 2015), and red palm weevil, *Rhyncophorus ferrugineus* Oliver. (Hou, 2016) .

Prodigiosin, a red colouring pigment produced by *S. marcescens* is known to have larvicidal activity (Patel *et al.*, 2011). The larvicidal activity of the pigment along with septicemia caused by the bacterium might be the reason for the rapid death of the infected insects. Reports of Ishii *et al.* (2014), suggests that *S. marcescens* are competent in producing adhesion-inhibitory factors against the host immune-surveillance cells thereby curbing the host cellular immunity leading to fast death.

5.1 PATHOGENICITY AND BIOASSAY

Pathogenicity is the qualitative ability of an infectious agent to cause disease or damage in a host. The prime requirement, for development of disease is the interaction between the host and the pathogen. However, the physiology of the host and pathogen is the key factor which determines the extent of disease development. Entomopathogenic fungi, unlike other entomopathogenic microbes possess a wide host range and exhibit wide variations in specificity between and within various genera and species. For any study using entomopathogenic fungi, knowledge of their host range, specificity and virulence is there for essential for promoting them as effective biocontrol agents.

In the present study, pathogenicity of the fungi *B. bassiana* (Bb5), *L. lecanii* (Vl 18), and *M. anisopliae* (Ma4) (obtained from National Bureau of Agricultural Insect Resources (NBAIR) Bengaluru and maintained at the Biocontrol Laboratory for Crop Pest Management, (College of Agriculture, Vellayani), KAU isolates *B. bassiana* (ITCC 6063) and *P. lilacinus* (ITCC 6064) were conducted against *B. cucurbitae, H. septima, D. indica*, *A. foveicollis* and *L. australis.* The results revealed that *L. lecanii* is not pathogenic to any of the test insects whereas

B. bassiana and *M. anisopliae* isolates were pathogenic to all the bitter gourd pests tested. The fungus *P. lilacinus* developed disease in adults and pupae of fruit fly and in larvae of *D. indica*.

Insects treated with various entomopathogenic fungi exhibited more or less similar symptoms for all the fungi tested. Normal feeding behavior and activity of the treated insects were observed initially whereas they followed reduced feeding pattern and sluggishness in the later stages of infection. Adults of epilachna and pumpkin beetles treated with various fungi followed a scattered pattern of egg laying as well. The dead insects got mummified and developed mycelial growth over them. The symptoms observed were typical of fungal infection in insects (McCoy *et al.*, 1988; Nilamudeen, 2015, Praveena, 2016)

White coloured puffy mycelial growth was observed in the treated insects two to three days post death in case of *B. bassiana*. In *M. anisopliae* infected insects, white coloured mycelia was seen covering the insect body initially which later turned yellowish and then green.

Virulence of different fungi was assessed against the test insects at a concentration of 1 x 10^8 spores mL^{-1}. *P.lilacinus* and *M. anisopliae* which caused a cumulative mortality of 93.54 per cent each at 7 DAT were the most virulant isolates against adults of fruit fly, *B. cucurbitae*. Also these treatments recorded the lowest adult emergence of 0.00 and 1.25 per cent respectively from *B.cucurbitae* pupae. Mortality obtained in *B. bassiana* (Bb5) and *B. bassiana* (ITCC 6063) were on par with 83.54 and 80 per cent adult mortality and 10 and 13.75 per cent adult emergence respectively at 7 DAT.

The results were in line with the findings of Thaochan and Sausa-Ard (2017) who reported 100 per cent mortality of fruit fly adults, *Zeugoderus cucurbitae* treated with *M. anisopliae* isolates, 7 DAT. Cent percent mortality of the adults of

Mediterranean fruit fly, *C. capitata* treated with *M. anisopliae* was observed by Castillo *et al.*, (2000). Amala (2010) found that the isolate of *P. lilacinus* (ITCC No. 6064) was more pathogenic to adults of *B. cucurbitae* compared to the indigenous *B. bassiana* isolate (ITCC 6063). The mortality percentages observed was 100 and 98.2 for adults and pupae treated with *P. lilacinus* whereas *B. bassiana* isolate (ITCC 6063) caused only 76.67 and 98.2 per cent mortality of adults and pupae respectively, 6 days post treatment.

The virulence of the fungal isolates tested against the pumpkin caterpillar, *D. indica* revealed that *M. anisopliae* is highly pathogenic to the insect causing 82.5 per cent mortality, 7 DAT. *B. bassiana* was the next best treatment and resulted in 55 per cent mortality. The infectivity of fungal pathogens to *D. indica* has been documented by Praveena (2016). In her studies it was seen that the performance of *M. anisopliae* was better than that of *B. bassiana* in controlling pumpkin caterpillar populations. At a concentration of 1 x 10^8 spores mL^{-1}, the mortality percentages of larvae of *D. indica* were 86.66 and 46.66 respectively for *M. anisopliae* and *B. bassiana* 7 DAT.

For *H. septima* grubs and adults, maximum mortality was obtained in insects treated with *M. anisopliae* followed by *B. bassiana* at 9 DAT. The adults were however less susceptible to both the fungi and the maximum mortality obtained was 20 per cent for *M. anisopliae* and 10 per cent for *B. bassiana*, 10 DAT. Similar to the present study, Viswanathan (2015) reported *M. anisopliae* to be more pathogenic to grubs and adults of *H. vigintioctopunctata*. She observed mortality percentage of 66.58 and 63.25 respectively for *M. anisopliae* and *B. bassiana* for epilachna grubs at 7 DAT. She also observed less susceptibility of adults to both the fungi. On the contrary, Joseph (2014) reported *B. bassiana* to be more pathogenic to *H. vigintioctopunctata* adults and grubs compared to *M. anisopliae.* The former caused a mortality of 64.48 and 35.52 for grubs and adults respectively while the latter caused only 42.21 per cent and 17.66 per cent mortality for grubs and adult. The less

susceptibility of epilachna adults to *B. bassiana* was also observed by Padmaja and Kaur (1998) and Rajendran and Gopalan (1999).

The effect of NBAIR isolates of *M. anisopliae* against *A. foveicollis* was on par with that of *B. bassiana* at 10 DAT. However, the significantly superior performance of *B. bassiana* was indicated from the cumulative mortality of 45 per cent observed 10 DAT compared to 37.50 per cent in *M. anisopliae*. The local isolate of *Beauveria, B. bassiana* (ITCC6063) was less pathogenic to *A. foveicollis* and caused only 22.50 per cent mortality. Earlier, Joseph (2014) reported the high pathogenicity of *B. bassiana* to *A. foveicollis* adults. High susceptibility of red pumpkin beetle to *B. bassiana* was also observed by Moorthi and Balasubramanian (2016).

M. anisopliae which caused a cumulative mortality of 78.12 per cent each at 8 DAT was the most virulant isolate against nymphs of *L. australis* and was superior to all the other fungi tested. *B. bassiana* reported a mortality percentage of 60.77, 8 DAT. Several morphological and physiological factors of the host and pathogens such as nutritional parameters, toxin production, production of defense chemicals, characters of insect integument etc. influence the pathogenicity of fungal pathogens (Roberts, 1981; Charnley, 2003; Goettel *et al.*, 2010; Butt *et al.*, 2001). Such factors might have contributed to the variable infectivity of the different isolates noted against the test insects in the present study.

According to Inglis *et al.*, (2001), a highly virulent pathogen will require only fewer propagules to incite disease and therefore, selection of virulent genotypes has obvious consequences for efficacious control of insects. A preliminary knowledge on the median lethal concentrations of the pathogens (LC_{50}) and also median lethal time (LT_{50}) required by the pathogen to kill the test insects are essential for effective utilization of different bio control agents. The minimum amount of fungal inoculum

required for disease development and the time required for bioagents to have an impact on the target organisms can be determined by dose-mortality studies (Butt *et al.*, 2001). Hence, bioassays were conducted to determine the dose mortality relationships of the fungal isolates at varying concentrations against the test insects. The mortality obtained in various test insects was dose dependent with the mortality increasing with increase in doses irrespective of the fungal pathogen tested.

Of the various fungi tested for dose mortality against *B. cucurbitae*, *P. lilacinus* recorded the lowest LC_{50} value of 1.05 x 10^9 spores mL^{-1} and the lowest LT_{50} value of 3.85 days at a concentration of 2.4 x 10^9 spores mL^{-1}.For *M. anisopliae* and *B. bassiana*, the LC_{50} varied from 1.55 x 10^9 to 1.65 x 10^9 spores mL^{-1} against adults of *B. cucurbitae* at 7 DAT. Amala (2010) observed an LC_{50} value of 1.3 x 10^8 and 1.8 x 10^8 spores mL^{-1} for *P. lilacinus* and *B. bassiana* against adults of *B. cucurbitae* respectively at 2 and 3 days of treatment. Jiji *et al.*, (2006) reported an LC_{50} value 5.0 x 10^6 spores mL^{-1} for *P. lilacinus*, 2 days post treatment.

The concentrations required for the pathogens, *M. anisopliae* and *B. bassiana* varied from 0.18 x 10^9 to 1.20 x 10^9 spores mL^{-1} to bring fifty per cent mortality in the second instar larvae of *D. indica* within a duration of seven days.

The LC_{50} of *M. anisopliae* required to bring fifty per cent mortality in the grubs of *H. septima* within the shortest period of five days was 3.93x 10^9 spores mL^{-1}, while 2.12 x 10^9 spores mL^{-1} was required against the adults to cause fifty per cent mortality at 12 DAT. *B. bassiana* could not develop fifty per cent mortality in the adults during the observed time of 14 DAT. For grubs, the LC_{50} value was 1.82 x 10^9 spores mL^{-1} at 7 DAT. the LT_{50} value obtained was 6.11 days for the highest dose of 2.7 x 10^9 spores mL^{-1}. Padmaja and Kaur, (1998) reported LT_{50} value of different isolates of *B. bassiana* on grubs and adults of *H. vigintioctopunctata* as 1.33 to 4.86 days for the grubs and 2.75 to 5.67 days for the prepupal stage. Joseph, (2014) reported LC_{50} values of *B. bassiana* and *M. anisopliae* as 0.48 x 10^7 and 3.33 x 10^7 spores mL^{-1} for

the grubs and 1.86 x 10^7 and 4.39 x 10^8 spores mL^{-1} respectively for adults at 10 DAT.

The LC_{50} varied from 1.55 x 10^9 to 2.34 x 10^9 spores mL^{-1} for *M. anisopliae* and *B. bassiana* against adults of *A. foveicollis* at 10 DAT. An analysis of the LT_{50} values showed that, to bring about fifty per cent mortality @ 10^9 spores mL^{-1}, *M. anisopliae* required only 5.56 days, compared to 8.83 days for *B. bassiana*. Joseph, (2014) reported LC_{50} of *M. anisopliae* and *B. bassiana* to adults of *A. foveicollis* as 11.42 x 10^8 and 5.27 x 10^8 spores mL^{-1} 7 days post treatment. On the contrary, Moorthi and Balasubramanian (2016) reported a lower LC_{50} value of 9 x 10^3 spores mL^{-1}, 12 days post treatment for *B. bassiana.*

5.2. COMPATIBILITY OF ENTOMOPATHOGENIC FUNGI

In an ecosystem, biocontrol agents of numerous insect pests and fungal pathogens do exist together but not much known about their interaction if used together. It is a general principle that complex associations in nature are more stable and there is a better chance of attaining successful biological control with a mixture of several antagonists than with a single one (Baker and Cook, 1982). Considering this, in vitro compatibility between different entomopathogenic fungi was tested using dual culture plate technique (Dennis and Webster, 1971) in the present study. Compatibility were assessed between *M. aniopliae* and *B. bassiana*,. *M. aniopliae* and *P.lilacinus, M. aniopliae* and *L. lecanii, B. bassiana* and *P.lilacinus, B. bassiana* and *L. lecanii* and *P.lilacinus* and *L. lecanii.* In dual culture studies, growth in the dual culture plates locked at the point of contact for *M. aniopliae* and *B. bassiana* leaving a margin of 3 to 5 mm at 14 DAI. Whereas in other combinations, growth in the dual culture plates stopped at 14 DAI leaving a margin of 1.0 to 1.5 cm between the colonies. The results of the present study states the relationship between various fungi tested as mutual inhibition from distance.

Similar to the present study, Claro and Silva (2010) reported that growth in dual culture plates *B. bassiana, M. anisopliae* and *L. lecanii* stopped by the third week after inoculation, leaving margins of 4 to 6 mm away from each other. They observed that germinations of conidia of different fungi were not affected by mixing of conidia and hence no adverse effect in pathogenicity of the fungi will occur due to mixing.

According to Krauss *et al.,* (2004), the interactions between various microbes, in-vitro are not necessarily an indicator of their in-vivo interactions and this could be an important issue in biocontrol of target pest. It is therefore important to test the in-vivo effect of different combinations of entomopathogenic fungi to know their combined effect in host mortality.

