Evaluation of Entomopathogenic Fungi against Pest Complex of Chilli (*Capsicum annuum* L.)

by

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THESIS

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DECLARATION

I, hereby declare that the thesis entitled **"Evaluation of Entomopathogenic Fungi against Pest Complex of Chilli (*Capsicum annuum* 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 to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Evaluation of Entomopathogenic Fungi against Pest Complex of Chilli (*Capsicum annuum* L.)"** is a record of research work done independently by Mr. Lokesh S. 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 him.

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Lokesh S.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

%	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
h	Hours
kg	Kilogram
l^{-1}	Per litre
m	Meter
mg	Milligram
ml	Millilitre
NS	Non Significant
Rs	Rupees
SC	Soluble Concentrate
Spp.	Species
Viz.	Namely

Introduction

1. INTRODUCTION

Biocontrol is taking centre stage in pest management programmes today. Realizing the adversities generated by the over and abuse of chemical pesticides, world over, Governments as well as scientists are forging ahead for containing pests in a safe and sustainable manner using bioagents. The feasibility of utilizing diverse groups of bioagents, including microbes is being explored.

Fungi centric management of pests was in vogue since 1879 when Elie Metshcnikoff used the fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin for the control of grain beetle, *Anisoplia austriaca* Hrbst. but the impetus gained during the latter half of the twentieth century with the realization that fungi are important population regulators of insects and mites. Entomopathogenic fungi are no more the under focused microbe. They have garnered the attention of researchers as they are the pathogens that infect insects through the cuticle and as they are effective against pests with sucking mouth parts unlike most other entomopathogens that infect their hosts through the gut.

Of the different entomopathogenic fungi, the ubiquitous white muscardine fungus *Beauveria bassiana* (Balsamo) Vuillimen and the green muscardine fungus, *M. anisopliae* seem set fair to be at the forefront of biological control programmes. The yellow muscardine fungus *Lecanicillium lecanii* Zare and Gams, is a well known pathogen of hemipteran pests including aphids and thrips (Christopher *et al.*, 2007; Ujjan and Shahzad, 2012).

Entomopathogenic fungi generally have wide host range but the existence of different isolates that vary considerably in their virulence are now known (Roberts and Humber, 1981) Isolation, identification and evaluation of isolates have relevance in this context. Selection of suitable isolates that ensure rapid kill of the targeted pests and that aptly fit to the desired ecosystems are important in the management of pests.

Virulence depends on both exposure dose and intrinsic features of the fungal isolate (Anderson *et al.*, 2011). Thus for the effective deployment of the isolates in pest management a thorough knowledge on the factors that affect virulence is a prerequisite.

Performance of these fungi in the field is dependent on the type of formulations. For the promotion of entomopathogenic fungi as biocontrol agents development of economically viable products of the fungi with long shelf life is mandatory and this is still a challenge to the scientists working in this arena of biological control.

Another aspect that needs to be resolved is the compatibility of these fungi with plant protection chemicals. The strategy that is widely sought after for containing crop pests is integrated pest management and this warrants knowledge on compatibility of the biological and chemical tools in IPM.

Chilli, *Capsicum annuum* L. is an indispensable vegetable in the Kerala cuisine as well as in the Indian. Apart from this, the fruits have medicinal and export values also. Green chilli is cultivated in an area of 1337 ha in Kerala (GOK, 2014). Chilli growers often face set backs from the tiny sucking insects, the thrips, aphids, whiteflies and mites that scourge the plants and reduce the yield drastically.

Scirtothrips dorsalis Hood, the chilli thrips feed on the leaves, flower buds and calyx of fruits. Softening of calyx consequent to thrips feeding causes premature falling of fruits (Kumar *et al.*, 2011). It is a polyphagous pest (Varadharajan and Veeravel, 1996; Panickar and Patel, 2001) that is widely distributed in Asia. It has invaded the different parts of the world too (Chang, 1995; Li *et al.*, 2004).

Polyphagotarsonemus latus Banks, the mite infesting chilli causes flower drop besides curling and rolling of leaves (Sudharma and Nair, 1999). It has numerous hosts from diverse families (Montasser *et al.*, 2011). The mite and thrips complex cause yield loss to the tune of 34.14 per cent in chilli. In extreme cases complete failure of the crop is not uncommon (Ahmad *et al.*, 1987).

The aphid infesting chilli, *Aphis gossypii* Glover besides being a sucking pest, vectors the chilli veinal mottle virus also (Hussein and Samad, 1993). The whitefly, *Bemisia tabaci* Gennadius is another devastating pest of chilli. The virus vectoring character and phoretic association of chilli mite and the white fly (Soroker *et al.*, 2003) further escalates the injury by the pest.

Many conventional insecticides are now known to induce resurgence of chilli pests besides causing problems of insecticide residues in green chilli (McKenzie and Cartwright, 1994; Rai *et al.*, 2007). This demands adoption of safe pest management tactics to the existing chemical measures in this crop too. Considering the aforesaid aspects of entomopathogenic fungi and the limited probes on the effect of these fungi to chilli pests, the present project was chalked out with the following objectives

- To isolate and identify indigenous strains of entomopathogenic fungi,
- assess the pathogenicity of *B. bassiana, L. lecanii, M. anisopliae* and indigenous isolates against major chilli pests,
- determine the LC_{50}, LC_{90} and LT_{50} of the fungi,
- develop products of fungi,
- assess the compatibility of these fungi with pesticides,
- evaluate the field efficacy of the fungal pathogens in comparison with newer molecules of pesticides against major pests.

Review of Literature

2. REVIEW OF LITERATURE

Entomopathogenic fungi are now gaining importance as biocontrol agents. The literature pertaining to the isolation and identification of fungi, pathogenicity, bioassay, products and compatibility of fungi with pesticides are reviewed below. Details on chilli pests and effect of entomopathogenic fungi and newer molecules on the pests and their natural enemies are also presented.

2.1 ISOLATION AND IDENTIFICATION OF ENTOMOPATHOGENIC FUNGI

2.1.1 Isolation

Many researchers have adopted the technique of isolating fungi from cadavers (Beevi, 1982; Hareendranath, 1989; Anitha, 1999; Sudharma and Rani, 2005; Moubasher, 2010). Cadavers were collected either directly from the field or from the culture maintained in the laboratories from the field collected insects

Insects collected were surface sterilized in two per cent sodium hypochlorite solution for three minutes, rinsed in plenty of sterile distilled water, then dried over filter paper. These cadavers were subsequently placed on to potato dextrose agar (PDA) containing 0.25 mg ml^{-1} chloramphenicol to inhibit growth of bacteria and incubated at 25°C. The fungal hyphae that grew and sporulated on the cadavers were cut and transferred to fresh PDA plates and incubated at 25°C (Assaf *et al.,* 2011). For surface sterilization of cadavers 0.1 per cent mercuric chloride was used by Hareendranath, 1989.

Other methods of isolating entomopathogenic fungi includes baiting using *Galleria mellonella* L. and large flour beetle, *Tribolium destructor* Uyttenboogaart and pink bark borer, *Acanthocinus oxalis* (Zimmerman, 1986; Sanchez-Pena *et al.*, 2011)

Yet another method of isolation of entomopathogenic fungi was from soil (Bidochka *et al.,* 2000). Hasan *et al.* (2012) collected soil from different habitats in Kurdistan region of Iraq and by dilution plate method, two species *B. bassiana* and *Isaria javanica* were isolated.

2.1.2 Identification of Fungi

2.1.2.1 Based on Morphological Characters

Samson *et al.* (1988) identified fungal isolates mainly based on the morphological characteristics of their reproductive structures with the aid of relevant taxonomic keys. Other researchers also identified entomopathogenic fungi based on morphological characters (Humber, 1997; Bielikova *et al.*, 2002; Draganova *et al.*, 2013; Assaf *et al.*, 2011).

2.1.2.2 Based on Molecular Characters

Fungi were identified based on polymorphism of DNA using RAPD-PCR technique (Caetano-annoles *et al.*, 1991; Mcdonald, 1997; Edel, 1998; St. Leger and Joshi, 1999; Gouli *et al.*, 2013). Identification of fungi was also done through rDNA ITS sequencing (Inglis and Tigano, 2006; Meyling, 2008; Tuininga *et al.*, 2009; Qadi *et al.*, 2010; Perez-González, 2014).

2.2 PATHOGENICITY

Entomopathogenic fungi are important natural regulators of insects (Fargues, 1975). *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium lecanii* Zare and Gams and *Metarhizium anisopliae* (Metschnikoff) Sorokin are the widely exploited species (Ferron, 1981; Hall, 1984) though several species in the genera *Aschersonia, Aspergillus, Fusarium, Hirsutella, Nomuraea* and *Paecilomyces* contain entomopathogenic fungi (Tanada and Kaya, 1993).

2.2.1 *B. bassiana*

The infectivity of *B. bassiana* to insects was reported from different parts of India (Wraight, *et al.*, 1998; Vimala and Hari, 2009; Sudharma and Archana, 2009; Jiji *et al.*, 2008).

Ekesi *et al.* (2000) reported *B. bassiana* as a pathogen of arachnids and insects. The fungus was reported to occur naturally in more than 700 species of hosts (Inglis *et al.*, 2001).

2.2.1.1 Pathogenicity of B. bassiana to Pests of Chilli.

2.2.1.1.1 Aphis gossypii Glover

Spore suspension of *B. bassiana* applied at 10^6 conidia ml^{-1} gave 100 per cent mortality on the seventh day after inoculation to *A. gossypii* (Roberts and Leger, 2004; Loureiro and Moino, 2006; Vu *et al.*, 2007).

Herlinda (2010) has reported that *B. bassiana* fungal isolates obtained from several insects were pathogenic to the nymphs of aphid, *A. gossypii*, with mortality rate ranging between 64 per cent and 94 per cent. Infected nymphs that were alive displayed lack of appetite and decreased mobility. Dead nymphs were hard and stiff.

Saranya *et al.* (2010) recorded 96.66 per cent mortality of *A. gossypii* when sprayed with *B. bassiana* at a concentration of 10^8 spores ml^{-1}.

2.2.1.1.2 Bemisia tabaci (Gennadius)

Post mortem hyphal growth and sporulation of *B. bassiana* in cadavers was relatively slow and usually confined to the region immediately surrounding the dead host. Nymphs treated with *B. bassiana* died and appeared slightly reddish. *B. tabaci* eggs also acquired infection (Wraight, 1998; Vicentini *et al.*, 2001; Ramos *et al.*, 2004).

Fourth instar *B. tabaci* treated with *B. bassiana* EABb 93/14-Tp isolate at 10^7 conidia ml^{-1} acquired infection and the mortality significantly increased with time. Mean mortality of nymphs eight days after inoculation ranged between 52.3±7.3 to 91.8±5.8 (Santiago Alvarez *et al.*, 2006).

Fourteen isolates of *B. bassiana* were highly pathogenic to the third instar nymphs of *B. tabaci* (Faria and Wriaght, 2007). Al Deghairi (2008) reported that *B. bassiana* caused higher mortality percentages in nymphs than in eggs. According to him the fungus was able to attach to the nymph cuticle, germinate and penetrate the cuticle to cause significant mortality.

2.2.1.1.3 Scirtothrips dorsalis (Hood)

There is only little information on the effectiveness of entomopathogenic fungi against *S. dorsalis*. Arthurs *et al.* (2013) reported that in greenhouse cages, three applications of *B. bassiana* GHA reduced the population of *S. dorsalis* in chilli by 81-94 per cent.

2.2.1.1.4 Polyphagotarsonemus latus (Banks)

Pena *et al.* (1996) reported that conidial suspensions of *B. bassiana* were able to infect when applied to detached bean leaves infested with *P. latus*. Infection by *B. bassiana* occurred two days after treatment with fungi at 1.16×10^{6} conidia ml^{-1} and reached peak infection after six days.

B. bassiana (8bGc) was able to cause significant mortality in the larvae, nymph and adults *P. latus*. Of the three fungi tested, *B. bassiana* was the most pathogenic against the mite and was followed by *P. fumosoroseus* (PfPp) and *M. anisopliae* (MaPs) (Nugroho, 2003).

Nugroho and Ibrahim (2004) reported that the infected *P. latus* sporulated outside on the body of mite. The mite entered the state of moribund two days after treatment and sporulation was observed over the surface of cadaver four to five days after treatment. *B. bassiana* sporulation over the mite took a whitish colour.

Other insects

Several researchers have reported the effect of *B. bassiana* to other thrips *viz.* western flower thrips, *Frankliniella occidentalis* Pergande, *F. intonsa* Trybom, *Thrips coloratus* Schmutz, *Thrips hawaiiensis* (Morgan), and *Thrips tabaci* Lindeman (Jacobson *et al.*, 2001; Sengonca *et al.*, 2006; Thungrabeab *et al.*, 2006). Second instar thrips of *F. occidentalis* that were exposed to conidia of *B. bassiana* within 24 h of the moult were more susceptible than thrips exposed 24 h after moult (Ugine *et al.*, 2005). Among the three fungal isolates of *B. bassiana* tested under laboratory conditions, the isolate KOG02 was

most virulent to all the above mentioned thrips when inoculated with conidial suspensions at a concentration of 1×10^7 conidia ml^{-1} (Abe and Ikegami, 2005).

Wu *et al.* (2014) reported that among the 28 isolates of *B. bassiana* tested for virulence against *F. occidentalis* in laboratory, the strain SZ-26 was the most potent causing 96 per cent mortality in adults at $1\times10^7 ml^{-1}$ conidia after four days.

2.2.2 *Fusarium* spp.

More than 13 *Fusarium* species are pathogenic to insects, and the genus had a hosts in the orders Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera (Hareendranath, 1989; Beevi, 1982; Teetor-Barsch and Roberts, 1983; Humber, 1992; Anitha *et al.*, 1999; Majumdar *et al.*, 2008).

A perusal of literature showed that there are no reports on the infection of *Fusarium* spp to *A. gossypii.*

2.2.3 *I. javanica*

I. javanica infect members of Lepidoptera or Coleoptera (Tzean *et al.*, 1997; Chen *et al.*, 2007; Hu *et al.*, 2007; Spacht *et al.*, 2009; Shimazu and Takatsuka, 2010). According to Cabanillas and Jones (2013) *Isaria* sp. tolerated high temperature (35°C), and that it was a naturally selected fungus in the subtropical semiarid environment where it could serve as an important natural control agent of the whitefly, *B. tabaci.* The pathogenicity of the fungus was also proved on insects in Hemiptera (Scorsetti *et al.*, 2008) and in Hymenoptera (Hu *et al.*, 2011).

2.2.3.1 Pathogenicity of I. javanica to B. tabaci

Scorsetti *et al.*, 2008; Cabanillas and Jones., 2013 reported the natural epizootic infection of *I. javanica* to *B. tabaci.*

Other Insects

Pathogenicity tests conducted against *T. vaporariorum* nymphs using conidial suspension of *I. javanica* at 10^7 conidia ml^{-1} revealed mortality rates between 26.6 per cent and 76.6 per cent after seven days. (Scorsetti *et al.*, 2008).

I. javanica was isolated from Iraqi soil and the pathogenicity studies on two aphid species indicated that the pathogen caused 66.7 per cent and 75.6 per cent mortality respectively (Hasan *et al.*, 2012).

2.2.4 *L. lecanii*

L. lecanii (*Verticillium lecanii*) an entomopathogenic fungus primarily infecting aphids and scales has been tested experimentally against a range of pests. Highly virulent and epizootic strains of *V. lecanii* have been developed as biocontrol agents against insects (Hall, 1981; Gardner *et al.*, 1984; Samson *et al.*, 1988; Yokomi and Gottwald, 1988; Pinna, 1992; Chandler *et al.*, 1993; Helyer, 1993; Hirte *et al.*, 1994; Rovesti *et al.*, 1997; Butt *et al.*, 2001; Alavo *et al.*, 2002; Sugimoto *et al.*, 2003).

2.2.4.1 Pathogenicity of L. lecanii to Chilli Pests

2.2.4.1.1 A. gossypii

A domestic strain of *V. lecanii* was tested for the control of *A. gossypii* in Korea (Kim *et al.*, 2001). Of the six fungi evaluated, *V. lecanii* caused the highest mortality of aphid.

The virulence of two strains of *V. lecanii* to *A. gossypii* was evaluated, the strain (V24) was reported to show higher virulence against the aphid species (Alavo *et al,* 2002).

Loureiro and Moino (2006) stated that *L. lecanii* exhibited delayed mortality in *A. gossypii* than *B. bassiana* which gave 100 per cent mortality on the seventh day after inoculation.

Nirmala *et al.* (2006) evaluated pathogenicity of isolates of *V. lecanii* against *A. gossypii* using detached leaf bioassay technique and they observed that the isolates were pathogenic to *A. gossypii* at a concentration of 10^7spores ml^{-1}.

At 25°C and 75 per cent relative humidity, *L. lecanii* 41185 showed high virulence to *A. gossypii* and the control attained was nearly 100 per cent at two days after treatment (Vu *et al.*, 2007).

2.2.4.1.2 B. tabaci

Thirty six isolates of *V. lecanii* and *Verticillium* sp. isolated from different hosts insects and geographical locations were analysed for virulence against *B. tabaci* (Mor *et al.*, 1996). The virulence of the isolates to nymphs of *B. tabaci* ranged from 0 to 83 per cent.

Park and Kim (2000) identified Btab01 and 4078 as the most effective strains against *B. tabaci* considering the high growth rate of hyphae and virulence of strains. Natural infections of *Verticillum* on *Bemisia* spp. have been noted by Faria and Wraight, (2001).

When four strains of *V. lecanii* (V20, V26, V07 and V17) were tested for pathogenicity to the third instar of *B. tabaci,* the insect was found highly susceptible to infection by all the isolates (Fatiha *et al.*, 2007).

2.2.4.1.3 S. dorsalis

The literature relating to pathogenicity of *L. lecanii* against *S. dorsalis* has not been documented.

2.2.4.1.4 P. latus

Parker *et al.* (1996) reported *V. lecanii* association with *P. latus.* Most work on mite pathogens were centered around fungal pathogens of eriophyids and spider mites and those related to *P. latus* is negligible.

Other Insects

Skinner *et al.* (1991) have reported that infection of *V. lecanii* in pear thrips produced pink, mummified larvae. According to Schreiter *et al.* (1994) conidia of *V. lecanii,* coated with mucilage, adhered to all surfaces of the *F. occidentalis.* The fungi colonized the surface of the insect prior to or concomitant with penetration of the host cuticle. Infected insects died before the pathogen had substantially colonized the haemocoel. Ahmadi *et al.* (2004) reported that the second larval stage of *T. tabaci* treated with *V. lecanii* at 10^3 to 10^7 conidia ml^{-1}

initiated mortality 24 h after treatment in the highest dose and two to seven days in the lowest dose.

Gindin *et al.*, (2000) reported that several strains of *V. lecanii* exhibited high pathogenicity to third instar nymphs of *Bemisia argentifolii*. Pineda (2007) evaluated the pathogenicity of six isolates of *L. lecanii* (VI_1, VI_2, VI_3, VI_4, VI_5, and VI_{10}) to eggs, second, third, and fourth nymphal instars of *Trialeurodes vaporariorum* (Westwood). Two of the six isolates, VI_3 and VI_5 produced 11–26 per cent infection on eggs while the remaining isolates had no significant effect.

Amjad *et al.* (2012) studied the effect of *V. lecanii* on *Tetranychus urticae* Koch. Three concentrations i.e. 10^6, 10^7 and 10^8 were used against eggs and adult mites. All tested fungal doses were pathogenic to both the eggs and adults. The mortality in adult increased with increase in conidial concentration while the percentage hatching in eggs decreased with increase in conidial concentration.

2.2.5 *M. anisopliae*

M. anisopliae was recorded as a common soil borne entomopathogenic fungus that occur worldwide (Bidochka *et al.*, 2000). It was responsible for the green muscardine disease (Santiago *et al.*, 2001). The immature and adult stages of several insect orders such as Lepidoptera, Hemiptera, Diptera and Coleoptera were infected by the fungus (Anitha *et al.*, 1999; Burgoni, 2005; Karthikeyan and Jacob, 2009).

2.2.5.1 Pathogenicity of M. anisopliae to Pests of Chilli

2.2.5.1.1 A. gossypii

Herlinda (2010) reported that different isolates of *M. anisopliae* infected *A. gossypii* and that they varied in their virulence, it was reported to range from 64 to 92 per cent. According to Tesfaye and Seyoum (2010) *Metarhizium* isolates exhibited infection on adult *A. gossypii* at 20°C, 25°C and 30°C temperature regimes.

2.2.5.1.2 B. tabaci

In a review, Faria and Wraight (2001) reported that isolates of *M. anisopliae* were highly virulent towards nymphs of *B. tabaci*. Norhelina *et al.* (2013) observed that some strains of *M. anisopliae* were twelve times more pathogenic to the second instar than the fourth instar of *B. tabaci*. Generally, there was a decrease in lethal time with increase in dosage. They further observed that most of the nymphs became moribund in two days of post treatment and were gradually covered by mycelia, which appeared pale yellowish white initially and light green to dark later.

2.2.5.1.3 S. dorsalis

There was no literature available on the pathogenicity of *M. anisopliae* to *S. dorsalis*.

2.2.5.1.4 P. latus

Nugroho and Ibrahim (2004) reported that *M. anisopliae* @ 1×10^8 conidia ml^{-1} gave 60 per cent mortality of *P. latus*. They further observed that the fungus exhibited rapid hyphal development and sporulation under moist conditions. Initially, hyphal strands emerged from the anal region of the broad mite cadaver which quickly covered the cadaver with profuse hyphal growth. Low infection on the eggs of the broad mite (10 per cent) was observed by *M. anisopliae* at a dosage of $1x\ 10^8$ conidia ml^{-1}. The fungus appeared greenish when sporulation occurred on the cadaver.

Maketon *et al.*, (2008) reported that *M. anisopliae* CKM- 048 was a virulent strain and it controlled both larvae and adults of *P. latus* at 2×10^8 conidia ml^{-1}. There was no ovicidal effect.

Other Insects

Loureiro and Moino (2006) recorded 100 per cent mortality of *Myzus persicae* (Sulzer) applied with 10^7 spores ml^{-1} of *M. anisopliae*.

Asi *et al.* (2009) found that the local strain of *M. anisopliae* infected the cabbage aphid *Brevicoryne barassicae* L.

The pathogenicty of *M. anisopliae* against the whitefly *T. vaporariorum* was observed by Malsam *et al.* (1998).

2.2.6 *Penicillium oxalicum* Currie & Thom

There are no reports on the infection of *P. oxalicum* on chilli pests. Kuruvilla *et al.* (1980) and Fang and Tan (1986) have reported the infectivity of *P. oxalicum* on *Cofana spectra* (Dist.) infesting rice and in sugarcane *Ceratovacuna lanigera* Zehntner.

2.3 BIOASSAY

2.3.1 Bioassay of *B. bassiana*

2.3.1.1 A. gossypii

The effect of isolates of *B. bassiana* against *A. gossypii* was studied by Nirmala *et al.* (2006). Mortality ranging from 14 to 80.8 per cent in *A. gossypii* was observed at different concentrations of the pathogen. The LC_{50} of *B. bassiana* (Bb5a) to three days old nymphs of *A. gossypii* was 6.57×10^5 spores ml^{-1}. The LT_{50} of *B. bassiana* (Bb5a) to three days old nymphs of *A. gossypii* was highest (9.67 days) for the lowest dose of 10^6 spores ml^{-1} tested. The highest dose 10^9 spores ml^{-1} recorded the lowest LT_{50} of 1.76 days.

In another study on the effect of *B. bassiana* to the third instar nymphs of *A. gossypii* by Loureiro and Moino (2006) it was seen that the fungus at 10^6 to 10^8 conidia ml^{-1} caused 100 per cent mortality on the seventh day after inoculation.

The virulence of four *Beauveria* isolates to adult *A. gossypii* was evaluated by Tesfaye and Seyoum (2010). The cumulative percentage mortality of *A. gossypii* at 25°C varied from 73.33 to 93.33 per cent and LT_{50} ranged from 3.83 to 4.98 days.

2.3.1.2 B. tabaci

Quesada-Moraga *et al.* (2006) evaluated isolates of *B. bassiana* and those were observed pathogenic to fourth instar nymphs of *B. tabaci* at a concentration of 10^7conidia ml^{-1}, the mortality varied from 3 to 85 per cent. The LC_{50} of the isolates varied from 1.1×10^5 to 6.2×10^6 conidia ml^{-1} (Zhu and Kim, 2011).

2.3.1.3 S .dorsalis

In the assay of commercial strains of *B. bassiana* GHA against *S. dorsalis*, LC_{50} was observed as 5.1×10^4 cfu ml^{-1}. Second instars were comparatively less susceptible to all isolates, with LC_{90} values of 1.1×10^8cfu spores ml^{-1} (Arthurs *et al.*, 2013).

2.3.1.4 P. latus

Nugroho and Ibrahim (2004) reported that *B. bassiana* caused mortality up to 80.88 per cent at a dose 1×10^8 conidia ml^{-1}. The effective concentration of *B. bassiana* to kill 50 per cent mite treated was 2.74×10^6 conidia ml^{-1}. The LT_{50} at comparable dosage 1×10^8 conidia ml^{-1} of *B. bassiana* was 3.4 days.

Other insects

Bioassay studies were carried with six different concentrations of *B. bassiana* against the adults of *A. craccivora.* The LC_{50} value of *B. bassiana* was $4.5x10^4$spores ml^{-1} and the LT_{50} value was 3.63 days. The LT_{50} values were found to be inversely proportional to the spore concentrations (Saranya *et al.*, 2010). In the bioassay studies conducted by Akmal *et al.* (2013) using different concentrations of *B. bassiana* i.e. 10^6, 10^7 and 10^8 spores ml^{-1} against *Lipaphis erysimi* (Kalt.), maximum mortality was observed at the highest concentration of 10^8 spores ml^{-1} with LC_{50} value of 1.36×10^6 spores ml^{-1}.

The mortality of larval stages of *F. occidentalis* treated with *B. bassiana* was dose dependent. Higher mortality was obtained with concentrations of 10^7

and 10^8 conidia ml^{-1}. The LC_{50} values ranged from 2.39×10^4 to 5.89×10^6 conidia ml^{-1} (Sengonca *et al.*, 2006).

2.3.2 Bioassay of *L. lecanii*

The literature relating to bioassay of *L. lecanii* against *A. gossypii, S. dorsalis* and *P. latus* has not been documented.

2.3.2.1 B. tabaci

Among the four strains of *V. lecanii* (V20, V26, V07 and V17) tested against third instars of *B. tabaci,* the LC_{50} values were 1.65×10^7, 1.87×10^7, 2.2×10^7 and 2.58×10^7 conidia ml^{-1} respectively. The least and highest LT_{50} values were 2.909 and 3.534 days noted for isolates V20 and V17 (Fatiha *et al.*, 2007).

Other Insects

The assay of *V. lecanii* at 10^8 spores ml^{-1}showed 100 per cent mortality in *A. craccivora.* Mortality declined with the decrease in concentrations. The LC_{50} value of *V. lecanii* was 2.5×10^4spores ml^{-1}. At the highest concentration of 10^8 spores ml^{-1}, the LT_{50} value was 3.90 days (Saranya *et al.*, 2010).

During bioassay, *L. lecanii* isolate PDRL922 showed significant mortality of *L. erysimi* population with LC_{50} of 5.0×10^3 spores ml^{-1}. A positive correlation between the spore concentration and the mortality of insects was observed (Ujjan and Shahzad, 2012).

2.3.3 Bioassay of *M. anisopliae*

2.3.3.1 A. gossypii

Loureiro and Moino (2006) reported that the at $1x10^6$ spores ml^{-1} caused 100 per cent mortality to *A. gossypii* with LT_{50} value of 3.90 days. Herlinda (2010) stated that the strain MPx of *M. anisopliae* at concentration 10^6 conidia ml^{-1} caused 92 per cent mortality of the aphid and that the LT_{50} was 35. 76 h.

2.3.3.2 B. tabaci

Taylor and Khan (2010) recorded that the LC_{50} of *M. anisopliae* against *B. tabaci* was 1.46×10^3 spores ml^{-1} and the LT_{50} was 1.69 days at 20°C.

Norhelina *et al.* (2013) reported that among the different isolates evaluated, the strain GJ4 of *M. anisopliae* recorded the lowest LT_{50} of 2.25 days at 10^7 ml^{-1} against the second and fourth nymphal instar of *B. tabaci.*

2.3.3.3 S .dorsalis

Literature regarding bioassay of *M. anisopliae* against *S. dorsalis* was unavailable.

2.3.3.4 P. latus

M. anisopliae caused 60 per cent mortality of *P. latus* at a dose $1x10^8$ conidia ml^{-1}. The LC_{50} of $2.77x10^7$ conidia ml^{-1} was recorded against *P. latus*. The LT_{50} at $1x10^8$ conidia ml^{-1} of *M. anisopliae* was 4.3 days (Nugroho and Ibrahim, 2004).

Maketon *et al.* (2009) reported that the LC_{50} values of *M. anisopliae* were 8.7×10^6 and 1.3×10^7conidia ml^{-1} and the LT_{50} were 2.4 and 3.8 days at 2×10^8 conidia ml^{-1} for larvae and adults of *P. latus* respectively.

Other insects

Saranya *et al.*, (2010) assessed the LC_{50} value of *M. anisopliae* as $8.9x10^5$spores ml^{-1} against *A. craccivora.* At the highest concentration of 10^8 spores ml^{-1}, the LT_{50} value was 5.54 days.

Based on the LC_{50} ($3x10^5$ spores ml^{-1}) and LT_{50} (2.1 days) values, *M. anisopliae* (PDRL526) was reported to be effective against the mustard aphid, *L. erysimi* whereas the strain PDRL738 was reported avirulent (Ujjan and Shahzad, 2012).

Vestergaard *et al.* (1995) reported that the LC_{50} of *M. anisopliae* treated against adult thrips *F. occidentalis* at 23°C was 3×10^5 conidia ml^{-1}. The LT_{50} values at 10^7 and 10^6 conidia ml^{-1} were 3 and 4.5 days.

2.3.4 Bioassay of *I. javanica*

2.3.4.1 B. tabaci

There are no references available on the bioassay i.e. LC_{50}, LC_{90} and LT_{50} of *I. javanica* to *B. tabaci.*

2.4. DEVELOPMENT OF PRODUCTS OF FUNGI

Roberts and Yendol (1971) described that spores can be applied as dusts, sprays or granule. The development of a suitable formulation is essential to the successful utilization of commercial mycoinsecticides (Daoust *et al.*, 1983). Mass production techniques of entomopathogenic fungi on different media for large scale application in the management of insects has been reviewed by Bartlett and Jaronski, 1988; Feng *et al.*, 1994; Goettel and Inglis, 1997; Jenkins *et al.*, 1998.

According to Pereira and Roberts (1990) the quality formulation depends primarily upon the fungal species. Many formulations can affect the conidial viability resulting in a short shelf life (Moore and Prior, 1993). Formulation technology must consider all stages from production of an organism to its eventual action on target (Jones and Burges, 1998).

Products of entomopathogenic fungi can be in form of either conidia or dry mycelium. Most of the technical powders of hypomycetes fungi (*B. bassiana* and *M. anisopliae*) contain aerial conidia and it is very difficult to suspend them in water because of their hydrophobic nature. Wraight *et al.* (2001) emphasized that dry mycelium remain viable in formulations when properly dried. Dust offer a slight advantage, in that they can be stored in a formulated condition, and their swirling in air jets tends to adhere them to lower as well as upper surfaces of foliage. Selection of appropriate diluents is important since some materials inhibit spore germination. Talc, flour and milk powder have served as suitable diluents

for dusts. Chen *et al.,* 2008 reported that the temperature was the most critical factor influencing the conidial storage ability. The shelf life of fungal entomopathogen can be affected by nutritional and environmental conditions present during production and drying (Jackson *et al.,* 2010).

2.4.1 Products of *B. bassiana*

Hidalgo *et al.,* (1998) reported that conidial viability of 83.3 per cent was maintained in the dustable powder formulation after 45 days of storage at 25°C. The bioefficacy of dustable powder formulation assessed against storage pest, *Sitophilus zeamais* Motsch. achieved up to 90 per cent control after 15 days at 25°C with an application of 20 g of dust per kg of maize.

Das *et al.,* (2013) reported that shelf life of talc based wettable powder formulation of *B. bassiana* at room temperature, lasted up to 180 days with 20.22×10^{7}conidia g^{-1}, and it exhibited 48 per cent pathogenicity.

2.4.2 Products of *L. lecanii*

Gulsar and Gopalakrishanan (2012) reported that *L. lecanii* exhibited no significant difference in the virulence of spores formulated in talc and oil. Among the different products evaluated by Banu (2013) the talc based formulation of sabouraud dextrose yeast broth maintained the maximum fungal viability followed by the product prepared from potato dextrose broth. Virulence studies revealed that media, temperature and storage duration of the products exerted effect on the test insect (mealy bug) mortality. At 180 days after storage PDB prepared talc gave a mortality of 45.33 per cent at room temperature.

2.4.3 Products of *M. anisopliae*

According to Moore and Higgins (1997) conidia survived better when stored as dry powder than when stored in a mixture of mineral and vegetable oils. Bagwan (2011) was of the opinion that Soya lecithin and neem oil formulations of *M. anisopliae* retained shelf life for 300 days, while vermicompost, deoiled castor cake and farmyard manure formulations retained shelf life for 200,190 and 160

days, respectively as compared to gypsum and talc powder where the cfu g^{-1} declined by 110 days after storage.

2.5. COMPATABILITY OF FUNGI WITH PESTICIDES.

Biological control is an important component for reducing the population density of pests in Integrated Pest Management (IPM) programs. The compatibility of entomopathogenic fungi with crop protection techniques such as the insecticides needs to be understood as it may inhibit to a smaller or larger extent the development and reproduction of pathogen (Quintela and McCoy, 1998).

The combined use of chemicals and biopesticides is an attractive strategy since the degree of intervention by the chemical agent is reduced by increasing levels of biological agents. However, the use of incompatible insecticides may inhibit the development and reproduction of these pathogens (Anderson and Roberts, 1983; Duarte *et al.*, 1992; Malo, 1993; Loureiro *et al.*, 2002; Andalo *et al.*, 2004). The inhibitory effects of agrochemicals on the germination and growth of entomopathogenic hyphomycetes often vary among taxa and strains (Vanninen and Hokkanen, 1988; Li and Holdom, 1994: Latteur and Jansen, 2002). The utilization of selective insecticides in association with pathogens can increase the efficiency of control, allowing the reduction of the amount of applied insecticides, minimizing environmental contamination hazards and the expression of pest resistance (Moino and Alves, 1998).

