# ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF INSECT PESTS IN RICE ECOSYSTEM

*by*

**MALINI NILAMUDEEN**

**(2011-21-109)**

**THESIS**

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**COLLEGE OF AGRICULTURE**

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I

## DECLARATION

I, hereby declare that the thesis entitled **"ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF INSECT PESTS IN RICE ECOSYSTEM"** is a bona fide 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, associateship, fellowship or other similar title, of any other University or Society.

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II

# CERTIFICATE

Certified that this thesis entitled **"ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF INSECT PESTS IN RICE ECOSYSTEM"** is a record of research work done independently by Mrs. Malini Nilamudeen under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF PLATES

## LIST OF APPENDICES

| Sl. No. | Title | Appendix No. |
|---|---|---|
| 1 | Estimation of benefit-cost ratio (Field trial- I) | I |
| 2 | Estimation of benefit-cost ratio (Field trial- II) | II |

# LIST OF ABBREVIATIONS

| | |
|---|---|
| % | Per cent |
| @ | At the rate of |
| µm | Micro metre |
| °C | Degree celsius |
| a.i. | Active ingredient |
| ANOVA | Analysis of variance |
| CD | Critical difference |
| Cm | Centimetre |
| $cm^{-2}$ | Per square centimetre |
| CTAB | Cetyl Trimethly Ammonium Bromide |
| DAI | Days after inoculation |
| DAS | Days after sowing |
| DAT | Days after treatment |
| dATP | Deoxyadenosinetriphosphate |
| dCTP | Deoxycytidinetriphosphate |
| dGTP | Deoxyguanosinetriphosphate |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| dTTP | Deoxythymidinetriphosphate |
| EC | Emulsifiable concentrate |

| | |
|---|---|
| *et al.* | And others |
| F | Forward |
| Fig. | Figure |
| g | Gram |
| GR | Granular |
| h | Hour |
| ha | Hectare |
| $ha^{-1}$ | Per hectare |
| *i.e.* | That is |
| kg | Kilogram |
| l | Litre |
| $l^{-1}$ | Per litre |
| Ltd. | Limited |
| m | Metre |
| mg | Milligram |
| min | Minute |
| ml | Millilitre |
| $ml^{-1}$ | Per millilitre |
| mm | Millimetre |
| $mm^{-2}$ | Per square millimetre |
| ng | Nanogram |
| NS | Non significant |

| | |
|---|---|
| PCR | Polymerase Chain Reaction |
| Pvt. | Private |
| R | Reverse |
| Rpm | Revolutions per minute |
| SC | Soluble concentrate |
| Sec | Second |
| SP | Soluble powder |
| sp. | Species |
| SPSS | Statistical Package for Social Sciences |
| Taq | *Thermus aquaticus* |
| TB | Tris buffer |
| UV | Ultra violet |
| V | Volt |
| *viz.* | Namely |
| WG | Wettable granules |

# *Introduction*

# 1. INTRODUCTION

Globally, many technologies have been developed for managing the most noxious pests - the insects that limit agricultural production, of which, many have been forsaken. Still, many technologies are being developed. Today, research converges to biological control by virtue of the effectiveness of the bioagents and their safety to non targets and environment. The naturally existing and disease causing microbes of insects are applauded as important biocontrol agents world over and research is spinning ahead for exploiting their potential. Of the different microbes, the entomopathogenic fungi having the ability to breach insect cuticle and enter insect body to cause infection in both chewing and sucking insects have instilled their role as biocontrol agents. Among the myriads of entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium lecanii* (Zimmermann) Zare and Gams and *Metarhizium anisopliae* (Metschnikoff) Sorokin are ahead of the pack.

High heterogeneity is demonstrated within the species complexes of entomopathogenic fungi (Mugnai *et al.*, 1989 and Bidochka *et al.*, 1994). Conventional taxonomic study is primarily based on morphological (Mugnai *et al.*, 1989) and physiological (St. Leger *et al.*, 1992) characters. Phenotypic characters are not sufficient to distinguish isolates of these entomopathogenic fungi and hence molecular analysis is demanded (Gaitan *et al.*, 2002 and Castrillo *et al.*, 2003). The ribosomal DNA repeated units which contain highly conserved DNA sequence as well as more variable DNA sequence regions have been used to detect genetic variations in populations (White *et al.*, 1990).

Soil and insect cadavers present in natural ecosystems are rich sources of entmopathogenic fungi (Ignoffo *et al.*, 1978). Isolation techniques of these fungi from these substrates have also been developed (Veen and Ferron, 1966 and

Zimmermann, 1986). It is now known that these fungi have many isolates that vary in their virulence and host range (Ignoffo and Garcia, 1985; Aizawa, 1987 and Fuxa and Tanada, 1987). Henceforth, isolation and molecular identification of fungi are indispensable. Further, to be successful down the track as prime bioagents in pest management programmes, the pathogenicity, host range, virulence, effective field dosé and moreover their performance under different agroecosystems needs elucidation.