5.3. LABORATORY EVALUATION OF CONSORTIUM

Assessment of virulence of various entomopathogens in the present study showed the fungi *M. anisopliae* and *B. bassiana* to be highly virulent causing more than 50 per cent mortality of all the insects tested except *H. septima* adults. The *P. lilacinus* isolate, ITCC No. 6064 also recorded high mortality of *B. cucurbitae* adults and pupae. The fungi, *M. anisopliae*, *B. bassiana* and *P. lilacinus* along with their combinations *i.e.* consortium 1 (*M. anisopliae* with *B. bassiana*), consortium 2 (*M. anisopliae* with *P. lilacinus*) and consortium 3 (*B. bassiana* with *P. lilacinus*)were tested for efficacy against the test insects at a spore concentration of 10^8 spores mL^{-1} . Consortium 1(*M. anisopliae* with *B. bassiana*) recorded the highest mortality of all the test insects among all the treatments except for *B. cucurbitae* adults and pupa where Consortium 1(*M. anisopliae* with *B. bassiana*) was on par with consortium 2 (*M. anisopliae* with *P. lilacinus*) (Fig.2). All the fungal combinations tested expressed higher mortality of the test insects than when treated individually.

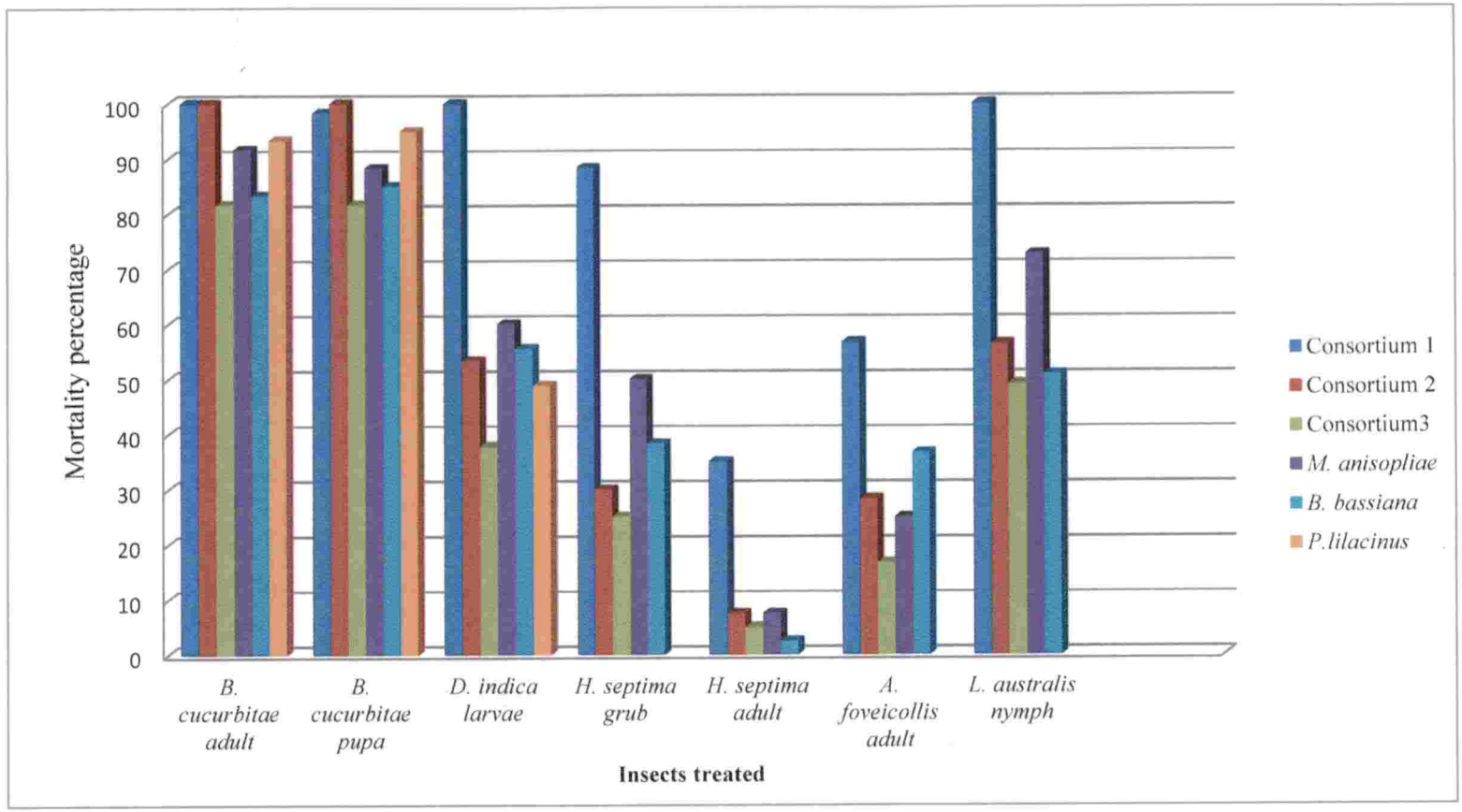


Fig 2. Effect of various consortium in the mortality of test insects

The mortality percentages observed for different insect treated with consortium I was 100, 98.33, 100, 100, 55.00, 64.91 and 100 respectively for adults and pupae of *B. cucurbitae*, larvae of *D. indica*, *H. septima* grubs and adults, *A. foveicollis* adult and *L. australis* nymph. Whereas *M. anisopliae* at the same duration, recorded a mortality of 91.67,88.33, 60, 51.67,22.5, 36.84 and72.73 per cent respectively for adults and pupae of *B. cucurbitae*, larvae of *D. indica*, *H. septima* grubs and adults, *A. foveicollis* adult and *L. australis* nymph. Comparitively lower mortality was observed for *B. bassiana i.e.*83.33, 85, 55.56, 43.33, 17.50, 43.86 and 50.91 per cent for adults and pupae of *B. cucurbitae*, larvae of *D. indica*, *H. septima* grubs and adults, *A. foveicollis* adult and *L. australis* nymph respectively.

Dal Bello *et al.*, (2001) opined high mortality (51.7 per cent) of rice weevil, *Sitophilus oryzae* when treated with a combination of fungal isolates, *B. bassiana* ARSEF 5500 and *M. anisopliae* ARSEF 2974 than the fungi treated alone. Similar results were obtained by Samira *et al.*, (2014) who observed high mortality (96.60 per cent) of Mediterranean fruit fly, *C. capitata* larvae in the soil when treated with a combination of *M. anisopliae* with *B. bassiana* at the concentration 1×10^{9} spores mL^{-1} compared to 73.80 and 65.70 per cent mortality obtained respectively for *M. anisopliae* and *B. bassiana* at the same dose. Lower tuber damage by sweet potato weevil *Cylas formicarius* was observed by Reddy *et al.*, (2014) when treated with a combination of *B. bassiana* and *M. brunneum*.

According to Geetha *et al.*, (2012), the efficacy of *M. anisopliae* was not adversely affected by the combination of *B. bassiana, B. brongniartii , Aspergillus* sp and *Pencillium* sp. but was complemented the most by the application of either *B. bassiana* (100 per cent) or *Aspergillus* sp. (93.33 per cent) prior to *M. anisopliae* against *Galleria mellonella*. However, sporulation of *M. anisopliae* was reduced by all species of fungi tested regardless of sequence of application. On the contrary, Umamaheswara Rao *et al.*, (2006) observed no synergistic effects in either

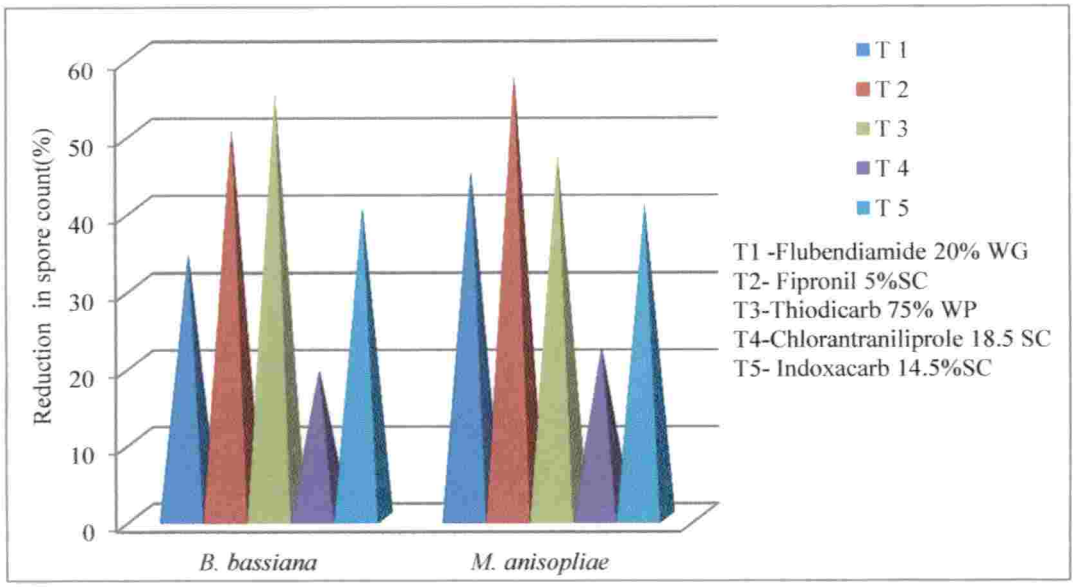
simultaneous or sequential combination treatments with *Nomuraea rileyi* and *B. bassiana* against *Spodoptera litura*.

The increase in mortality observed in fungal combinations may be because of the possible competitions that may arise for the same resources. Co-action of toxins produced by different fungi can be another reason for increased kill. Inglis *et al.* (1997) reported competitive colonization by *M. anisopliae* in combined treatments of *B. bassiana* and *M. anisopliae*. He also observed that the application of *B. bassiana* and *M. flavoviride* in combination may be a way to overcome some of the constraints of temperature on entomopathogenic Hyphomycetes especially where temperatures fluctuate or are high for a significant period of time.

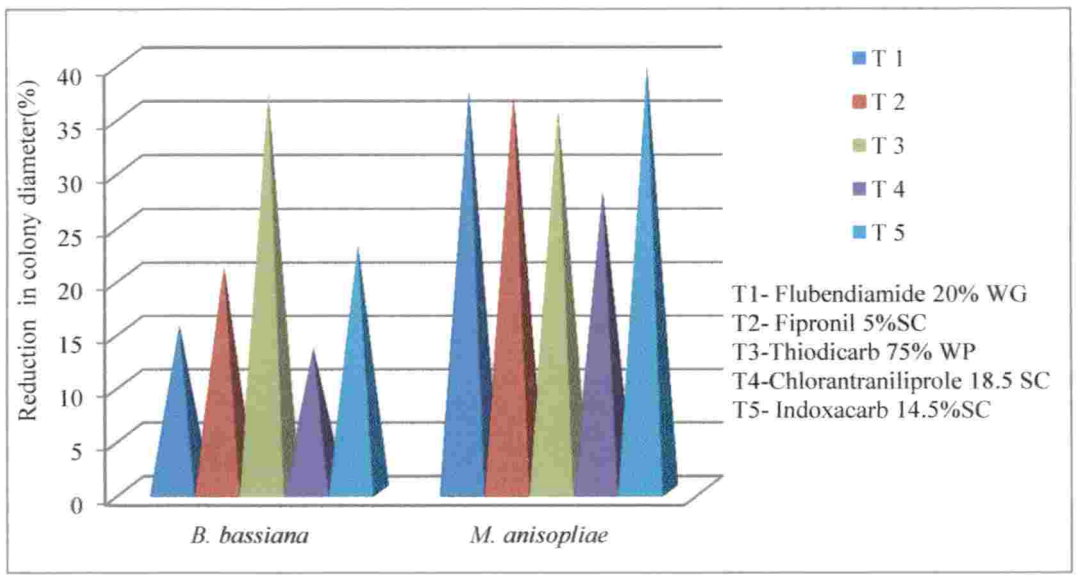
5.4. SHELF LIFE AND BIOEFFICACY OF TALC BASED FORMULATION OF CONSORTIUM

For the acceptance, commercialization and effective field use of any biological product, it is necessary to develop formulation systems that guarantee their viability, development capacity and storage as well as ensure greater or equal efficacy than chemical products. A talc based formulation of the most effective fungal combination, consortium 1(*M. anisopliae* with *B. bassiana*) was developed. Different doses of the formulation were tested against the second instar larvae of *D. indica* for selecting the ideal field dose. The higher doses tested, 35 and 40 g L^{-1} showed parity and recorded cent per cent mortality at seven DAT.Mona *et al.*, (2014) observed increase in mortality of cockroach, *Supella longipalpa* (F.) when exposed to surfaces treated with different doses of the conidia dust formulation of *M. anisopliae* in wheat flour. Cockroach mortality rates increased and survival times (ST_{50}) decreased with an increased proportion of conidia from 1% to 100%.