2.5.1 Compatibility of *B. bassiana* with Pesticides

Andalo *et al.* (2004) found that some insecticides *viz.*, thiamethoxam, imidacloprid, carbofuran and pencycuron were compatible; whereas some others glyphosate, dimetilurea, azafenidine, ouintozene, symazine + ametryne, 2,4-D, acetochlor and oxyfluorfen negatively affected vegetative growth and sporulation of *B. bassiana.*

Further Ambethgar (2009) studied the compatibility of *B. bassiana* with twelve insecticides at different concentrations. The chemical insecticides were

reported to inhibit the mycelial growth of *B. bassiana*. At 0.1× concentration, dimethoate exhibited 32.6 per cent mycelial inhibition.

Among the different insecticides tested for the compatibility, chlorpyriphos 20 EC was rated as relatively less toxic to *B. bassiana*, while acetamprid (20 per cent) was more toxic (Amutha *et al.*, 2010). Yue *et al.*, (2011) noted that all the pesticides evaluated inhibited sporulation and that inhibition of some isolates by emamectin benzoate was greatly reduced after diluting it 10 times.

2.5.2 Compatibility of *L. lecanii* with Pesticides

Wenzel *et al.* (2004) evaluated the compatibility of the insecticides imidacloprid and cyromazine with the fungi *L. lecanii*, in terms of vegetative growth, sporulation and conidial viability. The insecticides tested were compatible with *L. lecanii* in all the evaluated parameters.

Gonzalez *et al.* (2013) reported dicofol as very toxic, methamidophos as lightly toxic and abamectin and imidacloprid as compatible with the *L. lecanii*. This was supported by Adriana (2013) that imidacloprid was the only agrochemical that was compatible with *L. lecanii* among the different pesticides tested.

2.5.3 Compatibility of *M. anisopliae* with Pesticides

In accordance with the results of Mohammad *et al.*, (1987) spinosad and indoxacarb were the most compatible insecticides with *M. anisopliae*. Li and Holdom (1994) reported that chlorpyriphos was extremely detrimental to various developmental stages of *M. anisopliae*.

Dimethoate was found less detrimental to *M. anisopliae* with a spore count of 2.343×10^8 spores ml^{-1} and 30.8 per cent inhibition (Rachappa *et al.*, 2007). Asi *et al.* (2010) reported that proclaim (emamectin benzoate) was comparatively less toxic to mycelial growth with 36.78-48.67 per cent inhibition and conidial germination (40.32-49.97 per cent inhibition) of the fungal pathogens. Akbar *et al.* (2012) reported that acetamiprid and emamectin benzoate were less toxic to mycelial growth and spore production of *M. anisopliae*.

2.6. FIELD EXPERIMENT

2.6.1 Pests Infesting Chilli and Their Management

2.6.1.1 Pests

2.6.1.1.1 Aphid (A. gossypii)

A. gossypii is an important chilli pest because of its direct injury as well as its role in the transmission of viral diseases (Roff and Sharan, 1989; Slosser *et al.*, 1989; Roff and Ong, 1992 Hussein and Samad, 1993; Satar *et al.*, 1999; Vuong *et al.*, 2001; Martin and Fereres, 2003,). Yellowing and curling of the leaves, production of sooty mould on the aphid honeydew and stunting of plants are some of the symptoms of attack (Gundannavar *et al.*, 2007).

2.6.1.1.2 Whitefly (B. tabaci)

B. tabaci damages chilli plants by the removal of plant sap, excretion of honeydew upon which saprophytic fungi grow that taints foliage and fruit, and by transmission of yield limiting viruses (Gerling *et al.*, 1980; Alegbejo, 2000; Nomikou *et al.*, 2001; Faria and Wraight, 2001; Simon *et al.*, 2003).

2.6.1.1.3 Thrips (S. dorsalis)

The feeding of *S. dorsalis* in chilli resulted in upward curling of chilli leaves, followed by cupping of leaves (Ningappa, 1972). Similar observations were made by Nandihalli (1979), Reddy and Puttaswamy (1983). Murthy (1984) reported that plants attacked at earlier stages remained stunted and flower production and pod set were arrested. Borah (1987) Singh (1988) and Mikunthan and Manjunatha (2006) also noted similar symptoms of *S. dorsalis* infestation in chilli.

2.6.1.1.4 Chilli mite (P. latus)

The chilli mite, *P. latus* were found in large numbers on the ventral surface of leaves feeding on the plant sap. The mite feeding on the terminal and axillary

tender shoots of the chilli plants lead to the downward curling of leaves. Infestation in the flowering stage resulted in the withering of flowers. Infested fruits were malformed and did not attain normal size (Dhooria and Bindra, 1977; Nandihalli, 1979; Singh, 1988; Karuppuchamy *et al.*, 1993).

2.6.2 Management of Chilli Pests

2.6.2.1 B. bassiana against Pests of Chilli.

Many researchers have identified the potential of the pathogenic fungus, *B. bassiana* as an efficient biocontrol agent in IPM programs against many important pests, *A. gossypii, S. dorsalis, B. tabaci* and *P. latus* (Humber, 1989; Pena *et al.,* 1996). Nugroho and Ibrahim (2007) reported that the WP formulations of *B. bassiana* when applied on the chilli @ of 1×10^{10} conidia ml^{-1} significantly reduced the mite population and subsequently resulted in a high percentage of shoot recovery.

Seal and Kumar (2010) observed that botanigard applied to the foliage of jalapeno peppers reduced *S. dorsalis* larvae by about 50 per cent five days after treatment. In greenhouse three applications of *B. bassiana* reduced overall *S. dorsalis* populations on chilli by 81 to 94 per cent whereas in field four applications reduced thrips populations to the extent of 62 per cent (Arthurs *et al.,*2013).

2.6.2.2 L. lecanii against Pests of Chilli.

Ravensberg *et al.* (1990) reported that spraying of mycotal (*V. lecanii* formulated as WP) at weekly interval reduced infestation of glass house whitefly by approximately 90 percent and the infestation of thrips by 40 per cent. Field experiments in Germany by Pfrommer and Mendgen (1992) showed that control of aphids with *V. lecanii* was possible if the spores were mixed with additives such as polysaccharides and phospholipids.

Reddy and Kumar (2006) evaluated different IPM modules for the management of yellow mite, *P. latus* on sweet pepper grown under protected

cultivation at the Indian Institute of Horticultural Research, Bangalore, Karnataka, India. The results indicated that application of *V. lecanii* was marginally effective.

2.6.2.3 M. anisopliae against Pests of Chilli.

Weekly and biweekly applications of the fungus *M. anisopliae* (@1×10^{11} conidia ha^{-1}) revealed that, thrips density and damage were significantly low in the treatment using *M. anisopliae* (Maniania *et al.*, 2003).

The efficacy of wettable powder formulation of *M. anisopliae* (MaPs) was assessed against the *P. latus* in the field by Nugroho and Ibrahim (2007). He observed that *M. anisopliae* could not adequately suppress mite population.

Jagdish and Purnima (2011) reported that *M. anisopliae* treated in greenhouse was least effective compared to chemicals and botanicals with a reduction of 0.69, 12.30 and 30 per cent *S. dorsalis* per flower at one , three and five days after treatment respectively but Arthurs *et al.* (2013) reported that in greenhouse the chilli plants treated with *M. brunneum* reduced the populations by 84-93 per cent and mentioned that mycoinsecticides can be used in management strategies for low to moderate populations of *S. dorsalis* and provide resistance management tools for the limited number of insecticides that are effective against this pest.

2.6.2.4 Acetamiprid against Pests of Chilli.

Acetamiprid 20 SP @ 80 and 40 g a.i. ha^{-1} were effective in reducing the sucking pests of chilli followed by acetamiprid 20 SP @ 20 g a.i. ha^{-1} and these treatments recorded maximum yield (Jayewar *et al.*, 2003). According to Reddy *et al.* (2005) acetamiprid 20 SP (0.002 per cent) and dimethoate 30 EC (0.06 per cent) were effective against thrips.

Among the different newer molecules tested, acetamiprid 20 SP @ 200 g ha^{-1} and a combination product of indoxacarb 14.5 SC + acetamiprid 7.7 SC @ 500 ml ha^{-1} were on par in reducing *S. dorsalis* population in experiments conducted during 2005 and 2006 (Nandihalli, 2009). Acetamiprid 0.004 per cent

reduced 93.3 per cent population of thrips in chilli (Mandi and Senapati, 2009). Varghese and Mathew (2013) reported that the lowest mite population was recorded in acetamiprid treated plots and yield was higher next to spiromesifen.

2.6.2.5 Emamectin benzoate against Pests of Chilli.

Emamectin benzoate, spinosad, indoxacarb and fenvalerate proved significantly superior with a lower pod damage of 11.75, 12.92, 14.01 and 14.73 per cent, respectively. Highest pod yield of 15.14 q ha^{-1} was recorded in emamectin benzoate (Rekha and Mallapur, 2007). Tatagar *et al.* (2009) reported that five days after spraying emamectin benzoate 5 SC @ 11 g a.i. ha^{-1} recorded the lowest population of mite $leaf^{-1}$ among the different treatments observed. Vanisree *et al.* (2013) reported that emamectin benzoate 0.003 per cent reduced 52.61 per cent of *S. dorsalis* population.

2.6.2.6 Spiromesifen against Pests of Chilli.

Spiromesifen, a new insecticide molecule possessing potential activity and novel mode of action showed better efficacy than dicofol. Spiromesifen @ 72, 96 and 120 g a.i. ha^{-1} recorded 83.8, 86.6 and 91.7 per cent mean reduction of chilli mite population respectively. Among the different dosages applied, spiromesifen at higher dose, 120 g a.i. ha^{-1} was superior in controlling chilli mite but the lower dose of 72 g a.i ha^{-1} was also economically viable, if applied at the early stages of mite infestation. Spiromesifen had a long lasting efficacy on chilli and at 120 g a.i ha^{-1} reduced the leaf curl damage from 41.8 to 12.5 per cent (Kavitha *et al.*, 2006).

The lowest mean population of mite (8.17 mites $leaf^{-1}$) and the leaf curl index (1.61 LCI) was recorded in spiromesifen and it was significantly superior than the treatment with dicofol 18.5 EC (13.79 mites $leaf^{-1}$ and 2.14 LCI) (Nagaraj *et al.*, 2007). Varghese (2011) reported that spiromesifen treated plots recorded the lowest mite population compared to conventional insecticides.

2.6.2.7 Dimethoate against Pests of Chilli.

Bagle (1998) reported the efficacy of dimethoate 0.03 per cent and 0.05 per cent in reducing thrips population in chilli variety Pusa Jwala and G_4 and found that dimethoate 0.05 per cent was superior to dimethoate 0.03 per cent. The superiority of dimethoate 0.03 per cent when compared to neem products in controlling chilli thrips and aphids was reported by Mallikarjuna Rao *et al.* (1999 a and b), where a single round of dimethoate 0.03 per cent spray gave good control of the sucking pest. Dimethoate 0.03 per cent sprayed crop had only 42.38 per cent leaf curl incidence due to chilli mite compared to methyl -o-demeton 0.025 per cent water sprayed crop (Panickar and Patel, 2001). Dimethoate treated plots recorded a mean population 0.11 *S. dorsalis* leaf^{-1} at seven days after treatment and was similar to acetamiprid, imdacloprid, spiromesifen in control efficacy (Varghese, 2011).

2.6.2 Natural enemies

2.6.2.1 Effect of Entomopathogenic Fungi on Natural Enemies

Jacobson (2001) reported that *B. bassiana* when sprayed on to *Amblyseius cucumeris* (*Neoseiulus cucumeris*) Oudemans had no detrimental effect either in laboratory or glasshouse. Maniania *et al.*, 2003 reported that densities of nontarget organisms were higher in plots treated with *M. anisopliae* than in dimethoate treated plots.

2.6.2.1 Effect on Pesticides on Natural Enemies

Dimethoate at 250 g a.i. ha^{-1} resulted in 100 per cent mortality of the parasitoid *Aphidius* spp. within 24 h (Tonet *et al.*, 1997). Dimethoate was also toxic to *Coccinella sexmaculata* (Thayaalini and Raveendranath, 1998). Acharya *et al.* (2002) found that acetamiprid 20 g a.i. ha^{-1} was safe to predatory ladybird beetles.

Thamilvel (2004) observed that dimethoate 0.005 per cent was more toxic to coccinellid, syrphid, spider and braconid population. Spiromesifen 240 SC had no adverse effect on coccinellid beetles and green lace wings (Ameta *et al.*, 2010).

Varghese (2011) reported that spiromesifen was safe to coccinellid grubs and hermerobid larvae. Acetamiprid 20 g a.i. ha^{-1} was safer compared to dimethoate 300 g a.i. ha^{-1} which was toxic to *Amblyseius* spp, coccinellid beetles and spiders.

Materials and Methods

3. MATERIALS AND METHODS

Laboratory and field experiments were carried out for the evaluation of entomopathogenic fungi against pests infesting chilli. The laboratory experiments were carried out at Biocontrol Laboratory for Crop Pest Management, Department of Entomology, College of Agriculture, Vellayani and the field trial was conducted at the Instructional Farm, Vellayani during 2012-2014.

3.1. ISOLATION AND IDENTIFICATION OF INDIGENOUS STRAINS OF ENTOMOPATHOGENIC FUNGI.

3.1.1 Isolation of Fungi

Monitoring was done at monthly intervals during 2013-2014 for entomopathogenic fungi infecting chilli pests in twenty five farmer's fields in Thiruvananthapuram District. Dead insects collected from the field were brought to laboratory and the insects were then placed in moisture chamber for one to two days for the development of fungal mycelia, if any. The cadavers with mycelial growth were surface sterilized by keeping them in 0.01 per cent mercuric chloride for one minute, then washed using sterile water thrice. The cadavers were then placed in Potato Dextrose Agar (PDA) slants for development of mycelia. Hyphal tip culture was repeatedly done to obtain pure culture of the fungus. The fungi thus obtained were maintained in PDA under refrigerated conditions for further studies.

3.1.2 Identification of Fungi

3.1.2.1 Morphological Characters

Slide cultures of the fungi were prepared for morphological studies following the method of Harris (1986). The cultures were examined regularly for the fungal growth pattern, spore shape and spore size using Motic BA210 compound microscope under 40X magnification. The spore size was measured in

µm under 40X objective lens using motic images plus (Version 2.0ML). Images of the fungi were also captured.

3.1.2.2 Molecular Characterisation

The species level identification of the isolated fungi was done based on the ITS sequences of the fungal DNA using universal primers at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram adopting the following procedures

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

About 25 mg of the mycelium was homogenized using liquid nitrogen and the powdered tissue was transferred to a microcentrifuge tube. Four hundred microlitres of buffer PL1 was added and vortexed for one minute. Ten microlitres of RNase A solution was added and inverted to mix. The homogenate was incubated at 65°C for 10 minutes. The lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for two minutes. The flow through liquid was collected and the filter was discarded. Four hundred and fifty microlitres of buffer PC was added and mixed well. The solution was transferred to a Nucleospin Plant II column, centrifuged for one minute and the flow through liquid was discarded. Four hundred microlitre buffer PW1 was added to the column, centrifuged at 11000 x g for one minute and flow though liquid was discarded. Then 700 µl PW2 was added, centrifuged at 11000 x g and flow through liquid is 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°C for 5 minutes. The column was then centrifuged at 11000 x g for one minute to elute the DNA. The eluted DNA was stored at 4°C.

3.1.2.2.2 Agarose Gel Electrophoresis for DNA Quality Check

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. The samples were loaded

to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/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 visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.1.2.2.3 PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2mm each dNTPs (dATP, dGTP, dCTP and dTTP), 1 µl DNA, 0.2 µl PhireHotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5M Betaine, 5pM of forward and reverse primers.

3.1.2.2.4 Primers used

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

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

3.1.2.2.5 PCR Amplification Profile

ITS

98 °C	-	30 sec	
98 °C	-	5 sec	} 40 cycles
62 °C	-	10 sec	
72 °C	-	15 sec	
72 °C	-	60 sec	
4 °C	-	∞	

3.1.2.2.6 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 ethidiumbromide. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 h, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.1.2.2.7 ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

Five micro litres of PCR product was mixed with 2 µl of ExoSAP-IT and incubated at 37°C for 15 minutes followed by enzyme inactivation at 80°C for 15 minutes.

3.1.2.2.8 Sequencing using BigdyeTerminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol.

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 upto 10µl

The sequencing PCR temperature profile consisted of a 1st cycle at 96°C for two minutes followed by 30 cycles at 96°C for 30 sec, 50°C for 40 sec and 60°C

for four minutes for all the primers.

3.1.2.2.9 Post Sequencing PCR Clean up

1. Made master mix I of 10µl milli Q and 2 µl 125mM EDTA per reaction
2. Added 12µl of master mix I to each reaction containing 10µl of reaction contents and were properly mixed.
3. Made master mix II of 2 µl of 3M sodium acetate pH 4.6 and 50 µl of ethanol per reaction.
4. Added 52 µl of master mix II to each reaction.
5. Contents were mixed by inverting.
6. Incubated at room temperature for 30 minutes
7. Spinned at 14,000 rpm for 30 minutes
8. Decanted the supernatant and add 100 µl of 70% ethanol
9. Spinned at 14,000 rpm for 20 minutes.
10. Decanted the supernatant and repeat 70% ethanol wash
11. Decanted the supernatant and air dry the pellet.

The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.1.2.2.10 Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al.*, 2010).

3.2 PATHOGENICITY

The pathogenicity of *Beauveria bassiana* (Bb5a), *Lecanicillium lecanii* (Ll8), *Metarhizium anisopliae* (Ma4) and indigenous isolates *B.*bassiana (Bb21), *L. lecanii* (Llm) were evaluated against the major pests of chilli. The new indigenous isolates collected from dead cadavers were tested against their respective hosts.

The major pest of chilli selected for the study were

1. Aphid – *Aphis gossypii* Glover
2. Chilli thrips – *Scirtothrips dorsalis* Hood
3. Whitefly – *Bemisia tabaci* Gennadius
4. Chilli mite – *Polyphagotarsonemus latus* Banks

The experiments were laid out in completely randomized block design with three replications and an untreated check.

3.2.1. Stock Culture of Insects

The test insects/mite were reared on chilli plant. Seedlings of chilli *Capsicum annuum* variety Vellayani Athulya obtained from Department of Olericulture, College of Agriculture, Vellayani were sown in pots of 45cm diameter. Sequential planting was done to obtain enough number of chilli plants for rearing the test insects continuously.

3.2.1.1 A. gossypii

Gravid females of *A. gossypii* were collected from the field and released into the chilli plants in confined conditions. The emerging young ones were collected and transferred to new plants and two days later the second instar nymphs that emerged were collected carefully using a camel hairbrush and used for pathogenicity studies.

3.2.1.2 B. tabaci

Adults and nymphs of field collected *B. tabaci* were released into potted chilli plants kept in rearing cages and allowed to multiply. The adults obtained from the subsequent generations were used for pathogenicity studies.

3.2.1.3 S. dorsalis

The second instar nymphs were used for pathogenicity studies. The field collected thrips were released into potted plants and the first instar nymphs from the stock culture were then transferred to new plants and three days later the second instar nymphs that emerged were collected.

3.2.1.4 P. latus

The pathogenicity was tested against adult mites. Rearing was initiated from the field collected mites. Stock culture was maintained in potted plants as in 3.2.1.1.

3.2.2. Maintenance of Fungi

B. bassiana (Bb5a), *L. lecanii* (Ll8), and *M. anisopliae* (Ma4) obtained from National Bureau of Agriculturally Important Insects (NBAII) Bengaluru and maintained at the Biocontrol Laboratory for Crop Pest Management, College of Agriculture, Vellayani were used for the present investigation. The indigenous isolates *B. bassiana* (Bb21) and *L. lecanii* (Llm) was obtained from the Department of Microbiology, College of Agriculture, Vellayani. These cultures and the newly isolated indigenous isolates collected during the present investigation were maintained in PDA. The virulence of the pathogens was maintained by periodically passing the fungi through *A. gossypii*.

3.2.3. Assessment of Pathogenicity

The Leaf detached method (Yokomi and Gottwald, 1988) was followed to assess the pathogenicity of all the fungal pathogens. Tender leaf of chilli was detached and the leaf petiole was gently covered with sterile cotton wetted with sterile water to maintain the turgidity of the leaf and was kept upside down position in the Petri dish (9 cm diameter) with a filter paper moistened to saturation level. The insects/mite collected from the stock culture were transferred to the Petri dish individually using a soft and fine bristled brush moistened with water in case of *A. gossypii*, *S. dorsalis* and *P. latus*. In the case of *B. tabaci* the adults were collected in a test tube and kept in freezer for four to five minutes and later transferred to the Petri dish.

The spore suspension for application was prepared aseptically by pouring 10 ml of sterile water into heavily sporulating 14 day old culture grown on PDA in Petri dishes and with gentle scrapping the spores. The spore suspension was applied by using an atomizer on the nymphs of *A. gossypii* and *S. dorsalis* and on

adults of *P. latus*. In the case of *B. tabaci* the adults were transferred to leaves pre-treated with spore suspensions which reduced the risk of damage to wings. Care was taken not to drown the insects in spore suspension. Observations were recorded upto seven days after treatment. For confirmation of pathogenicity of the fungal cultures fungi were reisolated from the cadavers and examined under microscope.

3.2.4 Assessment of Virulence of Fungal Pathogens

The spore suspensions were prepared from 14 day old cultures grown in PDA and the spore load was standardised to a concentration of 1×10^8 spores ml^{-1} of the respective fungi. Application of spore suspension and assessment of pathogenicity done as in 3.2.3. The treated insects were observed for mortality at 24 h interval, till 100 per cent mortality was observed in any one of the treatment. Percentage mortality was corrected (Abbot, 1925) and statistically analysed using necessary transformations.

3.3. BIOASSAY

Bioassay to fix LC_{50}, LC_{90} and LT_{50} was conducted using fungal cultures that proved pathogenic to the test insects. Fungal cultures for the study were obtained by growing the fungi in Potato Dextrose Broth (PDB). For this 200g of potato was peeled cubed, boiled and cooked in 500ml water and sieved through muslin cloth to prepare potato extract. 20g dextrose was then stirred in 500ml of water. Afterwards the potato extract was mixed with dextrose solution. 650ml of the prepared media was then added to three litre fermenter flasks and sterilised at 121°C for 15 minutes at 1.06 kg cm^{-2} pressure. The sterilized media was inoculated with 30 ml of inoculum of the fungal culture in broth and kept aside for 14 days. Spores suspensions were prepared by blending in a mixer for 30 seconds and then sieving through muslin cloth. Spore concentrations were determined using a double ruled Neubauer's haemocytometer in a Motic BA210 compound microscope. The spore suspensions were then serially diluted to get five different concentrations of the respective fungi. The experiments were laid out in

completely randomized block design with three replications and an untreated check. The detached-leaf bioassay method (Yokomi and Gottwald, 1988) as mentioned in 3.2.3 was followed to conduct bioassay also. The spore suspensions were sprayed on insects/mite and leaves using an atomizer as described in 3.2.3. The mortality of test insects was determined and dose-mortality relationships were analysed using analytical software SPSS (Version 16.0) and LC_{50}, LC_{90} and LT_{50} were computed.

3.3.1 *A. gossypii*

Since the initial studies have proven that the fungal cultures *B.bassiana* (Bb5a), *L. lecanii* (Ll8), *M. anisopliae* (Ma4) and indigenous isolate *B. bassiana* (Bb21) caused mortality on aphids, these isolates were selected for bioassay. Five spore concentrations i.e 10^8, 10^7, 10^6, 10^5 and 10^4 spores ml^{-1} of all the above mentioned fungi were prepared and applied against second instar nymphs of *A. gossypii.* Symptoms and mortality of the insects were recorded at six hours interval.

3.3.2 *B. tabaci*

B.bassiana (Bb5a), *L. lecanii* (Ll8), *M. anisopliae* (Ma4) and indigenous isolate *B. bassiana* (Bb21) and *I. javanica* were pathogenic to *B. tabaci.* These isolates were selected and their efficacy was tested at concentrations of 10^9, 10^8, 10^7, 10^6 and 10^5 spores ml^{-1}. Symptoms and mortality of the insects were recorded every 24 h interval.

3.3.4. *S. dorsalis*

B. bassiana (Bb5a), *B. bassiana* (Bb21), *L. lecanii* (Ll8) and *M. anisopliae* (Ma4) were pathogenic to *S .dorsalis* and the bioassay was conducted at concentrations of 10^8, 10^7, 10^6, $10^5$and 10^4spores ml^{-1}.

3.3.3. *P. latus*

The fungal pathogens *B. bassiana* (Bb5a), *B. bassiana* (Bb21), *L. lecanii* (Ll8) and *M. anisopliae* (Ma4) were pathogenic and the bioassay was conducted at concentrations of 10^8, 10^7, 10^6, $10^5$and 10^4spores ml^{-1}.

3.4. DEVELOPMENT OF PRODUCTS OF FUNGI

3.4.1 Talc Based Products of Fungi

The fungal cultures were grown in PDB. The broth was blended in a mixer for two minutes and mixed with talc in 1:3 ratio. This was air dried to approximately eight per cent and 100 g of this talc based products of the fungi were packed in polypropylene cover and stored under room temperature.

3.4.2 Assessment of Shelf Life of Product

3.4.2.1 Assessment of Spore Count

One gram sample each of the talc based products was drawn from the stored packets and suspended separately in 10 ml of sterile water at 15 days after storage. The spore count in the talc suspension was assessed after making necessary dilutions using Neubauer's haemocytometer (Aneja, 1996). This was repeated at fortnightly intervals upto 90 days after storage.

3.4.2.2 Estimation of Colony Forming Units

Colony forming units (cfu) were estimated by dilution plate method using rose bengal medium. Spore suspension was prepared from one gram each of the talc based products stored for 15 days and serially diluted to obtain 10^{-6} concentrations. One ml of the spore suspension was poured into each Petri dish and 15 ml molten media was added and gently rotated for uniform spreading of spore suspension and incubated at room temperature. Three replications were maintained for each treatment. Colony forming units were estimated at 15 days interval until 90, days as follows

$$cfu = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{Weight of sample (g)}}$$

The bioefficay of the talc based products of fungi was assessed against *A. gossypii* at 15 days interval by spraying spore suspension prepared as mentioned in 3.4.2.1 and the mortality of the aphids was recorded and the percentage mortality was analysed statistically after correction (Abbot, 1925) and further transformations.

3.5. COMPATABILITY OF FUNGI WITH PESTICIDES.

The compatibility of four fungal isolates *viz., B.bassiana* (Bb5a), *L. lecanii* (Ll8), *M. anisopliae* (Ma4) and *B. bassiana* (Bb21) and four pesticides was assessed in the laboratory adopting poison food technique (Moorhouse *et al.*, 1992). The four pesticides used were acetamiprid 0.004 per cent, emamectin benzoate 0.002per cent, spiromesifen 0.02per cent and dimethoate 0.06per cent. The radial growth of fungi was recorded at 7, 14 and 21 days and spore count was assessed at 21st day after inoculation.

3.5.1 Assessment of Radial Growth

100 ml of PDA medium was sterilized separately in conical flasks and the insecticide at the above mentioned concentrations were incorporated into the melted sterile PDA aseptically, thoroughly mixed, poured into nine cm diameter sterile Petri dishes and allowed to solidify under laminar air flow chamber. An agar disc with mycelium of the respective fungi was cut from the periphery of 10 day old colony by using 5mm diameter cork borer and transferred into the centre of the Petri dish with pesticide amended media. PDA without insecticide but inoculated with mycelial disc served as untreated check. The plates were sealed with parafilm and incubated at room temperature for 21 days to allow maximum growth. The diameter of growing culture in each Petri dish was measured at 7, 14 and 21 days after inoculation (DAI). The data was further expressed as percentage inhibition in radial growth (Hokkanen and Kotiluoto, 1992).

$$X = \frac{Y-Z}{Y} \times 100$$

Where X, Y, Z stand for percentage of growth inhibition, radial growth of fungus in untreated check and radial growth of fungus in poisoned medium, respectively. The pesticides were further categorised on 1- 4 scoring index. 1 = harmless (<50 per cent inhibition in growth), 2 = slightly harmful (50-79 per cent), 3 = moderately harmful (80-90 per cent), 4 = harmful (>90 per cent) according to Hassan's classification scheme (Hassan, 1989).

3.5.2 Estimation of Spore Count

The spore count of the fungi was taken at 21 days after inoculation by adding 10 ml of sterile water in each plate and slightly scrapping the spores. One ml of spore suspension was estimated using haemocytometer (Aneja, 1996).

3.6. FIELD EXPERIMENT

A field experiment was conducted to evaluate the efficacy of fungal pathogens and newer pesticide molecules in comparison with the insecticide recommended in Package of Practices Recommendations of Kerala Agricultural University (KAU, 2011) against pests of chilli.

The chilli variety Vellayani Athulya was raised in the Instructional Farm, College of Agriculture, Vellayani adopting the Package of Practices Recommendations of Kerala Agricultural University (KAU, 2011). The experiment was laid out in randomized block design with ten treatments and three replications. One month old chilli seedlings were transplanted during the first week of January 2014 in plot size of 2.7m × 2.7m with a spacing of 45 × 45cm. The treatments were as follows.

T1 - Talc based product of *B. bassiana* (Bb5a) @ 20g l^{-1}

T2 - Talc based product of *L. lecanii* (Ll8) @ 20g l^{-1}

T3 - Talc based product of *M. anisopliae* (Ma4) @ 20g l^{-1}

T4- Indigenous isolate of *B. bassiana* (Bb21) @ 20g l^{-1}

T5 - Indigenous isolate of *L. lecanii* (Llm) @ 20g l^{-1}

T6 - Emamectin benzoate 5 SG @ 10 g a.i. ha^{-1}

T7 - Acetamiprid 20SP @ 20g a.i. ha^{-1}

T8 - Spiromesifen 240SC @ 100 mla.i. ha^{-1}

T9 - Dimethoate @ 300 ml a.i. ha^{-1}

T10 - Untreated

Need based application of the treatments was done at 45 days after transplanting. Spraying was done using hand sprayer.

3.6.1. Population of Pests

The population of *A. gossypii, B. tabaci, S. dorsalis* and *P. latus* were taken at weekly intervals with the help of a hand lens. For counting the pest population, five plants were randomly selected in each plot and tagged. From each plant five leaves were taken for assessing the pest count. Pre treatment count of pests was taken before treatment. The treatments were given during the evening hours. Post treatment observations on the number of *A. gossypii, B. tabaci, S. dorsalis* and *P. latus* were taken on the third, seventh, fourteenth and twenty first days after treatment.

3.6.2. Leaf Curl Index

Five plants were selected randomly in each plot and scored visually for leaf curl as given below.

Score	Symptom
0	No symptom
1	1-25% leaf curl
2	26-50% leaf curl
3	51-75% leaf curl, malformation of growing points and reduction in plant height
4	>75% leaf curl, severe/complete destruction of growing points, defoliation and severe malformation

3.6.3. Population of Natural Enemies

The plants selected for counting the pest populations were observed for recording the population of predatory mites, coccinellid predators and spiders per plant.

3.6.4. Yield and Benefit Cost Ratio

Total number of fruits harvested at different intervals (60, 90 and 120 days after transplanting) was recorded. The weight of chilli harvested was recorded and yield per plot was expressed as kg $plot^{-1}$ and converted to tons ha^{-1} to calculate the benefit cost ratio. The market price for pesticide free chilli was considered while calculating benefit cost ratio.

3.6.5 Statistical analysis

The data on population of pests, natural enemies and yield were subjected to ANOVA. Required transformations were also made.

Results

4. RESULTS

4.1 ISOLATION AND IDENTIFICATION OF INDIGENOUS STRAINS OF ENTOMOPATHOGENIC FUNGI.

The details on the fungi isolated from cadavers of chilli pests *viz. A. gossypii* and *B. tabaci* from the farmer's field in Thiruvananthapuram district are given in Table 1. The fungal isolates *viz. Fusarium solani* (Mart.) Sacc. (Hypocreales: Nectriaceae) and *Penicillium oxalicum* Currie & Thom (Eurotiales: Trichocomaceae) were isolated from *Aphis gossypii* Glover and the fungal isolate *Isaria javanica* (Friedrichs & Bally) Samson & Hywel-Jones (Hypocreales: Cordycipitaceae) and the yeast *Meyerozyma caribbica* (Vaughan-Martini, Kurtzman, S.A. Meyer & E.B. O'Neill) Kurtzman & M. Suzuki (Saccharomycetales : Debaryomycetaceae) were isolated from *Bemisia tabaci* Gennadius. The reports of these fungal pathogens on their respective hosts are new in India. The infection of the yeast *M. caribbica* on *B. tabaci* is a new report. Identification of the pathogens was done on the basis of morphological and molecular characters and the details are given below.

4.1.1 Identification

4.1.1.1 F. solani

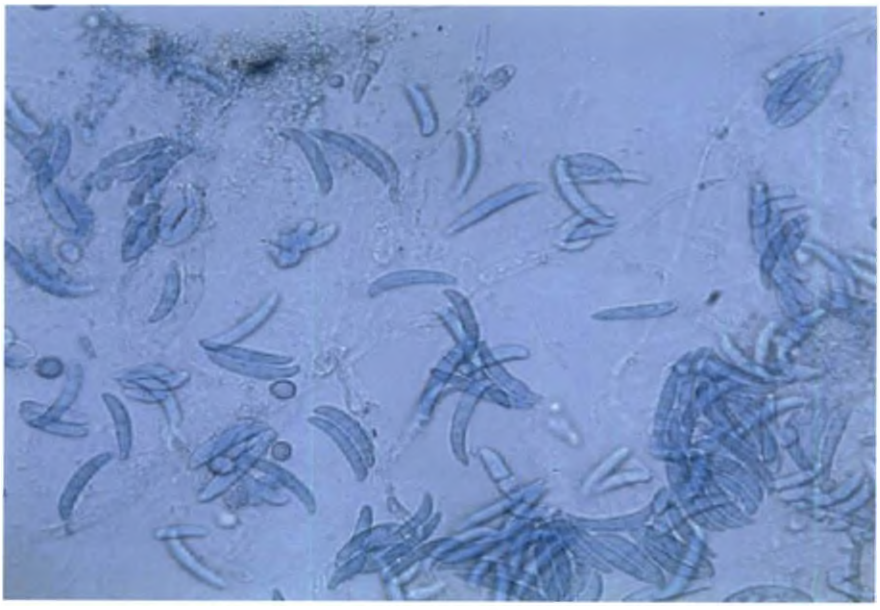
4.1.1.1.1 Morphological Characters

The mycelium of *F. solani* was slight pinkish in PDA and had a thin mat like appearance. The radial growth in diameter was 6.5-7.8 cm at 14 days after inoculation at a temperature of 28 ± 2 °C (Plate 1). Both macro and micro conidia were present. Macro spores were three to four septate sickle shaped, measured 10.2-15.0 µm. Micro spores were either single or aseptate, 4.4-5.3 µm. Chlamydospores, intercalary and terminal and measured 5.4-6.8 × 2.7-5.7 µm.