Food security is a critical issue. In Kerala, where the main caloric intake of people comes from rice, its production is also crucial. Insect pests that ravage the rice crop are one of the impediments in maximising its production. A sensible approach in any pest management programme is to select a tolerant / resistant variety that acts as the first line of defense in order to reduce the pest control cost. The rice variety Mo 16 (Uma), a relatively pest tolerant variety is cultivated in more than 60 per cent of the rice fields in the State (Anon., 2012). The crop is prone to the attack of two major rice pests, the leaf roller, *Cnaphalocrocis medinalis* Guen. and the rice bug, *Leptocorisa acuta* (Thunb). The leaf roller that appear in the vegetative phase as well as in the panicle initiation to booting stages cause 63 to 80 per cent yield loss in rice (Rajendran *et al*, 1986). The rice bug that directly desaps the grains in the milky stage reduces the yield from 10 to 50 per cent (Banerjee and Chatterjee, 1982). Even today, to contain them toxic insecticides are mainly depended upon. Henceforth, safer management strategies incorporating biocontrol agents are warranted in rice crop too to ward off the adversities of insecticides.

As information pertaining to pathogenicity and the field efficacy of the entomopathogenic fungi to rice pests is meager, studies are absolutely essential in this line. Temperature and relative humidity are the two physical factors having much impact on the development of fungal infections (Benz, 1987 and James *et al.*, 1998).

The microclimate prevailing in waterlogged paddy fields ensure high humidity and favour the use of entomopathogenic fungi for pest management in rice.

Now, the Government policies are switching too much in favour of organic farming in the State. At this cross road, a thoughtful approach is not to dismiss the chemical insecticides totally from the scenario of pest management but to keep in store the recommendations of chemical pesticides ready, of course, recommendations of safer chemicals with novel mode of action to overcome the exigencies during pest outbreaks and to safeguard the economy of the farmers in such situations.

Compatibility of the entomopathogens with other management tools needs to be addressed especially when they are concomitantly used with pesticides in integrated pest management programmes. A novel approach for the better utilization of bioagents in integrated pest management programmes is the development of pesticide tolerant strains. Though knowledge related to pesticide tolerant macrobials as exemplified by the parasitoid, *Trichogramma* sp. are present (Jalali *et al.*, 2006) information pertaining to pesticide tolerant microbials, especially, that of entomopathogenic fungi other than that of Shapiro *et al.* 2002; 2011 and Anis, 2014 are none. Considering the aforesaid aspects of entomopathogenic fungi and rice pest management, a project entitled "Entomopathogenic fungi for the management of insect pests in rice ecosystem" was undertaken with the following objectives:

- To isolate and identify indigenous strains of entomopathogenic fungi,
- assess the pathogenicity of *B. bassiana, M. anisopliae* and indigenous isolates against major rice pests and to determine the $LC_{50}$, $LC_{90}$ and $LT_{50}$ values of the fungi,
- evaluate the field efficacy of the effective fungal pathogens and newer molecules of pesticides against major pests,
- assess the compatibility of these fungi with pesticides and
- develop pesticide tolerant strains of *B. bassiana* and *M. anisopliae.*

# *Review of Literature*

# 2. REVIEW OF LITERATURE

Entomopathogenic fungi that cause epizootics in insect population are promising candidates as biocontrol agents. The literature pertaining to the isolation and identification, pathogenicity, bioassay and field evaluation of these fungi are reviewed below. Information available on compatibility of fungi with pesticides and pesticide tolerant strains are also reviewed below.

## 2.1 ISOLATION AND IDENTIFICATION OF INDIGENOUS STRAINS OF ENTOMOPATHOGENIC FUNGI

Prior (1989) stated that the use of biopesticides in many countries is limited mainly due to the poor efficacy of imported products under local conditions. Marohasy (1996) and McFayden (1998) applauded the inherent safety and stability of indigenous natural enemies, due to less host shift and negative impact on economy or environment. A number of *Beauveria* isolates have been studied due to their potential use as biopesticides (Smith *et al.*, 1999 and Devi *et al.*, 2001). Fungal species and sometimes isolates within the species can behave very differently with respect to host range and infection levels (Sierotzki *et al.*, 2000 and Pell *et al.*, 2001). Inglis *et al.* (2001) detailed that *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin have wider host ranges and that *B. bassiana* and *M. anisopliae* contain a diverse assemblage of genotypes and that within this taxa individual isolates can exhibit a substantially restricted host range. Variation in the infectivity of field collected isolates of *M. anisopliae* was reported by Rachappa *et al.* (2009) and Lopes *et al.* (2013). Fazeli-Dinan *et al.* (2012) was of the opinion that local isolates of *B. bassiana* varied in their infectivity.

### 2.1.1 Isolation of Fungi

#### *2.1.1.1 From Insect Cadavers*

The natural isolates of entomopathogenic fungi can be collected from mycosed insect cadavers (Beevi, 1982; Hareendranath, 1989; Anitha *et al*, 1999; Devi *et al.*, 2001; Ambethgar, 2002; Beegum, 2005; Sudharma and Rani, 2005;

Jiji *et al.*, 2008; Sivasundaram *et al.*, 2008; Assaf *et al.*, 2011; Ramanujam *et al.*, 2011; Anis, 2014 and Lokesh, 2014).