Shelf life studies of the formulation revealed that the talc based formulation maintained viability and efficacy upto three months of storage. The formulations



(A) Spore count



(B) Colony diameter

Fig.3. Effect of different insecticides on the spore count and colony diameter of entomopathogenic fungi

stored in refrigerated condition maintained high cfu and spore count compared to the formulation stored in room temperature. The cfu count ranged between 9.8×10^7 and 6.83×10^7 cfu g^{-1} for the formulation stored in room temperature while in refrigeration, cfu ranged from 11.13×10^7 to 9.20×10^7 cfu g^{-1} spores mL^{-1} upto 90 days of storage. Also spore counts of the stored at room temperature ranged from 5.77×10^9 to 2.59×10^9 spores mL^{-1} and for refrigeration, the spore count was 6.10×10^9 at 15 days of storage and 5.17×10^9 spores mL^{-1} at 90 days of storage.

Bioefficacy of the formulation varied for different pests. For *B. cucurbitae* adults and pupa, no significant difference in efficacy was observed because of storage. For *H. septima* grubs and adults, the mortality ranged between 100 to 86.67 per cent and 64.44 to 55.56 per cent for grubs and adults respectively. 100 per cent mortality was observed at 15 days of storage for *D. indica* larvae, while the mortality observed was 87.5 per cent at 90 days of storage. Significant reduction in the efficacy of formulation against *A. foveicollis* was noticed which ranged from 71.11 per cent at 15 DAS to 48.89 per cent at 90 DAS. Similar to the present study, reduction in the bioefficacy of talc based formulation of *B. bassiana* and *M. anisopliae* against adults of sweet potato weevil, *C. formicaricus* was observed by Joseph (2014). Also reduction in the bioefficacy of talc based formulation of *B. bassiana, M. anisopliae* and *L. lecanii* against *Aphis gossypii* with three months of storage was observed by Lokesh (2014).

5.5. COMPATIBILITY OF THE COMPONENTS OF CONSORTIUM WITH INSECTICIDES

The ability to evolve quickly to cope up with the adversities of environment is a characteristic feature of insects. Hence, dependence on a single tactic for managing their population is not wise. This recalls the necessity for integration of different tools in pest management. Assessment of compatibility between different tools of pest management should be worked out prior to their integration. The role of

synthetic chemicals in pest management cannot be forgotten. Moreover synthetic molecules are absolutely essential in containing pests in serious outbreaks. Considering all these aspects attempts were made to study the compatibility of the fungal isolates *B. bassiana* and *M. anisopliae* which are components of the most effective consortium with newer pesticide molecules *viz*, Flubendiamide 20 WG 0.01 % , Fipronil 5% SC 0.01% , Indoxacarb 14.5% SC 0.015% , Chlorantraniliprole 18.5 SC 0.006 % and Thiodicarb 75% WP 0.1 % .

The insecticide, Chlorantraniliprole 18.5 SC 0.006 % showed high compatibility with both the fungi and resulted in a colony diameter of 6 cm and 5.40 cm respectively for *M. anisopliae* and *B. bassiana* (Fig.3). The corresponding spore counts obtained were 4.89 x 10^8 and 5.61x 10^8 spores mL^{-1} respectively for *M. anisopliae* and *B. bassiana.* The mycelial growth of both the fungi were not much affected by Fipronil 5% SC 0.01%. However, the treatment caused the lowest spore production by both the fungi and hence classified as toxic to the growth of fungi. Similar to the present study toxicity of Fipronil to *B. bassiana* was reported by Xu *et al.*, (2002). Also reduction in the conidial yield of *M. anisopliae* by Fipronil was observed by Antonio *et al.*, (2001)

5.6. FIELD EVALUATION OF THE CONSORTIUM

The field conditions exhibit great contrariness compared to the controlled conditions prevailing in the laboratory. Therefore for the effectual adoption of the technologies developed in laboratories, field testing to assess its performance in natural ecosystems is a must. Padmaja and Kaur (1998) have stated that, the temperature and relative humidity prevailing in the field conditions are critical for fungal infection on insects. Thereupon, field experiment was conducted at instructional farm, College of Agriculture, Vellayani during July 2017 to September 2017 using Preethi variety of bitter gourd.

The treatments in the field experiment comprised of the talc based formulation of consortum I @ 35 gL^{-1}, chlorantraniliprole 18.5 SC @ 0.006% which is the compatible insecticide selected, consortum I @ 35 gL^{-1} followed by chlorantraniliprole 18.5 SC @ 0.006%, chlorantraniliprole 18.5 SC @ 0.006% followed by consortum I @ 35 gL^{-1}, malathion as chemical check and untreated control. The effect was evaluated in terms of the population of target pests, *B. cucurbitae*, *H. septima*, *D. indica,* and *A. foveicollis*, the extent of damage caused by these pests and yield.

In the field trial, from the observations taken at different intervals, the best treatment observed was consortum I @ 35 g L^{-1} followed by chlorantraniliprole 18.5 SC @ 0.006%. For *H. septima*, consortium I @ 35 gL^{-1}, offered a population reduction of 25.63, 81.89 and 64.89 per cent at 3rd, 7th and 14th DAT (Fig. 4). Maximum reduction in population was observed for consortum I @ 35 g L^{-1} followed by chlorantraniliprole 18.5 SC @ 0.006% where the population reduction ranged from 75 per cent at 3 DAT to 95.05 and 80.69 per cent at 7th and 14th DAT. The population reduction observed was 69.87 to 64.89 for chlorantraniliprole 18.5 SC @ 0.006% and 68.58 to 54.36 for chlorantraniliprole 18.5 SC @ 0.006% followed by consortum I @ 35 g L^{-1} at 3rd and 14th DAT.

Consortum I @ 35 g L^{-1} followed by chlorantraniliprole 18.5 SC @ 0.006%. caused 97, 96.77 and 97 per cent reduction in the population of *D. indica* over untreated on the 1st, 5th and 7th day after treatment (Fig. 5).The treatments consortum I @ 35 gL^{-1} and Chlorantraniliprole 18.5 SC @ 0.006% also offered high reduction in the population of the pest compared to the untreated control and also the chemical check. For consortum I @ 35 gL^{-1}, maximum reduction (95.83 per cent) in the population of the pest was observed at 7 DAT. *D. indica* population in the plots treated with two sprays of Chlorantraniliprole 18.5 SC @ 0.006% showed a slight increasing trend while the plots having consortum I @ 35 g L^{-1} followed by

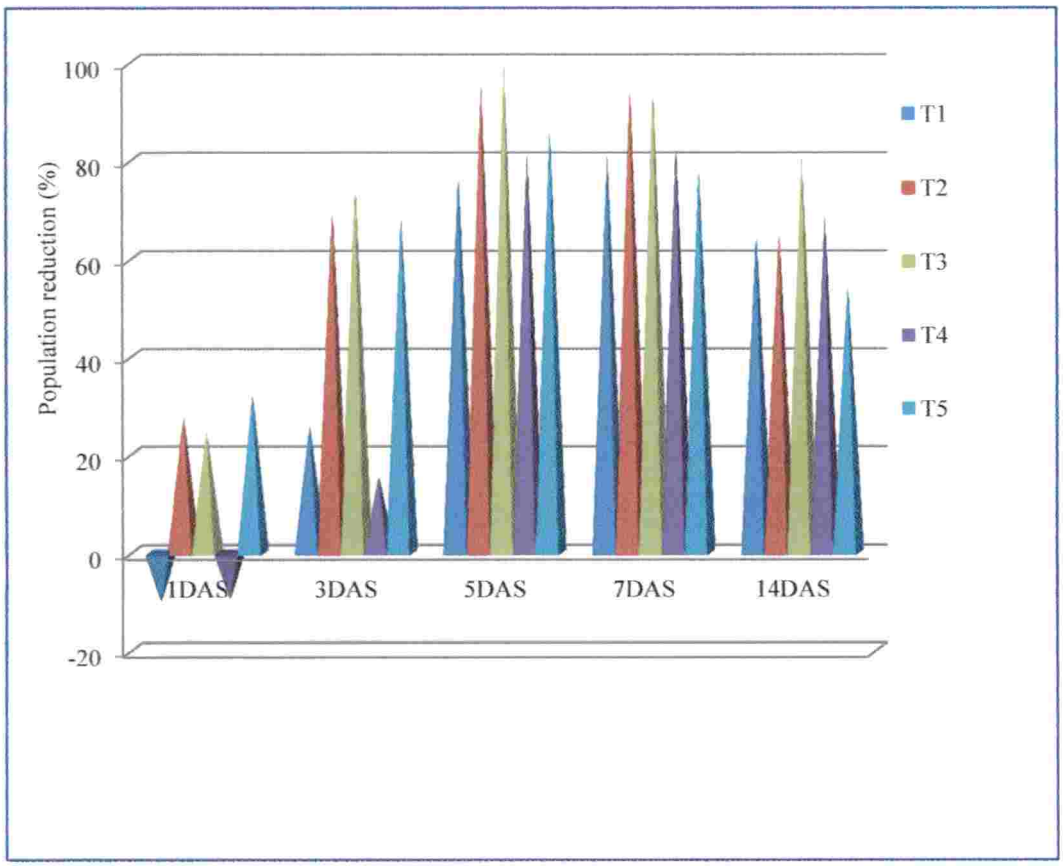


Fig.4. Reduction in the population of epilachna beetle *H. septima* in the field experiments

T1- Talc based formulation of consortium I @ 35 g L^{-1}

T2- Chlorantraniliprole 18.5 SC @ 0.006%

T3- T1 followed by T2

T4- T2 followed by T1

T5- Malathion

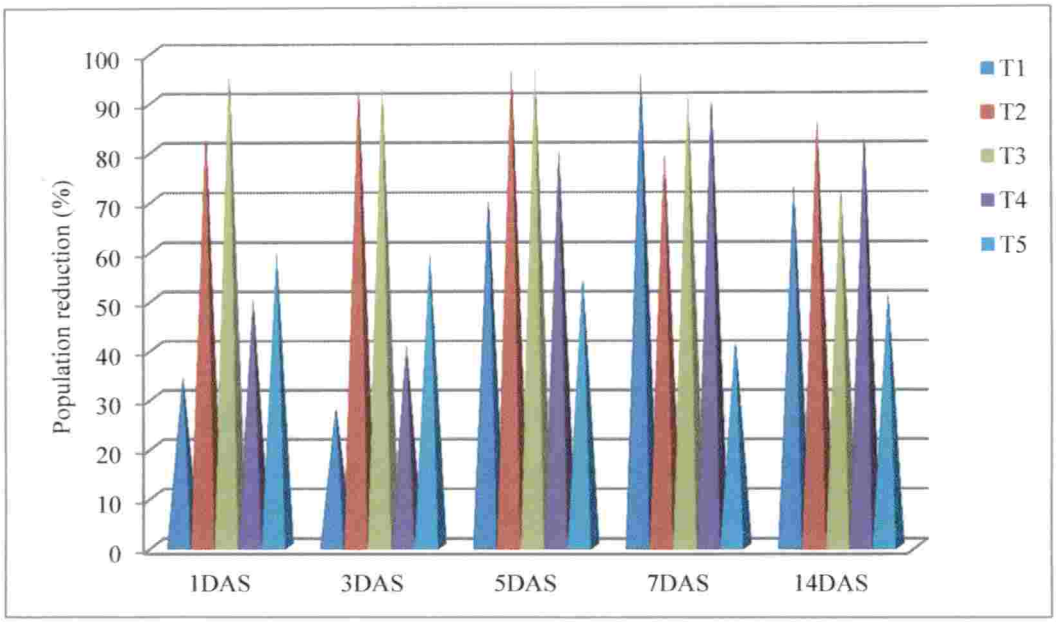


Fig.5. Reduction in the population of pumpkin caterpillar *D.indica* in field experiment

T1- Talc based formulation of consortium I @ 35 g L^{-1}

T2- Chlorantraniliprole 18.5 SC @ 0.006%

T3- T1 followed by T2

T4- T2 followed by T1

T5- Malathion

chlorantraniliprole 18.5 SC @ 0.006%, the reduction in pest population was high upto 7th DAT.

The per cent reduction in the population for *A. foveicollis* was high in the treatment consortum I @ 35 g L^{-1} followed by chlorantraniliprole 18.5 SC @ 0.006% and ranged between 97 to 91.5 per cent from 1st to 7th DAT (Fig 6) . At 14DAT, however, a reduction of 73.05 per cent was observed. For other treatments, the percentage reduction in population were 28.09, 96 and 73.05 for chlorantraniliprole 18.5 SC @ 0.006% , 40.82, 91.50 and 83.77 per cent for consortum I @ 35 g L^{-1} , 93.63, 79.00 and 86.36 per cent for chlorantraniliprole 18.5 SC @ 0.006% followed by consortum I @ 35 g L^{-1} .

The per cent reduction in fruit infestation by *B. cucurbitae* over untreated (60.92) was the lowest for consortum I @ 35 g L^{-1} at 7DAT (Fig.7). All the treatments offered more than 50 per cent reduction in the infestation compared to the untreated control. Also the treatments tested offered higher reduction in the fruit fly infestation compared to the chemical check malathion. At 14DAT, the per cent reduction in infestation was 40.35, 48.58, 47.32, 39.52 and 29.45 respectively for consortum I @ 35 g L^{-1}, chlorantraniliprole 18.5 SC @ 0.006%, consortum I @ 35 g L^{-1} followed by chlorantraniliprole 18.5 SC @ 0.006%, chlorantraniliprole 18.5 SC @ 0.006% followed by consortum I @ 35 g L^{-1} and Malathion.