Table 1. List of new indigenous fungi, their hosts and place of collection

SI No	Fungi	Host	Location in Thiruvananthapuram Dt.
1	*Penicillium oxalicum* Currie & Thom	*Aphis gossypii* Glover	Kalliyoor
2	*Fusarium solani* (Mart.) Sacc.	*A. gossypii*	Neyyattinkara
3	*Isaria javanica* (Friedrichs & Bally) Samson & Hywel-Jones	*Bemisia tabaci* Gennadius	Vellayani
4	*Meyerozyma caribbica* (Vaughan-Martini, Kurtzman, S.A. Meyer & E.B. O'Neill) Kurtzman & M. Suzuki	*B. tabaci*	Palappoor

1a. Mycelium of *Fusarium solani*



1b. Conidia of *Fusarium solani*

Plate 1. Mycelium and conidia of *Fusarium solani*

4.1.1.1.2 Molecular Characters

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

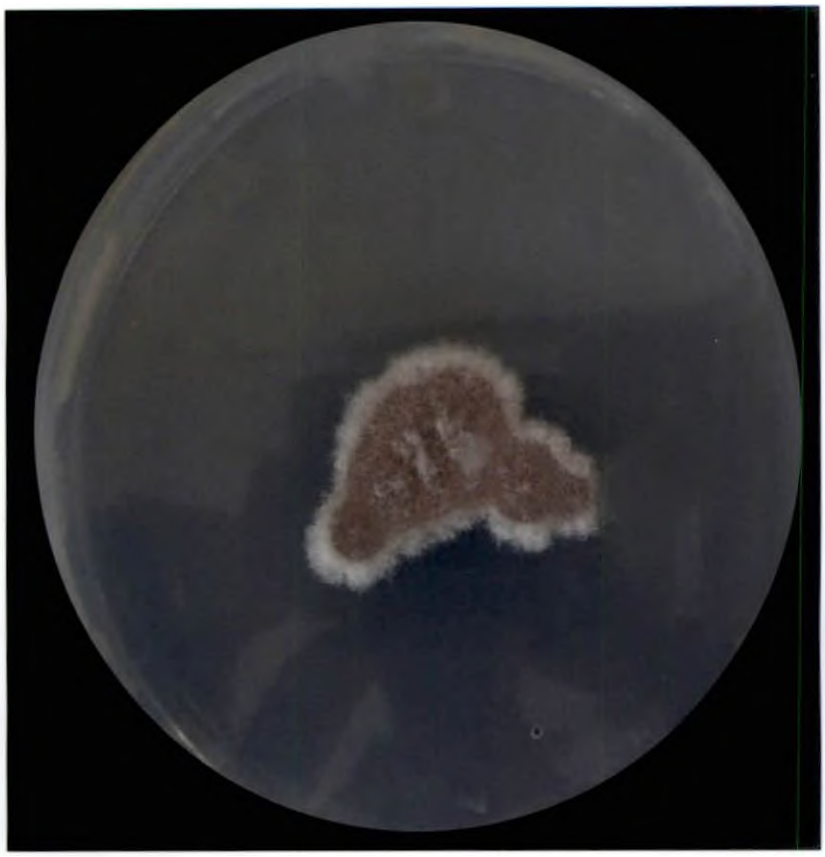
AGGGATCATTACCGAGTTATACAACTCATCAACCCTGTGAACATACCT
AAACGTTGCTTCGGCGGGAACAGACGGCCCCGTAACACGGGCCGCCC
CCGCCAGAGGACCCCCTAACTCTGTTTCTATAATGTTTCTTCTGAGTAA
AACAAGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGGCTCTGG
CATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG
AATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTA
TTCTGGCGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCC
GGGCCTGGCGTTGGGGATCGGCGGAGCCCCCCGCGGGCACACGCCGT
CCCCCAAATACAGTGGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAGC
TAACACCTCGCAACTGGAGAGCGGCGCGGCCACGCCGTAAAACCCCC
AACTTCTGAACGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTT
AA

The BLAST result showed that the fungus *F. solani* had 100 per cent similarity to *F. solani* isolate RSPG-229 having the accession number KC478532.1. The isolate also showed 100 per cent similarity with *Fusarium* sp., accession number AF178402.1.

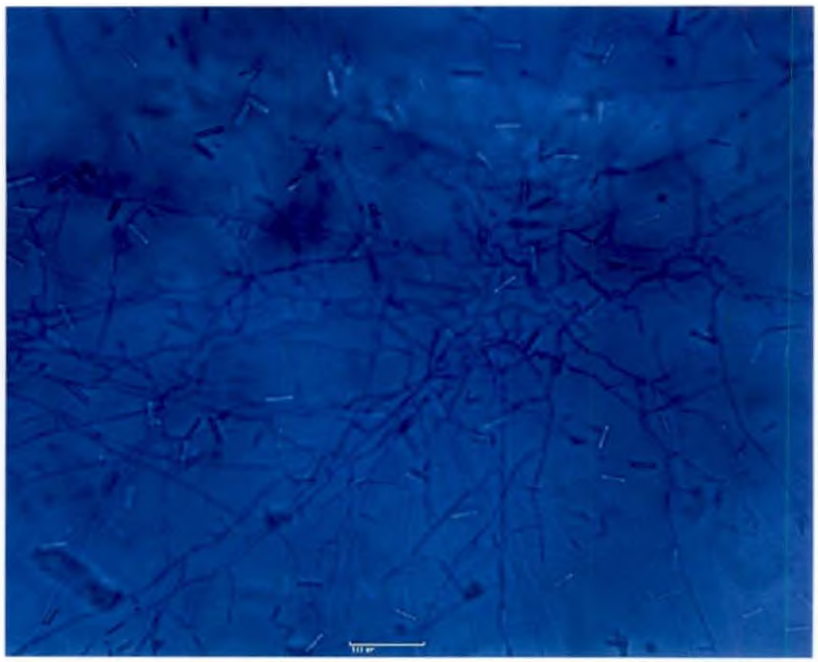
4.1.1.2 I. javanica

4.1.1.2.1 Morphological Characters

In PDA at 28 ± 2°C the fungal growth was grey in colour and attained a growth of 5.8-7 cm in 14 days (Plate 2). The conidia were cylindrical to oval shape measuring 3.3-6 x 1-1.5 µm. Chlamydospores were absent. Philiades 7.4-14.6 µm in length. On the insects the fungus usually produced a white powdery felt with numerous conidiophores.



2a. Mycelium of *Isaria javanica*



2b. Conidia of *Isaria javanica*

Plate 2. Mycelium and conidia of *Isaria javanica*

4.1.1.2.2 Molecular Characters

The molecular analysis gave the following sequence and the resultant sequence when subjected to nucleotide BLAST revealed the fungal isolate as *I. javanica.*

AGGGATCATTAACGAGTTTTTTCAACTCCCTAACCCTTTGTGAACATAC
CTATCGTTGCTTCGGCGGACTCGCCCCGGCGTCCGGACGGCCCTGCGC
CGCCCGCGACCCGGACCCAGGCGGCCGCCGGAGACCCACAAATTCTGT
TTCTATCAGTCTTTCTGAATCCGCCGCAAGGCAAAACAAATGAATCAA
AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAG
CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTT
CGAGCGTCATTTCAACCCTCGACACCCCTTCGGGGGAGTCGGCGTTGG
GGACCGGCAGCATACCGCCGGCCCCGAAATACAGTGGCGGCCCGTCC
GCGGCGACCTCTGCGTAGTACTCCAACGCGCACCGGGAACCCGACGCG
GCCACGCCGTAAAACACCCAACTTCTGAACGTTGACCTCGGATCAGGT
AGGACTACCCGCTGAACTTAA

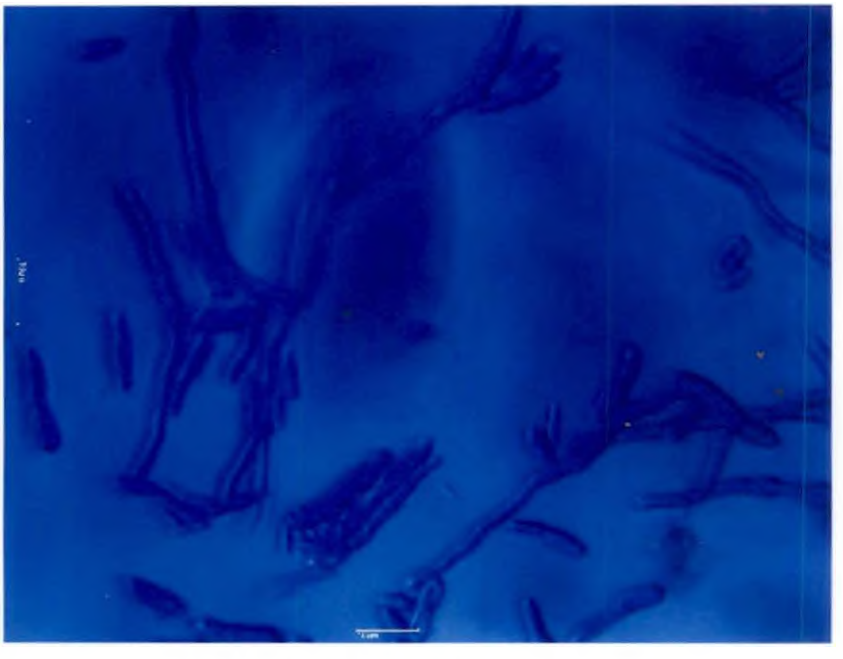
The BLAST result showed that the fungus *I. javanica* had 100 per cent similarity to *Isaria javanica* strain RSIj006 with the accession number KF373690.1. The isolate also showed cent per cent alignment with *Isaria fumosorosea* isolate NLUC (FJ765015.1) and *I. fumosorosea* NLHG-2 (FJ765013.1).

4.1.1.3 M. caribbica

4.1.1.3.1 Morphological Characters

M. caribbica grew upto 5.7-8.1 cm in diameter at a temperature 27 ± 2 °C after inoculating in PDA medium and was creamy white (Plate 3) in colour. The spores were ovoid or elongate, 3.2-7µm in length, single or in pairs. Pseudomycelium was also observed.

3a. Pseudomycelium of *Meyerozyma caribbica*



3b. Spores of *Meyerozyma caribbica*

Plate 3. Pseudomycelium and spores of *Meyerozyma caribbica*

4.1.1.3.2 Molecular Characters

The yeast when subjected to the ITS sequencing furnished the resultant sequence and when subjected to nucleotide BLAST it was identified as *M. caribbica.*

AAGGATCATTACAGTATTCTTTTGCCAGCGCTTAACTGCGCGGCGAAA
AACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGG
CCTAGAAATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTAT
TTTTATTGATAGTCAAATTTTGAATTAATCTTCAAAACTTTCAACAACG
GATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAG
TAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACAT
TGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTC
TCTCAAACCCCCGGGTTTGGTATTGAGTGATACTCTTAGTCGAACTAG
GCGTTTGCTTGAAAAGTATTGGCATGGGTAGTACTGGATAGTGCTGTC
GACCTCTCAATGTATTAGGTTTATCCAACTCGTTGAATGGTGTGGCGG
GATATTTCTGGTATTGTTGGCCCGGCCTTACAACAACCAAACAAGTTT
GACCTCAAATCAGGTAGGAATACCCGCTGAACTTAA

Genetic analysis of *M. caribbica* showed that the isolate had cent per cent similarity with *M. caribbica* strain CDFA887 having the accession number JX886024.1. Moreover it also showed cent per cent alignment with *Pichia caribbica* isolate CNRMA 200500808 (EU568999.1) and *Pichia guilliermondi* strain HK58-2 (EF197951.1)

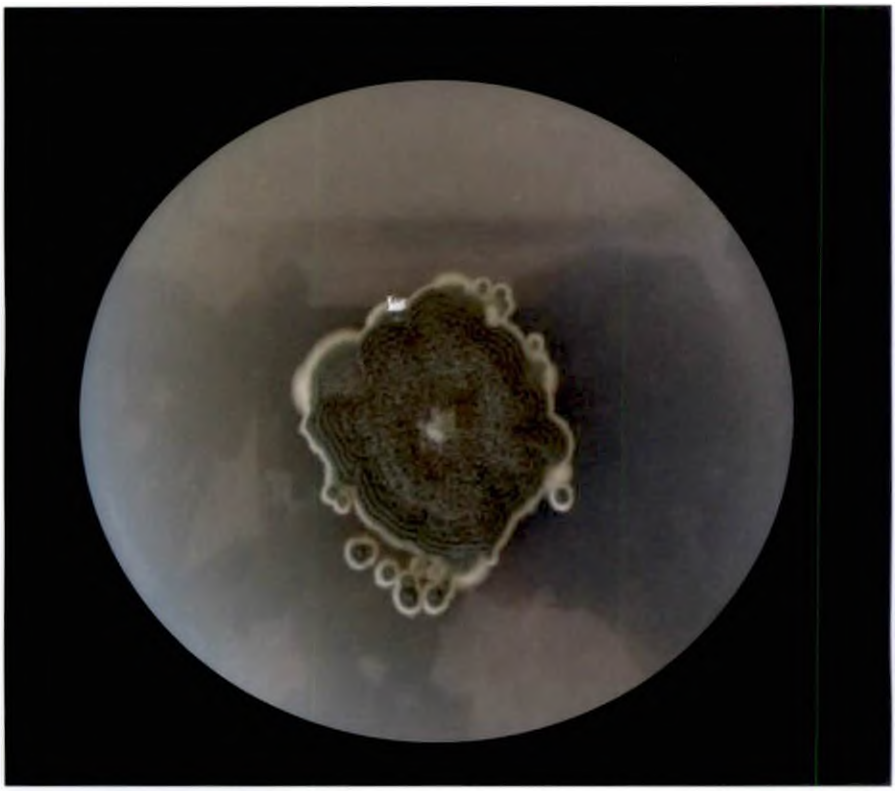
4.1.1.4 P. oxalicum

4.1.1.4.1 Morphological Characters

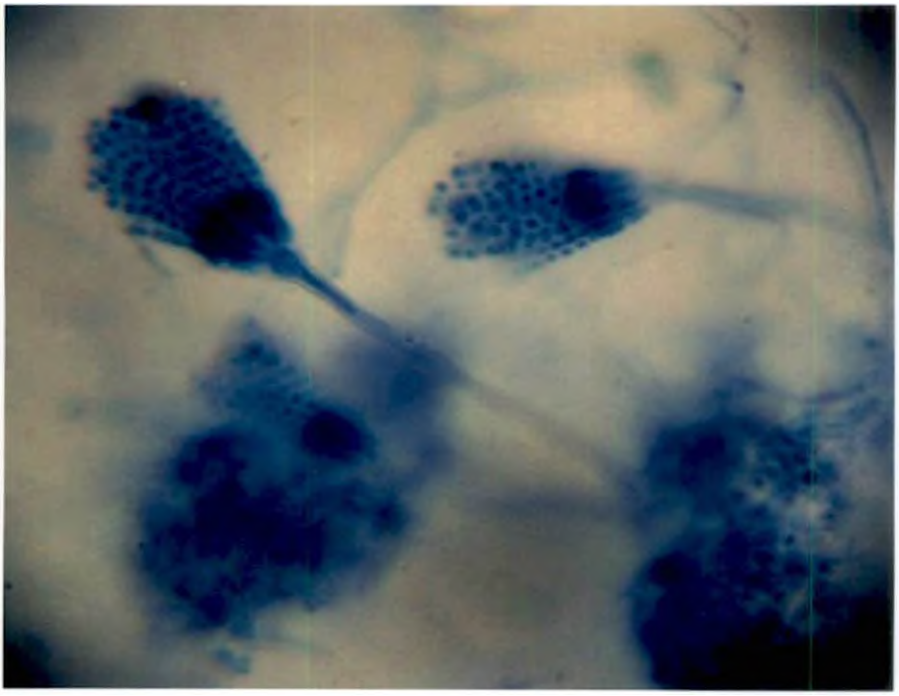
Radial growth of colonies attained 6.1-7.3 cm in 14 days at 28 ± 2°C in PDA medium (Plate 4). The fungal colony was dull green coloured. Monoverticileate or biverticileate. Phialides cylindrical. Conidia 2.3 - 6.0 µm.

4.1.1.4.2 Molecular Characters

The molecular sequence of the fungi obtained was as follows. The subsequent nucleotide BLAST revealed that the sequence was that of *P. oxalicum.*



4a. Mycelium of *Penicillium oxalicum*



4b. Conidia of *Penicillium oxalicum*

Plate 4. Mycelium and conidia of *Penicillium oxalicum*

AAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGT
GTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGG
GGGCATCCGCCCCCGGGCCCGCGCCCGCCGAAGACACACAAACGAAC
TCTTGTCTGAAGATTGCAGTCTGAGTACTTGACTAAATCAGTTAAAACT
TTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAA
ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTT
TGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGA
GCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCTCTCGCCCCC
CGCTTCCGGGGGGCGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGG
TCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTGTAGGCCCGGCCGGC
GCCCGCCGGCGAACACCATCAATCTTAACCAGGTTGACCTCGGATCAG
GTAGGGATACCCGCTGAACTTAA

BLAST results showed *P. oxalicum* had cent per cent alignment with *Penicillium* sp. PTN19 and *P. oxalicum* strain 114-1 having the accession numbers KF656713.1 and KF 152942.1

4.2. PATHOGENICITY

The pathogenicity of five fungal isolates *viz. B. bassiana* (Bb5a), *L. lecanii* (Ll8), *M. anisopliae* (Ma4) and *B. bassiana* (Bb21), *L. lecanii* (Llm) was tested against the three insect pests *viz. A. gosbbsypii, B. tabaci, S. dorsalis* and the mite, *P. latus* infesting chilli. The new indigenous fungi *viz. F. solani, I. javanica, M. caribbica* and *P. oxalicum* were tested against their respective hosts. All fungi except *L. lecanii* (Llm) were pathogenic to the treated insects/ mite and the pathogenicity was confirmed through Koch's postulates.

4.2.1 Pathogenesis

4.2.1.1 B. bassiana (Bb5a)

4.2.1.1.1 A. gossypii

Initially, *B. bassiana* (Bb5a) treated aphids were active, later they become sluggish. At 24 h the mortality of the aphids was recorded. Those alive were

immobile, feeding was also greatly reduced. In some aphids tremors were noticed in legs and they were unable to feed and move. Even after 48 h there was no change in the colour of the aphids. After the death of the insects most of their legs were tightly stuck to the plant surface and abdominal portions were shrunken. The mycelial growth was observed between 24-48 h after the death of the insect. Three to four days after the death i.e. at 72 and 96 h after treatment the cadavers were completely covered with fungal growth (Plate 5). However, one of the nymphs moulted and it completed its development and emerged as adult.

4.2.1.1.2 B. tabaci

The adults of *B. tabaci* released on *B. bassiana* (Bb5a) treated leaf were active on the first day of treatment. After 24 h they lost vigour and the movement was also reduced. 48 h after treatment death of the insects was observed. At 72 h there was a slight growth of the white thread like mycelium from the plural region of the thorax. At 96 h after treatment, mycelia was puffy and covered the entire body except the wings.

4.2.1.1.3 S. dorsalis

The thrips treated with *B. bassiana* (Bb5a) did not show any colour change but the fungal growth was evident on the cadaver. The mycelial growth was similar to that mentioned under 4.2.1.1.1.

4.2.1.1.4 P. latus

The development of symptoms of infection in *P. latus* were similar to that observed in infected *A. gossypii* except the time taken for initiation of mortality, which was observed 48 h after treatment.

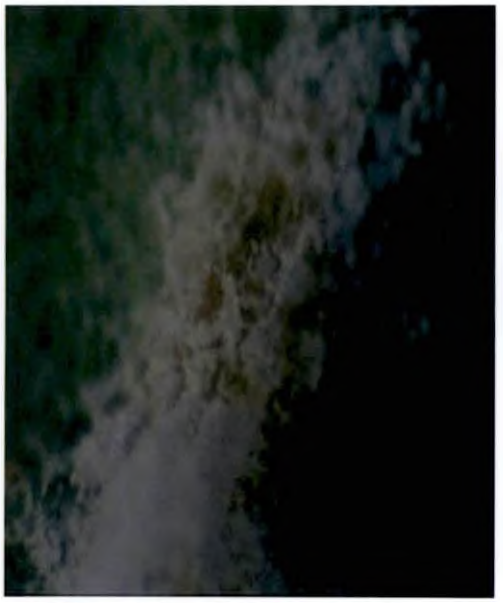
4.2.1.2 B. bassiana (Bb21)

4.2.1.2.1 A. gossypii

The sequence of symptom development in aphid nymphs was similar to that of *B. bassiana* (Bb5a).

Infected *Aphis gossypii*

Infected *Bemisia tabaci*



Infected *Scirtothrips dorsalis*

Infected *Polyphagotarsonemus latus*

Plate 5. *Beauveria bassiana* (Bb5a) infected insects and mite

4.2.1.2.2 B. tabaci

The symptom development of *B. bassiana* (Bb21) was similar to that observed with *B. bassiana* (Bb5a) infected *B. tabaci.*

4.2.1.2.3 S. dorsalis

The symptoms of pathogenesis of *B. bassiana* (Bb21) on *S. dorsalis* were similar to the symptoms expressed by *B. bassiana* (Bb5a) on *S. dorsalis* as described in 4.2.1.1.1. (Plate 6)

4.2.1.2.4 P. latus

The pathogenic symptoms developed by *B. bassiana* (Bb21) on *P. latus* were similar to that of the symptoms developed by *B. bassiana* (Bb5a) in *P. latus* as described under 4.2.1.1.4.

4.2.1.3 L. lecanii (Ll8)

4.2.1.3.1 A. gossypii

The nymphs treated were active and feeding was also normal initially but at 24 h after treatment mortality was observed and the remaining alive aphids completely lost their mobility and were unable to feed. At 48 h after treatment complete mortality of nymphs was recorded. After 72 h thread like mycelial out growths were observed on the cadavers. Within next 24-48 h these threads covered the body to form a mycelial mat (Table 7).

4.2.1.3.2 B. tabaci

Until death of whitefly the symptoms were similar as those described under 4.2.1.1.2. Death of whitefly initiated at 48 h after treatment. At 48 h the dead insects were stuck to the leaf surface and abdomen was shrunken. In the next 24 h the mycelial growth was observed from the abdominal region and by 96 h the mycelial mat covered the entire cadaver except the wings.

Infected *Aphis gossypii*

Infected *Bemisia tabaci*

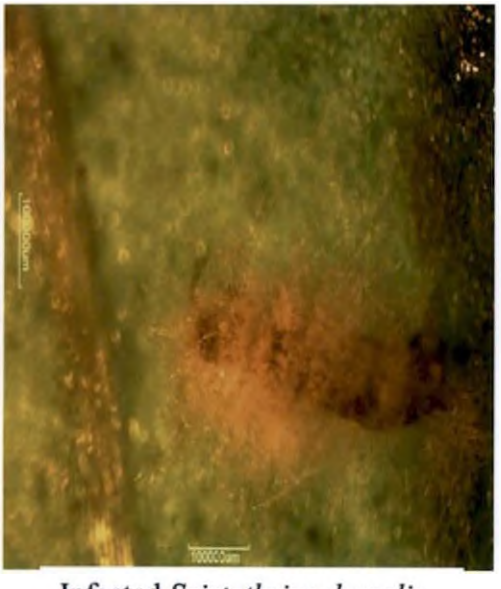
Infected *Scirtothrips dorsalis*

Infected *Polyphagotarsonemus latus*

Plate 6. *Beauveria bassiana* (Bb21) infected insects and mite

Infected *Aphis gossypii*

Infected *Bemisia tabaci*



Infected *Scirtothrips dorsalis*

Infected *Polyphagotarsonemus latus*

Plate 7. *Lecanicillium lecanii* (Ll8) infected insects and mite

4.2.1.3.3 S. dorsalis

The death of thrips was observed only after 48 h treatment. There was a slight colour change in body colour from yellow to slight brown. Profusely growing mycelia were observed on the cadavers. Mycelial growth was similar to that described in the case of *A. gossypii* infection.

4.2.1.3.4 P. latus

The death of mites was observed only after 48 h. There was no colour change in mite but the body was completely shrunken. A thread like mycelial growths was observed from the cadavers.

4.2.1.4 L. lecanii (Llm)

This isolate was not pathogenic to *A. gossypii, B. tabaci, S. dorsalis* and *P. latus*. The nymphs moulted normally and attained adult stage and reproduced.

4.2.1.5 M. anisopliae (Ma4)

4.2.1.5.1 A. gossypii

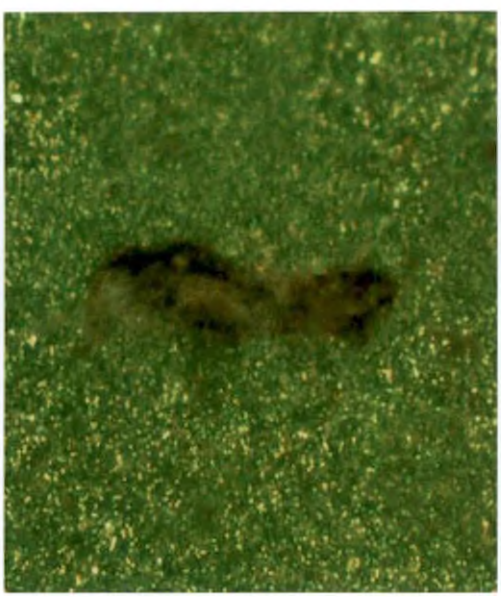
Until the death of the aphids the symptoms were similar to that mentioned under 4.2.1.1.1. At 24 h after treatment of *M. anisopliae* (Ma4) some of the nymphs attempted to the moult but they failed miserably and found to be dead at 48 h. There was a white mycelial outgrowth out of the cadaver that covered the entire body within 48 h of death and this white mat later turned to a greenish black.

4.2.1.5.2 B. tabaci

The symptoms were similar to that observed on *B. bassiana* (Bb5a) infected *B. tabaci* but the fungal growth pattern of *M. anisopliae* (Ma4) was evidently different from rest of the fungal isolates. The fully grown white mycelia turned into oval or round spore ball that was well clearly visible from the notal region of thorax (Plate 8).

Infected *Aphis gossypii*

Infected *Bemisia tabaci*



Infected *Scirtothrips dorsalis*

Infected *Polyphagotarsonemus latus*

Plate 8. *Metarhizium anisopliae* (Ma4) infected insects and mite

4.2.1.5.3 S. dorsalis

The thrips did not express any symptom or mortality until 24 h and were found to be actively feeding. On the second day, they showed a little colour change, turned pale greenish black and finally died. White mycelia was observed on the cadavers 24 h after the death. At 96 h profuse sporulation was noticed all over the cadaver.

4.2.1.5.4 P. latus

The symptoms of disease development and symptomology of infection were similar to that of *S. dorsalis* as described in 4.2.1.5.3.

4.2.1.6 F. solani

4.2.1.6.1 A. gossypii

The nymphs after treatment were found to be actively feeding till the end of 24 h. At 48 h of treatment insects were less active. After the death of the insects the fungal growth was observed only after 24 h. The cadavers were covered with pinkish mycelial growth (Plate 9a).

4.2.1.7 P. oxalicum

4.2.1.7.1 A. gossypii

The nymphs showed the symptoms of pathogenesis after 24-48 h treatment. After 48 h the nymphs became stiff and were found dead. 24 h after death the grey coloured mycelia was observed over the cadaver (Plate 9b).

4.2.1.8 I. javanica

4.2.1.8.1 B. tabaci

The treated adults were found actively feeding and flying around. With a lapse of 24 h they lost the vigour and showed less movement and some were unable to move. At 48 h the whiteflies were dead and there was a white to cement coloured mycelia coming out of the cadaver after 24 h of death later on covering the entire cadaver (Plate 10 a).

Plate 9a. *Fusarium solani* infected *Aphis gossypii*

Plate 9b. *Penicillium oxalicum* infected *Aphis gossypii*

Plate 10a. *Isaria javanica* infected *Bemisia tabaci*

Plate 10b. *Meyerozyma caribbica* infected *Bemisia tabaci*

4.2.1.9 M. caribbica

4.2.1.9.1 B. tabaci

The whiteflies did not show any symptom of disease until 24 h of treatment. Moreover there was no mortality for first 48 h. After 48 h the whiteflies became less active and mortality was observed. The whitefly cadavers were having thin layer of white pseudomycelia out of their body (Plate 10 b).

4.2.2 Assessment of Virulence of Fungal Pathogens

The data on the mortality of the test insects *viz. A. gossypii, B. tabaci, S. dorsalis* and *P. latus* treated with different fungi at spore concentration of 10^8 spores ml^{-1} are presented in Tables 2 to 5.

4.2.2.1 A. gossypii

Upon the exposure of aphid nymphs to the seven different fungal isolates at a concentration of 10^8 spores ml^{-1}, 24 h after treatment the highest mean per cent mortality of 33.23 per cent was observed with *L. lecanii* (Ll8) (Table 2) which was on par with the mortality percentage obtained with *B. bassiana, B. bassiana* (Bb21) (23.16 per cent) and *B. bassiana* (Bb5a) (22.14 per cent). *M. anisopliae* (Ma4), *F. solani* and *P. oxalicum* proved pathogenic to aphids with a mean mortality of 13.00, 1.38 and 1.38 per cent respectively. At 48 h the mortality was high in all the treatments and *L. lecanii* (Ll8) with cent per cent mortality was found significantly superior. The mortality of 73.44 in *B. bassiana* (Bb5a), 66.71 in *B. bassiana* (Bb21) and 57.31 in *M. anisopliae* (Ma4) were found to be on par at 48 h. 72 h *B. bassiana* (Bb5a) and *B. bassiana* (Bb21) attained a mortality of 98.61 per cent and 93.01 per cent respectively and were on par with *L. lecanii* (Ll8).

At 120 h after treatment *B. bassiana* (Bb5a) too attained 98.61 per cent mortality and was found to be on par with *B. bassiana* (Bb21) (93.01 per cent). *M. anisopliae* (Ma4), *F. solani* and *P. oxalicum* resulted in a mortality of 76.78, 70.30 and 63.37 per cent respectively and were on par.

Table 2. Virulence of fungal isolates to *Aphis gossypii*

Treatments @ 10^8 spores ml^{-1}	Mean mortality at different intervals after treatment (%)				
	24 h	48 h	72 h	96 h	120 h
B. bassiana (Bb5a)	22.14 (28.07)	73.44 (58.98)	98.61 (83.22)	98.61 (83.22)	98.61 (83.22)
M. anisopliae (Ma4)	13.00 (21.14)	57.31 (49.20)	76.78 (61.20)	76.78 (61.20)	76.78 (61.20)
L.lecanii (Ll8)	33.23 (35.20)	99.97 (89.06)	99.97 (89.06)	99.97 (89.06)	99.97 (89.06)
B. bassiana (21)	23.16 (28.77)	66.71 (54.76)	93.01 (74.67)	93.01 (74.67)	93.01 (74.67)
L.lecanii (Llm)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)
F. solani	1.38 (6.75)	19.30 (26.06)	39.83 (39.13)	70.30 (56.97)	70.30 (56.97)
P. oxalicum	1.38 (6.75)	4.75 (12.59)	23.16 (28.77)	49.97 (44.98)	63.37 (52.75)
CD (0.05)	(12.62)	(12.27)	(12.18)	(12.60)	(12.22)

Figures within parentheses are angular transformed values

Table 3. Virulence of fungal isolates to *Bemisia tabaci*

Treatments @ 10^8 spores ml^{-1}	Mean mortality at different intervals after treatment (%)				
	48 h	72 h	96 h	120 h	144 h
B. bassiana (Bb5a)	6.96 (15.30)	29.65 (32.99)	39.83 (39.13)	60.11 (50.83)	73.44 (58.98)
M. anisopliae (Ma4)	6.96 (15.30)	23.16 (28.77)	33.23 (35.20)	53.32 (46.90)	63.37 (52.75)
L.lecanii (Ll8)	13.00 (21.14)	26.50 (30.98)	36.57 (37.21)	49.97 (44.98)	66.71 (54.76)
B. bassiana (21)	4.75 (12.59)	19.30 (26.06)	33.23 (35.20)	53.32 (46.90)	66.71 (54.76)
L.lecanii (Llm)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)
I. javanica	13.00 (21.14)	33.23 (35.20)	49.97 (44.98)	67.05 (54.97)	83.61 (66.12)
M. caribbica	1.38 (6.75)	6.96 (15.30)	16.34 (23.85)	33.23 (35.20)	39.83 (39.13)
CD (0.05)	NS	(11.62)	(7.44)	(8.26)	(6.87)

Figures within parentheses are angular transformed values

4.2.2.2 B. tabaci

The mortality rate observed on *B. tabaci* was very less even after 48 h of treatment. The highest mortality observed was 13 per cent and it was recorded with *I. javanica* and *L. lecanii* (Ll8). The mortality observed with other isolates at this time was negligible ranging from 0.02 to 6.96 per cent. Even at the end of 72 h, none of the isolates resulted in 50 per cent mortality, the highest value having 33.33 per cent observed with *I. javanica* and was on par with that of *L. lecanii* (Ll8) (26.5 per cent). At 96 h the mortality values increased to 44.98 and 39.13 per cent with *I. javanica* and *B. bassiana* (Bb5a) respectively. It took 120 h for to cause more than 50 per cent mortality, highest being 67.05 per cent with *I. javanica*. At this point of time *B. bassiana* (Bb5a), *M. anisopliae* (Ma4) and *B. bassiana* (Bb21) was also found to be equally effective as *I. javanica*, the per cent mortality being 60.11, 53.32 and 53.32 per cent respectively.

Significant differences were observed among the isolates at 144 h of treatment. When *I. javanica* recorded the highest values of 83.61 per cent mortality, *B. bassiana* (Bb5a), *L. lecanii* (Ll8), *B. bassiana* (Bb21) and *M. anisopliae* (Ma4) were equally effective causing 73.44, 66.71, 66.71 and 63.37 per cent mortalities respectively.

4.2.2.3 S. dorsalis

The results obtained on the virulence of fungal isolates to *S. dorsalis* are presented in Table 4. At 48 h after treatment the mortality was noted at lesser rates in all isolates except *L. lecanii* (Llm), and mortality in treatments did not vary significantly. At different intervals of time (72, 96, 120 and 144 h after treatment) the treatments *B. bassiana* (Bb5a), *M. anisopliae* (Ma4), *L. lecanii* (Ll8) and *B. bassiana* (Bb21) were found to be on par. At 144 h the mean per cent mortality of *S. dorsalis* observed was 76.78 in *B. bassiana* (Bb5a), 73.44 in *M. anisopliae* (Ma4), 80.65 in *L. lecanii* (Ll8) and 77.81 in *B. bassiana* (Bb21).