Surface sterilisation of cadaver with one or two per cent sodium hypochlorite solution (Quesada-Moraga *et al.*, 2007; Assaf *et al.*, 2011; Anis, 2014; Lokesh, 2014 and Tkaczuk *et al.*, 2014) or 0.1 per cent mercuric chloride (Hareendranath, 1989; Sahayaraj and Namashivayam, 2008 or five per cent sodium hypochlorite and 75 per cent ethanol solution (Kumar *et al.*, 2014) followed by rinsing in sterile water, drying and incubating in medium was adopted by many researchers.

***2.1.1.2 From Soil***

Soil is the main reservoir of entomopathogenic fungi which have an essential influence on the occurrence and expansion of insect mycoses (Ignoffo *et al.*, 1978). Soil is considered as an excellent environmental shelter for entomopathogenic fungi since, in soil they are protected from ultra violet radiation and other adverse abiotic and biotic influences (Keller and Zimmerman, 1989). Entomopathogenic fungi are adapted to live as saprophytes as well as symbionts in the plant rhizosphere which make soil their major habitat (Hu and St. Leger, 2002). Serial dilution technique (Veen and Ferron, 1966 and Hasan *et al.*, 2012) or insect bait method (Vanninen *et al.*, 1989; Chandler *et al.*, 1997; Bidochka *et al.*, 1998; Klingen *et al.*, 2002 and Meyling and Eilenberg, 2006) was followed for the isolation of fungi from soil

***2.1.1.2.1 Using Insect Bait***

Many researchers used larvae of the wax moth, *Galleria mellonella* L. as bait for the isolation of entomopathogenic fungi from soil (Zimmermann, 1986; Vanninen, 1996; Keller *et al.*, 2003; Neuman and Shields, 2004; Sun *et al.*, 2008 and Rishi *et al.*, 2013).

Even though *G. mellonella* is the commonly used bait insect, a few reports on other bait insects are also available. Tkaczuk *et al.* (2000) used *Tribolium destructor* Uyttenboogaart as insect bait for isolating *M. anisopliae*.

Klingen *et al.* (2002) used larvae of the dipteran, *Delia floralis* (Fallen) as bait whereas David *et al.* (2003) isolated 22 fungal isolates of *B. bassiana* and *M. anisopliae* using pecan weevil *Curculio caryae* Horn as bait. Sivasundaram *et al.* (2008) collected three isolates of *B. bassiana* using *Cnaphalocrocis medinalis* Guen. larvae as bait. Sergio *et al.* (2010) used *Tenebrio molitor* (L) as the bait insect for isolating *B. bassiana* and *M. anisopliae* from soil.

***2.1.1.2.2 Using Selective Media***

Veen and Ferron (1966) formulated selective medium with dodine, chloramphenicol and cyclohexamide to isolate hypocrelean entomopathogenic fungi from soil and it was widely accepted as Veens semiselective medium. Many scientists used this medium for the isolation of entomopathogenic fungi form soil (Goettel and Inglis, 1997; Hu and St Leger, 2002 and Ibrahim *et al.*, 2011). Fungicides *viz.*, oxgall, cupric sulfate, cooper chloride, benomyl and dodine and antibiotics such as cholramphenicol, tetracycline and streptomycin have been used separately or in combination in selective media to isolate various entomopathogenic fungi (Beilhartz *et al.*, 1982; Shimazu and Sato, 1996; Mark and Douglas, 1997 and Shimazu *et al.*, 2002).

Strasser *et al.* (1996) modified Veens medium with streptomycin, and tetracycline. This medium was widely used by many researchers (Keller *et al.*, 2003; Kessler *et al.*, 2003; Enkerli *et al.*, 2004; Kessler *et al.*, 2004; Meyling and Eilenberg, 2006 and Tkaczuk *et al.*, 2014).

Luz *et al.* (2007) have opined that substitutes for dodine are necessary due to its unavailability. Ghanbary *et al.* (2009) used selective media with $KH_2PO_4$, $K_2HPO_4$, peptone, $MgSO_4$, dextrose, yeast extract, rose bengal and streptomycin sulphate for isolating *M. anisopliae* from soils. Fernandes *et al.* (2010) used a dodine free medium which consisted of potato dextrose agar plus yeast extract (PDAY) supplemented with chloramphenicol, thiabendazole and cycloheximide (CTC) for isolation of entomopathogenic fungi from soil. Rangel *et al.* (2010) reported that PDAY supplemented with dodine and gentamicin as an efficient

selective media. Posadas *et al.* (2012) used oat meal agar with chloramphenicol and Cetyl Trimethyl Ammonium Bromide (CTAB) to isolate *B. bassiana* and *M. anisopliae* from soils.

### 2.1.2 Identification of Fungi

#### *2.1.2.1 Morphological Identification*

There are several reports of morphological identification of fungi through microscopic inspection of the conidiogenous structure and conidial morphology (Samson *et al.*, 1988; Humber, 1997; Sivasundaram *et al.*, 2008; Assaf *et al.*, 2011; Ibrahim *et al.*, 2011 and Rishi *et al.*, 2013).