The efficacy of chlorantraniliprole 20 % SC was reported by Bharathi *et al.*, (2011) against *B. cucurbitae*, *D. indica* and *A. foveicollis* in bitter gourd. The effectiveness of chlorantraniliprole 18.5 SC 0.006 % against epilachna beetle in the present study was in line with the findings of Viswanathan (2015) who observed 100 per cent mortality of epilachna beetle, *H. vigintioctopunctata* in brinjal plants in the treated plants.

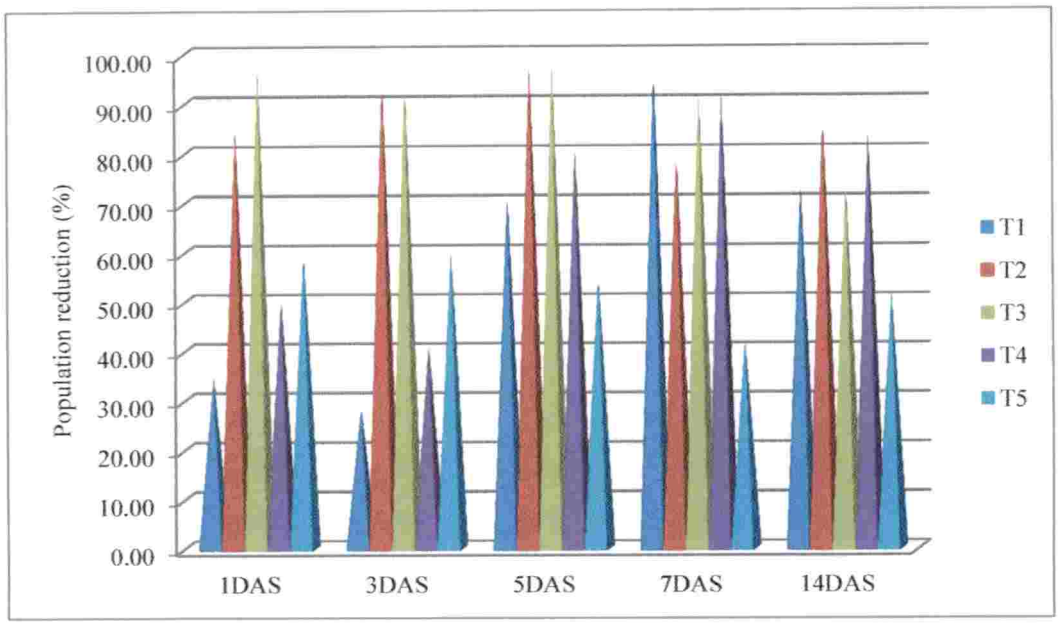


Fig.6. Reduction in the population of pumpkin beetle *A. foveicollis* in field experiment

T1- Talc based formulation of consortium I @ 35 g L^{-1}

T2- Chlorantraniliprole 18.5 SC @ 0.006%

T3- T1 followed by T2

T4- T2 followed by T1

T5- Malathion

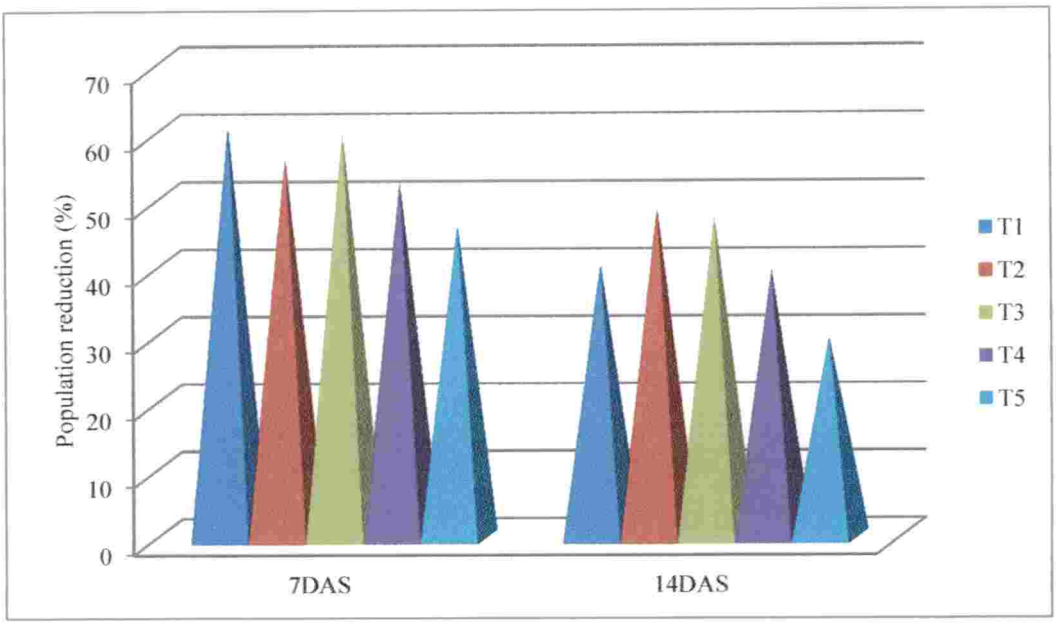


Fig. 7. Reduction in the infestation of fruit fly *B. cucurbitae* in field experiment

T1- Talc based formulation of consortium I @ 35 g L^{-1}

T2- Chlorantraniliprole 18.5 SC @ 0.006%

T3- T1 followed by T2

T4- T2 followed by T1

T5- Malathion

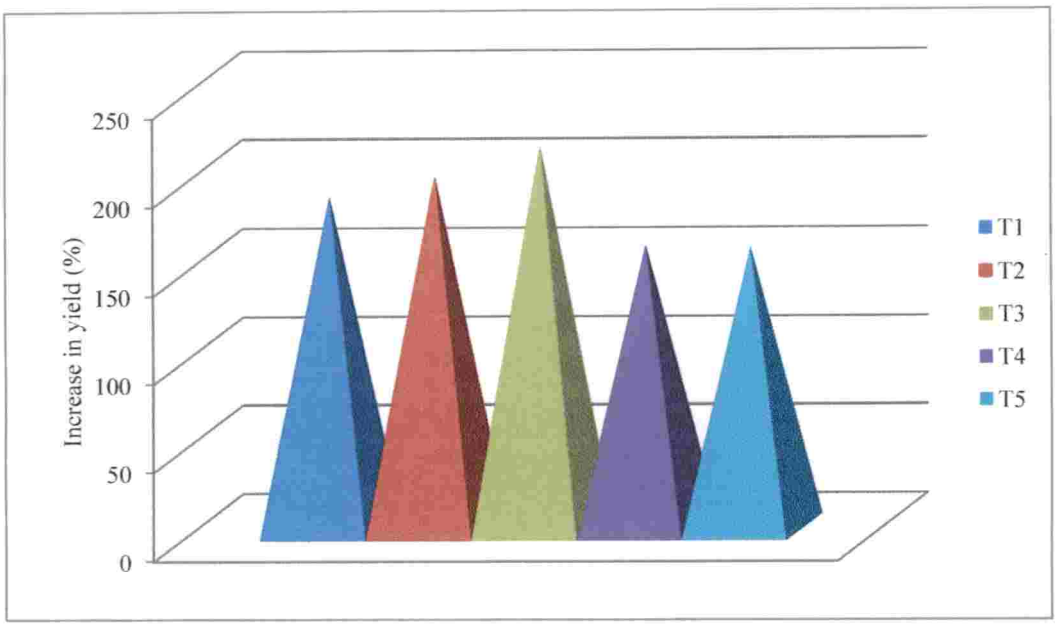


Fig.8. Increase in bitter gourd yield in different treatments compared to the untreated control.

T1- Talc based formulation of consortium I @ 35 g L^{-1}

T2- Chlorantraniliprole 18.5 SC @ 0.006%

T3- T1 followed by T2

T4- T2 followed by T1

T5- Malathion

Summary

6. SUMMARY

Synthetic chemical pesticides have been the mainstay of insect pest control. However, emerging problems like insecticide resistance, pest resurgence and concern over the environmental impact of agricultural inputs focuses a driving need to the search for alternative, biologically based forms of pest control. Entomopathogenic fungi are now recognized as important biocontrol agents of insect pests of crops. Host specificity, though an advantage of entomopathogenic fungus, demands the application of different microbes at the same ecosysytem for managing different pests. In this context, the study entitled "Microbial consortium for the management of insect pests of bitter gourd" was conducted to develop a fungal cocktail that could effectively contain different pests of bitter gourd.

- Two entomopathogens, one fungus from the mycosed cadavers of *H. septima* and *D. indica* and one bacterium from the phylloplane of bitter gourd were collected.

- The fungus isolated from the insect cadavers was identified as *Fusarium verticilloides* and the bacterium was identified as *Serratia marcescens.*

- Pathogenicity studies showed that the NBAIR isolates *M. anisopliae* (Ma4) and *B. bassiana* (Bb5) along with KAU isolate of *Beauveria, B. bassiana* (ITCC No.6063) to be pathogenic to all the test insects *viz.* fruit fly *B. cucurbitae*, *D. indica*, *H. septima*, *A. foveicollis* and *L. australis*. The isolate *P. lilacinus* (ITCC No. 6064) could develop disease only in adults and pupae of *B. cucurbitae.* The field isolates *F. verticilloides* and *S. marcescens* upon pathogenicity trials developed mortality only in *H. septima* grubs and *D. indica* larvae.

- Single dose assay of the fungal isolates *M. anisopliae* (Ma4), *B. bassiana* (Bb5 and ITCC No. 6063) and *P. lilacinus* at 10^8 spores mL^{-1} against adults of

B. cucurbitae , showed that *M. anisopliae* and *P. lilacinus* recorded 93.54 per cent mortality at 7 DAT and was significantly superior to other treatments. For *B. cucurbitae* pupa, *P. lilacinus* recorded maximum mortality of 100 per cent at 7 DAT and was on par with *M. anisopliae* which recorded 98.75 per cent mortality at 7 DAT.

- Fungal isolates *M. anisopliae* (Ma4), *B. bassiana* (Bb5 and ITCC No. 6063) , *P. lilacinus* (ITCC No.6064) and *F. verticilloides* at 10^8 spores mL^{-1} when tested against second instar larvae of *D. indica*, *M. anisopliae* recorded 82.50 per cent mortality at 7 DAT and was significantly superior to all the other treatments.

- Fungal isolates *M. anisopliae* (Ma4) and *B. bassiana* (Bb5 and ITCC No. 6063) at 10^8 spores mL^{-1} against second instar grubs of *H. septima* revealed 52.50 per cent mortality for grubs at 9 DAT by *M. anisopliae*. This was statistically superior to all other treatments. Adults of *H. septima* were less susceptible to different fungi with the highest mortality of 20 per cent at 10 DAT for *M. anisopliae*

- For adults of *A. foveicollis*, single dose assay of the fungal isolates *M. anisopliae* (Ma4) and *B. bassiana* (Bb5 and ITCC No. 6063) at 10^8 spores mL^{-1} showed that *B. bassiana* (Bb5) recorded a maximum mortality of 45 per cent at 10 DAT and was on par with *M. anisopliae* (Ma4) which caused 37.5 per cent mortality

- Nymphs of *L. austarlis* assayed with single dose of the fungal isolates *M. anisopliae* (Ma4) and *B. bassiana* (Bb5 and ITCC No. 6063) at 10^8 spores mL^{-1} showed that *M. anisopliae* (Ma4) recorded a maximum mortality of 78.12 per cent at 10 DAT and was statistically superior to all other treatments.

- The new indigenous fungal isolate *F. verticilloides* collected from the insect cadaver was inferior and caused only 30 per cent mortality for second instar larvae of *H. septima* and *D. indica* respectively at 9 DAT and 7 DAT.

- For *B. cucurbitae* adults, the lethal concentration required to bring fifty per cent mortality was 1.89 x 10^9 , 2.87 x 10^9 and 1.87x10^9 spores mL^{-1} respectively for *M. anisopliae* (MA4), *B. bassiana* (Bb5) *and P. lilacinus* (ITCC No. 6063). The corresponding LT_{50} values were 4.25,4.85 and 3.85 at a spore concentration of 10^9 spores mL^{-1}

- LC_{50} values of 0.18 x10^9 and 1.20 x10^9 spores mL^{-1} was obtained respectively for *M. anisopliae* and *B. bassiana* against second instar larvae of *D. indica* at 7 DAT. The time required to kill fifty per cent population at a spore concentration of 10^9 spores mL^{-1} was 3.76 days for *M. anisopliae* and 5.82 days for *B. bassiana.*

- *H. septima* grubs treated with different spore concentrations of various entomopathogenic fungi recorded a LC_{50} value of 2.2 x10^9 and 1.82x10^9 spores mL^{-1} respectively for *M. anisopliae* (Ma4) and *B. bassiana* (Bb5). Fifty per cent kill of the population was observed at 4.30 days and 6.11 days respectively for *M. anisopliae* and *B. bassiana* when treated with a spore concentration of 82 x 10^9 spores mL^{-1}.