Table 4. Virulence of fungal isolates to *Scirtothrips dorsalis*

Treatments @ 10^8 spores ml^{-1}	Mean mortality at different intervals after treatment (%)				
	48 h	72 h	96 h	120 h	144 h
B. bassiana (Bb5a)	9.05 (17.51)	22.14 (28.07)	43.13 (41.05)	60.11 (50.83)	76.78 (61.20)
M. anisopliae (Ma4)	4.75 (12.59)	19.30 (26.06)	33.23 (35.20)	56.66 (48.83)	73.44 (58.98)
L.lecanii (Ll8)	9.99 (18.43)	23.16 (28.77)	36.08 (36.92)	60.11 (50.83)	80.65 (63.90)
B. bassiana (21)	4.75 (12.59)	15.71 (23.35)	33.23 (35.20)	60.11 (50.83)	77.81 (61.90)
L.lecanii (Llm)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)
CD (0.05)	NS	(12.91)	(11.29)	(8.73)	(11.33)

Figures within parentheses are angular transformed values

Table 5. Virulence of fungal isolates to *Polyphagotarsonemus latus*

Treatments @ 10^8 spores ml^{-1}	Mean mortality at different intervals after treatment (%)				
	48 h	72 h	96 h	120 h	144 h
B. bassiana (Bb5a)	13.00 (21.14)	26.50 (30.98)	46.62 (43.06)	63.37 (52.75)	80.65 (63.90)
M. anisopliae (Ma4)	0.02 (0.91)	9.99 (18.43)	26.50 (30.98)	49.97 (44.98)	63.37 (52.75)
L.lecanii (Ll8)	6.96 (15.30)	16.34 (23.85)	26.50 (30.98)	53.32 (46.90)	73.44 (58.98)
B. bassiana (21)	4.75 (12.59)	15.71 (23.35)	29.65 (32.99)	49.97 (44.98)	70.30 (56.97)
L.lecanii (Llm)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)	0.02 (0.91)
CD (0.05)	(14.00)	(8.51)	(7.30)	(7.70)	(8.97)

Figures within parentheses are angular transformed values

4.2.2.4 P. latus

The effect of different fungal isolates at 10^8 spores ml^{-1} to *P. latus* are presented in Table 5. The mortality of insects just initiated at 48 h after treatment and the highest mean mortality was observed in *B. bassiana* (Bb5a) (13.00 per cent) which was on par with *L. lecanii* (Ll8) (6.96 per cent) and *B. bassiana* (Bb21) (4.75 per cent), but in *M. anisopliae* (Ma4) even after 48 h there was no mortality. At 72 h all the treatments except *L. lecanii* (Llm) were pathogenic to *P. latus* with the same trend of mortality rates as it was observed after 48 h. *M. anisopliae* (Ma4) recorded the lowest mortality of 9.9 per cent. Even after 96 h the mortality of *P. latus* was less than 50 per cent with a highest mean mortality of 46.62 per cent in *B. bassiana* (Bb5a) but was significantly superior to others. At 120 h after treatment the highest mean mortality was observed in *B. bassiana* (Bb5a) with 63.37 per cent which was statistically on par with *L. lecanii* (Ll8) (53.32 per cent). The highest mean mortality of 80.65 per cent on *P. latus* at 144 h was observed in *B. bassiana* (Bb5a) which was statistically on par with *L. lecanii* (Ll8) and *B. bassiana* (Bb21) (73.44 and 70.30 per cent respectively).

4.3 BIOASSAY

The data on the percentage mortality of the test insect at varying concentrations along with the LC_{50}, LC_{90} and LT_{50} are presented in Tables 6 to 22. The symptoms of infection observed during the bioassay were similar to that mentioned in pathogenicity studies.

4.3.1 *B. bassiana* (Bb5a)

4.3.1.1 A. gossypii

Aphids inoculated with different spore concentrations of *B. bassiana* (Bb5a) showed differences in mortality. As the spore concentration increased the mortality also increased. The mortality percentage ranged from 6.67 to 46.67 and 23.33 to 100 per cent at 24 and 42 h after treatment at spore concentrations ranging from 10^4 to 10^8 spores ml^{-1} (Table 6).

Table 6. Dose-mortality responses of second instar nymphs of *Aphis gossypii* treated with different spore concentrations of *Beauveria bassiana* (Bb5a)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	6 h	12 h	24 h	30 h	36 h	42 h	
10^8	13.33	36.67	46.67	70	86.67	100	20.739
10^7	0	6.67	33.33	46.67	66.67	80	30.846
10^6	0	6.67	26.67	33.337	46.67	53.33	38.092
10^5	0	0	16.67	26.67	33.33	36.67	44.110
10^4	0	0	6.67	13.33	20	23.33	52.314
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times10^8$ spores ml^{-1})	LC_{90} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times10^8$ spores ml^{-1})	χ^2	Regression equation
6	1.32	1.28-1.35	1.68	1.609-1.780	1.300	Y = 0.318 + 4.634 x
12	1.19	0.94-1.70	1.94	1.506-2.923	2.738	Y = 1.896 + 4.687 x
24	1.07	0.91- 1.22	2.68	2.369-3.134	6.482	Y = 2.940 + 6.325 x
30	0.50	0.39-0.61	1.63	1.414-1.950	6.518	Y = 2.092 + 4.566 x
36	0.18	0.096- 0.27	1.06	0.888-1.302	11.467	Y = 2.233 + 4.512 x
42	0.02	0.010-0.53	0.13	0.093-0.234	4.112	Y = 2.639 + 4.102 x

At the minimum time of 20.739 h *B. bassiana* (Bb5a) required a spore concentration of 10^8 spores ml^{-1} to kill 50 per cent of *A. gossypii* population. At the lowest dose of 10^4 spores ml^{-1} 52.314 h were required to kill of 50 per cent of aphids.

The LC_{50} values obtained at 6, 30 and 42 h after treatment are 1.32×10^8, 0.50×10^8 and 0.02×10^8 spores ml^{-1}. The corresponding LC_{90} values are 1.68×10^8, 1.63×10^8 and 0.13×10^8 spores ml^{-1} respectively.

4.3.2.1.2 B. tabaci

The data on bioassay of fungal pathogens against *B. tabaci* are recorded in Table 7. The percentage mortality of adult *B. tabaci* ranged from 3.33 per cent to 86.67 per cent at a highest concentration of 10^9 spores ml^{-1}. At lowest concentration of 10^5 spores ml^{-1} the mortality recorded was 3.33 to 16.67 per cent in the observations taken from 24 to 144 h.

The minimum time of 103.749 h was required by *B. bassiana* (Bb5a) at a spore concentration of 10^9 spores ml^{-1} to kill 50 per cent of *B. tabaci*. At the lowest dose of 10^5 spores ml^{-1} the time required to kill of 50 per cent of the insect was 194.432 h.

The LC_{50} values obtained with respect to 24, 72 and 144 h after treatment were 3.20×10^9, 1.66×10^9 and 0.19×10^9 spores ml^{-1}. The corresponding LC_{90} values were 5.10×10^9, 2.12×10^9 and 1.04×10^9 spores ml^{-1} respectively.

4.3.2.1.3 S. dorsalis

The data presented in Table 8 showed that at 168 h after treatment the concentrations 10^8, 10^6 and 10^4 spores ml^{-1} recorded the mortality of 83.33, 46.67 and 20 per cent respectively.

The time required for *B. bassiana* (Bb5a) to cause 50 per cent mortality of *S. dorsalis* second instar nymphs at the highest concentration of 10^8 spores ml^{-1} was 116.338 h while at the lowest spore load used (10^4 spores ml^{-1}) the time required was 207.142 h.

Table 7. Dose-mortality responses of adults of *Bemisia tabaci* treated with different spore concentrations of *Beauveria bassiana* (Bb5a)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	24 h	48 h	72 h	96 h	120 h	144 h	
10^9	3.33	6.67	20	43.33	63.33	86.67	103.749
10^8	0	3.33	16.67	33.33	50	70	118.772
10^7	0	3.33	16.67	30	36.67	46.67	139.907
10^6	0	0	10	16.67	23.33	30	168.280
10^5	0	0	3.33	6.67	16.67	16.67	194.432
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^9$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times10^9$ spores ml^{-1})	LC_{90} ($n\times10^9$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times10^9$ spores ml^{-1})	χ^2	Regression equation
24	3.20	3.02-3.39	5.10	4.74-5.64	0.878	$Y = 1.405 + 1.244 x$
48	3.12	2.80-3.33	6.42	5.79-7.36	1.934	$Y = 1.449 + 2.160 x$
72	1.66	1.62-1.71	2.12	2.04-2.25	3.385	$Y = 1.210 + 1.259 x$
96	1.20	1.02-1.39	3.10	2.73-3.63	7.333	$Y = 1.814 + 2.503 x$
120	0.57	0.43-7.11	1.99	1.72-2.40	7.547	$Y = 1.516 + 3.339 x$
144	0.19	1.10-2.77	1.04	0.88-1.28	15.817	$Y = 2.443 + 4.621 x$

Table 8. Dose-mortality responses of nymphs of *Scirtothrips dorsalis* treated with different spore concentrations of *Beauveria bassiana* (Bb5a)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	48 h	72 h	96 h	120 h	144 h	168 h	
10^8	6.67	16.67	33.33	53.33	76.67	83.33	116.338
10^7	3.33	13.33	33.33	43.33	56.67	66.67	134.499
10^6	0	6.67	13.33	26.67	40	46.67	164.238
10^5	0	0	6.67	20	30	33.33	180.291
10^4	0	0	3.33	10	20	20	207.142
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times10^8$ spores ml^{-1})	LC_{90} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times10^8$ spores ml^{-1})	χ^2	Regression equation
48	2.51	2.38-2.77	3.82	3.57-4.19	2.579	Y = 1.836 + 2.457 x
72	2.22	2.05-2.38	3.90	3.59-4.37	6.997	Y = 1.691 + 2.214 x
96	1.50	1.33-1.67	3.23	2.90-3.71	11.847	Y = 1.118 + 2.619 x
120	0.85	0.70-1.00	2.37	2.08-2.80	7.925	Y = 3.141 + 5.535 x
144	0.33	0.22-0.44	1.43	1.22-1.74	7.367	Y = 1.386 + 4.069 x
168	0.20	0.10-0.30	1.18	0.99-1.45	11.785	Y = 2.262 + 4.256 x

The LC_{50} values obtained based on the cumulative percentage mortality at 72, 120 and 168 h after treatment were 2.22×10^8, 0.85×10^8 and 0.20×10^8 spores ml^{-1} respectively. The corresponding LC_{90} values were 3.90×10^8, 2.37×10^8 and 1.18×10^8 spores ml^{-1} at 72, 120 and 168 h after treatment respectively.

4.3.2.1.4 P. latus

The percentage mortality of *P. latus* recorded using different spore concentrations of *B. bassiana* (Bb5a) are given in Table 9.

Mortality of *P. latus* was first noticed at 48 h after inoculation at a spore concentration of 10^8 spores ml^{-1} while it was seen only after 72 h at 10^7, 10^6 and 10^5 spores ml^{-1}. The mortality of *P. latus* ranged from 20 to 80 per cent in different spore concentrations at 168 h after treatment.

The time required for *B. bassiana* (Bb5a) to cause 50 per cent mortality of adult *P. latus* varied among the spore concentrations. At a spore concentration of 10^8 spores ml^{-1} 120.230 h was required to cause 50 per cent mortality to *P. latus* adults. The lowest dose (10^4 spores ml^{-1}) took 221.745 h for 50 per cent mortality of the test population.

As the lethal concentration increased time taken for mortality decreased. The LC_{50} values based on the cumulative percentage mortality at 72, 120 and 168 h were 2.39×10^8, 0.85×10^8 and 0.19×10^8 spores ml^{-1} respectively. The LC_{90} values were 4.29×10^8, 2.12×10^8 and 1.30×10^8 spores ml^{-1} at 72, 120 and 168 h after treatment.

4.3.2.2 B. bassiana (Bb21)

4.3.2.2.1 A. gossypii

The initial mortality of 13.33 per cent was observed in *A. gossypii* at the highest spore concentration of 10^8 spores ml^{-1} (Table 10). The mortality of aphids decreased as the spore concentration decreased. The mortality of second instar nymphs treated with *B. bassiana* (Bb21) at different intervals of time ranged from 13.33 to 96.67 and 0 to 13.33 per cent at a spore concentration of 10^8 and 10^4 spores ml^{-1}.

Table 9. Dose-mortality responses of adults of *Polyphagotarsonemus latus* treated with different spore concentrations of *Beauveria bassiana* (Bb5a)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	48 h	72 h	96 h	120 h	144 h	168 h	
10^8	3.33	16.67	40	53.33	66.67	80	120.230
10^7	0	13.33	26.67	46.67	63.33	73.33	129.925
10^6	0	6.67	10	16.67	30	53.33	166.846
10^5	0	3.33	10	13.33	20	30	199.900
10^4	0	0	3.33	6.67	13.33	20	221.745
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times10^8$ spores ml^{-1})	LC_{90} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times10^8$ spores ml^{-1})	χ^2	Regression equation
48	1.66	1.62-1.71	2.12	2.04-2.25	1.001	$Y = 0.568 + 2.332 x$
72	2.39	2.20-2.58	4.29	3.93-4.83	4.708	$Y = 1.991 + 2.607 x$
96	1.22	1.09-1.35	2.52	2.28-2.89	6.554	$Y = 1.198 + 3.478 x$
120	0.85	0.73-0.98	2.12	1.87-2.47	14.394	$Y = 1.866 + 3.737 x$
144	0.51	0.39-0.63	1.74	1.50-2.09	17.371	$Y = 2.530 + 4.278 x$
168	0.19	0.08-0.30	1.30	1.09-1.61	20.018	$Y = 1.842 + 3.890 x$

Table 10. Dose-mortality responses of nymphs of *Aphis gossypii* treated with different spore concentrations of *Beauveria bassiana* (Bb21)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	6 h	12 h	24 h	30 h	36 h	42 h	
10^8	13.33	26.67	43.33	60	80	96.67	23.477
10^7	0	6.67	20	36.67	53.33	70	34.828
10^6	0	0	10	20	40	53.33	39.954
10^5	0	0	6.67	20	26.67	36.67	45.265
10^4	0	0	0	6.67	10	13.33	57.492
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times 10^8$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times 10^8$ spores ml^{-1})	LC_{90} ($n\times 10^8$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times 10^8$ spores ml^{-1})	χ^2	Regression equation
6	1.32	1.28-1.35	1.68	1.61-1.78	0.937	Y = 1.314 + 4.641 x
12	1.37	1.06-2.15	2.17	1.62-3.65	4.626	Y = 2.219 + 4.072 x
24	1.11	1.01-1.21	2.14	1.94-2.14	5.932	Y = 1.387 + 4.308 x
30	0.75	0.64-0.86	1.86	1.65-2.18	6.443	Y = 3.860 + 6.409 x
36	0.36	0.27-0.45	1.27	1.09-1.53	11.454	Y = 3.509 + 4.750 x
42	0.11	0.53-0.16	0.66	0.55-0.82	16.465	Y = 2.069 + 4.377 x

The LT_{50} value of aphids at a concentration of 10^8 spores ml^{-1} was 23.477 h. At the lowest concentration of 10^4 spores ml^{-1} the time required to the kill of 50 per cent of the test population was 57.492 h respectively.

The LC_{50} values obtained at 6, 30 and 42 h after treatment were 1.32×10^8, 0.75×10^8 and 0.11×10^8 spores ml^{-1} respectively. The LC_{90} values were 1.68×10^8, 1.86×10^8 and 0.66×10^8 spores ml^{-1} at 6, 30 and 42 h after treatment respectively.

4.3.2.2.2 B. tabaci

Mortality of *B. tabaci* initiated 24 h after treatment with a mean mortality of 3.33 per cent at a spore concentration of 10^9 spores ml^{-1}. The mortality percentage ranged from 3.33 to 13.33, 6.67 to 46.67 and 23.33 to 83.333 per cent at 48, 96 and 144 h after treatment (Table 11).

At the highest concentration of 10^9 spores ml^{-1}, the time taken for 50 per cent kill of test insect was 103.765 h and the time increased as the concentration decreased. At a lowest concentration of 10^5 spores ml^{-1} the time required for the kill of 50 per cent of population was 178.733 h respectively.

The LC_{50} values corresponding to the 48, 96 and 144 h after treatment were 2.14×10^9, 1.05×10^9 and 0.13×10^9 spores ml^{-1}. The LC_{90} values based on cumulative percentage mortality were 5.53×10^9, 2.57×10^9 and 1.20×10^9 spores ml^{-1} at 48, 96 and 144 h after treatment.

4.3.2.2.3 S. dorsalis

Mortality of *S. dorsalis* initiated 48 h after inoculation in treatment with highest spore concentration of 10^8 spores ml^{-1}. As the concentration reduced the mortality decreased (Table 12). At 168 h after inoculation the mortality ranged between 16.67 to 83.33 at the spore concentration ranging from 10^8 to 10^4 spores ml^{-1}.

At the highest concentration of 10^8 spores ml^{-1}, the time taken for 50 per cent kill was 121.263 h. At a concentration of 10^4 spores ml^{-1} the time required for the kill of 50 per cent of population is 218.432 h.

Table 11. Dose-mortality responses of adults of *Bemisia tabaci* treated with different spore concentrations of *Beauveria bassiana* (Bb21)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	24 h	48 h	72 h	96 h	120 h	144 h	
10^9	3.33	10	23.33	46.67	60	83.33	103.765
10^8	3.33	13.33	23.33	36.67	50	70	115.889
10^7	0	3.33	13.33	23.33	40	53.33	135.167
10^6	0	0	6.67	13.33	26.67	36.67	155.879
10^5	0	0	3.33	6.67	16.67	23.33	178.733
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times 10^9$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times 10^9$ spores ml^{-1})	LC_{90} ($n\times 10^9$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times 10^9$ spores ml^{-1})	χ^2	Regression equation
24	3.77	3.58-3.96	5.75	5.34-6.31	2.799	$Y = -1.092 + 2.445 x$
48	2.14	2.99-3.44	5.53	5.01-6.19	8.484	$Y = 1.777 + 2.209 x$
72	2.28	2.05-2.52	4.67	4.21-5.35	6.137	$Y = 1.224 + 2.784 x$
96	1.05	0.90-1.20	2.57	2.28-3.00	8.177	$Y = 1.882 + 3.101 x$
120	0.61	0.45-0.78	2.30	1.98-2.78	7.540	$Y = 2.838 + 3.792 x$
144	0.13	0.02-0.23	1.20	0.99-1.50	12.327	$Y = 1.287 + 3.901 x$

Table 12. Dose-mortality responses of nymphs of *Scirtothrips dorsalis* treated with different spore concentrations of *Beauveria bassiana* (Bb21)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	48 h	72 h	96 h	120 h	144 h	168 h	
10^8	6.67	20	30	46.67	66.67	83.33	121.263
10^7	3.33	13.33	26.67	43.33	60	70	133.082
10^6	0	6.67	13.33	26.67	33.33	46.67	168.327
10^5	0	0	6.67	13.33	23.33	26.67	201.452
10^4	0	0	0	6.67	16.67	16.67	218.432
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times10^8$ spores ml^{-1})	LC_{90} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times10^8$ spores ml^{-1})	χ^2	Regression equation
48	2.51	2.38-2.77	3.82	3.57-4.19	2.579	$Y = 1.836 + 2.457 x$
72	1.91	1.77-2.08	3.35	3.07-3.76	6.832	$Y = 1.696 + 2.649 x$
96	1.68	1.50-1.85	3.18	3.14-3.99	7.664	$Y = 1.190 + 2.448 x$
120	1.04	0.87-1.20	2.71	2.39-3.18	11.986	$Y = 2.796 + 4.845 x$
144	0.49	0.36-0.62	1.80	1.55-2.17	12.274	$Y = 2.477 + 3.571 x$
168	0.24	0.15-0.33	1.15	0.97-1.41	18.332	$Y = 2.783 + 4.533 x$

The LC_{50} values corresponding to the 72, 120 and 168 h after treatment were 1.91×10^8, 1.04×10^8 and 0.24×10^8 spores ml^{-1}. The LC_{90} values based on cumulative percentage mortality were 3.35×10^8, 2.71×10^8 and 1.15×10^8 spores ml^{-1} at 72, 120 and 168 h after treatment.

4.3.2.2.4 P. latus

The mortality in adults of *P. latus* initiated at 48 h after treatment (Table 13). The mortality decreased as the spore concentration decreased. The mortality ranged from 0 to 20 and 6.67 to 80 per cent at a spore concentration of 10^4 and 10^8 spores ml^{-1}.

Based on the cumulative per cent mortality LT_{50} value of *P. latus* at a concentration of 10^8 spores ml^{-1} was 128.346 h. At a concentration of 10^4 spores ml^{-1} the time required for the kill of 50 per cent of population was 215.030 h.

The LC_{50} values obtained at 72, 120 and 168 h after treatment were 2.01×10^8, 1.35×10^8 and 0.26×10^8 spores ml^{-1} respectively. The LC_{90} values were 3.62×10^8, 3.35×10^8 and 1.28×10^8 spores ml^{-1} at 72, 120 and 168 h after treatment respectively.

4.3.2.3 L. lecanii (Ll8)

4.3.2.3.1 A. gossypii

The mortality in second instars of *A. gossypii* inoculated with *L. lecanii* (Ll8) was noticed from six h after treatment and the rate of mortality ranged from 0 to 16.67 per cent at the concentration of 10^4 to 10^8 spores ml^{-1} respectively (Table 14). The mortality increased with increase in time and the mortality ranged from 13.33 to 66.67 and 23.33 to 100 per cent at 30 and 42 h after treatments at concentrations ranging from 10^4 to 10^8 spores ml^{-1}.

The minimum time required for the mortality of the 50 per cent of the test insect at a concentration of 10^8 spores ml^{-1} was 20.046 h after treatment and at the lowest concentration 10^4 spores ml^{-1} was 51.170 h after treatment.

At six h after treatment the spore concentration required to kill 50 per cent of the *A. gossypii* was 1.75×10^8 spores ml^{-1} whereas the LC_{50} values obtained for

Table 13. Dose-mortality responses of adults of *Polyphagotarsonemus latus* treated with different spore concentrations of *Beauveria bassiana* (Bb21)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	48 h	72 h	96 h	120 h	144 h	168 h	
10^8	6.67	20	30	40	56.67	80	128.346
10^7	3.33	13.33	23.33	30	46.67	66.67	144.913
10^6	0	6.67	16.67	23.33	36.67	43.33	168.959
10^5	0	3.33	6.67	16.67	23.33	26.67	199.205
10^4	0	0	6.67	10	16.67	20	215.030
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times 10^8$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times 10^8$ spores ml^{-1})	LC_{90} ($n\times 10^8$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times 10^8$ spores ml^{-1})	χ^2	Regression equation
48	2.51	2.38-2.64	3.82	3.57-4.19	2.579	$Y = 1.836 + 2.457\ x$
72	2.01	1.23-8.95	3.62	2.14-17.20	4.570	$Y = 1.611 + 2.431\ x$
96	1.76	1.02-11.75	3.74	2..11-27.05	4.505	$Y = 1.142 + 2.250\ x$
120	1.35	0.79-6.85	3.35	1.93-18.77	3.534	$Y = 2.353 + 4.443\ x$
144	0.72	0.55-0.89	2.45	2.12-2.94	6.528	$Y = 2.776 + 4.305\ x$
168	0.26	0.16-0.36	1.28	1.09-1.57	13.237	$Y = 2.730 + 4.219\ x$

Table 14. Dose-mortality responses of nymphs of *Aphis gossypii* treated with different spore concentrations of *Lecanicillium lecanii* (Ll8)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	6 h	12 h	24 h	30 h	36 h	42 h	
10^8	16.67	36.67	53.33	66.67	86.67	100	20.046
10^7	6.67	13.33	33.33	53.33	73.33	86.67	28.076
10^6	0	0	10	23.33	46.67	53.33	39.060
10^5	0	0	6.67	13.33	26.67	33.33	46.535
10^4	0	0	3.33	13.33	20	23.33	51.170
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times10^8$ spores ml^{-1})	LC_{90} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times10^8$ spores ml^{-1})	χ^2	Regression equation
6	1.75	1.22-3.93	2.76	1.87-6.74	5.002	Y = 2.206+ 3.055 x
12	1.19	1.11-1.27	1.98	1.83-2.20	9.809	Y = 1.930 + 4.778 x
24	0.89	0.80-0.99	1.87	1.69-2.15	11.307	Y = 1.177 + 4.679 x
30	0.58	0.48-0.69	1.65	1.44-1.95	13.497	Y = 2.706 + 4.368 x
36	0.18	0.01-0.27	1.04	0.87-1.28	14.133	Y = 1.902 + 4.429 x
42	0.03	0.01-0.04	0.11	0.08-1.72	3.835	Y = 2.924 + 4.726 x

the 30 and 42 h after treatment were 0.58×10^8 and 0.03×10^8 spores ml^{-1} and the corresponding LC_{90} values obtained were 2.76×10^8, 1.65×10^8 and 0.11×10^8 spores ml^{-1} at 6, 30 and 42 h after treatment respectively (Table 14).

4.3.2.3.2 B. tabaci

The initiation of mortality of *B. tabaci* inoculated with *L. lecanii* (Ll8) was noticed from 24 h after treatment. At a concentration of 10^9 the mortality rate varied from 6.67 to 83.33 per cent at different intervals of time.

The minimum time required for the mortality of the 50 per cent of the test insect at a concentration of 10^9 spores ml^{-1} was 105.172 h after treatment and at the concentration of 10^5 spores ml^{-1} was 183.089 h after treatment (Table 15).

The LC_{50} values obtained at 24, 72 and 144 h after treatment were 2.21×10^9, 1.50×10^9 and 0.19×10^9 spores ml^{-1} respectively. At the above said intervals the LC_{90} values obtained were 4.12×10^9, 1.92×10^9 and 1.19×10^9 spores ml^{-1} respectively.

4.3.2.3.3 S. dorsalis

The mortality of the *S. dorsalis* inoculated with different spore concentrations of *L. lecanii* (Ll8) was noticed from 48 h after treatment and the mortality rate increased with increase in the spore concentration (Table 16). The mortality percentage ranged from 0 to 16.67, 10 to 50 and 20 to 80 per cent at 72, 120 and 168 h after treatment respectively at spore concentrations ranging from 10^4 to 10^8 spores ml^{-1}.

The shortest time span required for the mortality of half the population of test insect was 122.007 h at the highest concentration of 10^8 spores ml^{-1} and the longest duration of 207.142 h was required for the lowest spore concentration of 10^4 spores ml^{-1}.

The LC_{50} values obtained based on the probit analysis of dose- mortality response of *S. dorsalis* on 72, 120 and 168 h after treatment were 2.05×10^8, 0.96×10^8 and 0.25×10^8 spores ml^{-1} respectively. The corresponding LC_{90} values

Table 15. Dose-mortality responses of adults of *Bemisia tabaci* treated with different spore concentrations of *Lecanicillium lecanii* (LI8)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	24 h	48 h	72 h	96 h	120 h	144 h	
10^9	6.67	13.33	20	40	60	83.33	105.172
10^8	0	6.67	16.67	33.33	50	66.67	120.184
10^7	0	3.33	3.33	13.33	30	43.33	149.810
10^6	0	0	3.33	13.33	26.67	36.67	155.879
10^5	0	0	3.33	10	13.33	23.33	183.089
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^9$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times10^9$ spores ml^{-1})	LC_{90} ($n\times10^9$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times10^9$ spores ml^{-1})	χ^2	Regression equation
24	2.21	1.25-30.78	4.12	2.26-61.75	0.786	Y = 1.324 + 4.526 x
48	2.18	1.34-10.88	3.57	2.12-19.18	3.227	Y = 2.009 + 2.353 x
72	1.50	1.45-1.54	1.92	1.84-2.04	4.411	Y = 1.476 + 2.089 x
96	1.28	1.11-1.46	2.97	2.64-3.44	5.814	Y = 1.972 + 2.761 x
120	0.65	0.51-0.80	2.13	1.85-2.55	8.224	Y = 2.563 + 3.223 x
144	0.19	0.09-0.28	1.19	0.99-1.47	9.403	Y = 2.010 + 4.172 x

Table 16. Dose-mortality responses of nymphs of *Scirtothrips dorsalis* treated with different spore concentrations of *Lecanicillium lecanii* (Ll8)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	48h	72 h	96 h	120 h	144 h	168 h	
10^8	10	16.67	33.33	50	63.33	80	122.007
10^7	3.33	13.33	16.67	36.67	56.67	70	138.588
10^6	0	6.67	20	26.67	40	43.33	167.035
10^5	0	0	6.67	13.33	20	26.67	198.769
10^4	0	0	3.33	10	20	20	207.142
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} (spores $ml^{-1} \times 10^8$)	Fiducial limits for LC_{50} ($n \times 10^8$ spores ml^{-1})	LC_{90} ($n \times 10^8$ spores ml^{-1})	Fiducial limits for LC_{90} ($n \times 10^8$ spores ml^{-1})	χ^2	Regression equation
48	2.22	2.05-2.38	3.90	3.58-4.37	2.441	$Y = 2.466 + 4.366 x$
72	2.05	1.34-9.06	3.12	1.95-15.25	6.997	$Y = 1.691 + 2.214 x$
96	1.52	0.96-4.37	3.10	1.93-9.62	5.171	$Y = 1.222 + 2.804 x$
120	0.96	0.81-1.10	2.45	2.16-2.87	6.779	$Y = 1.822 + 3.187 x$
144	0.53	0.38-0.68	2.05	1.76-2.48	10.807	$Y = 1.447 + 3.135 x$
168	0.25	0.15-0.35	1.28	1.08-1.57	15.280	$Y = 2.564 + 4.174 x$

were 3.12×10^8, 2.45×10^8 and 1.28×10^8 spores ml^{-1} at 72, 120 and 168 h after treatment respectively.

4.3.2.3.4 P. latus

The mortality initiation in adults of *P. latus* inoculated with *L. lecanii* (Ll8) was noticed from 48 h after treatment. At 168 h the mortality rate ranged from 16.67 to 76.67 per cent at different concentrations.

The minimum time required for the mortality of the 50 per cent of the test insect at a concentration of 10^8 spores ml^{-1} was 133.315 h and at the lowest concentration of 10^4 spores ml^{-1} required 224.719 h (Table 17).

The corresponding LC_{50} values of *L. lecanii* (Ll8) at 72, 120 and 168 h were 3.38×10^8, 1.38×10^8 and 0.26×10^8 spores ml^{-1}. The LC_{90} values for the above mentioned intervals were 6.21×10^8, 3.42×10^8 and 1.46×10^8 spores ml^{-1} respectively.

4.3.2.4 M. anisopliae (Ma4)

4.3.2.4.1 A. gossypii

Death of aphids inoculated with *M. anisopliae* initiated at 12 h after treatment at the highest concentrations 10^8 (13.33 per cent) and 10^7 spores ml^{-1} (6.67 per cent). The mortality rates obtained at different concentrations are presented in the Table 18. It ranged from 0 to 83.33 per cent at a concentrations ranging from 10^4 to 10^8 spores ml^{-1} at different intervals of time.

The minimum time (29.114 h after treatment) required for 50 per cent kill of the test insect was obtained at a spore concentration of 10^8 spores ml^{-1}. At the lowest dose of 10^4 spores ml^{-1}, 50.275 h was required to achieve 50 per cent kill of *A. gossypii*.

The LC_{50} values at 12, 30 and 42 h after treatments were 1.97×10^8, 0.89×10^8 and 0.16×10^8 spores ml^{-1}, respectively. The LC_{90} values at the corresponding time intervals were 3.11×10^8, 2.09×10^8 and 1.18×10^8 spores ml^{-1} respectively.

Table 17. Dose-mortality responses of adults of *Polyphagotarsonemus latus* treated with different spore concentrations of *Lecanicillium lecanii* (Ll8)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	48 h	72 h	96 h	120 h	144 h	168 h	
10^8	6.67	13.33	20	40	56.67	76.67	133.315
10^7	3.33	13.33	16.67	26.67	36.67	63.33	154.703
10^6	0	10	10	23.33	33.33	50	167.092
10^5	0	0	10	16.67	23.33	33.33	192.145
10^4	0	0	6.67	13.33	16.67	16.67	224.719
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^8$ spores ml^{-1})	Fiducial limit for LC_{50} ($n\times10^8$ spores ml^{-1})	LC_{90} ($n\times10^8$ spores ml^{-1})	Fiducial limit for LC_{90} ($n\times10^8$ spores ml^{-1})	χ^2	Regression equation
48	2.51	2.38-2.64	3.82	3.57-4.19	2.579	Y = 1.836 + 2.457 x
72	3.38	3.10-3.66	6.21	5.67-7.01	5.087	Y = 1.529 + 3.308 x
96	3.15	2.82-3.47	6.48	5.84-7.43	1.423	Y = 1.209 + 3.248 x
120	1.38	0.80-7.77	3.42	1.94-21.36	1.673	Y = 2.305 + 3.865 x
144	0.77	0.47-1.80	2.34	1.49-6.12	3.113	Y = 3.054 + 4.975 x
168	0.26	0.14-0.38	1.46	1.23-1.80	13.138	Y = 2.312 + 3.724 x

Table 18. Dose-mortality responses of nymphs of *Aphis gossypii* treated with different spore concentrations of *Metarhizium anisopliae* (Ma4)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment					LT_{50} (h)
	12 h	24 h	30 h	36 h	42 h	
10^8	13.33	26.67	53.33	73.33	83.33	29.114
10^7	6.67	16.67	33.33	50	70	35.706
10^6	0	6.67	20	36.67	50	40.908
10^5	0	3.33	13.33	20	33.33	47.177
10^4	0	3.33	6.67	23.33	23.33	50.275
Control	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} (n× 10^8 spores ml^{-1})	Fiducial limits for LC_{50} (n×10^8 spores ml^{-1})	LC_{90} (n× 10^8 spores ml^{-1})	Fiducial limits for LC_{90} (n×10^8 spores ml^{-1})	χ^2	Regression equation
12	1.97	1.30-6.20	3.11	1.98-10.64	5.166	Y = 2.200 + 2.637 x
24	1.66	1.08-4.47	3.10	1.97-8.90	4.111	Y = 1.484 + 2.893 x
30	0.89	1.86-2.45	2.09	0.77-3.25	6.130	Y = 1.087 + 3.918 x
36	0.43	0.32-0.52	1.53	1.32-1.84	5.834	Y = 2.499 + 4.145 x
42	0.16	0.06-0.26	1.18	0.99-1.47	12.407	Y = 1.660 + 4.056 x

4.3.2.4.2 B. tabaci

The initial mortality of *B. tabaci* was observed at 48 h after treatment. The highest mean mortality of 76.67 per cent was observed at a concentration of 10^9 spores ml^{-1}. The mortality of *M. anisopliae* (Ma4) treated insects ranged from 20 to 76.67 at 144 h after treatment at a concentration ranging from 10^5 to 10^9 spores ml^{-1}.