#### *2.1.2.2 Molecular Identification*

According to Bruns *et al.* (1991), the use of molecular techniques allowed quicker and accurate identification of broader range of fungal species compared to the identification through morphological characteristics alone. Butt *et al.* (2001) opined that the application of molecular methods is shedding new light on generic and species concepts within this group of entomopathogenic fungi.

Random Amplified Polymorphic DNA (RAPD) was followed to identify entomopathogenic fungi by several researchers (Bidochka *et al.*, 1994; Leal *et al.*, 1994; Fungaro *et al.*, 1996; Urtz and Rice, 1997; Freire *et al.*, 2001; Jensen *et al.*, 2001 and Castrillo *et al.*, 2003).

Restriction Fragment Length Polymorphism (RFLP) fingerprints has been used to distinguish between species of entomopathogenic fungi (Hegedus and Khachatourians, 1995).

Species or strain specific, Sequence Characterized Amplified Region (SCAR) were also used to detect various isolates (Schilling *et al.*, 1996., Abbasi *et al.*, 1999., Li *et al.*, 1999., Lecomte *et al.*, 2000 and Castrillo *et al.*, 2003).

For entomopathogenic fungi, attempts were made to sequence the Internal Transcribed Spacer (ITS) region using general fungal primer sets (Driver *et al.*, 2000; Muro *et al.*, 2003; Glare, 2004). ITS sequencing led to the isolation of

different species of *Aspergillus* (Henry *et al.*, 2000 and Das *et al.*, 2013). Ramanujam *et al.* (2011) identified thirty one isolates of *Lecanicillium* sp. using ITS1, ITS2 and 5.8 S gene of rRNA sequence.

ITS sequencing of ribosomal DNA (rDNA-ITS) was adopted by many scientists to characterise entomopathogenic fungi (Coates *et al.*, 2002; Gaitan *et al.*, 2002; Muro *et al.*, 2003; 2005; Wada *et al.*, 2003; Anis, 2014 and Lokesh, 2014).

Destefano *et al.* (2004) analyzed ITS1 and 2 regions for the identification of *M. anisopliae.* The ITS region of *M. anisopliae* isolate Ma 69 was sequenced and analyzed by BLASTN (Nucleotide Basic Local Alignment Search Tool) to confirm its identity by Pattemore *et al.* (2014).

Rehner and Buckley (2005) used nuclear ribosomal ITS and elongation factor 1-alpha (EF1-a) sequences to investigate molecular phylogenetic diversity of 86 isolates of *Beauveria* and related *Cordyceps* sp. from diverse geographic origins, habitats and insect hosts. Carneiro *et al.* (2008) characterized twenty four *Beauveria* isolates through rDNA-ITS sequencing. Wang and Zheng (2012) identified the isolate *B. bassiana* from *Frankliniella occidentalis* Perganda using ITS and partial sequence of EF1-a.

## 2.2 PATHOGENICITY

Entomogenous fungi are promising agents in pest management because they act by contact and do not require ingestion and are quite host specific (Burges, 1981; Robert and Humber, 1984; Carruthers and Soper, 1987; McCoy *et al.*, 1988; Tanada and Kaya, 1993; Hajek and St. Leger, 1994; Maddox, 1994 and Shahid *et al.*, 2012).

### 2.2.1 *B. bassiana*

The entomopathogenic fungus *B. bassiana* is widely regarded as a potential biocontrol agent. Many scientists reported the suitability of this pathogen for pest management (Feng *et al.*, 1994; Muro *et al.*, 2003; Jiji *et al.*, 2008; Sudharma and

Archana, 2009; Vimala and Hari, 2009; Karthikeyan and Jacob, 2010; Anis, 2014 and Lokesh, 2014).

***2.2.1.1 B. bassiana against Rice Pests***

***2.2.1.1.1 C. medinalis***

Pathogenicity of *B. bassiana* against *C. medinalis* was reported by many researchers (Rao, 1989; Ambethgar, 1997; Alice *et al.*, 2003; Sivasundaram *et al.*, 2008 and Kirubakaran *et al.*, 2013).

Ambethgar *et al.* (2007) recorded the pathogenicity of 22 isolates of *B. bassiana* @ $10^7$ spores $ml^{-1}$ against *C. medinalis* larvae and the mortality varied from 52.22 to 95.55 per cent at 10 days after treatment (DAT). Sivasundaram *et al.* (2008) reported the pathogenicity of 13 isolates of *B. bassiana* @ $10^8$ spores $ml^{-1}$ against *C. medinalis* larvae and the percentage mortality varied from 23.33 to 73.30 per cent. Kirubakaran *et al.* (2013) observed that *B. bassiana* MTCC 7690 @ $10^8$ spore $ml^{-1}$ caused 83 per cent mortality of larvae of *C. medinalis.*

Symptoms under in vitro conditions included altered feeding behavior of larva, reduced pupal weight, prolonged pupation period and malformed pupa and adult (Sivasundaram *et al.*, 2008).