- *B. bassiana* could not produce 50 per cent mortality of the adults of *H. septima* in any of the concentrations tried. For *M. anisopliae*, the LC_{50} value obtained against adults was 2.12 x 10^9 spores mL^{-1} and the LT_{50} value was 11.25 days at a spore concentration of 10^9 spores mL^{-1}

- For *A. foveicollis* adults, the lethal concentration required to bring fifty per cent mortality was 1.55 x 10^9 and 2.34 x 10^9 spores mL^{-1} respectively for *M.*

anisopliae (Ma4) and *B. bassiana* (Bb5). The corresponding LT_{50} values were 5.56 and 8.83 at a spore concentration of 10^9 spores mL^{-1}

- In vitro compatibility studies were conducted between the different entomopathogenic fungi *viz. B. bassiana*, *M. anisopliae*, *P. lilacinus* and *L. lecanii.* In dual cultures, colony growth of *B. bassiana* and *M. anisopliae* got locked at the point of contact after two weeks of inoculation leaving a space of 3 to 5 mm apart from each other.

- In dual culture combinations of *B. bassiana* with *P. lilacinus* and *L. lecanii* and *M. anisopliae* with *P. lilacinus* and *L. lecanii*, the colony growth stopped at two weeks after inoculation and a space of 1.0 cm to 1.5 cm was observed between different fungi.

- In vivo studies of different microbial combinations viz. *M. anisopliae* with *B. bassiana* (consortium 1), *M. anisopliae* with *P. lilacinus* (Consortium 2) and *B. bassiana* with *P. lilacinus* (Consortium 3) revealed that the fungal combinations were more efficient in reducing the pest populations than their fungal components when treated individually.

- Among the different combinations tried, consortium 1 resulted in the highest mortality of all the test insects, with 100 per cent mortality for *D. indica* six DAT, 100 per cent mortality for *H. septima* grubs seven DAT, 55 per cent mortality for adults of *H. septima* at 11 DAT, 64.91 per cent mortality for *A. foveicollis* adults at 10 DAT and 100 per cent mortality for nymphs of *L. australis* at seven DAT. For *B. cucurbitae* adults and pupae, the mortality obtained in consortium 1 was on par with that obtained in consortium 2 the mortality per cent being 100 for both the treatments for adults. For *B. cucurbitae* pupae, 100 per cent mortality was obtained by consortium 2 and was on par with consortium 1 which resulted in 98.33 per cent mortality.

- The talc based formulation of consortium 1 when evaluated for efficacy against the second instar larvae of *D. indica* to fix the suitable dose; it was observed that the higher doses, consortium 1 @ 40 g L^{-1} and 35 g L^{-1} showed parity in mortality percentages of the test insects with cent per cent mortality at seven days after treatment.

- Shelf life studies of the talc based formulation of the consortium resulted in an increased cfu and spore count for the formulation stored in refrigeration than that stored in room temperature. The cfu varied between 9.87 x 10^7 spores g^{-1} to 6.83 x 10^7 spores g^{-1} in room temperature and 11.13 x 10^7 spores g^{-1} to 7.23 x 10^7 spores g^{-1} in refrigeration at 15 and 90 DAS. A spore count of 5.77 x 10^9 spores mL^{-1} to 2.59 x 10^9 spores mL^{-1} was observed for the formulation stored in room temperature at 15 and 90 DAS. The corresponding spore count for the formulation stored in refrigerated condition was 6.10 x 10^9 spores mL^{-1} and 5.17 x 10^9 spores mL^{-1}.

- The bioefficacy of the talc based formulation of consortium 1 showed no significant difference with storage against adults and pupae of *B. cucurbitae*. For *D. indica* larvae, the mortality percentages varied from 100 to 87.5 at 15 DAS and 90 DAS. The maximum mortality observed varied between 100 to 86.67 per cent and 64.44 to 55.56 per cent respectively for grubs and adults of *H. septima* at 15 DAS and 90 DAS. Considerable variation in efficacy with storage was noticed against adults of *A. foveicollis*. At 15 DAS, the mortality obtained for *A. foveicollis* adults was 71.11 per cent which then reduced to 48.89 per cent at 90 DAS.

- Different insecticides that are normally used for pest control in bitter gourd at their field dose were evaluated for their compatibility with *B. bassiana* and *M. anisopliae*. The insecticide, Chlorantraniliprole 18.5 SC 0.006% was compatible with both the fungi with the least reduction in conidial load and

colony diameter. Fipronil 5% SC 0.01 % offered less reduction in the colony diameter of both the fungi. But the spore production was greatly affected and hence found incompatible.

- The results of the field experiments showed that all the treatments effectively reduced the population of different test insects in the field compared to the untreated control. The treatment consortium I followed by Chlorantraniliprole 18.5 SC 0.006% was the best among all the treatments as it effectively reduced the population of different target pests *viz. H. septima*, *D. indica* and *A. foveicollis* in the field. Also the treatment offered 59.84 per cent reduction fruit damage by *B. cucurbitae* compared to the untreated control and recorded the highest yield of 14.53 t ha^{-1} with a B: C ratio of 2.41.

References

7. REFERENCES

Abbot, W. S. 1925. A method for computing the effectiveness of insecticides. *J. Econ. Entomol.* 18: 265-267

Aemprara, S. 2007. Entomopathogenic fungi screening against fruit fly in Thailand *KMITL Sci. Tech. J.* 7 (S2):122-126

Aggarwal, C., Paul. S., Tripathi, V., Paul, B., and Aslam, M.D.K. 2015. Chitinolytic activity in *Serratia marcescens* (strain SEN) and potency against different larval instars of *Spodoptera litura* with effect of sublethal doses on insect development. *BioControl.* 60(2) (in press).

Akbar, S., Freed, S., Hameed, A., Tahira, H. G., Akmal, M., Malik, M. N., Muhammad, N. and Khan, M. B. 2012. Compatibility of *Metarhizium anisopliae* with different insecticides and fungicides. *Afr. J. Microbiol. Res.* 6(17): 3956-3962

Akutsu, K., Hirat, M., Yamamota, M., Hirayae, K., Okuyama, S., and Hibi, T.1993. Growth inhibition of *Botrytis spp.* by *Serratia marcescens* B2 isolated from tomato phylloplane. *Ann. Rev. Phytopathol. Soc. Japan.* 59:18-25.

Alves S.B., Monino Jr A. and Almeida J.E.M. 1998. Produtos fitos- sanitarios e entomopatogênicos. p. 289–370. In: "Controle Microbiano de Insetos" (S.B. Alves, ed.). São Paulo, Fealq, 1163pp.

Alves, R. T., Bateman, R. P., Gunn, J., Prior, C., and Leather, S. R. 2002. Effects of different formulations on viability and medium-term storage of *Metarhizium anisopliae* conidia. *Neotrop. Entomol.* 31(1): 91-99.

Andrews, J. H.1992 Biological control in the phyllosphere. *Annu. Rev. Phytopathol.* 30: 603–635.

Antonio, B.F., Almeida, J.E.M. and Clovis. L. 2001. Effect of Thiamethoxam on entomopathogenic microorganisms. *Neotropic. Entomol.* 30(3): 437-447.

Amala, U. 2010. Management of melon fly (*Bactrocera cucurbitae* Coquillet) using local isolates *Beauveria bassiana* (Bals.) Vuill, *Paecilomyces lilacinus* (Thom.) Samson and *Aspergillus candidus* Link: Fries. M.Sc.(Ag) thesis, Kerala Agricultural University, Thrissur, 131p

Amutha, M., Banu, G., Surulivelu, T. and Gopalakrishna, N. 2010. Effect of commonly used insecticides on the growth of white Muscardine fungus, *Beauveria bassiana* under laboratory conditions. *J. Biopesticides* 3(1): 143-146.

Amutha, M and Banu, G. J. 2012. Compatability of *Metarhizium anisopliae* and *Pochonia lecanii* with insecticides. *Annl Plant Prot. Sci.* 20 : 354 – 357.

Aneja, K. R. 1996. Experiments in Microbiology, Plant Pathology, Tissue culture and Mushroom cultivation. Vishwa Prakashan, New Delhi, 451p.

Aneja, K. R. 2003. Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International Limited (P) Ltd, New Delhi, 460p.

Assaf, L. H., Haleem, R. A., and Abdullah, S. K. 2011. Association of entomopathogenic and other opportunistic fungi with insects in dormant locations. *Jordan J. Biol. Sci.* 4(2): 87-92.

Ayyar, T. V. R. 1923. Some insect pests of fruit crop trees in India. *Agric. J. India*.18:50-59.

Bacci, L., Picanco, M. C., Gonring, A. H. R., Guedes, R. N. C., and Crespo, A. L. B. 2006. Critical yield components and key loss factors of tropical cucumber crops. *Crop Prot.* 25: 1117–1125.

Bai, N. S., Remadevi, O. K., Sasidharan, T. O., Balachander, M., and Dharmarajan, P. 2012. Cuticle degrading enzyme production by some isolates of the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.). *J. Bio-sci.* 20 : 2532.

Baker, K. and Cook, R.J. 1982. Biological control of plant pathogens. The American Phytopathological Society, St. Paul, MN, USA

Balikai, R.A. and Mallapur, C.P. 2017. Bio-efficacy of Flubendiamide 480 SC (Fame 480 SC) against Fruit Borer in Gherkin, *Int. J. Hort.*7(28): 250-261

Banu, J. G. 2013. Effect of different storage conditions on spore viability of *Lecanicillium lecanii* formulations and infectivity to mealybug, *Paracoccus marginatus*. *Int. J. Plant Prot.* 6(2): 334-337.

Banu, J. G. and Gopalakrishnan, N. 2012. Development of formulations of a native entomopathogenic fungus, *Lecanicillium lecanii* and testing virulence against mealybug, *Paracoccus marginatus* infesting cotton. *Indian J. Plant Prot.* 40(3): 182-186.

Barta, M. 2010. Preliminary evaluation of insect-pathogenic Hypocreales against *Leptoglossus occidentalis* (Heteroptera: Coreidae) in laboratory conditions. *Folia Oecologica*, 37 (2): 1336-5266

Bassi, A. 1836. *Del Mal del Segno Calcinaccio O Moscardino*. Part Sceonda, Practica, Lodi, Orcesi, p. 1-67

Beevi, S.N. and Jacob, A. 1982. Studies on relative susceptibility of stages of *Henosepilachna vigintioctopunctata* (F.) to infection by *Fusarium moniliformae* var. *subglutinans* and its use in the control of the pest. *Entomon* 7(2): 237-238.

Bell, D.K., Wells, H.D. and Markham, B.B. 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathol.* 72: 379-382.

Bharathi, K., Mohanraj, A. and Rajavel, D. S. 2011. Field efficacy of Chlorantraniliprole 20% SC against pests of bitter gourd. *Pestology* 35(7): 32-35

Bhagat, K.C. and Munshi, S.K. 1999. *Fusarium solani* (Mart.) Sacc. - an entomogenous fungus infecting the spotted epilachna beetle, *Henosepilachna vigintioctopunctata* Fabr. *Pest Manag. Econ. Zool.* 5(2): 139-142.

Borgia, M.N. and Miranda, M.T.P. 2016. Bionomics, damage caused, biology and morphometrics of 28-spotted lady beetle *Epilachna vigintioctopunctata* (Fab.) (Coleoptera: coccinellidae) – a serious pest of bitter gourd, *Momordica charantia* in Kerala. *Int. J. Applied Pure Sci. Agri.* 2(6): 2394-5532

Butani, D. K. and Jotwani, M. J. 1984. Insects in Vegetables. Periodical Expert Book Agency, Vivek-Vihar, Delhi, India, pp. 69–79

Butt, T.M., Jackson, C.W., and Magan, N. 2001. Introduction-Fungal Biological Control Agents: Progress, Problems and Potential. In: Butt, T.M., Jackson, C.W., and Magan, N. (eds), Fungi as Biocontrol Agents: Progress, Problems and Potential. CABI Publishing, Wallingford, UK, pp. 1-9.

Castillo, M.A., Moya, P., Hernandez, F. and Primo- Yufera, E. 2000. Susceptibility of *Ceratitis capitata* Weidemann (Diptera: Tephritidae) to entomo- pathogenic fungi and their extracts. *Biol. Control* 19: 274–282.

Charnley, A.K. 1989. Mycoinsecticides : Present Use and Future Prospects. Progress and prospects in insect control. *Br. Crop Prot. Council Monogr.* 43: 165-181.

Charnley, A. K. 2003. Fungal pathogens of insects: cuticle degrading enzymes and toxins. *Adv. Bot. Res.* 40: 241–321.