The shortest time span recorded for the kill of 50 per cent population of the test insect was 105.371 h at a spore concentration of 10^9 spores ml^{-1}. The longest duration of 176.839 h was required for the lowest spore concentration of 10^5 spores ml^{-1} (Table 19).

A spore concentration of 2.05×10^9 spores ml^{-1} was recorded as the LC_{50} value at 48 h after treatment. The LC_{50} values at 96 and 144 h after treatments were 0.98×10^9 spores ml^{-1} and 0.19×10^9 spores ml^{-1}, respectively. The LC_{90} values on 48, 96 and 144 h after treatment were 3.12×10^9, 2.06×10^9 and 1.04×10^9 spores ml^{-1}, respectively.

4.3.2.4.3 S. dorsalis

The initial mortality of *S. dorsalis* inoculated with different concentrations of *M. anisopliae* (Ma4) was observed at 48 h after treatment. The highest mean mortality of 76.67 per cent was observed at a concentration of 10^8. The mortality of treated insects ranged from 20 to 76.67 at 168 h after treatment (Table 20).

The shortest time span required for the mortality of 50 per cent population of the test insect was 121.016 that was obtained at a spore concentration of 10^8 spores ml^{-1}. The longest duration of 200.660 h was required to kill 50 per cent of test insect at the lowest spore concentration of 10^4 spores ml^{-1}.

A spore concentration of 1.81×10^8 spores ml^{-1} was recorded as the LC_{50} value at 72 h after treatment. The LC_{50} values at 120 and 168 h after treatments were 0.95×10^8 and 0.29×10^8 spores ml^{-1}. The LC_{90} values on 72, 120 and 168 h after treatment were 3.11×10^8, 2.24×10^8 and 1.44×10^8 spores ml^{-1}, respectively.

Table 19. Dose-mortality responses of adults of *Bemisia tabaci* treated with different spore concentrations of *Metarhizium anisopliae* (Ma4)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment					LT_{50} (h)
	48 h	72 h	96 h	120 h	144 h	
10^9	10	23.33	50	60	76.67	105.371
10^8	3.33	10	23.33	46.67	63.33	126.977
10^7	0	3.33	16.67	26.67	43.33	148.241
10^6	0	3.33	10	23.33	26.67	169.800
10^5	0	0	3.33	10	20	176.839
Control	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^9$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times10^9$ spores ml^{-1})	LC_{90} ($n\times10^9$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times10^9$ spores ml^{-1})	χ^2	Regression equation
48	2.05	1.34-9.06	3.12	1.95-15.25	2.441	$Y = 2.466 + 3.270 x$
72	1.66	1.13-3.66	2.85	1.90-6.71	3.090	$Y = 1.783 + 3.198 x$
96	0.98	0.71-1.61	2.06	1.48-3.59	4.544	$Y = 1.158 + 4.243 x$
120	0.57	0.43-0.71	1.99	1.72-2.40	8.715	$Y = 1.666 + 3.587 x$
144	0.19	0.11-0.28	1.04	0.88-1.28	11.656	$Y = 2.869 + 3.976 x$

Table 20. Dose-mortality responses of nymphs of *Scirtothrips dorsalis* treated with different spore concentrations of *Metarhizium anisopliae* (Ma4)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	48 h	72 h	96 h	120 h	144 h	168 h	
10^8	6.67	20	33.33	50	70	76.67	121.016
10^7	3.33	13.33	23.33	36.67	46.67	63.33	145.424
10^6	0	0	10	20	33.33	40	172.401
10^5	0	0	6.67	13.33	26.67	33.33	188.555
10^4	0	0	0	3.33	10	20	200.660
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times10^8$ spores ml^{-1})	LC_{90} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times10^8$ spores ml^{-1})	χ^2	Regression equation
48	2.51	2.38-2.77	3.82	3.57-4.19	2.579	Y = 1.836 + 2.457 x
72	1.81	1.69-1.94	3.11	2.87-3.48	7.724	Y = 1.792 + 2.866 x
96	1.42	1.28-1.55	2.78	2.52-3.16	8.184	Y = 1.331 + 3.215 x
120	0.95	0.83-1.08	2.24	1.99-2.60	9.924	Y = 2.951 + 3.656 x
144	0.51	0.40-0.62	1.61	1.40-1.92	8.266	Y = 2.595 + 4.189 x
168	0.29	0.18-0.40	1.44	1.22-1.76	10.007	Y = 2.680 + 3.886 x

4.3.2.4.4 P. latus

Mortality of *P. latus* inoculated with the highest concentration of *M. anisopliae* was observed from 48 h after treatment and at the lowest spore dose mortality initiated at 96 h after treatment. At different concentrations mortality rate ranged from 3.33per cent to 23.33 per cent and increased to 23.33 to 76.67 at 96 and 168 h after treatment.

The minimum time (137.813 h after treatment) required for 50 per cent kill of the test insect was obtained at a spore concentration of 10^8 spores ml^{-1}. At the lowest dose of 10^4 spores ml^{-1}, 202.955 h was required to achieve 50 per cent kill of *P. latus*.

The LC_{50} values obtained in the probit analysis at 48, 96 and 168 h were 2.51×10^8, 2.06×10^8 and 0.23×10^8 spores ml^{-1} and the corresponding LC_{90} values are 3.82×10^8, 3.40×10^8 and 1.7×10^8 spores ml^{-1} respectively are presented in the Table 21.

4.3.2.5 I. javanica

4.3.2.5.1 B. tabaci

The mortality initiation of *B. tabaci* inoculated with *I. javanica* was observed from 48 h after treatment and the rate of mortality ranged from 0 to 6.67 per cent. The mortality increased as the concentration increased. At the concentration of 10^5, 10^7 and 10^9 the highest mean percentage mortality obtained was 16.67, 46.67 and 93.33 per cent at 144 h after treatment.

The minimum time required for mortality of 50 per cent of the test insect at a concentration of 10^9 spores ml^{-1} was 91.878 h while at the lowest concentration of 10^5 spores ml^{-1} it was 172.394 h.

The LC_{50} values corresponding to the different intervals (48, 96 and 144 h after treatment) were 2.51×10^9, 0.82×10^9 and 0.16×10^9 spores ml^{-1} whereas the LC_{90} values obtained for the 48, 96 and 144 h after treatment were 3.82×10^9, 1.88×10^9 and 0.80×10^9 spores ml^{-1} respectively (Table 22).

Table 21. Dose-mortality responses of adults of *Polyphagotarsonemus latus* treated with different spore concentrations of *Metarhizium anisopliae* (Ma4)

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment						LT_{50} (h)
	48 h	72 h	96 h	120 h	144 h	168 h	
10^8	6.67	13.33	23.33	33.33	60	76.67	137.813
10^7	3.33	6.67	16.67	30	50	66.67	145.294
10^6	0	3.33	13.33	23.33	40	50	159.462
10^5	0	0	3.33	6.67	20	30	189.211
10^4	0	0	3.33	6.67	16.67	23.33	202.955
Control	0	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times10^8$spores ml^{-1})	LC_{90} ($n\times10^8$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times10^8$ spores ml^{-1})	χ^2	Regression equation
48	2.51	2.38-2.64	3.82	3.57-4.19	2.579	Y = 1.836 + 2.457 x
72	2.18	2.04-2.32	3.57	3.31-3.97	3.227	Y = 2.009 + 3.353 x
96	2.06	1.18-19.56	3.40	2.22-40.84	4.585	Y = 1.363 + 2.156 x
120	2.55	2.21-2.88	5.96	5.30-69.21	7.153	Y = 1.958 + 3.312 x
144	0.79	5.85-9.95	2.88	2.48-3.48	10.393	Y = 2.310 + 3.941 x
168	0.23	1.04-3.48	1.7	1.23-1.82	12.065	Y = 1.958 + 3.591 x

Table 22. Dose-mortality responses of adults of *Bemisia tabaci* treated with different spore concentrations of *Isaria javanica*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment					LT_{50} (h)
	48 h	72 h	96 h	120 h	144 h	
10^9	6.67	33.33	56.67	76.67	93.33	91.878
10^8	3.23	20	36.67	60	73.33	112.499
10^7	0	10	23.33	30	46.67	144.961
10^6	0	3.23	10	16.67	23.33	182.250
10^5	0	0	0	6.67	16.67	172.394
Control	0	0	0	0	0	

Probit analysis

Hours after treatment	LC_{50} ($n\times10^9$ spores ml^{-1})	Fiducial limits for LC_{50} ($n\times10^9$ spores ml^{-1})	LC_{90} ($n\times10^9$ spores ml^{-1})	Fiducial limits for LC_{90} ($n\times10^9$ spores ml^{-1})	χ^2	Regression equation
48	2.51	2.38-2.64	3.82	3.58-4.19	2.579	$Y = 1.836 + 2.457 x$
72	1.38	1.26-1.50	2.61	2.37-2.96	7.725	$Y = 1.437 + 3.496 x$
96	0.82	0.72-0.92	1.88	1.68-2.18	13.582	$Y = 1.991 + 4.392 x$
120	0.44	0.35-0.52	1.32	1.15-1.56	19.329	$Y = 2.641 + 5.004 x$
144	0.16	0.10-0.23	0.80	0.68-0.98	18.918	$Y = 2.750 + 4.936 x$

4.4. DEVELOPMENT OF TALC BASED PRODUCT OF FUNGI

The data on mean spore count, colony forming units and bioefficacy of the talc based products of the fungi are presented in Tables 23 to 26.

4.4.1 Talc Based Product of *B. bassiana* (Bb5a)

4.4.1.1 Spore Count and cfu

At 15 Days After Storage (DAS the spore count was 6.51×10^8 spores ml^{-1}. This was decreased to 6.08×10^8 spores ml^{-1} at 90 DAS. However that decrease in spore load was not significantly lowered (Table 23) even after three months of storage.

The highest number of colonies (129.19×10^6 colonies g^{-1}) was observed at 15 DAS. As the period of storage increased from 30 to 60 and 90 days, the cfus observed was 124.66, 103.65 and 66.24×10^6 colonies g^{-1} which were not significantly different.

4.4.1.2 Bioefficacy

The data on bioefficacy talc based *B. bassiana* (Bb5a) was evaluated against *A. gossypii* at fortnightly intervals are presented in the Table 23. The highest mortality of test insect was attained at 15 DAS with a mortality of 93.28 per cent and was found to be par with those at 30 and 45 DAS. The mean per cent mortality of aphids at 60 DAS was 82.17 per cent which was on par with 75 and 90 DAS with a mortality of 77.68 and 75.56 percent respectively.

4.4.2 Talc Based Product of *B. bassiana* (21)

4.4.2.1 Spore Count and cfu

The shelf life of talc based product of *B. bassiana* (Bb21) was assessed at fortnight intervals and its spore count and cfu are given in the Table 24. The spore count of 30.25×10^8 spores ml^{-1} was observed at 15 DAS and it declined to 16.14×10^8 spores ml^{-1} at 90 DAS, but the spore count was not significantly lowered over these days.

Table 23. Mean spore count and cfu in talc based product of *Beauveria bassiana* (Bb5a) at different intervals after storage and its effect on mortality of *Aphis gossypii*

Days after storage	cfu (10^6)	Spore count (n × 10^8 spores ml^{-1})	Mortality (%)
15	129.19 (11.41)	6.51 (2.74)	93.28 (9.71)
30	124.66 (11.21)	5.92 (2.63)	86.61 (9.36)
45	127.82 (11.35)	4.24 (2.29)	84.38 (9.24)
60	103.65 (10.23)	5.30 (2.51)	82.17 (9.12)
75	86.98 (9.38)	5.60 (2.57)	77.68 (8.87)
90	66.24 (8.20)	6.08 (2.66)	75.56 (8.75)
CD (0.05)	(1.25)	NS	(0.48)

Figures within parentheses are $\sqrt{x+1}$ transformed values

Table 24. Mean spore count and cfu in talc based product of *Beauveria bassiana* (Bb21) at different intervals after storage and its effect on mortality of *Aphis gossypii*

Days after storage	cfu (10^6)	Spore count (n × 10^8 spores ml^{-1})	Mortality (%)
15 DAS	142.52 (11.98)	30.25 (5.59)	90.97 (9.59)
30 DAS	131.25 (11.50)	25.21 (5.12)	86.61 (9.36)
45DAS	130.33 (11.46)	19.88 (4.57)	84.38 (9.24)
60 DAS	118.68 (10.94)	20.34 (4.62)	84.38 (9.24)
75 DAS	102.02 (10.15)	21.75 (4.77)	75.56 (8.75)
90 DAS	96.61 (9.88)	16.14 (4.14)	77.68 (8.87)
CD (0.05)	(0.82)	NS	(0.50)

Figures within parentheses are $\sqrt{x+1}$ values

The highest number of colonies (142.52×10^6 colonies g^{-1}) was observed at 15 DAS and this was on par with the cfu at 30 and 45 DAS with mean number of 131.25×10^6 and 130.33×10^6 colonies g^{-1}. At 60 days the cfu observed was significantly reduced to 118.68×10^6 colonies g^{-1} and was on par with that observed at 75 DAS (102.02×10^6 colonies g^{-1}). At 90 DAS a mean number of 66.24×10^6 colonies g^{-1} was observed.

4.4.2.2 Bioefficacy

The data on bioefficacy of stored talc based product of *B. bassiana* (Bb21) was evaluated on *A. gossypii* at fortnightly intervals and are presented in the Table 24. The highest mortality of test insect was achieved at 15 DAS with a mortality of 90.97 per cent and was statistically on par at 30, 45 and 60 DAS with a mean per cent mortality of 86.61, 84.38 and 84.38 per cent respectively and hence forth this shows the efficacy of product with good mortality at longer period of time. Even after 90 DAS a mean mortality of 77.68 percent was observed in *B. bassiana* (Bb21).

4.4.3 Talc Based Product of *L. lecanii* (Ll8)

4.4.3.1 Spore Count and cfu

The mean spore count of 3.33×10^8 spores ml^{-1} observed at 15 DAS and 3.37×10^8 spores ml^{-1} at 90 DAS. The spore count observed did not significantly vary even after 90 DAS and the data are presented in the Table 25.

The viability of spores when observed at fortnight intervals a mean number of 84.75 and 81.26×10^6 colonies g^{-1} was observed at 15 and 30 DAS respectively. As the storage days increased reduction in number of colonies was observed. At 60 DAS 68.89×10^6 colonies g^{-1} was observed and it was on par with that on 90 DAS with a mean number of 65.26×10^6 colonies g^{-1} respectively.

4.4.3.2 Bioefficacy

The highest mortality of 97.80 per cent was observed at 15 DAS and which was on par with 30 and 45 DAS with a mortality of 95.43 and 90.97 per cent

Table 25. Mean spore count and cfu in talc based product of *Lecanicillium lecanii* (Ll8) at different intervals after storage and its effect on mortality of *Aphis gossypii.*

Days after storage	cfu (10^6)	Spore count (n × 10^8 spores ml^{-1})	Mortality (%)
15 DAS	84.75 (9.26)	3.33 (2.08)	97.80 (9.94)
30 DAS	81.26 (9.07)	4.43 (2.33)	95.43 (9.82)
45DAS	72.62 (8.58)	4.15 (2.27)	90.97 (9.59)
60 DAS	68.89 (8.36)	3.58 (2.14)	88.87 (9.48)
75 DAS	68.89 (8.36)	3.00 (2.00)	82.17 (9.12)
90 DAS	65.26 (8.14)	3.37 (2.09)	79.82 (8.99)
CD (0.05)	(0.56)	NS	(0.42)

Figures within parentheses are $\sqrt{x+1}$ values,

DAS- days after storage

respectively. The mortality of the test insect reduced as the storage days prolonged. At 60 DAS 88.87 per cent mortality of test insect was observed which was found to be on par with 75 and 90 DAS with a mortality of 82.17 per cent and 79.82 per cent respectively (Table 25).

4.4.4 Talc Based Product of *M. anisopliae* (Ma4)

4.4.4.1 Spore Count and cfu

The spore counts taken at different intervals ranged from 5.35 to 2.61×10^8 spores ml^{-1}. The spore count assessed at fortnight intervals to check the shelf life of talc based product was statistically not significant and are presented in the table (Table 26).

The highest mean number of colonies 70.40×10^6 g^{-1} was observed at 15 DAS which was on par with the 30 and 45 DAS with a mean number of 64.45×10^6 colonies g^{-1} and 56.61×10^6 colonies g^{-1}. The viable spores reduced after 45 DAS and the data are presented in the Table 26. At 60 DAS the mean number of 44.70×10^6 colonies g^{-1} was observed and was on par with 75 DAS. The lowest number of 26.46×10^6 colonies g^{-1} was observed at 90 DAS.

4.4.4.2 Bioefficacy

The bioefficacy of talc based product of *M. anisopliae* (Ma4) against the test insect *A. gossypii* obtained at different intervals are presented in the Table 26. At 15 DAS a highest mean mortality of 86.61per cent was observed and this was on par with that stored for 30 and 45 DAS with a corresponding mortality of 82.17 per cent and 79.82 per cent. At 60 days after storage 73.30 per cent of mortality was observed in the test insect and was on par with that on 75 and 90 DAS with a mortality of 68.89 and 66.57 per cent.

4.5. COMPATABILITY OF FUNGI WITH PESTICIDES

The entomopathogenic fungi were tested for its compatibility with pesticides. It was assessed based on the radial growth of mycelia and spore count. The data on compatibility are presented in Tables 27 to 30.

Table 26. Mean spore count and cfu in talc based product of *Metarhizium anisopliae* (Ma4) at different intervals after storage and its effect on mortality of *Aphis gossypii*

Days after storage	cfu (10^6)	Spore count (n × 10^8 spores ml^{-1})	Mortality (%)
15 DAS	70.40 (8.45)	5.35 (2.52)	86.61 (9.36)
30 DAS	64.45 (8.09)	4.34 (2.31)	82.17 (9.12)
45DAS	56.61 (7.59)	5.50 (2.55)	79.82 (8.99)
60 DAS	44.70 (6.76)	4.76 (2.40)	73.30 (8.62)
75 DAS	30.92 (5.65)	2.69 (1.92)	68.89 (8.36)
90 DAS	26.46 (5.24)	2.61 (1.90)	66.57 (8.22)
CD (0.05)	(1.27)	NS	(0.59)

Figures within parentheses are $\sqrt{x+1}$ values

4.5.1 Compatibility of *B. bassiana* (Bb5a)

Among the different pesticides tested at 7 Days After Inoculation (DAI) acetamiprid recorded the highest radial growth of 1.50 cm and was found to be on par with emamectin benzoate 0.002 per cent (1.30 cm) and spiromesifen 0.02 per cent (1.33 cm). At 14 DAI spiromesifen 0.02 per cent recorded the highest radial growth and was on par with emamectin benzoate 0.002 and acetamiprid 0.004 per cent. Out of four pesticides, emamectin benzoate 0.002 per cent was found to be least inhibitory having the highest radial growth of 3.50 cm at 21 DAI. This was on par with those of acetamiprid 0.004 per cent and spiromesifen 0.02 per cent (3.23 and 3.03 cm respectively) (Plate 11a). Dimethoate 0.06 per cent was highly inhibitory to *B. bassiana* (Bb5a) with a mean radial growth of 0.17cm, 0.25cm and 0.40cm in diameter at 7, 14 and 21 DAI. At different intervals of time 7, 14 and 21 DAI the mean radial growth observed was highest in control with a diameter of 2.93cm, 5.03cm and 6.85cm respectively.

The spore count of the fungus treated with different pesticides were significantly lower when compared to untreated control. At 21 days after inoculation the spore count in untreated media was 40.86×10^8 spores ml^{-1}. It was less in emamectin benzoate 0.002 per cent treated media (4.76×10^8 spores ml^{-1}) which was on par with acetamiprid 0.004 per cent (3.58×10^8 spores ml^{-1}) and spiromesifen 0.02 per cent (3.33×10^8 spores ml^{-1}). Least sporulation was observed in media poisoned with dimethoate 0.06 per cent.

4.5.2 Compatibility of *B. bassiana* (Bb21)

The compatibility of *B. bassiana* (Bb21) with different pesticides, its radial growth and spore count are presented in the Table 28. The four pesticides tested had significant effect on the growth of *B. bassiana* (Bb21). At 7 and 14 DAI the highest radial growth of 1.65 and 2.75 cm was observed respectively in media mixed with emamectin benzoate. This was found to be on par with that of spiromesifen 0.02 per cent. The treatment spiromesifen 0.02 per cent was having lesser inhibition of *B. bassiana* (Bb21), the mean growth being 4.38 at 21 DAI.

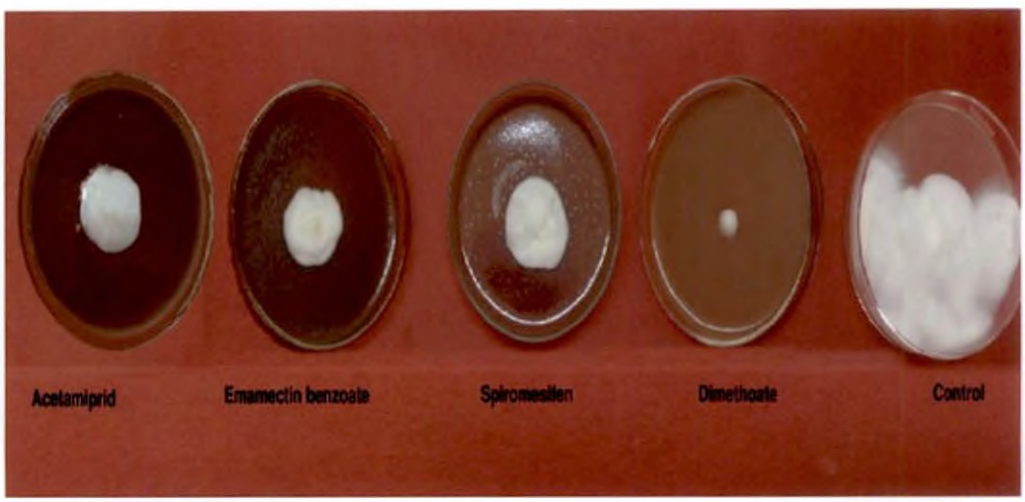


Plate 11a. Radial growth of *Beauveria bassiana* (Bb5a) in pesticide poisoned media at 21 DAI

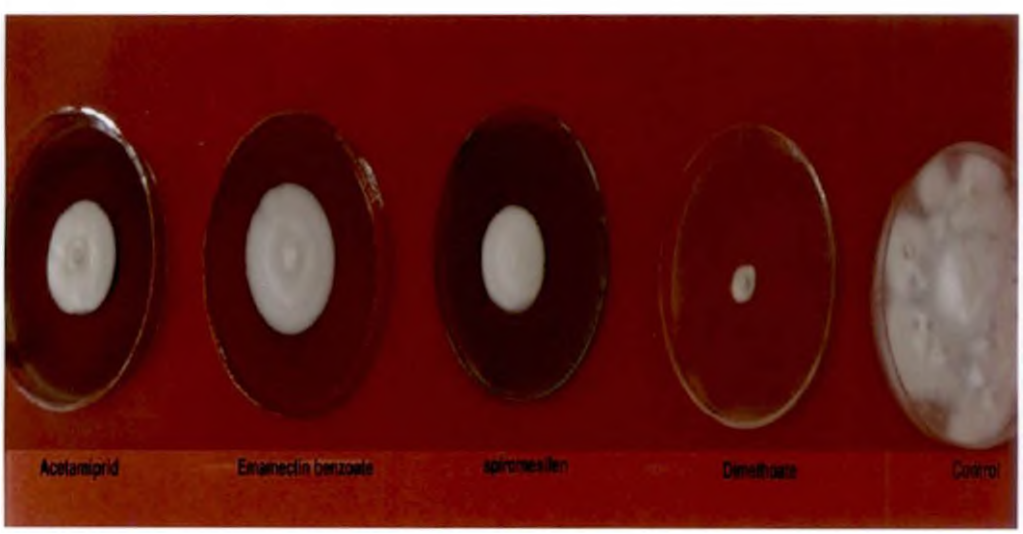


Plate 11b. Radial growth of *Beauveria bassiana* (Bb21) in pesticide poisoned media at 21 DAI

Table 27. Compatibility of *Beauveria bassiana* (Bb5a) with different pesticides

Treatments	Radial growth of fungus at different intervals after inoculation (cm)			Spore count at 21 DAI ($n \times 10^8$ spores ml^{-1})
	7 DAI	14 DAI	21 DAI	
Acetamiprid (0.004%)	1.50	2.38	3.23	3.58 (2.14)
Emamectin benzoate (0.002%)	1.30	2.80	3.50	4.76 (2.40)
Spiromesifen (0.02%)	1.33	2.38	3.03	3.33 (2.08)
Dimethoate (0.06%)	0.25	0.40	0.60	0.17 (1.08)
Control	2.93	5.03	6.85	40.86 (6.47)
CD (0.05)	0.45	0.56	0.78	(0.55)

Figures within parentheses are $\sqrt{x+1}$ values,

DAI- days after inoculation

Table28. Compatibility of *Beauveria bassiana* (Bb21) with different pesticides

Treatments	Radial growth of fungus at different intervals after inoculation (cm)			Spore count at 21 DAI ($n \times 10^8$ spores ml^{-1})
	7 DAI	14 DAI	21 DAI	
Acetamiprid (0.004%)	1.18	2.30	3.20	5.35 (2.52)
Emamectin benzoate (0.002%)	1.65	2.75	3.90	6.67 (2.77)
Spiromesifen (0.02%)	1.45	2.68	4.38	7.01 (2.83)
Dimethoate (0.06%)	0.58	1.03	1.28	0.19 (1.09)
Control	2.85	3.75	7.10	91.93 (9.64)
CD (0.05)	0.42	0.51	0.74	(0.21)

Figures within parentheses are $\sqrt{x+1}$ values,

DAI- days after inoculation

This was observed to be on par with that of emamectin benzoate 0.002 per cent (3.9 cm) (Plate 11b). Lowest growth of the fungus was observed in the case of dimethoate 0.06 percent (1.28cm). In control, the mean radial growth of *B. bassiana* (Bb21) was 7.10cm at 21 DAI.

Based on the spore count assessed at 21 DAI, the treatment with spiromesifen 0.02 per cent was found to be best among the different pesticides evaluated with a mean spore count 7.01×10^8 spores ml^{-1} and it was on par with emamectin benzoate 0.002 per cent (6.67×10^8 spores ml^{-1}). A mean spore count of 5.35×10^8 spores ml^{-1} was observed in case of acetamiprid 0.004 per cent. In the treatment with dimethoate 0.06 per cent spore count of 0.19×10^8 spores ml^{-1} was observed. The highest mean spore count was observed in control (91.93×10^8 spores ml^{-1}).

4.5.3 Compatibility of *L. lecanii* (Ll8)

The results indicated that the pesticides inhibited the mycelial growth and sporulation of the fungus. Initially on 7 and 14 DAI the radial growth in emamectin benzoate poisoned media recorded the highest growth (1.95 and 2.80 cm respectively) but it eventually reduced. Significant lower growth of 1.53 and 2.23 cm was recorded in spiromesifen 0.02 per cent at 7 and 14 DAI respectively. The mycelial growth on media poisoned with acetamiprid 0.004 per cent (3.40 cm) was on par with those of emamectin benzoate 0.002 per cent (3.33 cm) and spiromesifen 0.02 per cent (3.03 cm) (Plate 12a). Dimethoate was highly inhibitory to *L. lecanii* (Ll8) and there was no growth at all. The mean radial growth observed in control was 6.35cm.

The spore count of the fungus grown in media mixed with pesticide and untreated control was having significant difference (Table 29). Acetamiprid was observed to have 4.81×10^8 spores ml^{-1} which was on par with those of emamectin benzoate and spiromesifen mixed media (4.52×10^8 and 3.93×10^8 spores ml^{-1} respectively). Whereas, in untreated media the spore count was 10.16×10^8 spores ml^{-1}.

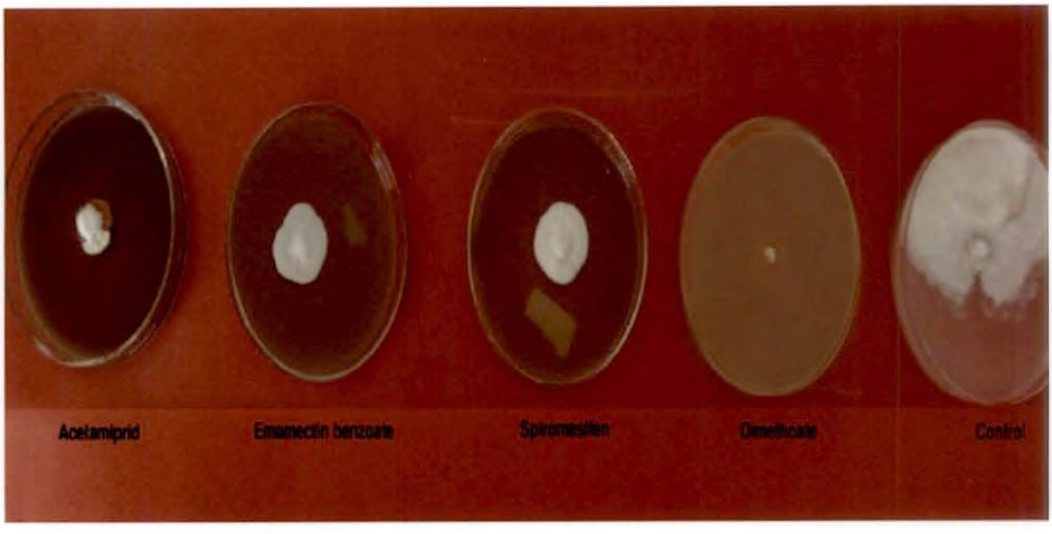


Plate 12a. Radial growth of *Lecanicillium lecanii* (Ll8) in pesticide poisoned media at 21 DAI

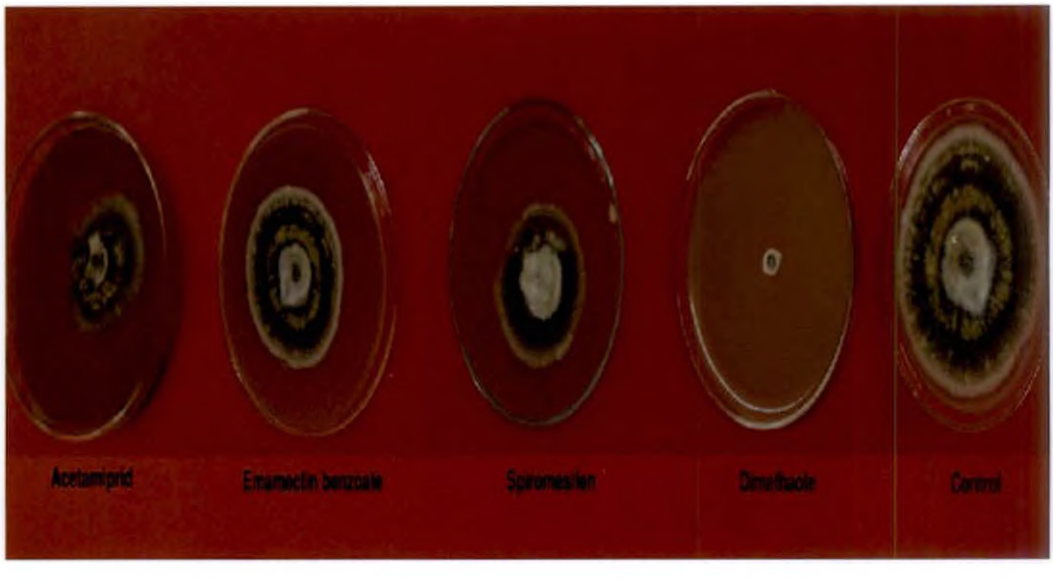


Plate 12b. Radial growth of *Metarhizium anisopliae* (Ma4) in pesticide poisoned media at 21 DAI

Table 29. Compatibility of *Lecanicillium lecanii* (Ll8) with different pesticides

Treatments	Radial growth of fungus at different intervals after inoculation (cm)			Spore count at 21 DAI ($n \times 10^8$ spores ml^{-1})
	7 DAI	14 DAI	21 DAI	
Acetamiprid (0.004%)	1.13	2.28	3.40	4.81 (2.41)
Emamectin benzoate (0.002%)	1.95	2.80	3.33	4.52 (2.35)
Spiromesifen (0.02%)	1.53	2.23	3.03	3.93 (2.22)
Dimethoate (0.06%)	0.00	0.00	0.00	0.00 (1.00)
Control	2.65	4.20	6.35	10.16 (3.34)
CD (0.05)	0.33	0.53	0.52	(0.16)

Figures within parentheses are $\sqrt{x+1}$ values,

DAI- days after inoculation

Table 30. Compatibility of *Metarhizium anisopliae* (Ma4) with different pesticides

Treatments	Radial growth of fungus at different intervals after inoculation (cm)			Spore count at 21 DAI ($n \times 10^8$ spores ml^{-1})
	7 DAI	14 DAI	21 DAI	
Acetamiprid (0.004%)	1.80	3.53	5.03	0.32 (1.15)
Emamectin benzoate (0.002%)	1.90	3.33	5.08	0.66 (1.29)
Spiromesifen (0.02%)	1.78	3.30	4.53	0.21 (1.10)
Dimethoate (0.06%)	0.00	0.00	0.00	0.00 (1.00)
Control	2.58	5.30	7.53	1.66 (1.63)
CD (0.05)	0.20	0.46	0.49	(0.08)

Figures within parentheses are $\sqrt{x+1}$ values,

DAI- days after inoculation

4.5.4 Compatibility of *M. anisopliae* (Ma4)

The radial growth at different intervals and spore count of *M. anisopliae* (Ma4) are presented in Table 30. Radial growth of 1.9 cm was observed at 7 DAI in emamectin benzoate 0.002 per cent and was found to be on par with acetamiprid (1.80 cm) and spiromesifen (1.78 cm). A similar trend of growth was seen at 14 DAI but here acetamiprid 0.004 per cent (3.53 cm) recorded the highest growth. In emamectin benzoate the mean radial growth at 21 DAI was 5.08cm and it was on par with 5.03cm growth obtained in acetamiprid poisoned media. In spiromesifen, a mean radial growth of 4.53cm in diameter was observed at 21 DAI (Plate 12b). Dimethoate was having a still higher inhibition on *M. anisopliae* (Ma4) and it completely inhibited the growth of fungi. The highest mean radial growth of *M. anisopliae* (Ma4) was observed in case of control with a mean radial growth of diameter 7.53cm at 21 DAI.