***2.2.1.1.2 Leptocorisa* sp.**

Twelve isolates of *B. bassiana* @ $10^8$ spore $ml^{-1}$ were found pathogenic to *L. acuta* and mortality was reported to range from 57.50 to 77.70 per cent at 10 DAT (Loc and Chi, 2005). Similarly, Herlinda *et al.* (2008) reported mortality of 12 other different isolates of *B. bassiana* and the extent of infection was recorded as 46 to 93 per cent against nymphs of *Leptocorisa oratorius* (Fab.).

Symptoms such as loss of appetite and slow movement were shown by infected insects. White coloured fungal hyphae appeared from their stiff and dried bodies (Herlinda *et al.*, 2008).

*2.2.1.1.3 Other Rice Pests*

Puzari and Hazarika (1992) reported 90 per cent mortality to adults of *Dicladispa armigera* (Olivier) by *B. bassiana*. Dhuyo and Soomro (2008) reported the pathogenicity of isolate no. 274 and 373 of *B. bassiana* at concentrations ranging from $10^5$ to $10^9$ spores $ml^{-1}$ to egg, larva and pupa of *Scirpophaga incertulas* (Walker). Phukan *et al.* (2008) observed a decrease in the total haemocyte count in *B. bassiana* infected adults of *D. armigera*. According to Karthikeyan and Jacob (2010) the cumulative mortality of *Leptispa pygmae* Baly ranged from 56.67 to 80.00 per cent at $10^5$ to $10^9$ spores $ml^{-1}$ of *B. bassiana*. Fazeli-Dinan *et al.* (2012) recorded 81.03 per cent mortality of eggs and 50.76 per cent mortality of larvae of *Naranga aenescens* Moore by the isolate DEBI003 when applied @ $10^7$ spores $ml^{-1}$.

**2.2.2 *M. anisopliae***

*Metarhizium* sp. are cosmopolitan entomopathogenic fungi reported to infect more than 300 arthropod species belonging to several insect orders (Alves, 1998). Pathogenicity of *M. anisopliae* to pests infesting various crops have been reported earlier by many scientists (Anitha *et al.*, 1999; Bidochka *et al.*, 2000; Santiago *et al.*, 2001; Burgoni, 2005; Anis, 2014 and Lokesh, 2014).

*2.2.2.1 M. anisopliae against Rice Pests*

*2.2.2.1.1 C. medinalis*

Shahid *et al.* (2003) recorded the pathogenicity of *M. anisopliae* against leaf folder. Ambethgar *et al.* (2007) reported 37.77 to 63.33 per cent mortality of *C. medinalis* larvae by four isolates of *Metarhizium* sp. @ $10^7$ spore $ml^{-1}$. *M. anisopliae* MTCC 4104 @ $10^8$ spore $ml^{-1}$ caused 79 per cent mortality of larvae of *C. medinalis* (Kirubakaran *et al.*, 2013).

*2.2.2.1.2 Leptocorisa* **sp.**

Loc and Chi (2005) found that *M. anisopliae* caused infection in *L. acuta* and that the mortality percentage ranged from 74.40 to 87.00 per cent at10 DAT. The infection of five isolates of *Metarhizium* sp. in *L. oratorius* that caused 50 to

62 per cent mortality in the nymphs was reported by Herlinda *et al.* (2008).

Symptoms of fungal infection were more or less similar to that caused by *B. bassiana*, but the mycelia colour was greenish white (Herlinda *et al.*, 2008).

***2.2.2.1.3 Other Rice Pests***

Rombach *et al.* (1987) reported the pathogenicity of *M. anisopliae* on *Nephotettix virescens* (Uhler) and *Cofana spectra* (Distant). Martins *et al.* (2004) and Rampoletti *et al.* (2007) reported epizootics by *M. anisopliae* on populations of rice stink bug, *Tibraca limbativentris* Stal. Jin *et al.* (2008) observed that the isolates Ma 456 and Ma 576 caused more than 50 per cent mortality of nymphs of *Nilaparvata lugens* Stal.

**2.2.3 Other Entomopathogenic Fungi against Rice Pests**

Holdom *et al.* (1988) reported the pathogencity of *Erynia delphacis* (Hori) to *N. lugens* and that of the *E. delphacis*, Zoophthora *radicans* (Brefeld) and *Entomophaga* sp. on *Sogatodes oryzicola* (Sogata).

Puzari and Hazarika (1992) reported that *Aspergillus flavus Link* and *Fusarium heterosporum* Nees were pathogenic to adults of *D. armigera* and mortality percentage was fifty and seven, respectively.

Yasodha and Narayanasamy (2004) observed the pathogenicity of *Fusarium* sp., *Mucor* sp., *Scopularopsis* sp. @ $10^7$ spores $ml^{-1}$ against lepidopteran rice pests.

Ambethgar *et al.* (2007) reported the pathogenicity of *Aspergillus* sp, *Entomophthora* sp., *Fusarium* sp., *Nomuraea rileyi* (Farlow), *Paecilomyces* sp. and *Z. radicans* against *C. medinalis*.