Charnley, A. K. and Collins, S.A., 2007. Entomopathogenic fungi and their role in pest control. In: Howard, D.H., Miller, J.D. (Eds.), *The Mycota IV: Environmental and Microbial Relationships*. Springer- Verlag, Berlin, Heidelberg, pp. 159–187.

Chattopadhayay, P. and Sen, S.K. 2013. Systemic infestation of *Serratia entomophila* AB2 through plant tissue inferred protection against insect pest and fungal pathogens. *Afr. J. Microbiol. Res.* 7(21): 2651-2655.

Chaudary, D. K. R., Kanaujia and Rathore, R. R. S. 2001. Mycopesticidal dust formulations: Evaluation of diluents for viability of *Beauveria bassiana* (Balsamo) Vuillemin. In: Proceedings of symposium on biocontrol based pest management for quality crop protection in the current millennium. 87: 18-19.

Chen, A., Shi, Z., and Zhang, L. 2008. The effects of some storage conditions on viability of *Lecanicillium lecanii* conidia to whitefly (Homoptera: *Trialeurodes vaporariorum*). *Biocontrol Sci. Technol.* 18: 267-278.

Claro, E. O. and Silva, E. A. I. 2010. *In vitro* microbial evaluation of biopesticide active ingredients from species mixtures of Cuban entomopathogenic fungi. *Fitosanidad* 14 (2): 102-109

Clavijo, A. J., Munroe, E., and Arias, C. Q. 1995. The genus *Diaphania* Hubner (Lepidoptera: Crambidae); key to the economically important species. *Agronomia Trop. Maracay* 45: 347–358.

Das, P., Hazarika, L. K., Bora, D., Puzari, K. C. and Kalita, S. 2013. Influence of storage conditions on viability and infectivity of talc based WP formulation of

Beauveria bassiana against rice hispa, *Dicladispa armigera* (Olivier). *J. of Biol. Control* 21(3): 229–233

Dash, N. 2016.Studies on evaluation of new molecules of insecticides against hadda beetle in brinjal, M. Sc. (Ag.) thesis, Orissa University of Agriculture and Technology, Bhubaneswar, 57p.

Dal Bello, G., Padin, S., LoÂ pez Lastra, C..and Fabrizio, M. 2001. Laboratory evaluation of chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. *J. Stored Prod. Res.* 37:77-84

Dennis, C., and Webster, J. 1971. 'Antagonistic Properties of Species Groups of *Trichoderma* 1. Production of Non-Volatile Antibiotics', *Trans. British Mycol. Soci.* 57: 363-369.

Derakhshan, A., Rabindra, R. J., and Ramanujam, B. 2008. Effect of storage of formulations on viability of *Verticillium lecanii* (Zimmermann) Viegas and its virulence to *Brevicorne brassicae* (L). *J. Biol. Sci.* 8: 498-501.

De La Rosa, W., F. L. López, and P. Liedo. 2002. *Beauveria bassiana* as a pathogen of the Mexican fruit fly (Diptera: Tephritidae) under laboratory conditions. *J. Econ. Entomol.* . 95: 36-43.

De La Rosa, W., Alatorre, R., Barrera, J.F. and Toriello, C. 2000. Effect of *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycetes) upon the coffee berry borer (Coleoptera: Scolytidae) under field conditions. *J. Econ. Entomol.* 93: 1409– 1414.

Devi, U.K., Padmavathi, J., Rao, U.M.C., Khan, A.A.P., and Mohan, M.C. 2008. A study of host specificity in the entomopathogenic fungus *Beauveria bassiana* (Hypocreales, Clavicipitaceae). *Biocontrol Sci. Technol.* 18(9 – 10): 975-989.

Dhillon, M.K., Naresh, J.S., Singh, R. and Sharma, N.K. 2005. Evaluation of bitter gourd (*Momordica charantia* L.) genotypes for resistance to melon fruit fly, *Bactrocera cucurbitae*. *Indian J. Plant. Prot.* 33(1):55-59.

Dupo, H. and Aganon, T. 2009. Indoxacarb (Steward 30WDG): an effective insecticide for the control of *Diaphania spp.* on bittergourd. *Philippine Entomologists (Philippines)* 20(2):183-185

Faria, M.R.D. and Wraight, S. P. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biol. Control* 43: 237- 256

Feng, M. G., Johnson, B. J. and Kish, L. P. 1990. Virulence of *Verticillium lecanii* and an aphid-derived isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) for six species of cereal-infesting aphids (Homoptera: Aphididae). *Environ. Entomol.* 19(3): 815-820.

Ferron, P., Fargues, J. and Riba, D. 1991 insecticides against pests. In: Handbook of Applied Mycology: Humans, Animals and insects. Arora, D. K., Mukherji, K. G. and Drouhel, E. (Eds.), New York Marcel Dekker ISBN O-824784359. 2: 665-705.

Geetha, N., Praseetha, M., Hari, K., Santhalakshmi, G. and Subadra, B. K. 2012. In vivo interactions of entomopathogenic fungi, *Beauveria* spp. and *Metarhizium anisopliae* with selected opportunistic soil fungi of sugarcane ecosystem. *J. Environ. Biol.* 33: 721-727

Ghatak, S. S. and Mondal, S. 2008. Feasibility of using plant products and bio-pesticide against epilachna beetle, *Henosepilachna vigintioctopunctata* Fabr. infesting brinjal. *Indian J. Entomol.* 70(3): 278-291.

Ghiasian, S. A., Rezayat, S.M., Kord-Bacheh, P., Maghsood, A. H., Yazdanpanah, H. and Shepard, G. S. 2005. Fumonisin production by *Fusarium* species isolated from freshly harvested corn in Iran. *Mycopathol.* 159: 31-40.13.

Ghosh, S.K. and Chakraborty, G. 2012. Integrated field management of *Henosepilachna vigintioctopunctata* (Fabr.) on potato using botanical and microbial pesticides. *J. Biopest.* 5: 151-154.

Ghule, T. M., Devi, L. L. and Jha, S. 2014. Seasonal incidence of Epilachna beetle (*Henosepilachna septima* Dieke) in relation to weather parameters on pointed gourd (*Tricosanthes dioica* Roxb.), *J. Crop Weed*, 10(2):430-432

Glare T.R. and O'Callaghan M. 2000. *Bacillus thuringiensis*: Biology, ecology and safety. Wiley, Chichester, UK.

Goettel M.S. and Inglis G.D. (1997). Fungi: Hyphomycetes. In Manual of techniques in insect pathology. Lacey L.A. (ed.). San Diego, Academic Press, pp. 211–249.

Goettel, M. S., Eilenberg, J., and Glare, T. 2010. Entomopathogenic fungi and their role in regulation of insect populations. In: Gilbert, L. I. and Gill, S. S. (eds), *Insect Control: Biological and Synthetic Agents*. Academic Press, Amesterdam, pp. 387-432.

Gopalakrishnan, C. and Narayanan, K. 1989. Occurrence of two entomofungal pathogen *Metarhizium anisopliae* (Metschnikoff) Sorokin var. minor Tulloch and *Nomuraea rileyi* in *Heliothis armigera. Curr. Sci.* 57: 167-168.

Gupta, J.N. and Verma, A.N. 1978. Screening of different cucurbit crops for the attack of the melon fruit fly, *Dacus cucurbitae* Coq. (Diptera: Tephritidae). *Haryana J. Hort. Sci.*7: 78-82.

Gupta, R. B. L., Shashi, S and Yadav, C. P. S. 2000. Determination of shelf life and virulence of talc formulation of *Metarhizium anisopliae* against *Holotrichia consanguinea*. *Indian J. Agri. Sci.*70 (6): 422-424

Hajek, A. E. and St. Leger, R. J. 1994. Interactions between fungal pathogens and insect hosts. *Annual Rev. Entomol.*39: 293–322.

Hareendranath, V.1989. Control of *Aphis craccivora* Koch with fungal pathogens and their impact on the natural enemies of the pest. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 161 p.

Hou, Y.M. and Pu, Y.C. 2016. Isolation and identification of bacterial strains with insecticidal activities from *Rhynchophorus ferrugineus* Oliver (Coleoptera: Curculionidae). *J Appl. Entomol.* 140(8):617-626.

Inglis, G. D., Johnson, D. L., Cheng, K. J. and Goettel, M. S. 1997. Use of pathogen combinations to overcome the constraints of temperature on entomopathogenic hyphomycetes against grasshoppers. *Biol. Control* 8:143–52

Inglis, G. D., Goettel, M.S., Butt, T.M. and Strasser, H. 2001. Use of hyphomycetous fungi for managing insect pests. In: Fungi as biocontrol agents: progress, problems and potential. Ed. by Butt TM, Jackson C, Magan N, CAB International, Wallingford, UK, 23–69.

Ishii, K., Adachi, T., Hamamoto, H., and Sekimizu, K. 2014. *Serratia marcescens* suppresses host cellular immunity via the production of an adhesion-inhibitory factor against immunosurveillance cells. *J. Biol. Chem.* 289: 5876–5888.

Jhala, R. C., Patel, Y. C., Dabhi, M. V., and Patel, H. M. 2004. Upsurge of pumpkin caterpliiar, *Margaronia indica* (Saunders) in cucurbits in Anand district of Gujarat. *Insect Environ.* 10: 122.

Jiji,T., Praveena, R., Naseema, A. and Anitha, N. 2006. Three promising fungi pathogenic to fruit flies. In: Proc. of Seventh Int. Sym. of Fruit flies of Economic Importance, Salvador, Bahia, Brazil, 5p.

Jiji, T., Praveena, R., Naseema, A., Anitha, N. and Simna, T. 2008. Entomopathogenic fungus, *Beauveria bassiana* (B.) V. for the management of key insect pest of vegetables. *Green farming* 1(10-11): 54-56.

Joseph, A.R. 2014. Evaluation of entomopathogenic fungi for the management of coleopteran pests and characterization of pesticide tolerant strains. Ph.D thesis, Kerala Agricultural University, Thrissur, p. 335.

Karthikeyan, K. and Jacob, S. 2010. Biological efficacy of *Beauveria bassiana* against Rice blue beetle, *Leptispa pygmaea* Baly (Coleoptera: Chrysomelidae). *Int. Res. J. Plant. Sci.* 1(2): 026-033

KAU (Kerala Agricultural University). 2016. Package of Practices Recommendations: Crops (16th Ed.). Kerala Agricultural University, Thrissur, 360p.

Klochko, M.D. 1969. The use of entomopathogenic fungi for controlling *Epilachna vigintioctopunctata. Inst. Zashch. Rast.* 1: 26-31.

Kodandaram, M.H., Haldar, J. and Rai, A.W. 2014. New insecticides molecules and entomopathogens against hadda beetle, *Henosepilachna vigintioctopunctata* infesting vegetable cowpea. *Indian J. Plant Prot.* 42 (4): 333-337

Krauss, U., Hidalgo, E., Arroyo, C. and Piper, S.R., 2004. Interaction between the entomopathogens *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* and the mycoparasites *Clonostachys* spp., *Trichoderma harzianum* and *Lecanicillium lecanii*. *Biocontrol Sci. Technol.* 14: 331-346

Kuruvila, S. and Jacob, A. 1980. Pathogenicity of the entomogenous fungus *Paecilomyces farinosus* (Dickson ex Fries) to several insect pests. *Entomon* 5(3): 175-176.

Kushwaha, K.S., Pareek, B.L and Noor, A. 1973. Fruit fly damage in cucurbits at Udaipur. *Udaipur Uni. Res. J.*11: 22-23.

Lall, B.S. and Singh, B.N. 1969. Studies on the biology and control of melon fly, *Dacus cucurbitae* (Coq.) (Diptera: Tephritidae). *Labdev J. Sci. Technol.*7B: 148-153.

Lauzon, C.R., Bussert, T.G., Sjogren, R.E., and Prokopy, R.J. 2003. *Serratia marcescens* as a bacteria pathogen of *Rhagoletis pomonella* flies (Diptera: Tephritidae). *Eur. J. Entomol.* 100: 87-92.

Lenin, N. 2011. Infestation of the pumpkin caterpillar, *Diaphania indica* Saunders in cucurbits and its management. M.Sc. (Ag) thesis, Kerala Agricultural University. 50p.

Lezama-Gutiérrez, R., Trujillo-de la Luz, A.,Ochoa, M. J., Rebolledo-Domínguez, O., Pescador, A. R., Edwards, L. M. and Aluja, M. 2000. Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hypho- mycetes) on *Anastrepha ludens* (Diptera: Tephritidae): Laboratory and field trials. *J. Econ. Entomol.* 93: 1080-1084.

Liu, L., Zhan, R., Yang, L., Liang, C., Zeng, D., and Huang, J. 2012. Isolation and identification of *Metarhizium anisopliae* from *Chilo venosatus* (Lepidoptera: Pyralidae) cadaver. *Afr. J. Biotechnol.* 11(30): 7609-7617.

Liz, J. M. 2017. Management of major chewing pests, *Henosepilachna septima* (Dieke) and *Diaphania indica* (Saund) infesting bitter gourd with bacterial bioagents. M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 130p.