The treatment with emamectin benzoate was found to be less inhibitive to sporulation of *M. anisopliae* (Ma4) among the pesticides with a mean spore count 0.66×10^{8} spores ml^{-1}. The spore count in other treatments were significantly lower. A mean spore count of 0.32×10^{8} spores ml^{-1} was observed in the case of acetamiprid and it was on par with spiromesifen which supported a mean spore count of 0.21×10^{8} spores ml^{-1}. At 21 DAI, the highest mean spore count was observed in control (1.66×10^{8} spores ml^{-1}).

4.6. FIELD EXPERIMENT

Field experiment conducted to evaluate the effectiveness of fungal pathogens and newer molecules of pesticides in the management of pests of chilli are presented in Tables 31 to 37.

4.6.1 Population of Pests

4.6.1.1 P. latus

The population of *P. latus* recorded in the treated plots at different intervals of time are presented in Table 31. Pretreatment count of *P. latus* showed no

significant difference in various plots. Third day after treatment acetamiprid 20 g a.i. ha^{-1} treated plots recorded the lowest mite population of 0.42 five $leaves^{-1}$ was found to be the best treatment and was significantly superior to others. Next best treatment was spiromesifen 100 ml a.i. ha^{-1} with a mean population of 0.90 mites five $leaves^{-1}$. Significantly lower reduction of mites was recorded in the treatments with emamectin benzoate 10 g a.i. ha^{-1}, dimethoate 100 ml a.i. ha^{-1}, *B. bassiana* (Bb5a) and *L. lecanii* (Ll8) with the mean population of 1.22, 2.06, 3.80 and 4.38 mites five $leaves^{-1}$ respectively. The population levels in treatments *L. lecanii* (Llm) and untreated control plots were significantly higher than other treatments.

On 7^{th} day after treatment the lowest mite population was observed in acetamiprid 20 g a.i. ha^{-1} treated plot with a mean population of 0.12 mites five $leaves^{-1}$ and it was on par with 0.28 mites five $leaves^{-1}$ in spiromesifen 100 ml a.i. ha^{-1} treatment. A significantly higher population of 1.16 mites five $leaves^{-1}$ was observed in emamectin benzoate 10 g a.i. ha^{-1} treatment and it was on par with *B. bassiana* (Bb5a) (1.28 mites five $leaves^{-1}$). In dimethoate 300 ml a.i. ha^{-1} the mean population of 1.43 mites five $leaves^{-1}$ was observed and was significantly better than the treatments *L. lecanii* (Ll8), *M. anisopliae* (Ma4), *B. bassiana* (Bb21) the mite population recorded were 1.82, 3.04 and 2.65 mites five $leaves^{-1}$. However the populations in these treatments were significantly lower than those in the treatment *L. lecanii* (Llm) and untreated control.

On 14^{th} day the lowest pest population (0.74, 0.88 and 1.04 mites five $leaves^{-1}$) was recorded in acetamiprid 20 g a.i. ha^{-1}, *B. bassiana* (Bb5a) and spiromesifen 100 ml a.i. ha^{-1} treated plots and these were on par. In treatments *L. lecanii* (Ll8) and emamectin benzoate 1.25 mites five $leaves^{-1}$ was observed and were on par with *B. bassiana* (Bb21) (1.62 mites five $leaves^{-1}$). *L. lecanii* (Llm) treated and untreated plot population levels were significantly higher with a mean population of 11.96 and 11.89 mites five $leaves^{-1}$ respectively.

The treatment *B. bassiana* (Bb5a) recorded the lowest pest population (1.82 mites five $leaves^{-1}$) on 21^{st} day after treatment and the treatments *L. lecanii* (Ll8)

Table 31. Mean population of *Polyphagotarsonemus latus* five leaves^{-1} in different treatments on chilli under field conditions

Treatment	Dosage	Number of *P. latus* five leaves $^{-1}$			
		3 DAT	7 DAT	14 DAT	21 DAT
Talc based product of *B. bassiana* (Bb5a)	20g l^{-1}	3.80 (2.19)	1.28 (1.51)	0.88 (1.37)	1.82 (1.68)
Talc based product of *L. lecanii* (Ll8)	20g l^{-1}	4.38 (2.32)	1.82 (1.68)	1.25 (1.5)	1.96 (1.72)
Talc based product of *M. anisopliae* (Ma4)	20g l^{-1}	5.50 (2.55)	3.04 (2.01)	2.1 (1.76)	2.69 (1.92)
Indigenous isolate of *B. bassiana* (21)	20g l^{-1}	5.40 (2.53)	2.65 (1.91)	1.62 (1.62)	2.57 (1.89)
Indigenous isolate of *L. lecanii* (Llm)	20g l^{-1}	10.83 (3.44)	13.21 (3.77)	11.96 (3.6)	15.81 (4.1)
Emamectin benzoate 5 SG	10 g a.i ha^{-1}	1.22 (1.49)	1.16 (1.47)	1.25 (1.5)	2.88 (1.97)
Acetamiprid 20 SP	20 g a.i ha^{-1}	0.42 (1.19)	0.12 (1.06)	0.74 (1.32)	2.24 (1.8)
Spiromesifen 240 SC	100 g a.i ha^{-1}	0.90 (1.38)	0.28 (1.13)	1.04 (1.43)	2.53 (1.88)
Dimethoate 30 EC	300 g a.i ha^{-1}	2.06 (1.75)	1.43 (1.56)	2.28 (1.81)	4.52 (2.35)
Untreated	Water spray	10.83 (3.44)	13.67 (3.83)	11.89 (3.59)	15.97 (4.12)
CD (0.05)		(0.09)	(0.08)	(0.14)	(0.15)

Figures within the parentheses are $\sqrt{x+1}$ values,

DAT- days after treatment

and acetamiprid 20 g a.i. ha^{-1} with a mean population of 1.96 and 2.24 mites five $leaves^{-1}$ were on par.

The treatments spiromesifen 100 ml a.i. ha^{-1}, *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and emamectin benzoate 10 g a.i. ha^{-1} were on par with a mean population of 2.53, 2.57, 2.69 and 2.88 ranged from mites five $leaves^{-1}$.

Overall observations on population of mites revealed that the synthetic pesticides acetamiprid 20 g a.i. ha^{-1}, spiromesifen 100 ml a.i. ha^{-1}, emamectin benzoate 10 g a.i. ha^{-1} and dimethoate 300 ml a.i. ha^{-1} were effective in controlling the mite population within a shorter period of seven days but the population of *P. latus* was in an increasing phase seven days after treatment.

The population of the *P. latus* was in a decreasing trend in *B. bassiana* (Bb5a), *L. lecanii* (Ll8), *B. bassiana* (Bb21) and *M. anisopliae* (Ma4) at a long run period. However the treatments *B. bassiana* (Bb5a) and *L. lecanii* (Ll8) were statistically on par with acetamiprid 10 g a.i. ha^{-1} and spiromesifen 100 ml a.i. ha^{-1} at 14 days after treatment.

4.6.1.2 S. dorsalis

The data on the population of *S. dorsalis* is presented in Table 32. The pretreatment population count of *S. dorsalis* was not significant. Third day after treatment, the lowest mean population of 0.66 five $leaves^{-1}$ was observed in plots treated with acetamiprid 10 g a.i. ha^{-1} and in spiromesifen 100 ml a.i. ha^{-1} a mean population of 0.85 thrips five $leaves^{-1}$ was observed and these were on par. The treatments, emamectin benzoate 10 g a.i. ha^{-1} and dimethoate 300 ml a.i. ha^{-1} were on par with populations of 1.4 and 1.79 thrips five $leaves^{-1}$ respectively. In *B. bassiana* (Bb21), *B. bassiana* (Bb5a), *L. lecanii* (Ll8) and *M. anisopliae* (Ma4) the mean population of 3.41, 3.58, 3.75 and 4.11 thrips five $leaves^{-1}$ was observed. The population levels were higher in *L. lecanii* (Llm) and untreated control plots (4.57 and 5.15 thrips five $leaves^{-1}$ respectively).

The lowest thrips population on seventh day after treatment was recorded in spiromesifen 100 ml a.i. ha^{-1} and acetamiprid 10 g a.i. ha^{-1} treated plot with a mean population of 0.12 and 0.44 thrips five $leaves^{-1}$ and were on par. A mean

Table 32. Mean population of *Scirtothrips dorsalis* five leaves^{-1} in different treatments on chilli under field conditions

Treatment	Dosage	Number of *S. dorsalis* five leaves $^{-1}$			
		3 DAT	7 DAT	14 DAT	21 DAT
Talc based product of *B. bassiana* (Bb5a)	20g l^{-1}	3.58 (2.14)	0.96 (1.4)	0.82 (1.35)	1.72 (1.65)
Talc based product of *L. lecanii* (Ll8)	20g l^{-1}	3.75 (2.18)	1.56 (1.6)	1.07 (1.44)	1.86 (1.69)
Talc based product of *M. anisopliae* (Ma4)	20g l^{-1}	4.11 (2.26)	1.92 (1.71)	1.34 (1.53)	2.20 (1.79)
Indigenous isolate of *B. bassiana* (Bb21)	20g l^{-1}	3.41 (2.1)	1.79 (1.67)	1.28 (1.51)	2.13 (1.77)
Indigenous isolate of *L. lecanii* (Llm)	20g l^{-1}	4.57 (2.36)	7.41 (2.9)	4.86 (2.42)	8.67 (3.11)
Emamectin benzoate 5 SG	10 g a.i ha^{-1}	1.4 (1.55)	0.93 (1.39)	0.88 (1.37)	2.35 (1.83)
Acetamiprid 20 SP	20 g a.i ha^{-1}	0.66 (1.29)	0.44 (1.2)	0.42 (1.19)	1.37 (1.54)
Spiromesifen 240 SC	100 ml a.i ha^{-1}	0.85 (1.36)	0.12 (1.06)	0.32 (1.15)	1.40 (1.55)
Dimethoate 30 EC	300 ml a.i ha^{-1}	1.79 (1.67)	1.25 (1.5)	0.90 (1.38)	1.99 (1.73)
Untreated	Water spray	5.15 (2.48)	9.76 (3.28)	6.02 (2.65)	9.30 (3.21)
CD (0.05)		(0.18)	(0.24)	(0.18)	(0.12)

Figures within the parentheses are $\sqrt{x+1}$ values,

DAT- days after treatment

population of 0.93 thrips five leaves^{-1} was observed in emamectin benzoate 10 g a.i. ha^{-1} and was on par with *B. bassiana* (Bb5a), dimethoate 300 ml a.i. ha^{-1} and *L. lecanii* (Ll8) with a population of 0.96, 1.25 and 1.56 thrips five leaves^{-1}.

On 14th day, the lowest pest population (0.42 and 0.32 thrips five leaves^{-1}) was recorded in acetamiprid 10 g a.i. ha^{-1} and spiromesifen 100 ml a.i. ha^{-1} treated plots and were on par. The treatments *B. bassiana* (Bb5a), *L. lecanii* (Ll8), *M. anisopliae* (Ma4), *B. bassiana* (Bb21), emamectin benzoate and dimethoate were statistically on par and the mean population was 0.82, 1.07, 1.34, 1.28, 0.88 and 0.90 five leaves^{-1} respectively. In *L. lecanii* (Llm) treated and untreated control plots, population levels were significantly higher with mean populations of 4.86 and 6.02 thrips five leaves^{-1} respectively.

The treatment acetamiprid recorded the lowest pest population (0.32 thrips five leaves^{-1}) on 21st day. The plots treated with spiromesifen and *B. bassiana* (Bb5a) were on par the mean population being 1.96 and 1.72 thrips five leaves^{-1}. The treatments *L. lecanii* (Ll8), *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and dimethoate were on par with mean populations 1.86, 2.20, 2.13 and 1.99 thrips five leaves^{-1} respectively. The populations were significantly higher in plots treated with *L. lecanii* (Llm) and untreated control.

4.6.1.2 Other Pests

The population of aphids, *A. gossypii* (0.03 to 0.07 five leaves^{-1}) and whitefly, *B. tabaci* (0.04 to 0.11 five leaves^{-1}) were very low during the crop period and were observed in only a few plants (Table 33). There was no fruit borer infestation in the field throughout the period of observation.

4.6.2 Leaf Curl Incidence

An assessment of the chilli leaf curl symptom based on five grades revealed that there was no leaf curl incidence in plants treated with *B. bassiana* (Bb5a) 20 g l^{-1}, *L. lecanii* (Ll8) 20 g l^{-1}, emamectin benzoate 10 g a.i. ha-1, acetamiprid 20g a.i. ha^{-1} and spiromesifen 100 ml a.i. ha^{-1} 15 DAT while *B. bassiana* (Bb21) 20 g l^{-1} and *M. anisopliae* (Ma4) 20 g l^{-1} and dimethoate 300 ml a.i. ha^{-1} treated plants

Table 33. Mean population of *Aphis gossypii* and *Bemisia tabaci* five leaves^{-1} in different treatments on chilli under field conditions

Treatment	Dosage	Number of *A. gossypii* five leaves $^{-1}$			Number of *B. tabaci* five leaves $^{-1}$		
		7 DAT	14 DAT	21 DAT	7 DAT	14 DAT	21 DAT
Talc based product of *B. bassiana* (Bb5a)	20g l^{-1}	0	0	0	0	0.04	0.04
Talc based product of *L. lecanii* (Ll8)	20g l^{-1}	0	0.03	0	0.04	0.04	0.07
Talc based product of *M. anisopliae* (Ma4)	20g l^{-1}	0.07	0.03	0.07	0.04	0	0.07
Indigenous isolate of *B. bassiana* (Bb21)	20g l^{-1}	0.04	0.07	0.04	0.04	0.04	0.07
Indigenous isolate of *L0. lecanii* (Llm)	20g l^{-1}	0.04	0.07	0.04	0.04	0.04	0.07
Emamectin benzoate 5 SG	10 g a.i ha^{-1}	0	0	0	0	0	0.07
Acetamiprid 20 SP	20 g a.i ha^{-1}	0	0	0	0	0	0.04
Spiromesifen 240 SC	100 ml a.i ha^{-1}	0	0	0	0	0	0.07
Dimethoate 30 EC	300 ml a.i ha^{-1}	0	0.03	0.07	0	0.04	0.07
Untreated	Water spray	0.07	0.03	0.07	0.07	0.04	0.07

Table 34. Leaf curl incidence in chilli at different intervals after treatment

Treatment	Dosage	Leaf curl incidence (score)		
		60 DAT	90 DAT	120 DAT
Talc based product of *B. bassiana* (Bb5a)	20g l^{-1}	0	0	0
Talc based product of *L. lecanii* (Ll8)	20g l^{-1}	0	0	0
Talc based product of *M. anisopliae* (Ma4)	20g l^{-1}	1	0	0
Indigenous isolate of *B. bassiana* (Bb21)	20g l^{-1}	1	0	0
Indigenous isolate of *L. lecanii* (Llm)	20g l^{-1}	2	2	1
Emamectin benzoate 5 SG	10 g a.i ha^{-1}	0	0	0
Acetamiprid 20 SP	20 g a.i ha^{-1}	0	0	0
Spiromesifen 240 SC	100 ml a.i ha^{-1}	0	0	0
Dimethoate 30 EC	300 ml a.i ha^{-1}	1	1	0
Untreated	Water spray	2	2	1

were in the grade 1 i.e. they were observed to have the leaf curl damage ranging from 1 to 25 per cent and the scores are presented in the Table 34. The treatment *L. lecanii* (Llm) and control recorded the maximum leaf curl index with score 2 i.e. plants observed were having the leaf curl damage ranging from 25 to 50 per cent. In the subsequent observations at 90 and 120 days after transplanting there was no further increase in the leaf curl symptom either in the treatments or in control.

4.6.3 Population of Natural Enemies

The natural enemy population was also recorded in the field along with the pest population. The data on number of spiders present in the different treatments are presented in the Table 35.

4.6.3.1 Spiders Plant $^{-1}$

The population densities of spiders prior to treatment and after treatments were taken. The pre treatment count was statistically insignificant. The post treatment count taken at different intervals after treatment are presented in the Table 35.

On the third day after treatment the population of spiders were significantly different in the treated plots. Spiders were completely absent in dimethoate 300 ml a.i. ha^{-1} treated plots and it was on par with acetamiprid 20 g a.i. ha^{-1} treated plot with a mean population of 0.06 spiders plant^{-1}. The spider population were unaffected by the application of entomopathogenic fungi *viz. B. bassiana* (Bb5a), *L. lecanii* (Ll8), *M. anisopliae* (Ma4) and *B. bassiana* (Bb21).

A similar trend was noticed on seventh and fourteenth day after treatment in all the treatments except in acetamiprid 20 g a.i. ha^{-1} treated plots where the spider populations was increased and found to be on par with that of emamectin benzoate after seventh day.

Dimethoate was observed to have an adverse effect on spider population. In acetamiprid 20 g a.i. ha^{-1} treated plots though the population was very low initially eventually the population increased. The highest number of population

Table 35. Mean population of spiders plant^{-1} in different treatments in chilli under field conditions

Treatment	Dosage g a.i.ha^{-1}	Spiders count plant^{-1}			
		3 DAT	7 DAT	14 DAT	21 DAT
Talc based product of *B. bassiana* (Bb5a)	20g l^{-1}	1.13 (1.46)	1.79 (1.67)	2.20 (1.79)	1.79 (1.67)
Talc based product of *L. lecanii* (Ll8)	20g l^{-1}	1.31 (1.52)	1.99 (1.73)	2.28 (1.81)	1.72 (1.65)
Talc based product of *M. anisopliae* (Ma4)	20g l^{-1}	1.53 (1.59)	1.86 (1.69)	2.35 (1.83)	1.86 (1.69)
Indigenous isolate of *B. bassiana* (Bb21)	20g l^{-1}	1.46 (1.57)	1.99 (1.73)	2.31 (1.82)	1.72 (1.65)
Indigenous isolate of *L. lecanii* (Llm)	20g l^{-1}	1.92 (1.71)	2.61 (1.90)	2.61 (1.90)	2.46 (1.86)
Emamectin benzoate 5 SG	10 g a.i ha^{-1}	0.32 (1.15)	0.74 (1.32)	1.72 (1.65)	1.66 (1.63)
Acetamiprid 20 SP	20 g a.i ha^{-1}	0.06 (1.03)	0.54 (1.24)	1.66 (1.63)	1.72 (1.65)
Spiromesifen 240 SC	100 ml a.i ha^{-1}	0.46 (1.21)	0.93 (1.39)	1.99 (1.73)	1.59 (1.61)
Dimethoate 30 EC	300 ml a.i ha^{-1}	0.00 (1.00)	0.00 (1.00)	0.59 (1.26)	0.93 (1.39)
Untreated	Water spray	1.86 (1.69)	2.80 (1.95)	2.80 (1.95)	2.46 (1.86)
CD (0.05)		(0.12)	(0.09)	(0.11)	(0.10)

Figures within the parentheses are $\sqrt{x+1}$ values,

DAT- days after treatment

was observed in treatment *L. lecanii* (Llm), untreated control and *M. anisopliae* (Ma4) with a mean population of 1.92, 1.86 and 1.53 spiders plant^{-1}.

4.6.3.2 Other Natural Enemies

The population of other natural enemies *viz.,* predatory mites and coccinellid predators were meagre and it ranged from 0.06 to 0.13 plant^{-1}.

4.6.4 Yield

The data on number of fruits harvested at different intervals after treatment are given in the Table 36. The total yield plot^{-1} and benefit cost ratio calculated are presented in the Table 37.

4.6.4.1 Number of Fruits

The maximum number of fruits was obtained from acetamiprid 20 g a.i. ha^{-1} treated plots followed by spiromesifen 100 ml a.i. ha^{-1} treated plots. The mean fruit number at 60, 90 and 120 days after treatment in both the treatments were 28.7, 26.14, 18.89 fruits plant^{-1} in acetamiprid 10 g a.i. ha^{-1} and 27.09, 24.70 and 19.16 fruits plant^{-1} in spiromesifen100 ml a.i. ha^{-1} respectively.

At 60 and 90 days after transplanting the treatments, emamectin benzoate and *B. bassiana* (Bb5a) were on par with a mean number of 22.23, 23.50 and 22.62, 23.11 fruits plant^{-1}. The lowest number of fruits was recorded in *L. lecanii* (Llm) treated and untreated control plot at all the intervals.

In the treatments *L. lecanii* (Ll8), *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and dimethoate 300 ml a.i. ha^{-1} the mean number of fruits at 60 days after treatment were 18.10, 17.23, 16.64 and 12.62 fruits plant^{-1}. At 90 days 21.56, 21.09, 21.37 and 20.90 fruits plant^{-1} respectively were obtained.

At 120 days treatments emamectin benzoate, *M. anisopliae* (Ma4), *B. bassiana* (Bb5a) and dimethoate were found to be on par with mean number of 17.58, 17.40, 16.98 and 16.64 fruits plant^{-1} respectively.

Table 36. Mean number of fruits plant^{-1} at different intervals after planting in the different treatments in chilli

Treatment	Dosage g a.i.ha^{-1}	Number of fruits plant^{-1}		
		60 DAT	90 DAT	120 DAT
Talc based product of *B. bassiana* (Bb5a)	20g l^{-1}	22.62 (4.86)	23.11 (4.91)	16.98 (4.24)
Talc based product of *L. lecanii* (Ll8)	20g l^{-1}	18.10 (4.37)	21.56 (4.75)	16.14 (4.14)
Talc based product of *M. anisopliae* (Ma4)	20g l^{-1}	16.64 (4.2)	21.37 (4.73)	17.40 (4.29)
Indigenous isolate of *B. bassiana* (Bb21)	20g l^{-1}	17.23 (4.27)	21.09 (4.70)	15.81 (4.10)
Indigenous isolate of *L. lecanii* (Llm)	20g l^{-1}	10.29 (3.36)	15.16 (4.02)	10.36 (3.37)
Emamectin benzoate 5 SG	10 g a.i ha^{-1}	22.23 (4.82)	23.50 (4.95)	17.58 (4.31)
Acetamiprid 20 SP	20 g a.i ha^{-1}	28.70 (5.45)	26.14 (5.21)	18.89 (4.46)
Spiromesifen 240 SC	100 g a.i ha^{-1}	27.09 (5.3)	24.70 (5.07)	19.16 (4.49)
Dimethoate 30 EC	300 g a.i ha^{-1}	12.62 (3.69)	20.90 (4.68)	16.64 (4.20)
Untreated	Water spray	8.99 (3.16)	14.13 (3.89)	9.43 (3.23)
CD (0.05)		(0.18)	(0.16)	(0.13)

Figures within the parentheses are $\sqrt{x+1}$ values,

DAT- days after treatment

4.6.4.2 Benefit Cost Ratio

Among the fungal pathogens evaluated *B. bassiana* (Bb5a) recorded the highest yield. This was followed by *L. lecanii* (Ll8), *B. bassiana* (Bb21) and *M. anisopliae* (Ma4) the total mean yield of 4.93, 4.9 and 4.87 kg chilli per 7.29m^2 plots respectively. The treatments except *L. lecanii* (Llm) resulted in significantly higher yield when compared to control plot (2.75 kg). Acetamiprid 20 g a.i. ha^{-1} (6.24 kg) and spiromesifen 100 ml a.i. ha^{-1} (6.07 kg) recorded the highest compared to other treatments. A significant lower yield of 5.59 and 5.52 kg was recorded from treatments emamectin benzoate and *B. bassiana* (Bb5a) and were on par with each other. Among the pesticides used dimethoate 300 ml a.i. ha^{-1} treated plot recorded the lowest mean yield of 4.52 kg but it was significantly superior than control.

The data on benefit cost ratio (Table 37) revealed that *B. bassiana* (Bb5a) gave the maximum returns with a benefit cost ratio 2.62. The next economical treatment was from acetamiprid 20 g a.i. ha^{-1}, *L. lecanii* (Ll8) @ 20g l^{-1}, *B. bassiana* (Bb21) @ 20g l^{-1}, *M. anisopliae* (Ma4) @ 20g l^{-1} and spiromesifen 100 g a.i. ha^{-1} which gave a B: C ratios of 2.22, 2.15, 2.12, 2.10 and 2.07 respectively. In emamectin benzoate10 g a.i. ha^{-1} and dimethoate 300 ml a.i. ha^{-1} the B: C ratios were 1.75 and 1.10 respectively.

Table 37. Yield and Benefit: Cost (B: C) ratio of different treatments in the field experiment on chilli

Treatment	Mean yield (kg 7.29 m^2 $plot^{-1}$)	B:C ratio
Talc based product of *B. bassiana* (Bb5a)	5.52	2.62
Talc based product of *L. lecanii* (Ll8)	4.93	2.15
Talc based product of *M. anisopliae* (Ma4)	4.87	2.10
Indigenous isolate of *B. bassiana* (Bb21)	4.90	2.12
Indigenous isolate of *L. lecanii* (Llm)	3.12	0.73
Emamectin benzoate 5 SG	5.59	1.75
Acetamiprid 20 SP	6.24	2.22
Spiromesifen 240 SC	6.07	2.07
Dimethoate 30 EC	4.52	1.10
Untreated	2.75	
CD (0.05)	0.321	

Discussion

5. DISCUSSION

Ecological impact of chemical pesticides has long captured the attention of scientists world over and ways and means to counter the adversities through the identification of alternatives to the chemicals are being sought. The recent issues bombarded in Kerala, consequent to the widespread application of insecticides for pest management triggered actions from the Government too in the promotion of biocontrol, one of the safer alternatives for pest management. Of the different groups of bioagents, entomopathogenic fungi is now the cynosure of researchers in view of their efficacy, safety to non targets, easiness in mass production and the rising demand for mycopesticides. Among the entomopathogenic fungi, the white muscardine fungus *Beauveria bassiana* (Balsamo), the green muscardine fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin and the white halo fungus *Lecanicillium lecanii* Zare and Gams are in the foreground (Butt *et al.*, 2001; Roberts and St.Legar, 2004; Wang *et al.*, 2004; Chavan and Kadam, 2009).

The existence of different isolates within a species of these fungal pathogens is now evident (Roberts and Humber, 1981) and it is imperative that we identify these isolates, select the right one against the pest and that befits the desired ecosystems. To achieve the targeted benefits of these fungi it is inevitable to contemplate various aspects of these fungi such as their virulence, effective dose, suitability to the ecosystems and compatibility with other management tools.

Non availability of formulations with good shelf life often constraints the utilization of fungi based pesticides for pest management. Though pragmatic research on entomopathogenic fungi could bring forth formulations with good shelf life, patent related issues often make the technologies unravel for production by others. This demands further research in this line.

It is worth remembering the fact that to steer clear of chemical pesticides in the immediate future is not possible, henceforth, continuous selection of effective and less hazardous pesticides preferably from the newer molecules that are compatible with other pest management tools such as the entomopathogens is important.

The effect displayed by these microbial agents in the laboratories need not be reiterated in the field. This necessitates assessment of their field performance also.

Chilli (*Capsicum annuum* L.) is an important vegetable grown in Kerala for domestic consumption as well as for export. Cultivation of the crop is often hampered by the infestation of a multitude of pests of which the major pests are the mite, *Polyphagotarsonemus latus* Banks (Sudharma, 1996; Varghese, 2011) thrips, *Scirtpthrips dorsalis* Hood (Ananthakrishnan, 1993; Vasundarajan, 1994; Kumar, 1995), white fly, *Bemisia tabaci* Gennadius (Byrne and Bellows,1991; Saad *et al.,* 2013) and aphid, *Aphis gossypii* Glover (Nair, 1998). In extreme infestation of the mites and thrips complete failure of the crop is not uncommon (Borah, 1987), *B. tabaci* is a polyphagous pest. Besides chilli, it infests over 500 different plant species in many tropical and subtropical cropping systems (Greathead, 1986). The indirect injury by way of vectoring plant viruses in addition to the removal of sap by the whitefly is vast. Yet another role played by *B. tabaci* is its phoretic relationship (Soroker *et al.*, 2003) with the broad mite *P. latus* which further aggravates the injury. *A. gossypii* on its own can cause enough damage to reduce the productivity of chilli up to 25 per cent (Miles, 1987). It also serves as a vector of many different viruses and can transmit 76 viral diseases (Satar *et al.*, 1999).

Conventional pesticides still form the major tool for tackling these pest problems in chilli. The downside of such chemical protection, problems of pesticide residues in chilli (Awasthi *et al.*, 2001; Dhotre *et al.*, 2001) and resurgence of pests, with conventional insecticides (David, 1991) and even with the newer insecticide imidacloprid (Srinivasalu *et al.*, 2002) underlines the need for development of alternative technologies for pest management.

Considering the importance of entomopathogenic fungi in the current scenario of pest management and the importance of safe pest management in chilli, the present project entitled "Evaluation of entomopathogenic fungi against pest complex of chilli (*Capsicum annuum* L.) was slated with the objectives: To isolate and identify indigenous strains of entomopathogenic fungi, assess the

pathogenicity of *B. bassiana*, *L. lecanii*, *M. anisopliae* and indigenous isolates against chilli pests, fix LC_{50}, LC_{90} and LT_{50} values through bioassay, develop products of fungi, determine the compatibility of the fungi with pesticides and to evaluate the fungal pathogens and newer molecules of pesticides in the field.

Initially, monitoring was done at monthly intervals in twenty five chilli fields in Thiruvananthapuram District during 2013 to 2014 to collect dead insects and mites. The method of isolation of entomopathogenic fungi from cadavers of chilli pests was followed even though other isolation techniques of fungi exists because of the probability of getting more virulent isolates of the pests. Two fungi *viz. Fusarium solani* (Mart.) Sacc (Hypocreales : Nectriaceae) and *Penicillium oxalicum* Currie and Thom (Eurotiales : Trichocomaceae) were isolated from *A. gossypii*. Another fungus *Isaria javanica* (Friederichs & Bally) Brown & Smith (Hypocreales : Cordycipitaceae) and an yeast *Meyerozyma carribica.* (Vaughan-Martini, Kurtzman, S.A. Meyer & E.B. O'Neill) Kurtzman & M. Suzuki (Saccharomycetales : Debaryomycetaceae) were isolated from *B. tabaci*. In India, the present reports on the pathogenicity of these fungi on their respective hosts are new. No reports are there on the pathogenicity of *M. carribica* to *B. tabaci* from any of the places in India or abroad.

The morphological characters of the newly isolated fungi were studied in the laboratory. For further identification molecular characterization of the isolate was done through ITS sequencing of the fungal DNA using partial sequencing of 18 S ribosomal RNA gene (ITS-1), 28 S ribosomal RNA gene and complete sequence of 5.8 ribosomal RNA gene (ITS -2). Subsequently through NCBI BLAST results the fungus *F. solani* isolated from *A. gossypiii* was found to have 100 per cent similarity to *F. solani* isolate RSPG-229 having the accession number KC478532.1. The isolate also showed 100 per cent similarity with *Fusarium* sp. having the accession number AF178402.1.

Fusarium spp. as entmopathogens are evident from the earlier reports of Hareendranath, 1989; Anitha 2000; Mikunthan and Manjunatha, 2006).

The other fungal isolate identified, *P. oxalicum* from *A. gossypii* on molecular characterization and further NCBI BLAST showed cent per cent alignment with *Penicillium* sp. PTN19 and *P. oxalicum* strain 114-1 having the accession numbers KF656713.1 and KF 152942.1 respectively.

During a survey on the pathogenic fungi of insect pests in Kerala, Kuruvila *et al.* (1980) and Fang and Tan (1986) in China, isolated *P. oxalicum* from the cicadellid, *Cofana spectra* (Dist.) and the aphid *Ceratovacuna lanigera* Zehntner respectively.

I. javanica, the fungus isolated from *B. tabaci* was previously known as *Paecilomyces javanicus* (Friedrichs & Bally) A.H.S. Br. & G. Sm as well as *Spicaria javanica* Bally). Molecular characterization and basic local alignment search adopting NCBI BLAST done for *I. javanica* revealed that the isolate was 100 per cent similar to *I. javanica* strain RSIj006.af with the accession number KF373690.1. The isolate also showed cent per cent alignment with *Isaria fumosorosea* isolate NLUC (FJ765015.1) and *I. fumosorosea* NLHG-2 (FJ765013.1). Internal transcribed spacer (ITS) regions 1TS1 AND ITS 2 (ITS1-5.8-ITS2) sequences have been used to characterize *Isaria* isolates from Argentina, Mexico and Brazil as well as to study phylogenetic relationships among these isolates and other related fungi from Hypocreales.

The occurrence of *I. javanica,* known as grey muscardine was recorded earlier on *Lonomia obliqua* Walker (Lepidoptera: Saturniidae) caterpillars by Shimazu and Takatsuka (2010) and on *Trialeurodes vaporarium* by Scorosetti *et al.*, (2008).

Genetic analysis of the yeast *M. caribbica* showed that the isolate had cent per cent alignment with *M. caribbica* strain CDFA887 having the accession number JX886024.1. Moreover, it also showed cent per cent alignment with *Pichia caribbica* isolate CNRMA 200500808 (EU568999.1) and *Pichia guilliermondi* strain HK58-2 (EF197951.1)

Though there are no reports on the infectivity of *M. caribbica* to insects, there are earlier reports on the ability of *M. caribbica* to control post harvest diseases of fruit crops (Daniel *et al.*, 2009). The present identification of *M. caribbica* as an entomopathogen reveals the dual biological properties of *M. caribbica.* Such dual properties has been demonstrated in the case of *V. lecanii* and *B. bassiana ie.* they are natural enemies of pests as well as plant pathogens (Goettel *et al.* 2008; Bonnie *et al.*, 2009). Even though *M. caribbica* possess dual biocontrol properties, for the exploitation of this yeast in biological control programmes, further in depth studies are required.

Pathogenicity, the qualitative ability of a pathogen to cause disease is determined by the physiology of the host and the pathogen. Among the arthropod pathogens, fungal pathogens relatively have wide host range. Species such as *B. bassiana* and *M. anisopliae* have numerous hosts spanning several orders in the class Insecta. It is now recognized that *B. bassiana* and *M. anisopliae* contain a diverse assemblage of genotypes and probably comprise species complex and it is probable that within these taxa individual isolates can exhibit a substantially restricted host range (Inglis *et al.* 2001). Literature pertaining to the pathogenicity of these fungi against insects and mite pests of chilli are scanty. Hence in the present studies pathogenicity of three fungal pathogens *M. anisopliae* (Ma4), *B. bassiana* (Bb5a), *L. lecanii* (Ll8) obtained from National Bureau of Agriculturally Important Insects (NBAII), Bengaluru and two fungal pathogens *viz. B. bassiana* (Bb21) and *L. lecanii* (Llm) obtained from Department of Microbiology, College of Agriculture, Vellayani was assessed against the three major insects *viz. A. gossypii, B. tabaci* and *S. dorsalis* and the mite, *P. latus.* The pathogenicity was assessed against the second instar nymphs of *A. gossypii* and *S. dorsalis* whereas in the case of *B. tabaci* and *P. latus* the effect was tested against the adults. The results revealed that all the fungi except *L. lecanii* (Llm) were pathogenic to all the chilli pests tested.