*Verticillium lecanii* (Zimmermann) @ $10^8$ spores $ml^{-1}$ was reported as pathogenic to *N. lugens* (Reddy *et al.*, 2013).

Kumar *et al.* (2014) reported the pathogenicity of *Aspergillus flavus* Link, *Aspergillus fumigatus* (Fres.) and *Aspergillus niger* (L) to three rice grass hoppers *Oxya velox* (Fab.), *Oxya hyla hyla* (Serville) and *Hieroglyphus nigrorepletus*

(Bolivar). Infection of *Z. radicans* on *C. medinalis* was observed by Senthilkumar *et al.* (2014).

2.3 BIOASSAY

**2.3.1 Bioassay of *B. bassiana***

***2.3.1.1. C. medinalis***

Ambethgar *et al.* (2007) recorded $LC_{50}$ of *B. bassiana* isolates, BbOn KKL and BbCmKKL as 2.2 x $10^4$ and 3.7 x $10^5$ spores $ml^{-1}$, respectively against *C. medinalis* larvae and $LT_{50}$ value of these isolates @ $10^7$ spores $ml^{-1}$ as 5.56 and 6.20 days, respectively. Sivasundaram *et al.* (2008) reported $LC_{50}$ and $LT_{50}$ of another *B. bassiana* isolate, as 3.39 x $10^4$ spores $ml^{-1}$ and 4.4 days against *C. medinalis* while Kirubakaran *et al.* (2013) recorded the $LC_{50}$ value as 9.09 x $10^4$ spores $ml^{-1}$ for *B. bassiana* against this pest.

***2.3.1.2 Leptocorisa* sp.**

$LT_{50}$ of 3.52 and 10.36 days for the KBC and SLSS isolates of *B. bassiana* against *L. oratorius* was observed by Herlinda *et al.* (2008).

***2.3.1.3 Other Rice Pests***

Dhuyo and Soomro (2008) noted variations in the $LC_{50}$ for the two isolates of *B. bassiana* (274 and 373), the values being $10^5$, $10^5$ and $10^6$ spores $ml^{-1}$, respectively against eggs, larvae and pupae of *S. incertulas* and the corresponding values for the isolate 373 was $10^6$, $10^8$ and $10^8$ spores $ml^{-1}$. The $LT_{50}$ values of the isolate *B. bassiana* (274) @ $10^9$ spores $ml^{-1}$ against larvae and pupae of *S. incertulas* was 4.3, 3.8 days, respectively whereas for the isolate *B. bassiana* (373) it was 6.0 and 3.4 days, respectively. Karthikeyan and Jacob (2010) noted 2.26 x $10^4$ spores $ml^{-1}$ as the $LC_{50}$ of *B. bassiana* against *L. pygmaea.* In 2012, Li *et al.* found 2.9 x $10^7$ spores $ml^{-1}$ as the $LC_{50}$ of *B. bassiana* against the brown plant hopper, *N. lugens.*

**2.3.2 Bioassay of *M. anisopliae***

***2.3.2.1. C. medinalis***

It was noted by Kirubakaran *et al.* (2013) that a concentration of 6.08 x $10^5$ spores $ml^{-1}$ of *M. anisopliae* was required to bring fifty per cent mortality in *C. medinalis*

***2.3.2.2 Leptocorisa* sp.**

Herlinda *et al.* (2008) reported that the lethal time to bring fifty per cent mortality in the rice bug, *L. oratorius* as 5.75 and 7.46 days, respectively for the two isolates Mtm and Mpx of *M. anisopliae.*

***2.3.2.3 Other Rice Pests***

Jin *et al.* (2008) has stated the $LC_{50}$ of *M. anisopliae* as $10^{16}$ conidia $mm^{-2}$ against *N. lugens.* Fazeli-Dinan *et al.* (2012) recorded $LC_{50}$ value of 1 x $10^7$ spores $ml^{-1}$ against larva of *N. aenescens.*

2.4 FIELD EVALUATION

**2.4.1 Crop Loss**

***2.4.1.1 C. medinalis***

*C. medinalis* was reported as a major pest of rice in Kerala by Nair (1978); Nadarajan and Skaria (1988); Nalinakumari *et al.* (1996) and Lekha (2003). *C. medinalis* larvae damage rice leaves by scraping the green matter, resulting in reduced photosynthesis and subsequent yield reduction (Fraenkel and Fallil, 1981). The pest has been recorded to cause 63 to 80 per cent yield loss in rice (Rajendran *et al.*, 1986 and Murugesan and Chelliah, 1987). According to Nanda and Bisoi (1990) every unit of increase in infestation by *C. medinalis* decreased the yield by 14 and 1.46 per cent during summer and wet season, respectively. The pest as an important production constraint of rice in South Asia and other parts of the world was documented by Dale (1994). Pathak and Khan (1994) reported five to 60 per cent yield loss due to rice leaf roller infestation. Arshad

*et al.* (2012) observed 20 to 80 per cent yield loss due to *C. medinalis* infestation in rice.