Lokesh, S. 2014. Evaluation of entomopathogenic fungi against pest complex of chilli (*Capsicum annuum* L.). M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 126p.

Maniruzzaman, F.M., 1981. Plant Protection in Bangladesh. National Book Centre Bangladesh, Purana Paltan and Dhaka, pp. 249–251.

Marri, D., Gomez, D. A. M. A., Wilson, D.D., Bilah, M., Yeboah, S. and Osae, M. 2016. Evaluation of the Efficacy of a commercial formulation of *Beauveria bassiana* for the control of the invasive fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae) *Biopestic. Int.* 12(1): 9-18

McCoy, C. W., Samson, R. A., and Boucias, D. G. 1988. Entomogenous fungi. In: Ignoffa, C. M. and Mandava, N. B. (eds), *Handbook of Natural Pesticides*. CRC press Inc. Florida, 259p.

Metschnikoff, K. 1879. Disease of larvae of grain weevil. Insects harmful to agriculture. Odessa Zemstrob Office. Odessa. 32p.

Meyling, N. V. 2007. *Methods for Isolation of Entomopathogenic Fungi from the Soil Environment. Laboratory Manual* [on-line]. Available: http://orgprints.org/11200/1/11200.pdf. [21 September 2014].

Misra, S and Sarangi, P.K. 2014. Relative efficacy of bio-pesticides against epilachna beetle, *Henosepilachna septima* infesting bitter gourd. *Agrotechnol.* 2(4): 261

Mohamed, M. M. A., Elabdeen, M. H. Z., and Ali, S. A. I. 2013. Host preference of the melon worm, *Diaphania hyalinata* L. (Lepidoptera: Pyralidae), on cucurbits in Gezira state, Sudan. *Persian Gulf Crop Prot.* 2(3): 55-63

Mona, S., Muhammad, S.M. Babak, V. and Seyed, M.L. 2014. Evaluation of conidia-dust formulation of the entomopathogenic fungus, *Metarhizium anisopliae* to

biocontrol the brown-banded cockroach, *Supella longipalpa* F., *Jundishapur J. Microbiol.* 7(6): 10721

Moorthi, P.V. and Balasubramanian, C. 2016. *Aulacophora foveicollis*, a natural diet to entomopathogenic fungus, *Beauveria bassiana*. *J. Basic Appl. Zool.* 73: 28–31

Mote, U.N. 1975. Control of fruit fly (*Dacus cucurbitae*) on bitter gourd and cucumber. *Pesticides* 9: 36-37.

Muñoz R., J. A. 2000. Patogenicidad de *Beauveria bassiana* (Bals.) Bullí. sobre la mosca del mediterráneo, *Ceratitis capitata* (Wied.) en condiciones de laboratorio. Tesis de Licenciatura. Facultad de Ciencias Agrícolas. Univ. Aut. de Chiapas. Huehuetán, Chis., México. 55 p.

Nagaraju, M.C., Nadagouda, S., Hosamani, A.C. and Hurali, S. 2018. Field evaluation of insecticides for the management of cucumber moth, *Diaphania indica* (Saunders) (Lepidoptera: Crambidae) on bitter gourd. *J. Entomol Zool. Studies* 6(2): 79-82

Nahar, P., Yadav, P., Kulye, M., Hadapad, A., Hassani, M., Tuor, U., Keller, S., Chandele, A. G., Thomas, B., and Deshpande, M.V. 2004. Evaluation of indegenous fungal isolates on *Metarhizium anisopliae* M34412, *Beauveria bassiana* B3301 and *Nomuraea rileyi* for control of *Helicoverpa armigera* (Hubner) in pigeon pea field. *J. Biol. Control.* 18:1-8.

Negasi, A., Parker, B.L. and Brownbridge, M. 1998. Screening and bioassay of entomopathogenic fungi for the control of silver leaf whitefly, *Bemisia argentifolii*. *Insect Sci. Application* 18: 37-44.

Nilamudeen, M. 2015. Entomopathogenic fungi for the management of insect pests in rice ecosystem. Ph. D thesis, Kerala Agricultural University, Thrissur, 252p

Nitin. 2014. Studies on mass production of *Beauveria bassiana* ,its efficacy and compatibility with some new generation insecticides against *Helicoverpa armigera* on chickpea (*Cicer arietinum* L.)".M.Sc.(Ag) thesis, JNKVV, Jabalpur. 130p.

Nithya, P. R. 2015. Improved Formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams and its evaluation against sucking pests. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 201p.

Otsu, Y., Matsuda, H., Mori, H., Ueki, T., Nakajima, K., Fujiwara, M., Matsumoto, N., Azuma, K., Kakutani, T., Nonomura, Y., Sakuratani, T., Shinogi, Y., Tosa, S., Mayama, S., and Toyoda, H. 2004. Stable phylloplane colonization by entomopathogenic bacteria Pseudomonas fluorescens KPM – 018P and biological control of phytophagous ladybird beetle *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae). *Biocontrol Sci. Technol.* 14(5): 427 – 439.

Otsu, Y., Matsuda, Y., Shimizu, H., Ueki, H., Fujwara, K., Nakajima, T., Nonomura, T., Sakuratani, Y., Tosa, T., Mayama, S., and Toyoda, H. 2003. Biological control of phytophagous ladybird beetle *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae) by chitinolytic phylloplane bacteria *Alcaligenes paradoxus* entrapped in alginate beads. *J. Appl. Ent.* 127(8): 441-451.

Padmaja, V. and Kaur, G. 1998. Relative susceptibility of brinjal spotted beetle, *Henosepilachna vigintioctopunctata* (Fabricius) to certain isolates of *Beauveria bassiana* (Bals.) Vuill. *J. Biol. Control* 12(2): 147-151.

Patel, C.D., Patel, S.V., Salunke, B.K., and Salunkhe, R.B. 2011. Prodigiosin produced by *Serratia marcescens* NMCC 46 as a mosquito larvicidal agent against *Aedes aegypti* and *Anopheles stephensi*. *Parasitol. Res.* 109(4): 1179- 1187.

Pelizza, S. A., Stenglein, S. A., Cabello, M. N., Dinolfo, M.I. and Lange, C. E. 2011. First record of *Fusarium verticillioides* as an entomopathogenic fungus of grasshoppers. *J. Insect Sci.* 11(70):1-8.

Prasad, K. and Kumar, P. 2002. Insect pest status on summer vegetables in hilly tracts. *Karnataka J. Agric. Sci.* 15 (1): 156–157.

Prasad, A. and Syed, N. 2010. Evaluating prospects of fungal biopesticide *Beauveria bassiana* against *Helicoverpa armigera*; An ecosafe strategy for pesticidal pollution. *Asian J. Exp. Biol. Sci.* 1(3): 596-601

Praveena. 2016. Pathogenicity of indigenous entomopathogenic fungi against select lepidopteran pests. M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 195p.

Rachappa, V. 2003. Occurrence of entomopathogenic fungi and utilization of *Metarhizium anisopliae* (Metschnikoff) Sorokin in the management of selected crop pests in north Karnataka. Ph. D. Thesis, University of Agricultural Sciences, Dharwad.

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

Rabindranath, K. and Pillai, K.S. 1986. Control of fruit fly of bitter gourd using synthetic pyrethroids. *Entomon* 11: 269-272.

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

Rajendran, B. 2002. Preliminary studies on the effect of green muscardine fungus, *Metarhizium anisopliae* (Metsch.) Sorokin on egg plant spotted beetle, *Henosepilachna vigintioctopunctata* Fab. [Coleoptera: Coccinellidae]. *Pest Manag. in Hortic. Ecosyst.* 8(2): 127-130.

Rajendran, B. and Gopalan, M. 1999. Effect of biopathogens (*Bacillus thuringiensis* Berliner and *Beauveria bassiana* Vuillemin) on different stages of egg plant spotted beetle, *Henosepilachna vigintioctopunctata* (Fab.) (Coleoptera: Coccinellidae). *J. Entomol. Res.* 23(1): 9-14.

Ramanujam, B., Balachander, M., Roopa, G., Rangeshwaran, R., and Karmakar, P. 2011. ITS sequencing of Indian isolates of *Lecanicillium* species. *J. Biol. Control* 25 (4): 337–341.

Ramaraje Urs, N. V. Govindu, H. C. and Sasthri, S. K. S. 1967. The effect of certain insecticides on the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*. *Invert. Pathol.* 9:398-403

Ramarethinam, S., Marimuthu, S., Loganathan, S and Murgesan, N. V. 2002. Potentials of entomopathogenic fungal based commercial formulations on some important pests of selected vegetable crops in India. *Pestology* 26(7): 17-21.

Rashid, M., Garjan, A. S., Naseri, B., Ghazavi, M and Barari, H. 2012. Compatibility of the entomopathogenic fungus *Beauveria bassiana* with the insecticides fipronil, pyriproxyfen and hexaflumuron. *J. Entomol. Soc. Iran* 31(2): 29-37

Rashid, M., Baghdadi, A., Sheikhi, A., Hamid-Reza, P. and Gazavi, M. 2010. Compatibility of *Metarhizium anisopliae* (ascomycota: hypocreales) with several insectisides *J. Plant Prot. Res.* 50 (1): 22-26

Rath L. K., 2005. Antibiosis mechanism in eggplant against Epilachna beetle, *Henosepilachna vigintioctopunctata* (Fabr.). *Indian J. Plant Prot.* 33(1):82-84.

Ravi, K. C. 1998. Management of Insect pests of gherkins with particular reference to the fruit borer *Diaphania indica* Saunders (Lepidoptera: Pyralidae). Ph.D thesis, University of Agricultural Sciences, Bangalore, 45p.

Reddy, G. V. P., Zhao, Z. and Humber, R. A. 2014. Laboratory and field efficacy of entomopathogenic fungi for the management of the sweetpotato weevil, *Cylas formicarius* (Coleoptera: Brentidae). *J. Invert. Pathol.* 122: 10–15.

Reshma, M. 2017. Field evaluation of some new insecticides against major insect pests of brinjal. M. Sc. (Ag.) thesis, Orissa University of Agriculture and Technology, Bhubaneswar, 93p.

Riveros, A.S., Giraldo, C.I., and Gamboa, A. 2002. Microbiological control of black leaf streak disease. In: Mycosphaerella leaf spot disease of bananas: present status and outlook. Proc. 2nd Int. Workshop Mycosphaerella Leaf Spot Dis. May 20-23, 2002, San Jose, CA, USA. pp: 287-296.

Roberts, D. R. 1981. Toxins of entomopathogenic fungi. In: Burges, H. D. (ed.), *Microbial Control of Pests and Diseases, 1970–1980.* Academic Press, London, pp. 441–464.

Rodrigues-Destéfano, R. H., I. J. Bechara, C. L. Messias, and Piedrabuena, A. E. 2005. Effectiveness of *Metarhizium anisopliae* against immature stages of *Anastrepha fraterculus* fruitfly (Diptera: Tephritidae). Brazil. *J. Microbiol.* 36: 94-99.

Samira A. K., Mohammad W. K., Hussain, F. A., Ali, K. S. and Sabreen, A. H. 2014. Efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* to control Mediterranean fruit fly, *Ceratitis capitata*. *Int. J. Entomol. Res.* 2 (3): 169-173

Santharam, G., Easwaramoorthy, E., and Jayaraj, S. 1978. Preliminary laboratory evaluation of *Cephalosporium lecanii* Zimm, as a pathogen of brinjal leaf beetle, *Henosepilachna vigintioctopunctata* (Fabr.). *Curr. Sci.* 47(13): 477.

Schreiner, I. H. 1991. Damage threshold for *Diaphania indica* Saunders (Lepidoptera: Pyralidae) on cucumbers in Guam. *Trop. Pest Manag.* 37: 17-20.

Shooker, P., Khayrattee, F. and Permalloo, S. (2006). Use of maize as a trap crops for the control of melon fly, *Bactrocera cucurbitae* (Diptera:Tephritidae) with GF-120. Bio-control and other control methods [Online]. Available on: http\\www.fcla. edu/FlaEnt/fe87 p354.pdf. [Retrieved on: 20th Jan. 2008].

Singh, H. S. and Naik, G. 2006. Seasonal dynamics and management of pumpkin caterpillar, *Diaphania indic*a Saunders and fruit fly, *Bactrocera cucurbitae* Conq. in bitter gourd. *Veg. Sci.* 33: 203- 205.

Sookar, P., Bhagwant, S. and Ouna, A. E. 2008. Isolation of entomopathogenic fungi from the soil and their pathogenicity to two fruit fly species (Diptera: Tephritidae). *J. Appl. Entomol.* 132: 778-788

Srinivasan, P.M. 1959. Guard your bitter gourd against the fruit fly. *Indian Farming* 9: 8.

Srikanth, J., Santhalakshmi, G and Tamizharasi, V. 2006.Viability and Virulence of selected *Beauveria brongniartii* formulations against *Holotrichia serrate. Sugar Technol.* 8(2 & 3): 152-154.