The development of symptom due to the infection of *B. bassiana* (Bb5a) and *B. bassiana* (Bb21) were more or less similar in *A. gossypii, B. tabaci, S. dorsalis* and *P. latus.* The treated *A. gossypii* were initially active but later they

became sluggish. Feeding was greatly reduced. 24 h after treatment death of aphids was noticed. Those alive were inactive. Abdomen of the cadavers shrunk and white puffy mycelia growth appeared over the body. Mycelial growth completely covered the body three to four days after infection. Though the symptom development by the two isolates of *B. bassiana* in adult *B. tabaci* and *P. latus* was similar to that in *A. gossypii*, the death occurred only 48 h after treatment and later the body was covered with white mycelial growth. The present observation agrees with that of Pena *et al.*, (1996) who observed that the broad mite, *P. latus* developed symptoms of infection by *B. bassiana* two days after treatment when sprayed with conidial suspension of the fungus. Nugroho and Ibrahim (2004) reported that the broad mite entered the state of moribund two days after treatment and fungal spores of *B. bassiana* appeared on the surface of the cadaver four to five days after treatment.

The insects *viz. A. gossypii, B. tabaci* and *S. dorsalis* and the mite, *P. latus* treated with *L. lecanii* (Ll8) exhibited symptoms similar to that of the symptoms in *B. bassiana* infection. However, comparatively an early infection was noticed in *A. gossypii*. The period for attaining mortality in *L. lecanii* (Ll8) treated *P. latus* was also longer as in *B. bassiana* infection. The isolate *L. lecanii* (Llm) neither caused symptoms of infection nor mortality in all the insects and mite tested.

The sequence of development of symptoms in *M. anisopliae* infected test insects and mite were similar to that observed for *B. bassiana* but a slower action compared to *B. bassiana* was evident. The mycelial growth on the cadaver though white in the early stage of infection turned greenish black later and in the case of *B. tabaci* mycelia and spores clumped and the entire mass erupted between the wings. Similar symptom development consequent to *M. anisopliae* infection was reported by Herlinda, (2010).

An important consideration in selecting a fungal strain for biological control programme is its virulence which is the quantitative amount of disease that a pathogen can incite in a group of insects (Inglis *et al.*, 2001). In order to gather information on the virulence of the different fungal isolates they were tested at a

known concentration of 1 x 10^8 spores ml^{-1} against the chilli pests. *L. lecanii* (Ll8) that caused 33.33and 99.97 per cent mortality at 24 and 48 h after treatment respectively to *A. gossypii* was the most virulent among the different isolates and it was significantly superior to other isolates (Fig 1). This was followed by *B. bassiana* (Bb5a), *B. bassiana* (Bb21), *M. anisopliae* (Ma4), *F. solani* and *P. oxalicum* with a mean mortality of 73.44, 66.71, 57.31, 19.30 and 4.75 per cent at 48 h after treatment. Cent per cent mortality of *A. gossypii* by *L. lecanii* previously known as *Verticillium lecanii* @ 10^6 - 10^7 spores ml^{-1} was highlighted in the studies of Yokomi and Gottwald (1988). The higher virulence of *L. lecanii* compared to *B. bassiana* and *M. anisopliae* was evident from an earlier study conducted by Saranya *et al.*, (2010) against cowpea aphid, *Aphis craccivora* Koch. After a lapse of 120 h, the effect of *B. bassiana* (Bb5a) came on par with *L. lecanii* (Ll8) in the present study. Similar observations were made by Ekesi, (2000) and Saranya *et al.*, (2010) during their investigations using the aphid, *A. craccivora*. These results indicate a stable and reproducible virulent character of *L. lecanii* and *B. bassiana* to aphid species in the laboratory. *M. anisopliae* (Ma4) and the indigenous isolates, *F. solani* and *P. oxalicum* were significantly less virulent. The mortality caused was 76.78 and 70.30and 63.37 per cent respectively. The isolate *L. lecanii* (Llm) was ineffective.

The effect of NBAII isolates *L. lecanii* (Ll8), *B. bassiana* (Bb5a) and *M. anisopliae* (Ma4) to *B. tabaci* @10^8 spores ml^{-1} were on par with the new indigenous white fly isolate *I. javanica* at 48 h after treatment. However, the significantly superior performance of *I. javanica* was indicated from the cumulative mortality of 83.61 per cent (Fig 2) observed 144 h after treatment compared to 73 .44, 63.37 and 66.71 per cent in *B.bassiana* (Bb5a), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8). Previously, Eyal *et al.* (1994) found that *B. bassiana* @ 4×10^6 conidia ml^{-1} caused 98 per cent mortality of *B. tabaci*. The performance of the yeast *M. caribbica* though a white fly isolate was only moderate as evident from the cumulative mortality of 39.83 per cent. The isolate *L. lecanii* (Llm) was not pathogenic to *B .tabaci*.

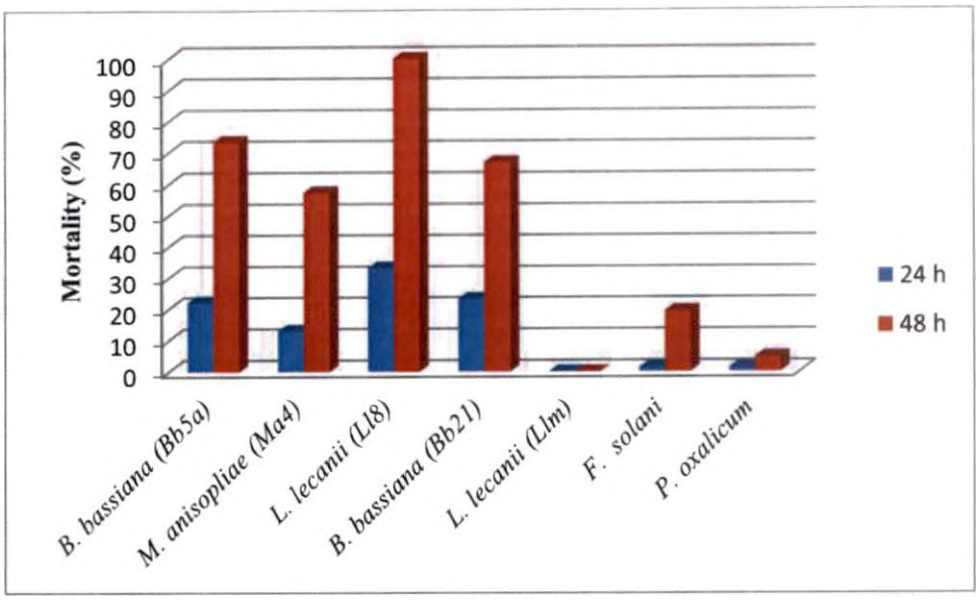


Fig. 1. Mortality of *Aphis gossypii* at different intervals on treatment with fungal pathogens

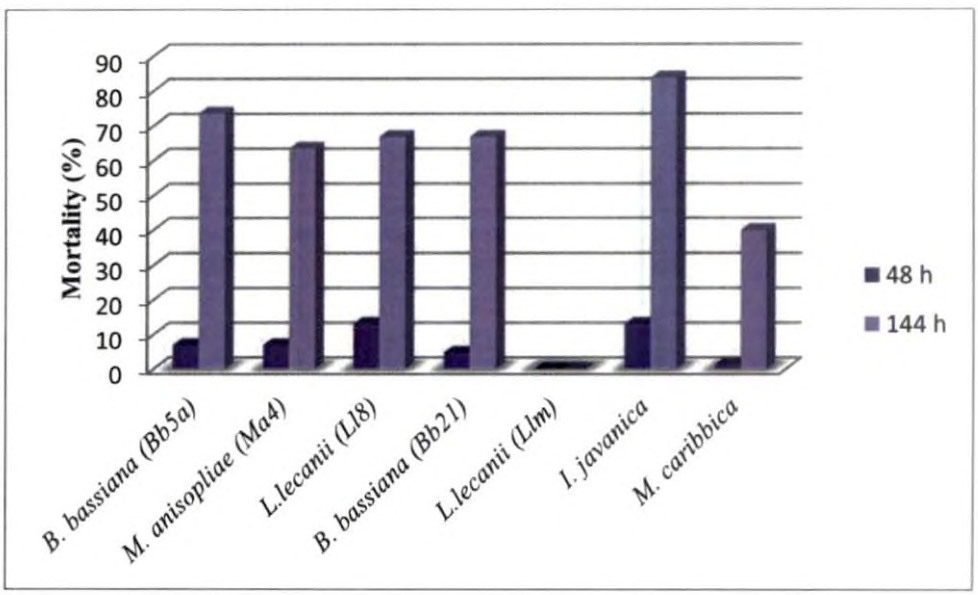


Fig. 2. Mortality of *Bemisia tabaci* at different intervals on treatment with fungal pathogens

The virulence of the fungal isolates tested against the thrips, *S. dorsalis* revealed that the isolates *L. lecanii* (Ll8), *B. bassiana* (Bb21), *B. bassiana* (Bb5a) and *M. anisopliae* (Ma4) were equally pathogenic to *S. dorsalis* (Fig 3). *L. lecanii* (Llm) was not pathogenic to *S. dorsalis*. The infectivity of fungal pathogens to chilli thrips has been documented by Arthurs *et al.* (2013). In their studies it was seen that the performance of *B. bassiana* was better than *Metarhizium.*

Assessment of the virulence of the fungal isolates against the mite, *P. latus* revealed that the isolates *viz. L. lecanii* (Ll8), *B. bassiana* (B21), *B. bassiana* (Bb5a) and *M. anisopliae* (Ma4) were on par, the maximum mortality of 80.65 was seen in *L. lecanii* (Ll8). Though the initial mortality at 48 h was low (4.7 to 9.9) with extended period of infection the mortality rates also increased (Fig 4). The isolate *L. lecanii* (Llm) was not pathogenic. The findings of Nugroho and Ibrahim (2004) was slightly different, another isolate of *B. bassiana*, BbGc which caused 80.88 per cent mortality to *P. latus* was more virulent than *M. anisopliae* isolate MaPs that caused 60 per cent mortality. A perusal of the comparative performance of the isolates to the three test insects and mite showed that .the virulence varied with pests. A shorter period was taken by all the isolates to produce mortality in *A. gossypii* compared to *B. tabaci*, *S. dorsalis* and *P. latus*.

Selection of virulent genotypes has obvious consequences for efficacious control of insects and a highly virulent pathogen will require only fewer propagules to incite disease (Inglis *et al.*, 2001). As such, knowledge on the median lethal concentrations of the pathogens (LC_{50}) and further their LC_{90} values and also on the median lethal time (LT_{50}) are essential for fixing their field doses. Hence, bioassays were conducted to determine the dose mortality relationships of the fungal isolates at varying concentrations except the nonpathogenic *L. lecanii* (Llm) to *A. gossypii*, *B. tabaci*, *S. dorsalis* and *P. latus*. It was observed that spore concentrations of 1.19 x 10^8, 1.37x10^9, 1.97x 10^8 and 1.19 x 10^8 spores ml^{-1} in *B. bassiana* (Bb5a), *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) was required to achieve fifty percent mortality in 12 h. It was also seen that still lower concentrations was enough to obtain fifty per cent mortality in 42 h, the LC_{50} values ranged between 0.02 to 0.16 x10^8 spores ml^{-1} in the various isolates.

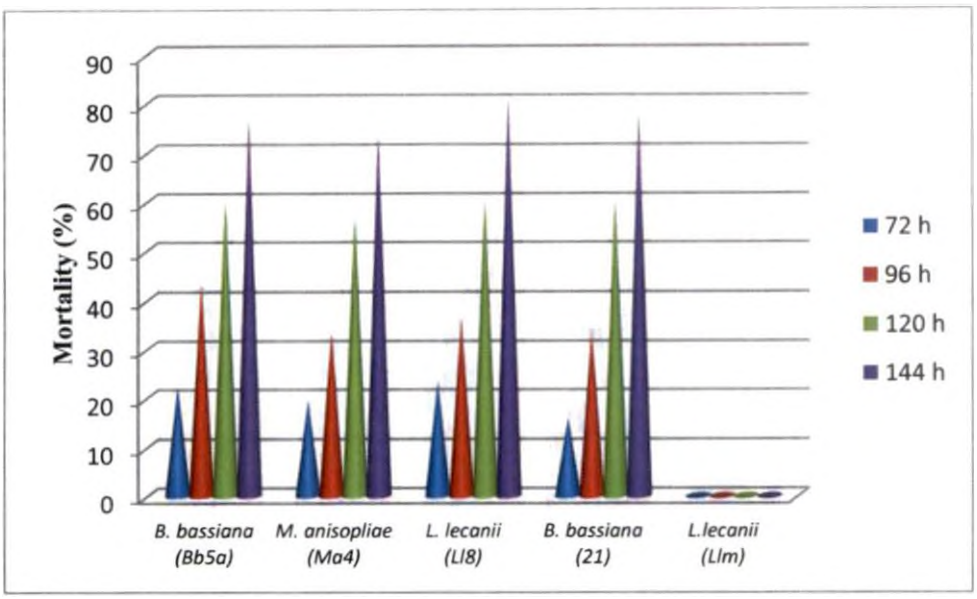


Fig. 3. Mortality of *Scirtothrips dorsalis* at different intervals on treatment with fungal pathogens

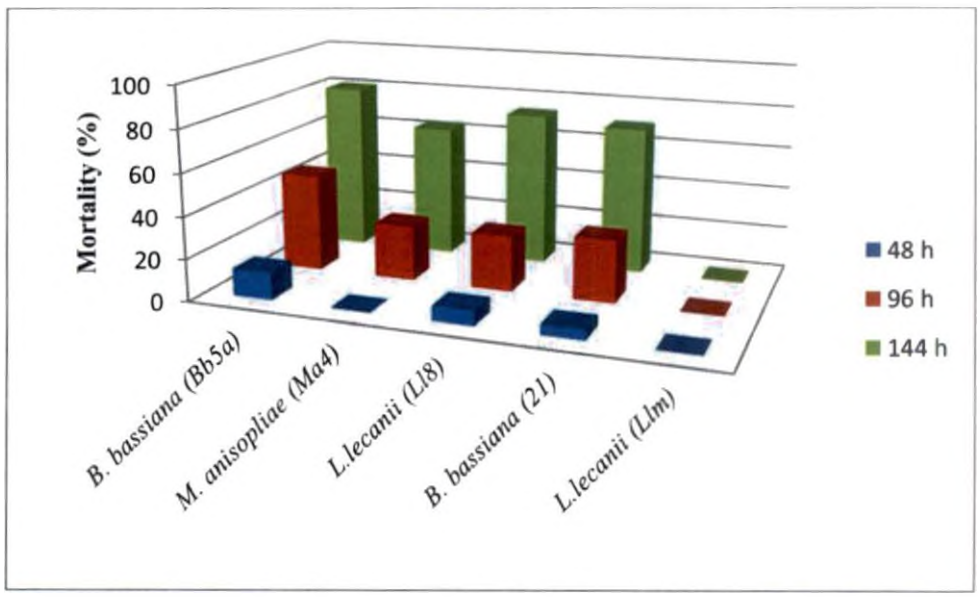


Fig. 4. Mortality of *Polyphagotarsonemus latus* at different intervals on treatment with fungal pathogens

At 42 h after inoculation the LC_{90} values ranged from 0.11 x 10^8 in *L. lecanii* (Ll8) to 1.18x 10^8 in *M. anisopliae* (Ma4). On analysis of the lethal time to achieve fifty per cent mortality it was seen that the shortest time was recorded by *L. lecanii* (Ll8) that took 20.046 h at the highest concentration of 10^8 spores ml^{-1} compared to 20 .739, 23.477 and 29.114 in *B. bassiana* (Bb5a), *B. bassiana* (Bb21) and *M. anisopliae* (Ma4). Considering all these values *L. lecanii* (Ll8) is adjudged as the most virulent isolate to *A. gossypii* but it was also noted that the other isolates evaluated were also highly virulent and could be recommended very well for management of *A. gossypii* in chilli. The higher virulence of *L. lecanii* compared to *B. bassiana* and *M. anisopliae* was evident from the reports of Saranya *et al.* (2010) against another aphid species *A. craccivora* in which the LC_{50} value was fixed as 2.5x 10^4 for *L. lecanii* and 4.5 x10^4 and 8.9 x 10^5 for *B. bassiana* and *M. anisopliae* respectively.

A dose dependent pathogenicity of the isolates was also evident in the studies on bioassay (Fig. 5 & 6). As spore concentrations increased mortality also increased. Strong dosage mortality and dosage mycosis responses were observed in insects treated with hyphomycetous fungi in the earlier studies on the aphid species *viz. Diuraphis noxia* Kurdjumov (Mesquita *et al.*, 1996), *Aphis craccivora* Koch (Saranya *et al.* 2010), Spiralling whitefly, *Aleurodicus disperus* Russell (Aiswarya *et al.*, 2007) and *S. dorsalis* (Arthurs *et al.*, 2013). All these findings apprise the need for fixing and selecting the optimum dosage of the pathogens for field application in order to achieve the desired pest regulation.

The effect of the isolates including the new indigenous isolate *I. javanica* was evaluated against the white fly *B. tabaci.* It was seen that the shortest LT_{50} of 91.78 h was recorded in *I. javanica* and the highest duration of 105.371 h was taken by *M. anisopliae* (Ma4) at 10^9 spores ml^{-1}. It was also observed that a higher spore concentration was needed for the white fly, *B. tabaci* to achieve fifty per cent mortality when compared to other test insects. The LC_{50} values ranged from 2.05 x 10^9 to 3.77 x 10^9 spores ml^{-1} whereas the LC_{90} values were observed to range from 3.12 x 10^9 to 5.75 x 10^9 spores ml^{-1} in the different isolates. Norhelina *et al.* (2013) studied the infectivity of five strains of the fungus,

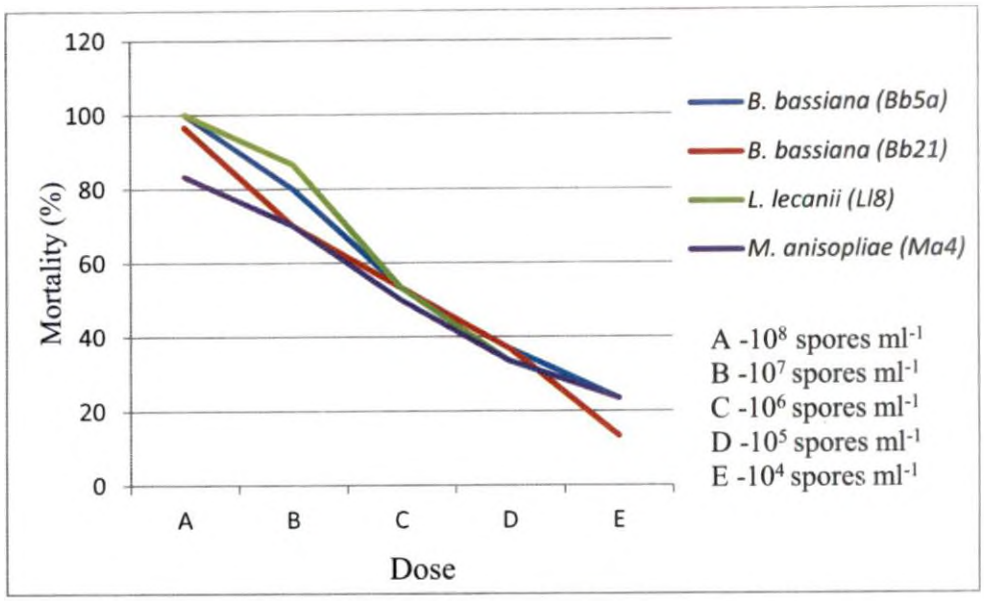


Fig. 5a. *Aphis gossypii*

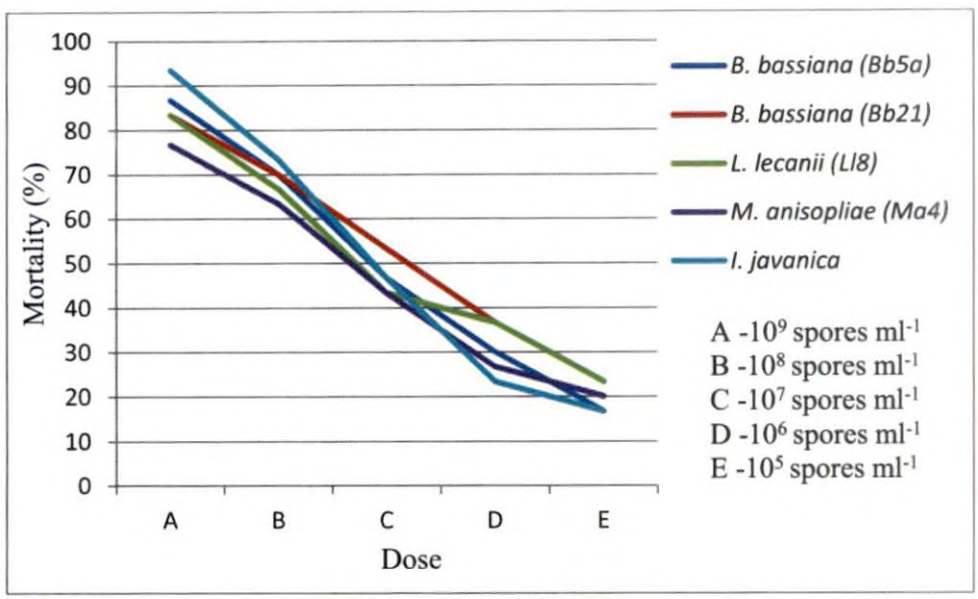


Fig. 5b. *Bemisia tabaci*

Fig. 5. Dose - Mortality relationships of fungal pathogens in pests

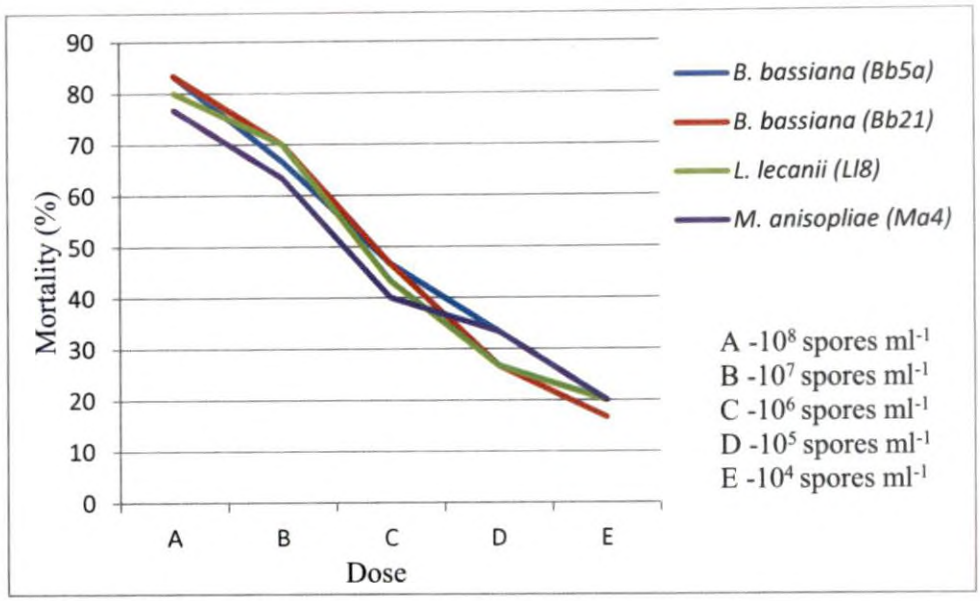


Fig. 6a. *Scirtothrips dorsalis*

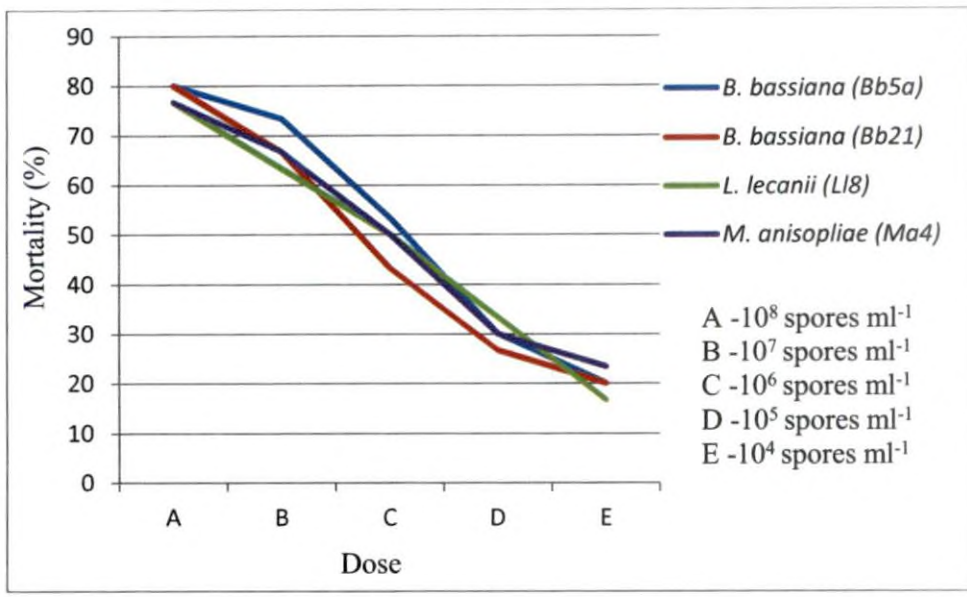


Fig. 6b. *Polyphagotarsonemus latus*

Fig. 6. Dose - Mortality relationships of fungal pathogens and pests

M. anisopliae against *B. tabaci.* Among the different strains, GJ4 was found to be the most virulent with the lowest LT_{50} of 2.16 days. He further opined that the time needed to cause fifty per cent mortality decreased with increasing dosage of the conidia.

The effect of the pathogens slightly varied with pests (Fig. 7). It was seen from the probit analysis of the dose mortality responses of the second instar nymphs of *S. dorsalis* that *B. bassiana* (Bb5a) was the best isolate as it recorded the minimum LT_{50} of 116.338 h at a concentration of 10^8 spores ml^{-1} compared to 121.263, 121.016 and 122.007 h in *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) but it was also noted that there was not much difference in the lethal time between the isolates. At 48 h the LC_{50} values ranged from 2.51 x 10^8, to 2.22 x 10^8 spores ml^{-1} for these isolates. Correspondingly, concentrations ranging from 3.82 x 10^8 to 3.9 x10^8 spores ml^{-1} were required to obtain ninety per cent mortality in these isolates. Studies from Thailand on the potential of different isolates of entomopathogenic fungi as biological control agents against another species of thrips, the flower thrips, *Frankliniella occidentalis* (Pergande) by Thungrabeab *et al.* (2006) revealed that LC_{50} values of *Beauveria* spp. ranged from 2.39× 10^4 to 5.89× 10^6 conidia ml^{-1} which was lower than the one recorded in the present study and this may be due to the higher susceptibility of *F. occidentalis* or due to the variations in the pathogenicity of the isolates. In another study by Arthurs *et al.* (2013) it was seen that the LC_{50} value of *B. bassiana* GHA isolate was 5.1 x10^4 cfu ml^{-1} against adults which was also a lower concentration. Pathogen, isolate, pest and dose dependent mortality was evident from the present results and earlier publications.

Against *P. latus* it was seen that *B. bassiana* (Bb5a) that caused fifty per cent mortality of adults in a period of 120.230 h was the best isolate and a concentration of 1.66 x 10^8 spores ml^{-1} was required to bring mortality in 48 h after treatment. The corresponding LC_{90} value was 2.12 x 10^8 spores ml^{-1}. For *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) the values for LT_{50} were 128.46, 137.813 and 133.315 respectively. No variations in the LC_{50} and LC_{90} values were seen for the *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and

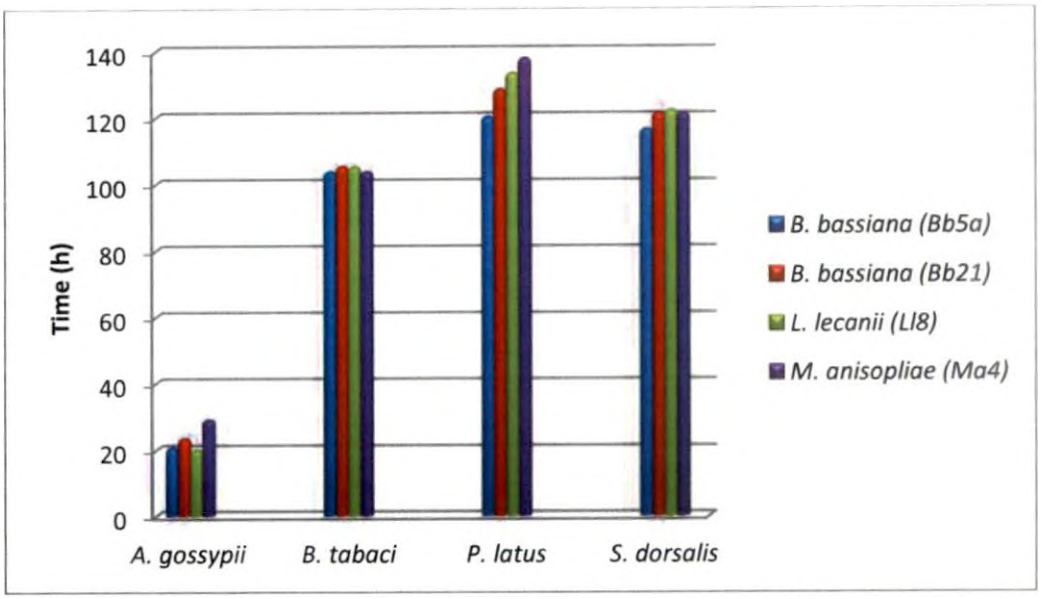


Fig. 7. LT_{50} values of fungal isolates to different pests

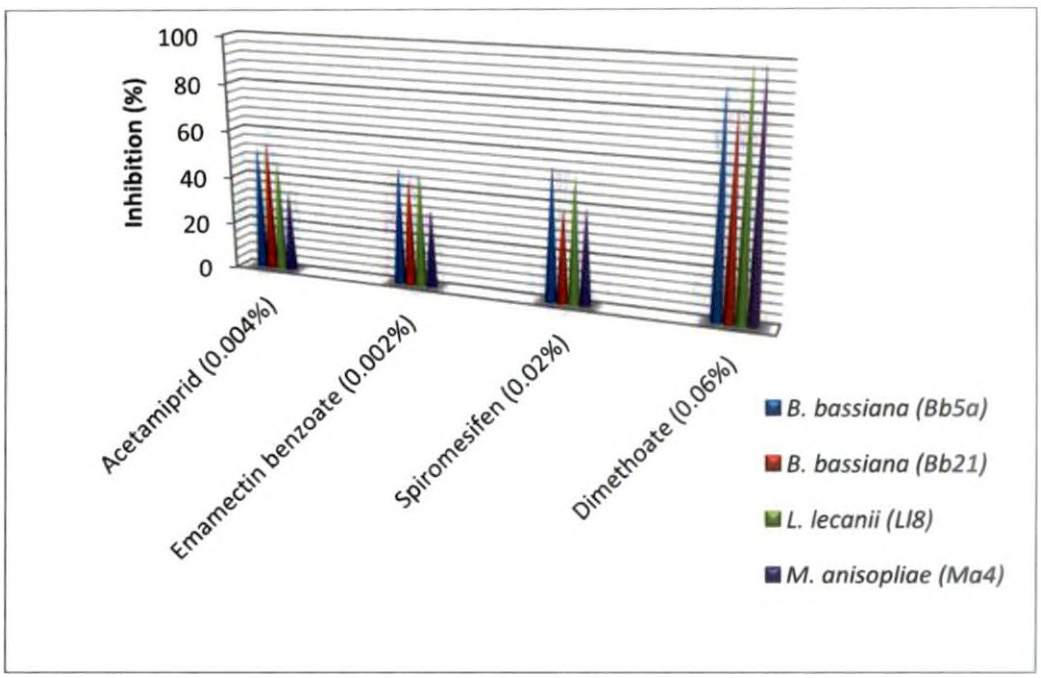


Fig. 8. Inhibition in growth of fungal isolates in pesticide poisoned media

L. lecanii (Ll8) at 48 h after treatment. In the investigations carried out by Pena *et al.* (1996) it was seen that the LC_{50} value of *B. bassiana* against *P. latus* was 1.16 x 10^{6} conidia ml^{-1}. Further in 2004, Maketon *et al.* (2008) while evaluating fungi against *P. latus* observed that *M. anisopliae* CKM-048 was the most virulent strain in controlling both larvae and adult mites at concentrations of $2x10^{8}$ conidia ml^{-1} and that the median lethal time (LT_{50}) for the adults and larvae were 2.4 and 3.8 days respectively. The present finding that *B. bassiana* is more infective to *P. latus* is in agreement with the findings of Nugroh and Ibrahim (2004)

Despite the efficacy of entomopathogenic fungi, and the easiness in production, their commercialization is constrained even today owing to the lack of products with good shelf life. This demands development of technologies and their refinement in continuum. Hence attempts were made to develop talc based products of fungal isolates *B. bassiana* (Bb5a), *B. bassiana* (Bb21), *M. anisopliae* (Ma4), and *L. lecanii* (Ll8) during the course of the investigation. At fifteen days interval on the basis of spore count, cfu and mortality to *A. gossypii* the shelf life was assessed. The spore count of 6.51 $x10^{8}$ spores ml^{-1} and cfu of 129.19 $x10^{6}$ g^{-1} observed initially at 15 days after storage (DAS) declined to 6.08 $x10^{8}$ and 66.24 x 10^{6} in *B. bassiana* (Bb5a) at 90 DAS. The values for spore count at 15 DAS and 90 DAS for *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) ranged between 30.25 $x10^{8}$ to 16.14 $x10^{8}$, 5.35x 10^{8} to 2.61x 10^{8} and 3.33to 3.37x 10^{8} spores ml^{-1}. Corresponding values of cfu ranged from 142.52 to 96.61 x 10^{6}, 70.40 $x10^{6}$ to 26.46x 10^{6} and 84.75x 10^{6} to 65.26x 10^{6} g^{-1} respectively.