*2.4.1.2 L. acuta*

*L. acuta* was also reported as a major rice pest in Kerala by Nair (1978). Gupta *et al.* (1993) observed that nymphs and adults of *L. acuta*, suck milk from the developing rice grains, and they reported 2.5 to 6.21 per cent and 1.72 to 5.23 per cent damage during wet and dry seasons, respectively. Dale (1994) opined that the quality of damaged grains reduced and lowered the market value as the damaged grains retained the buggy odour even after cooking. Sugimoto and Nugaliyadde (1995) recorded 10 per cent damage due to rice bug infestation. Krishnakumar and Visalakshi (1996) and Nalinakumari *et al.* (1996) recorded *L. acuta* as a major pest in rice fields of Thiruvananthapuram and Kuttanad in Kerala. Panda and Rath (2003) reported 25 to 51 per cent yield loss due to rice bug infestation and they observed that the damage by nymphs was more severe as they preferred grains at milky stage. Kalita *et al.* (2009) reported 13.60 per cent grain damage due to the infestation of *L. acuta* whereas Tiwari *et al.* (2014) reported 30 per cent yield loss in rice.

**2.4.2 Management of Rice Pests**

***2.4.2.1 Entomopathogenic Fungi***

***2.4.2.1.1 B. bassiana***

***2.4.2.1.1.1 C. medinalis***

Sivasundaram *et al.* (2008) reported significantly low damage by *C. medinalis* in *B. bassiana* @ $10^8$ spores $ml^{-1}$ treated plots.

***2.4.2.1.1.2 Leptocorisa* sp.**

The extent of mortality of *L. acuta* observed by Loc and Chi (2005) in the field on application of *B. bassiana* @ 6 x $10^{12}$ spores $ha^{-1}$ was 45.30 to 74.90 per cent whilst a high mortality of 85 to 100 per cent of rice bug nymphs was observed by the application of *B. bassiana* @ $10^9$ spores $ml^{-1}$ (Herlinda *et al.*,

2008). The reduction in rice bug damaged grains noted by Kalita *et al.* (2009) on application of *B. bassiana* was 5.15 per cent.

***2.4.2.1.1.3 Other Rice Pests***

Rombach *et al.* (1986a and 1986b) reported the effectiveness of conidial suspension of *B. bassiana* @ 5 x $10^{12}$ conidia $ha^{-1}$ in reducing the population of *Scotinophara coarctata* (F.) and *N. lugens*. Puzari and Hazarika (1992) observed that *B. bassiana* @ $10^7$ spores $ml^{-1}$ caused 81 per cent mortality to *D. armigera*. Karthikeyan and Jacob (2010) observed reduction in damage by *L. pygmaea* by the application of *B. bassiana* @ $10^7$ spores $ml^{-1}$ and it was to the tune of 61 to 72 per cent.

***2.4.2.1.2 M. anisopliae***

***2.4.2.1.2.1 C. medinalis***

Padmaja and Kaur (2001) reported that *M. anisopliae* @ $10^8$ spores $ml^{-1}$ caused 60 to 70 per cent reduction in the population of *C. medinalis*. Effectiveness of *M. anisopliae* in reducing the population of *C. medinalis* when applied @ 250 g $acre^{-1}$ was reported by Shahid *et al.* (2003).

***2.4.2.1.2.2 L. acuta***

Loc and Chi (2005) found 63.60 to 86.60 per cent mortality of *L. acuta* by the application of *M. anisopliae* isolates @ 6 x $10^{12}$ spores $ha^{-1}$. Still higher mortality of 85 to 100 per cent of rice bug nymphs was recorded by Herlinda *et al.* (2008) by the application of *M. anisopliae*. Kalita *et al.* (2009) also reported the effectiveness of *M. anisopliae* in reducing the damage by *L. acuta* to the extent of 4.89 per cent.

***2.4.2.1.2.3 Other Rice Pests***

Rombach *et al.* (1986 a) recorded 63 to 98 per cent mortality of *N. lugens* by the application of conidial suspension of *M. anisopliae* @ 5 x $10^{12}$ conidia $ha^{-1}$. Population reduction of the rice stem borer, *S. incertulas* by the application of *M. anisopliae* @ 250 g $acre^{-1}$ was also reported by Shahid *et al.* (2003).

*2.4.2.1.3 Other Entomopathogenic Fungi*

Rombach *et al.* (1986a) reported that population of *S. coarctata* was significantly reduced by the application of conidial suspension of *Paecilomyces lilacinus* (Thom.) @ 2.5 x $10^{12}$ spores $ha^{-1}$. Conidial suspension of *Hirsutella citriformis* Speare @ 5 x $10^{12}$ conidia $ha^{-1}$ was found effective in reducing the population of *N. lugens* by 63 to 98 per cent (Rombach *et al.*, 1986b).

*2.4.2.2 Chemical Pesticides*

*2.4.2.2.1 C. medinalis*

The efficacy of acephate @ 750 g a.i $ha^{-1}$ against *C. medinalis* was reported by many researchers (Saroja and Raju, 1982; Rao *et al.*, 1985; Korat *et al.*, 1999; Zhong *et al.*, 2002 and Smitha, 2004). Murali *et al.* (2013) observed reduction in the population of *C. medinalis* by the application of combination product of chlorantraniliprole 5 per cent GR and thiamethoxam 10 per cent GR @ 6 kg $ha^{-1}$. Sarao and Kaur (2013) reported significant reduction in leaf roller incidence in rice by the application of chlorantraniliprole 0.4 per cent GR.