Swaminathan, R., Manjoo, S., and Hussain, T. 2010. Anti-feedant activity of some biopesticides on *Henosepilachna vigintioctopunctata* (F.) (Coleoptera: Coccinellidae). *J. Biopest.* 3(1) (Special Issue) : 077 – 080

Tanada, Y. and Kaya, H.K. 1993. Insect pathology. In: *Bacterial Infection : Bacillaceae.* Academic press Inc. San Diego, California, pp. 83 – 122.

Thamilarasi, N. 2016. Management of pests of cowpea and salad cucumber in polyhouse, M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 196p.

Tara, J.S. and Sharma, S. 2017. Biology and life cycle of *Henosepilachna vigitioctopunctata* Fabricius, a serious defoliator of bitter gourd (*Momordica charantia*) in Jammu region (Jammu & Kashmir) India. *Indian J. Sci. Res.* 13(1):199-203

Thilagam, P., Sivasubramanian, P, Kuttalam, S. and Kubendran, G. 2010. Compatibility of Flubendiamide 480 SC (Fame®) against *Trichoderma viride* (Persoon) and *Beauveria bassiana* (Balsamo). *Pestology* 34(11):53-55 ·

Thaochan, N. and Sausa-Ard, W. 2017. Occurrence and effectiveness of indigenous *Metarhizium anisopliae* against adults *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) in Southern Thailand. *Songklanakarin J. Sci. Technol.* 39 (3): 325-334

Thurkathipana, N. and Mikunthan, G. 2008. Eco-friendly management of hadda beetle using *Beauveria bassiana* in Brinjal. *Commun. Agric. Appl. Biol. Sci.* 73(3): 597-602.

Toledo, J., Liedo, P., Flores, S., Campos, S. E., Villaseñor, A. and Montoya, P. 2006. Use of *Beauveria bassiana* and *Metarhizium anisopliae* for fruit fly control: a novel approach. Fruit Flies of Economic Importance: From Basic to Applied Knowledge Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance 10-15 September 2006, Salvador, Brazil pp. 127-132

Toledo, A. V., Simurro, M. E., and Balatti, P. A. 2013. Morphological and molecular characterization of a fungus, *Hirsutella* sp., isolated from planthoppers and psocids in Argentina. *J. Insect Sci.* 13: 18.

Tripathi, R. and Pandy P. 1973. A non-cucurbitaceous food plant of *Diaphania indica*. *J. Sci. Technol.* 11(3): 80.

Umamaheswara Rao, U., Uma Devi, K. and Alikhan, A.P. 2006. Effect of combination treatment with entomopathogenic fungi, *Beauveria bassiana* and *Nomuraea rileyi* (Hypocreales) on *Spodoptera litura* (Lepidoptera: Noctuidaeae). *Biocontrol Sci. Technol.* 16:2 21-232

Visalakshy, P. N. G. 2005. Natural enemies of *Diaphania indica* (Saunders) (Pyralidae: Lepidoptera) in Karnataka. *Entomon* 30(3): 261-262.

Vishwakarma, R., Prasad, P. H., Ghatak, S. S., and Mondal, S. 2011. Bio-efficacy of plant extracts and entomopathogenic fungi against epilachna beetle, *Henosepilachna vigintioctopunctata* (Fabricius) infesting bottle gourd. *J. Insect Sci.* 24(1): 65-70.

Viswanathan, A. 2015. Management of epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) with phylloplane and pathogenic microorganisms. M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 195p.

Wang, C.S., Li, Z.Z. and Butt, T.M. 2002. Molecular studies of co-formulated strains of the entomopathogenic fungus, *Beauveria bassiana*. *J. Invert. Pathol.* 80: 29–34.

Xu, S. T., Ying, S. H. and Feng, M. G. 2002. Biological compatibility of ten commercial pesticides with *Beauveria bassiana* conidia. *Acta Phytophylacica Sinica* 29: 158–162.

Zentmeyer G.A. 1955. A laboratory method for testing soil fungicides with *Phytopthora cinnamoni*, a test organism. *Phytopathol.* 45:398-404.

Zhang, A. W., Liu, W. Z., Nong, X. Q., Deng, C. S., Guo, W. L., and Jang, B. 1992. A trial production of wettable powder of *Beauveria bassiana*. *Chin. J. Biol. Control* 8(3): 118-120.

Zhang, S., Cheng, Y. J. Bin, Y. and Shuo, W. G. 2006. Toxicity of the mixture of *Metarhizium anisopliae* and *Beauveria bassiana* to *Dendrolimus punctataus*. *J. Fujian Agric. Forestry University*, 35(3): 254-257

Zimmermann, G., 2008. The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. *Biocontrol Sci. Technol.* 18: 865–901.

Appendices

APPENDIX-I

COMPOSITION OF MEDIA USED

1. Nutrient Agar

Peptone - 5g

NaCl - 5g

Beef extract - 3g

Agar - 20g

Distilled water - 1000 ml

Peptone, NaCl and beef extract were dissolved in 500 ml distilled water and volume made up to 1000 ml. 20 g agar-agar was added into this mixture and autoclaved at 15 lsb pressure and 121 °C for 15 min.

.

2. Potato Dextrose Agar

Peeled and sliced potatoes - 200g

Dextrose ($C_6H_{12}O_6$) - 20g

Agar-agar - 20g

Distilled water - 1000 ml

Potatoes were boiled in 500 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was dissolved separately in 500 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was dissolved in to the mixture. The volume was made up to 1000 ml with distilled water and medium was sterilized at 15 lsb pressure and 121 °C for 15 min.

Microbial Consortium for the Management of Insect Pests of Bitter gourd (*Momordica charantia* L.)

212

by

NAVEEDA S.

(2013-21-107)

Abstract of the Thesis
Submitted in partial fulfilment of the
requirement for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE
Faculty of Agriculture

Kerala Agricultural University
Department of Agricultural Entomology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695 522
KERALA, INDIA

2018

ABSTRACT

The study entitled "Microbial consortium for the management of insect pests of bitter gourd" was undertaken in the Department of Agricultural Entomology at College of Agriculture, Vellayani during the period 2013 – 2018 with an objective to isolate and identify microbial pathogens of pests of bitter gourd, develop a microbial consortium, test its compatibility with new pesticide molecules and to evaluate the efficacy of the microbial consortium against major pests of bitter gourd.

Important pests of bitter gourd viz. fruit fly, *Bactrocera cucurbitae*, pumpkin caterpillar *Diaphania indica*, epilachna beetle *Henosepilachna septima* and red pumpkin beetle *Aulacophora foveicollis* were monitored from bitter gourd fields in Thiruvananthapuram and Kollam districts. One entomopathogenic fungi from diseased epilachna grub and pumpkin caterpillar larvae and one phylloplane bacteria were isolated. Kotch's postulates were proved for both the fungi. Upon preliminary screening for pathogenicity to various pests of bitter gourd, the fungi showed infectivity only towards epilachna grubs and pumpkin caterpillar whereas, the bacteria was found to be infective to epilachna grub, pumpkin caterpillar and adults of red pumpkin beetle. The fungus from both the insects was identified as *Fusarium verticilloides* and the bacteria was identified as *Serratia marcescens*

Pathogenicity studies were conducted using isolates of *Metarhizium anisopliae* (Ma4) *Beauveria bassiana* (Bb5) and *Lecanicillium lecanii* obtained from NBAIR, *Paecilomyces lilacinus* isolate (ITCC 6064), *Beauveria bassiana* isolate (ITCC 6063) and the indigenous isolates. The fungi *M. anisopliae* and *B. bassiana* were infective to all the test insects selected whereas the *P. lilacinus* was found infective only to pumpkin caterpillar and fruit flies. *Lecanicillium lecanii* was not infective to any of the test insects. *M. anisopliae* was more virulent to the test insects compared to *B. bassiana* and reported a mortality of 93.54%, 82.5 %, 45%, 5 %,

25%, 61.05% respectively in *B. cucurbitae* adults, *D. indica* larvae, grubs and adults of *H. septima*, adults of *A. foveicollis* and nymphs of *L. australis* seven days after treatment at a dose of 10^8 spores mL^{-1}. Lethargic movement followed by cessation of feeding was common symptom of mycosis in all the species.

Bioassay was conducted against the adults of *B. cucurbitae*, *H. septima* and *A. foveicollis* and the larvae of *D. indica* and *H. septima* using *M. anisopliae* (Ma4), *B. bassiana* (Bb5) and *P. lilacinus* (ITCC 6064). The LC 50 values for *M. anisopliae* were 1.55 x 10^9, 0.18 x 10^9, 1.56 x 10^9 and 4.70 x 10^9 spores mL^{-1} respectively for adults of *B. cucurbitae*, *D. indica* larvae, grubs and adults of *H. septima*, adults of *A. foveicollis* at seven days after treatment. With respect to *B. bassiana*, LC 50 values were 1.65x10^9, 1.20 x 10^9, 1.05 x 10^9, 5.48 x $10^9$and 2.94 x 10^9 spores mL^{-1}respectively was required for the adults of *B. cucurbitae*, *D. indica* larvae, grubs and adults of *H. septima*, adults of *A. foveicollis* respectively .

In vitro compatibility studies were conducted between the different entomopathogenic fungi *viz. B. bassiana*, *M. anisopliae*, *P. lilacinus* and *L. lecanii*. In dual cultures, colony growth of *B. bassiana* and *M. anisopliae* got locked at the point of contact after two weeks of inoculation leaving a space of 3 to 5 mm apart from each other. In dual culture combinations of *B. bassiana* with *P. lilacinus* and *L. lecanii* and *M. anisopliae* with *P. lilacinus* and *L. lecanii*, the colony growth stopped at two weeks after inoculation and a space of 1.5 cm to 2 cm was observed between different fungi. The interactions between various microbes *in-vitro* is not necessarily an indicator of their *in-vivo* interactions. Hence in vivo studies were conducted to assess the effect different microbial combinations viz. *M. anisopliae* with *B. bassiana* (consortium 1), *M. anisopliae* with *P. lilacinus* (Consortium 2) and *B. bassiana* with *P. lilacinus* (Consortium 3) on test insects.

Laboratory evaluation of the fungal consortium showed that the mixtures of various fungi were more pathogenic to the test insects in comparison with the fungi

using individually. The fungal consortium 1 (Ma4 +Bb5) was highly virulent to all the test insects and resulted in per cent mortality of 100, 100 , 88.33, 36.67, 36.67 and 100 respectively for adults of *B. cucurbitae*, *D. indica* larvae, grubs and adults of *H. septima*, adults of *A. foveicollis* and nymphs of *L. australis* seven days after treatment.

Talc based formulations of the consortium maintained the required standards of colony forming units in the formulation and retained bioefficacy against various test insects. Cfu at three months after storage was 0 .83 x 10^{7} and 7.23 x 10^{7}cfu g^{-1} respectively for room temperature and refrigeration. The components of the consortium I, *B. bassiana* and *M. anisopliae* were tested for the compatibility with five insecticides commonly used for pest management in bitter gourd in order to evaluate the suitability of integrating the product with pesticides in pest management programmes. Compatibility of both the fungi was observed for the insecticide Chlorantraniliprole 18.5 SC 0.006 % .

Field experiment was conducted in bitter gourd, variety Preethi to evaluate the talc based formulation of consortium I along with insecticide Chlorantraniliprole 18.5 SC and Malathion as check. The treatments were two application of Consortium I @ 35 g L^{-1}, consortium followed by Chlorantraniliprole 18.5 SC 0.006 %, Chlorantraniliprole 18.5 SC 0.006 % followed by Consortium I @ 35 g L^{-1}, two applications of Chlorantraniliprole 18.5 SC 0.006 % and two applications of Malathion and untreated check. Results showed that the treatment, consortium 1 followed by Chlorantraniliprole 18.5 SC 0.006 % effectively managed populations of *D. indica* (0.08 larvae /10 leaves) , *H. septima* (0.11 grubs/ 10 leaves) and *A. foveicollis* (1.5 adults/ plant) at five days after treatment. The percentage infestation of fruits by *B. cucurbitae* also got reduced from 52.18 % during pre-treatment count to 9.13% after treatment and produced the highest yield of 14.53 t ha^{-1}

To conclude, the fungal isolate which caused natural epizootic in epilachna grub and pumpkin caterpillar larvae was identified as *F. verticilloides*. A bacterium *S. marcescens* was obtained from the phylloplane of bitter gourd and found to be pathogenic to larvae of *D. indica* and grubs of *H. septima*. The fungi *B. bassiana* and *M. anisopliae* were pathogenic to all the test insects. The combination of both resulted in a higher mortality of the test insects and increased the speed of kill than when treated alone. However, in dual culture studies, the fungi were incompatible. Talc based formulation of the consortium maintained the required standards of cfu in the formulations and efficacy upto three months after storage. Highest mortality of the test insects were noticed at the dose 40 g L^{-1} of the formulation. In the field study, consortium 1 (Ma4 + Bb5) followed by Chlorantraniliprole 18.5 SC 0.006 % was effective in managing the insect population and resulted in a high yield.