Further, the efficacy of the talc based product was assessed against the aphid *A. gossypii* by applying spore suspensions prepared from the products stored for different periods. The aphid mortality of 93.28, 90.97, 86.61 and 97.8 per cent was observed when sprayed with spore suspension from 20 g l^{-1} of talc based product of *B. bassiana* (Bb5a and Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) respectively that were stored for 15 days. When sprayed with spore suspensions from products stored for 90 days the mortality observed was 75.56, 77.68, 66.57 and 79.82 respectively in *B. bassiana* (Bb5a), *B. bassiana* (Bb21), *M. anisopliae*

(Ma4) and *L. lecanii* (Ll8) respectively. As per the standards fixed, a minimum of 10^8 cfu g^{-1} is essential in a mycopesticide. In the talc based products prepared it was seen that the required cfu of 10^8 was retained upto 60 days in *B. bassiana* (Bb5a) and 75 days in *B. bassiana* (Bb21). With respect to *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) even after 15 days the cfu content was below the minimum requirement. Interestingly, it was also observed that even if the products did not meet the standard fixed for cfu, mortality of the test insect *A. gossypii* to the tune of 86.61 and 97.8 per cent was seen after 15 DAS and 66.57 and 79.82 per cent at 90 DAS in *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) respectively, which may be accounted to the effect of the fungal spores as well as the toxins present in the products. While analyzing the shelf life of talc based wettable powder formulation of *B. bassiana* at room temperature (24±1°C), Das *et al.* (2013) observed that the viability of conidia lasted up to 180 days with 20.22×10^7 conidia $g^{-1}$and that showed 48 per cent pathogenicity.

One basic attribute with biological pesticides is long shelf life with no loss of bioefficacy. At least 18 months or even longer shelf life has been suggested by Couch and Ignoffo, 1981; Moore and Prior, 1993 but less could be acceptable for control operations (Moore *et al.*, 1996). These authors suggested that maintenance of the shelf life covering two cropping seasons, would be desirable and that long term storage would be more convenient for the manufacturers than the farmers. In this perspective, the talc based product prepared with a shelf life of two months can find a place for pest management on *in situ* production and provision in local markets.

Insects, the most dominant group of organisms have the ability to evolve quickly and they often gain measures to counter the adversities in the environment as evident from the development of pesticide resistant strains and biotypes in many insect species (Perring, 2001; Vicentni *et al.,* 2001). Hence, dependence on a single tactic, even on the seemingly safe entomopathogens is not wise. This recalls the need to integrate different tools in pest management. Before integrating the different tools it is of prime importance to elucidate their compatibility. The fact that synthetic molecules still claim the lion's share of the

pesticide market also cannot be over looked. Moreover, they 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* (Bb5a), *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) with newer pesticide molecules *viz*, emamectin benzoate 0.002 per cent, acetamiprid 0.004 per cent, spiromesifen 0.02 per cent and the conventional insecticide dimethoate 0.06 per cent. The results (Fig. 8) revealed that emamectin benzoate 0.002 per cent was the least inhibitory to all the fungal pathogens tested in which the extent of inhibition over control varied between 32.43 to 49 per cent only and it was ranked harmless to the fungal pathogens. Acetamiprid 0.004 per cent was slightly harmful to both the isolates of *B. bassiana* (Bb5a and Bb21), the percentage of inhibition was 46.21 to 52.34 per cent in *B.bassiana* isolates but it was graded as harmless to *M. anisopliae* and *L. lecanii* in which inhibition in growth over control was 33.2 and 46.21 per cent respectively. The inhibition in spiromesifen pesticide media varied from 38.43 to 55.46 per cent. The relative safety of the newer molecules acetamiprid and emamectin benzoate to *M. anisopliae* was recognized by Akabar *et al.* (2012) also. Dimethoate 0.06 per cent was harmful to the growth of the fungal pathogens as inhibition ranging from 82 per cent to 100 per cent was noticed. According to Oliveira and Neves (2004) dimethoate 0.06 per cent, the acaricide belonging to the organophosphorous group significantly reduced the growth of *B. bassiana*. Contrastingly, the reports of Raj *et al.* (2011) stated that dimethoate exhibited minimum inhibitory effect among the different pesticides evaluated. Among the four fungal isolates, the growth of *M. anisopliae* (Ma4) was least affected by the pesticides tested.

The spore count of the fungi estimated on the twenty first day after inoculation in pesticide media revealed that *B. bassiana* (Bb5a) in emamectin benzoate 0.002 per cent had the highest spore count of 4.76×10^8 spores ml^{-1} but it was on par with that in acetamiprid 0.004 per cent and spiromesifen 0.02 per cent (3.58 and 3.33×10^8 spores ml^{-1}). Slight difference was observed with respect to the spore production in *B. bassiana* (Bb21). This fungus recorded the highest spore production in spiromesifen 0.02 per cent

amended media (7.01×10^8 spores ml^{-1}) but it was on par with emamectin benzoate 0.002 per cent. The spore production in *L. lecanii* (Ll8) was different from that of *B. bassiana* (Bb5a) and *B. bassiana* (Bb21) as acetamiprid 0.004 per cent recorded the highest spore count of 4.81×10^8 spores ml^{-1} and was on par with emamectin benzoate 0.002 per cent (4.52×10^8 spores ml^{-1}). The performance of *M. anisopliae* (Ma4) was similar to that in *B. bassiana* (Bb5a). In *M. anisopliae* (Ma4) emamectin benzoate recorded highest spore count of 0.66×10^8 spores ml^{-1}. Dimethoate was observed to be the most detrimental pesticide which completely inhibited spores of *L. lecanii* (Ll8) and *M. anisopliae* (Ma4) and with lower spore counts of 0.17 and 0.19×10^8 spores ml^{-1} in *B. bassiana* (Bb5a) and *B. bassiana* (Bb21) respectively. Earlier research findings also indicate that the inhibitory effects of agrochemicals on the entomopathogenic hyphomycetes often vary among taxa and strains (Vanninen and Hokkanen, 1988; Anderson *et al.*, 1989; Majchrocwicz and Poprawski, 1993; Li and Holdom, 1994; Rachappa *et al.*, 2007 and Asi *et al.*, 2010).

For the fruitful adoption of the technologies developed in laboratories their reiteration in the agroecosystems is crucial. So in order to evaluate the fungal pathogens in comparison with the newer pesticide molecules a field experiment using the chilli variety Vellayani Athulya was carried out.

The effect of the treatments under field conditions was assessed on the basis of the pest population. The results revealed that *B. bassiana* (Bb5a) was the best treatment among the fungal pathogens tested against *S. dorsalis*. The mean population of thrips was 0.96 five $leaves^{-1}$ and it recorded the maximum population reduction of 81.51 to 90.16 per cent over control. This was followed by *L. lecanii* (Ll8), *B. bassiana* (Bb21) and *M. anisopliae* (Ma4) in which population reductions of 80.0 to 84.02, 77.1 to 81.66 and 76.34 to 80.33 per cent (Fig. 9) respectively were observed. With respect to *S. dorsalis* spiromesifen 100 ml a.i. ha^{-1} with a mean population 0.12 and 0.32 thrips five $leaves^{-1}$ at seven and 14 days after treatment was on par with acetamiprid 20 g a.i. ha^{-1} (0.44 and 0.42 thrips five $leaves^{-1}$), the percentage reduction in population over control ranged from 84.5 to 98.77 per cent in spiromesifen 100 ml a.i. ha^{-1} while population

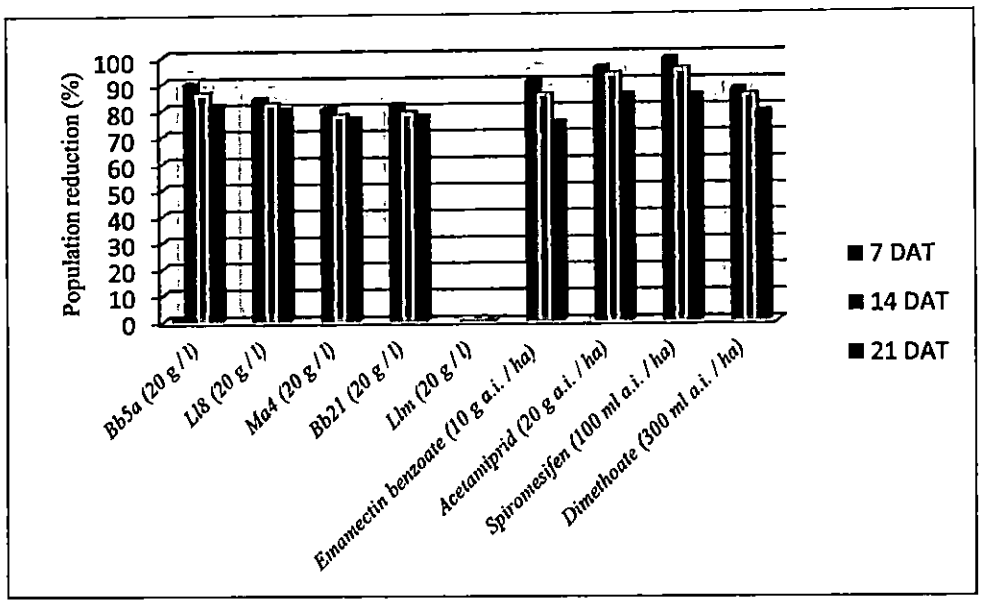


Fig. 9. Population reduction of *Scirtothrips dorsalis* in the different treatments under field conditions

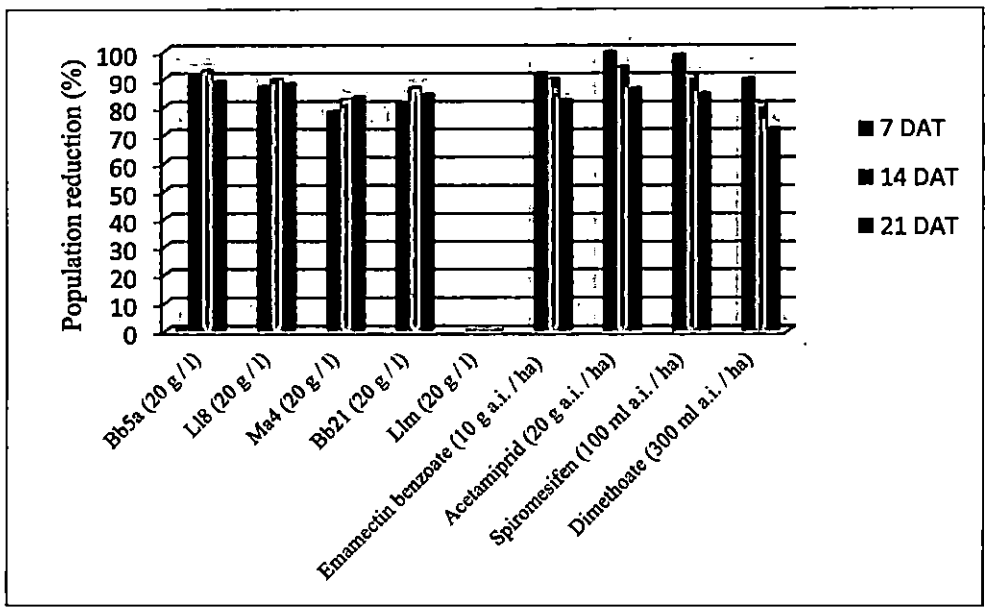


Fig. 10. Population reduction of *Polyphagotarsonemus latus* in the different treatments under field conditions

reduction ranging from 85.27 to 95.49 was observed in acetamiprid 20 g a.i. ha^{-1} and these two treatments were significantly better than the fungal pathogen *B. bassiana* (Bb5a) which indicated the maximum population reduction among the fungal pathogens. At 14 days after treatment all the fungal treatments were on par with emamectin benzoate 10 g a.i. ha^{-1} and dimethoate 300 ml a.i. ha^{-1} treated plot that recorded a mean population of 0.88 and 0.90 thrips five $leaves^{-1}$ with 85.38 and 85.05 per cent reduction in population over control. As in the laboratory, *L. lecanii* (Llm) had no effect neither on *S. dorsalis* nor on *P. latus*. The results are in corroboration with that of Varghese and Mathew (2013) that spiromesifen followed by acetamiprid significantly reduced *S. dorsalis* population in chilli. Relatively there are only few references on the effect of fungal pathogens to *S. dorsalis* infesting chilli. Arthurs *et al.* (2013) highlighted the potential of mycoinsecticides for the management of *S. dorsalis* in chilli. They found that *B. bassiana* applied at label rates reduced population of thrips. However, there are reports on the infectivity and performance of *B. bassiana* to other species of thrips, *Thrips tabaci* Lindeman infesting onion under field situations. According to Kumar *et al.* (2012) application of *B. bassiana* @ 10^9 spores ml^{-1} was the best treatment followed by *V. lecanii* @10^9 spores ml^{-1} that brought about population reductions of 64.15 and 49.89 per cent respectively in the field experiment.

With respect to the efficacy on the mite, *P. latus* also *B. bassiana* (Bb5a) that harbored only 1.28 mites five $leaves^{-1}$ at seven DAT ranked topmost among the fungal pathogens. It brought about 90.64, 92.6 and 88.6 per cent reduction over control of the pest in the observations taken at 7, 14 and 21 days after treatment (Fig. 10). This was followed by *L. lecanii* (Ll8), *B. bassiana* (Bb21) and *M. anisopliae* (Ma4) which recorded population reductions of 86.69 to 89.49 per cent, 80.61 to 86.38 and 77.76 to 83.16 per cent respectively. Attempts to evaluate mycopesticides in the field was under way in the different parts of the world. The significant effect of wettable powder formulations of *B. bassaina* @ 1x 10^{10} conidia ml^{-1} for the population reduction of *P. latus* in chilli was

documented by Nugroho and Ibrahim (2007). They further stated that the effect of *B. bassiana* was better than that of *M. anisopliae*.

Of all the chemicals and fungi evaluated, at 7 and 14 days after treatment, acetamiprid 20 g a.i. ha^{-1} recorded the lowest incidence of 0.12 and 0.74 mites five $leaves^{-1}$ and showed the highest reduction in population over control (99.12 and 93.78 per cent) but it was on par with spiromesifen 100 g a.i. ha^{-1}. However, in the subsequent observation at 21 DAT the control exerted by acetamiprid 20 g a.i. ha^{-1} (85.97 per cent) was less than that in *B. bassiana* (Bb5a) (88. 6) and *L. lecanii* (Ll8) (87.73) at 20 g l^{-1}. Similarly emamectin benzoate 10 g a.i. ha^{-1} that recorded 91.51 per cent population reduction of *P. latus* at seven DAT was significantly better than *B. bassiana* (Bb5a) initially but in the subsequent observations *B. bassiana* (Bb5a) showed better performance. Among the chemical treatments dimethoate 300 ml a.i. ha^{-1} recorded the lowest reduction of 89.54 per cent. The present observation that acetamiprid exerted significant control of *P. latus* was supported by the findings of Bretschneider *et al.*, (2003); Kavitha *et al.* (2006); Nagaraj *et al.* (2007); Varghese and Mathew (2013).

An assessment of the chilli leaf curl symptom based on five grades revealed that there was no leaf curl incidence in plants treated with *B. bassiana* (Bb5a) 20 g l^{-1}, *L. lecanii* (Ll8) 20 g l^{-1}, emamectin benzoate 10 g a.i. ha^{-1}, acetamiprid 20 g a.i. ha^{-1} and spiromesifen 100 ml a.i. ha^{-1} 15 DAT while *B. bassiana* (Bb21) 20 g l^{-1} and *M. anisopliae* (Ma4) 20 g l^{-1} and dimethoate 300 ml ha^{-1} treated plants were in the grade 1 (1 to 25 per cent). The treatment *L. lecanii* (Llm) and control recorded the maximum leaf curl index of 2 (25 to50 per cent). In the subsequent observations at 90 DAT and 120 DAT there was no further increase in the leaf curl symptom either in the treatments or in control which can be attributed to the low incidence of the thrips and mites during the period.

Assessment of the population of other chilli pests *viz. A. gossypii* and *B. tabaci* during the period of observation revealed only meager incidence of the pests. A mean population of 0.04 to 0.18 aphids and 0.04 to 0.07 whitefly five $leaves^{-1}$ observed in treatments did not differ from that in untreated control. There was no fruit borer incidence in chilli during the period of observations.

An analysis of the effect of the treatments on natural enemy population was also done. The spider population exhibited significant differences between treatments. The treatment found comparatively safe to the spiders was *L. lecanii* (Ll8) and *B. bassiana* (Bb21) as it recorded significantly higher population of 1.99 spiders plant^{-1}, however this was lower than that observed in untreated control and *L. lecanii* (Llm) that recorded 2.80 and 2.61 spiders plant^{-1}. Dimethoate 300 ml a.i. ha^{-1} inhibited the population of spiders completely at seven DAT. According to Varghese and Mathew (2013) spiromesifen 100 ml a.i. ha^{-1} was safe to spiders but dimethoate 300 g a.i. ha^{-1} was detrimental.

A perusal of the yield data showed that the highest yield of chilli, on the basis of the number and weight of fruits was highest in acetamiprid 20 g a.i. ha^{-1} with a mean number of 73.73 fruits plot^{-1} and 6.24 kg plot^{-1} and was on par with spiromesifen 100 ml a.i. ha^{-1} (70.95 fruits plot^{-1} and 6.07 kg plot^{-1}). This was followed by emamectin benzoate 10 g a.i. ha^{-1}, *B. bassiana* (Bb5a) 20 g l^{-1}, *L. lecanii* (Ll8) 20 g l^{-1}, *B. bassiana* (Bb21) 20 g l^{-1} and *M. anisopliae* (Ma4) 20 g l^{-1} with of 5.59, 5.52, 4.93, 4.9 and 4.87 kg plot^{-1} respectively. Dimethoate recorded the lowest number and weight of fruits among all the treatments except *L. lecanii* (Llm). The observations that acetamiprid and spiromesifen treated plots yielded highest was supported by the findings of Varghese (2011).

In consonance with the premium price prevalent in the market for chemical free chilli, benefit ratios for the different treatments were worked out and it was seen that the highest benefit cost ratio of 2.62 was recorded for *B. bassiana* (Bb5a). This was followed by acetamiprid 20 g a.i. ha^{-1}, *L. lecanii* (Ll8) 20 g l^{-1}, *B. bassiana* (Bb21) 20 g l^{-1}, *M. anisopliae* (Ma4) 20 g l^{-1} and spiromesifen 100 ml a.i. ha^{-1} with benefit ratios of 2.22, 2.15, 2.12, 2.10 and 2.07 respectively. Dimethoate 300 ml a.i. ha^{-1} was observed to have the lowest benefit cost ratio of 1.10 and was less than for all the fungal treatments except *L. lecanii* (Llm).

Based on the information gathered on the different aspects of the entomopathogenic fungi in the present study, the highlights are the following

- Three fungi *viz. Fusarium solani* (Mart.) Sacc and *Penicillium oxalicum* Currie and Thom infecting *A. gossypii* and *Isaria javanica* (Friederichs &

Bally) Brown & Smith infecting *B. tabaci* were isolated and the reports on these insects are new in India. *I. javanica* offers scope for the management of whitefly, *B. tabaci.*

- The report on the yeast *M. caribbica* as an entomopathogen of *B. tabaci* is new.
- The field dose of the fungal pathogens *viz. B. bassiana* (Bb5a and Bb21), *L. lecanii* (Ll8) and *M. anisopliae* (Ma4) against *A. gossypii, B. tabaci, P. latus* and *S. dorsalis* were determined from LC_{90} values.
- Talc based products of fungal pathogens effectively managed chilli pests in the field. Benefit cost ratio was highest for the treatment with *B.* bassiana (Bb5a) 20 g l^{-1}.
- Acetamiprid (0.004%) and spiromesifen (0.02%) ranked top for the management of *P. latus* and *S. dorsalis.*

Application of the talc based products of *B. bassiana* (Bb5a / Bb21), *L. lecanii* (Ll8) and *M. anisopliae* (Ma4) at 20 g l^{-1} is recommended to keep the chilli pest at bay.

Summary

6. SUMMARY

In the past two decades adversities created by synthetic pesticides widely caught the attention of the researchers and recently that of the policy makers too. Initiatives to identify alternative measures to tackle pests brought the bioagents to the foreground. The potential of the entomopathogenic fungi as biocontrol agents is now globally recognized and there is an unprecedented increase in the attempts to exploit the potential of the classic members *viz. Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Lecanicillium lecanii* Zare & Gams. Since the isolates of these fungi are known to vary in their virulence, only with the deployment of the right isolates at their effective concentrations the targeted pest management can be achieved. Further, assessment of the performance of the fungal products in the field and their compatibility with other management tools especially with chemical pesticides needs elucidation before large scale adoption of mycopesticides.

Chilli (*Capsicum annuum* L.) the indispensible vegetable is valued as a foreign exchange earner also. The chilli plant is prone to attack by a spectrum of pests. Development of safe pest management measures are necessary in this crop to ward off problems of pesticide residues since the consumers now prefer to opt pesticide free chillies. The importance of the relatively safe newer molecules of synthetic pesticides in chilli pest management was also reckoned with while chalking out the project entitled "Entomopathogenic fungi against the pest complex of chilli (*Capsicum annuum* L.) with the following objectives to isolate and identify indigenous strains of entomopathogenic fungi, assess the pathogenicity of *B. bassiana, L. lecanii, M. anisopliae* and indigenous isolates against major chilli pests, determine the LC_{50}, LC_{90} and LT_{50} of the fungi for major susceptible pests, develop products of fungi, assess the compatibility of these fungi with pesticides and evaluate the field efficacy of the fungal pathogens in comparison with newer molecules of synthetic pesticides against major chilli pests.

- Monitoring in farmers fields in Thiruvanathapuram District during 2013-2014 brought to light the infection of three new fungal pathogens and one yeast in chilli pests.
- Morphological and molecular characters revealed the identity of the fungi isolated from *A. gossypii* as *Fusarium solani* (Mart.) Sacc (Hypocreales: Nectriaceae) and *Penicillium oxalicum* Currie and Thom (Eurotiales: Trichocomaceae).
- The fungal pathogen *viz. Isaria javanica* (Friederichs & Bally) (Hypocreales : Cordycipitaceae) Brown & Smith and the yeast, *M. caribbica* (Vaughan-Martini, Kurtzman, S.A. Meyer & E.B. O'Neill) Kurtzman & M. Suzuki (Saccharomycetales: Debaryomycetaceae) were isolated from *Bemisia tabaci* Gennadius.
- The reports on the infection of the three fungi in chilli pests are new in India. Infection of the yeast *M. caribbica* on *B. tabaci* is new and it has not been documented yet from any of the places in India or abroad.
- The fungal pathogens *viz. B. bassiana* (Bb5a and Bb21), *L. lecanii* (Ll8), *M. anisopliae* (Ma4) were pathogenic to *A. gossypii*, *B. tabaci*, *S. dorsalis* and *P. latus*.
- *L. lecanii* (Ll8) that caused 33. 33and 99.97 per cent mortality at 24 and 48 hours after treatment respectively to *A. gossypii* was the most virulent among the different isolates and it was significantly superior to other isolates.
- Cumulative percentage mortality revealed that *I. javanica* was significantly superior to *B. bassiana* (Bb5a), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) in its infectivity to the white fly, *B. tabaci.*
- The virulence of the fungal isolates tested against the thrips, *S. dorsalis* revealed that the isolates *L. lecanii* (Ll8), *B. bassiana* (Bb21), *B. bassiana* (Bb5a) and *M. anisopliae* (Ma4) were equally pathogenic to *S. dorsalis.*

- Assessment of the virulence of the fungal isolates against the mite, *P. latus* revealed that the isolates *viz. L. lecanii* (Ll8), B21, *B. bassiana* (Bb5a) and *M. anisopliae* (Ma4) were on par, the maximum mortality of 80.65 was seen in *L. lecanii* (Ll8).
- Spore concentrations of $1.19x\ 10^8$, $1.37x\ 10^8$, $1.97x\ 10^8$ and $1.19 x\ 10^8$ spores ml^{-1} in *B. bassiana* (Bb5a), *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) was required to achieve fifty percent mortality in 12 hours. Corresponding LC_{90} values were $1.94x\ 10^8$, $2.17x\ 10^8$ $3.11 x\ 10^8$ and $1.98 x\ 10^8$ respectively.
- Based on the LT_{50} *L. lecanii* (Ll8) is adjudged as the most virulent isolate to *A. gossypii.* The virulence was in the order *L. lecanii* (Ll8) > *B. bassiana* (Bb5a) > *B. bassiana* (Bb21) > *M. anisopliae* (Ma4).
- The LC_{50} of the different fungal isolates with respect to *B. tabaci* ranged from $2.05 x\ 10^9$ to $3.77 x\ 10^9$ spores ml^{-1} and the LC_{90} values ranged from $3.12 x\ 10^9$ to $5.75 x\ 10^9$ spores ml^{-1}.
- Analysis of the median lethal time to bring fifty per cent mortality in in *B. tabaci* showed that the shortest LT_{50} of 91.78 hours was recorded by *I. javanica* and the highest LT_{50} 105.371 h was recorded by *M. anisopliae* (Ma4).
- *I. javanica* offers scope for management of whitefly, *B. tabaci.*
- The LC_{50} values of *B. bassiana* (Bb5a), *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) against *S. dorsalis* ranged from 2.51 $x\ 10^8$ to $2.22 x\ 10^8$ spores ml^{-1} at 48 hours.
- The LC_{90} values of *B. bassiana* (Bb5a), *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) against *S. dorsalis* ranged from 3.82 to $3.90 x\ 10^8$ spores ml^{-1} for the isolates at 48 hours.
- At a concentration of 10^8 spores ml^{-1} the LT_{50} to *S. dorsalis* were 116.338, 121.263, 121.016 and 122.007 hours in *B. bassiana* (Bb5a), *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) respectively.

➢ Against the chili mite, *P. latus B. bassiana* (Bb5a) that caused fifty per cent mortality of adults in a period of 120.230 hours was the best isolate and a concentration of 1.66 x 10^{8} spores ml^{-1} was required to bring mortality in 48 hours after treatment. The corresponding LC_{90} value was 2.12 x 10^{8} spores ml^{-1}. No variations in the LC_{50} and LC_{90} values were seen for the *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) at 48 hours after treatment.

➢ Assessment of the spore count in talc based product of *B. bassiana* (Bb5a) showed that the spore load of 6.51 x10^{8} spores ml^{-1} at 15 days after storage declined to 6.08 x10^{8} at 90 days after storage. The spore count at 90 days after storage in *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) based products ranged between, 16.14 x10^{8}, 2.61x 10^{8} and 3.37x 10^{8} spores ml^{-1}.

➢ Talc based products of *B. bassiana* (Bb5a) and *B. bassiana* (Bb21) maintained the required cfu of 10^{8} spores ml^{-1} upto two months.

➢ When sprayed with spore suspensions prepared from talc based *B. bassiana* (Bb5a), *B. bassiana* (Bb21), *M. anisopliae* (Ma4) and *L. lecanii* (Ll8) that was stored for stored for 90 days, the mortality of *A. gossypii* observed was 75.56, 77.68, 66.57 and 79.82 per cent respectively.

➢ The compatibility studies revealed that emamectin benzoate 0.002 per cent was least inhibitory to all the fungal pathogens tested. The extent of inhibition over control varied between 32.43 to 48.86 per cent.

➢ Acetamiprid 0.004 per cent was slightly harmful to both the isolates of *B. bassiana* (Bb5a and Bb21), the percentage of inhibition was 46 .21 to 52.34 per cent in *B.bassiana* isolates. In *M. anisopliae* and *L. lecanii* inhibition in growth over control was 33.2 and 46.21 per cent.

➢ Among the four fungal isolates, the growth of *M. anisopliae* (Ma4) was least affected by the pesticides.

➢ In the field experiment, the effect assessed on the basis of the population of thrips, *S. dorsalis* revealed that *B. bassiana* (Bb5a) 20 g l^{-1} was

the best treatment among the fungal pathogens tested. The mean population of thrips was 0.96 five leaves^{-1} and it recorded the maximum population reduction of 81.51 to 90.16 per cent over control.

- The percentage reduction in population of *S. dorsalis* over control ranged from 84.5 to 98.77 per cent in spiromesifen 100 ml a.i. ha^{-1}. Population reduction ranging from 85.27 to 95.49 was observed in acetamiprid 20 g a.i. ha^{-1}.
- With respect to the efficacy on the mite, *P. latus* also *B. bassiana* (Bb5a) 20 g l^{-1} that harboured only 1.28 mites five leaves^{-1} at seven DAT ranked topmost among the fungal pathogens. It brought about 90.64, 92.6 and 88.6 per cent reduction over control of the pest in the observations taken at 7, 14 and 21 days after treatment
- Of all the chemicals and fungi evaluated, at seven and 14 days after treatment, acetamiprid 20 g a.i. ha^{-1} recorded the lowest incidence of 0.12 and 0.74 mites five leaves^{-1} and showed the highest reduction in population over control (99.12 and 93.78 per cent) and it was on par with spiromesifen 100 g a.i. ha^{-1}.
- The treatment found comparatively safe to the spiders was *L. lecanii* (Ll8) 20 g l^{-1} and *B. bassiana* (Bb21) 20 g l^{-1} as it recorded significantly higher population of 1.99 spiders plant^{-1}. Dimethoate 300 ml a.i. ha^{-1} inhibited the population of spiders completely at seven DAT.
- The highest yield of chilli, on the basis of the number and weight of fruits was highest in acetamiprid 20 g a.i. ha^{-1} with a mean number of 73.73 fruits plot^{-1} and 6.24 kg plot^{-1} and was on par with spiromesifen 100 ml a.i. ha^{-1} (70.95 fruits plot^{-1} and 6.07 kg plot^{-1}).
- Considering the premium price prevalent in the market for chemical free chilli benefit ratios for the different treatments were worked out and it was seen that the highest benefit cost ratio of 2.62 was recorded for *B. bassiana* (Bb5a) 20 g l^{-1}. This was followed by acetamiprid 20 g a.i. ha^{-1},

L. lecanii (Ll8) 20 g l^{-1}, *B. bassiana* (Bb21) 20 g l^{-1}, *M. anisopliae* (Ma4) 20 g l^{-1} and spiromesifen 100 ml a.i. ha^{-1} with benefit ratios of 2.22, 2.15, 2.12, 2.10 and 2.07 respectively.

Considering the various aspects of the fungal pathogens, it is inferred that *B. bassiana* (Bb5a) 20 g l^{-1}, *L. lecanii* (Ll8) 20 g l^{-1}, *M. anisopliae* (Ma4) 20 g l^{-1} and *B. bassiana* (Bb21) 20 g l^{-1} are effective and economical for the management of pest complex of chilli besides being safe to natural enemies and ideal for integration with newer pesticide molecules *viz.*, acetamiprid (0.004%), emamectin benzoate (0.002%) and spiromesifen (0.02%).

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Abstract

Evaluation of Entomopathogenic Fungi against Pest Complex of Chilli (*Capsicum annuum* L.)

by

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Abstract of the thesis

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ABSTRACT

An investigation entitled "Evaluation of entomopathogenic fungi against pest complex of chilli (*Capsicum annuum* L.)" was carried out during 2012-14 at College of Agriculture, Vellayani. The objectives were to evaluate the efficacy of *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium lecanii* Zare & Gams and *Metarhizium anisopliae* (Metschnikoff) Sorokin against pests of chilli, assess the compatibility of the fungi with pesticides and develop products of the fungi.

Three fungi *viz. Fusarium solani* (Mart.) Sacc and *Penicillium oxalicum* Currie and Thom infecting *Aphis gossypii* Glover and *Isaria javanica* (Friederichs & Bally) Brown & Smith infecting *Bemisia tabaci* Gennadius were isolated and the reports on these insects are new in India.

An yeast, *Meyerozyma caribbica* (Vaughan-Martini, Kurtzman, S.A. Meyer & E.B. O'Neill) Kurtzman & M. Suzuki infecting *B. tabaci* has been isolated and the report of the yeast is new.

The pathogenicity of fungal isolates was evaluated against *A. gossypii, B. tabaci, P. latus* and *S. dorsalis*. All the treated insects showed symptoms of infection by all the isolates except Llm. The virulence of the fungi was evaluated @ 10^8 spores ml^{-1}. At 48 hours after treatment, the highest mortality of 99.97 per cent to *A. gossypii* was caused by *L. lecanii* (Ll8). *I. javanica* caused the maximum mortality of 83.61 per cent to *B. tabaci.* With respect to *P. latus*, the highest mortality of 80.65 per cent was recorded in *B. bassiana* (Bb5a) and it was on par with the mortality in *L. lecanii* (Ll8) and *B. bassiana* (Bb21). The effect of all the fungi except *L. lecanii* (Llm) to *S. dorsalis* was on par.

The LC_{50}, LC_{90} and LT_{50} values of the fungal pathogens to *A. gossypii, B. tabaci, P. latus* and *S. dorsalis* were worked out. To achieve control of these sucking pests in the shortest period, the field doses fixed for *B. bassiana* (Bb5a), *B. bassiana* (Bb21), *L. lecanii* (Ll8) and *M. anisopliae* (Ma4) ranged from 1.68×10^8

to 5.10× 10^9, 1.68× 10^8 to 5.75× 10^9, 2.76× 10^8 to 4.12× 10^9 and 3.11× 10^8 to 3.12× 10^9 spore ml^{-1} respectively.

Talc based products of the fungi were prepared and their shelf life was determined. The cfu observed at 60 days after storage was 1.03× 10^8 ml^{-1}, 0.44× 10^8 ml^{-1}, 0.68× 10^8 ml^{-1} and 1.18× 10^8 ml^{-1} for *B. bassiana* (Bb5a), *M. anisopliae* (Ma4), *L. lecanii* (Ll8) and Bb21 respectively.

The compatibility of the fungal pathogens with pesticides was assessed. Dimethoate (0.06%) was the most inhibitory to all the fungal isolates. *M. anisopliae* (Ma4) was least inhibited by the pesticides.

Field experiment was conducted to evaluate the fungal pathogens and newer molecules of insecticides against pests of chilli. The treatments *B. bassiana* (Bb5a) and *L. lecanii* (Ll8) @ 20 g l^{-1} and acetamiprid (0.004%) were found to be on par in reducing the *P. latus* population. *B. bassiana* (Bb5a) 20 g l^{-1} was found on par with acetamiprid (0.004%), spiromesifen (0.02%) and emamectin benzoate (0.002%) in their effectiveness to *S. dorsalis* at 7 DAT. The fungal isolates did not affect the spider population. The benefit cost ratio was highest for the treatment with *B. bassiana* (Bb5a) 20 g l^{-1} (2.62).

To conclude, the fungal pathogens *viz. B. bassiana* (Bb5a and Bb21), *L. lecanii* (Ll8) and *M. anisopliae* (Ma4) @ 20 g l^{-1} are safe, economical and ideal for integration with newer pesticide molecules *viz.* emamectin benzoate (0.002%), spiromesifen (0.02%) and acetamiprid (0.004%) for management of pest complex of chilli.