*2.4.2.2.2 L. acuta*

Effectiveness of acephate @ 750 g a.i $ha^{-1}$ was reported for the management of *L. acuta* (Kay *et al.*, 1993 and Smitha, 2004). Krishnakumar and Visalakshi (1996) reported 75.72 per cent reduction in the population of *L. acuta* by the application of 0.15 per cent malathion. Ashokappa (2011) recommended the use of thiamethoxam @ 25 g a.i $ha^{-1}$ and malathion 575 g a.i $ha^{-1}$ for the management of *L. acuta.*

*2.4.2.2.3 Other Rice Pests*

Lakshmi *et al.* (2010) recorded 56 per cent mortality to *N. lugens* and cent per cent mortality to *Sogatella furcifera* (Horvath) by the application of acephate @ 750 g a.i $ha^{-1}$. Murali *et al.* (2013) observed reduction in the population of *N. lugens* and *N. virescens* by the application of a combination product of chlorantraniliprole 5 per cent GR and thiamethoxam 10 per cent GR @ 6 kg $ha^{-1}$.

### 2.4.3 Natural Enemies

#### *2.4.3.1 Entomopathogenic Fungi*

##### *2.4.3.1.1 B. bassiana*

Pingel and Lewis (1996) opined that *B. bassiana* was safe to coccinellid predators. The safety of *B. bassiana* to predatory insects and spiders in rice ecosystem was recorded by Chi *et al.* (2005). Significantly higher spider population in *B. bassiana* treated plots compared to chemical treated plots was recorded by Reddy *et al.* (2013).

##### *2.4.3.1.2 M. anisopliae*

Rao (1989) reported that *M. anisopliae* was safe to the predators *Cyrtorhinus lividipennis* Reuter, *Coccinella arcuata* Fabricius, *Lycosa pseudoannulata* (Boesenberg et Strand) and parasitoids *viz.*, *Trichogramma japonicum* Ashm and *Platygaster oryzae* (Cameron). Chi *et al.* (2005) observed significantly higher population of predatory spiders and insects in *M. anisopliae* @ $10^9$ spores $ml^{-1}$ treated plots. Reddy *et al.* (2013) also reported the safety of *M. anisopliae* @ $10^8$ spores $ml^{-1}$ to natural enemies in rice ecosystem.

##### *2.4.3.1.3 Other Entomopathogenic Fungi*

Reddy *et al.* (2013) recorded higher spider population in *V. lecanii* @ $10^8$ spores $ml^{-1}$ treated rice plots.

#### *2.4.3.2 Chemical Pesticides*

Low toxicity of acephate to natural enemies was reported (Sun *et al.*, 2002 and Preetha *et al.* 2010). Contrastingly toxicity of acephate @ 750 g a.i $ha^{-1}$ to predators in rice ecosystem was reported by many researchers (Smitha, 2004; Lakshmi *et al.*, 2010 and Reddy *et al.*, 2013). The selectivity of chlorantraniliprole @ 30 g a.i $ha^{-1}$ to beneficial insects was earlier reported by Bassi *et al.* (2007) and Dinter *et al.* (2008). Harmful effect of thiamaethoxam @ 25 g a.i $ha^{-1}$ to parasitoids and predators was documented by Cloyd and Bethke (2011) and Prabhaker *et al.* (2011). Safety of the insecticide, chlorantraniliprole

@ 30 g a.i $ha^{-1}$ to spiders in rice ecosystem was also reported by Jaafar *et al.* (2013) and Karthick *et al.* (2014).

## 2.5 COMPATIBILITY OF FUNGI WITH PESTICIDES

### 2.5.1 *B. bassiana*

Filho *et al.* (2001) reported the compatibility of *B. bassiana* with thiamethoxam 0.005 per cent, diafenthiuron 0.1 per cent and acephate 0.15 per cent. They also reported the moderate incompatibility of the fungus with imidacloprid 0.005 per cent and total incompatibility with carbosulfan 0.1 ppm. The compatibility of the fungus with alphamethrin 0.008 per cent, cypermethrin 0.006 per cent, deltamethrin 0.002 per cent and phosphamidon 0.005 per cent and incompatibility with dichlorvos 0.12 per cent was observed by Puzari *et al.* (2006). The increase in the radial growth of *B. bassiana* in acetamiprid 0.003 per cent poisoned media was documented by Dhar and Kaur (2009) as an evidence for the compatibility of *B. bassiana* and insecticide. Pandey *et al.* (2009) reported high compatibility with dimethoate 500 ppm as it recorded higher spore production of 6.67 x $10^5$ spores ml $1^{-1}$. They also recorded 65.60 per cent spore germination in dimethoate 700 ppm. Rajanikanth *et al.* (2010) reported high compatibility with imidacloprid 0.005 per cent and spinosad 0.018 per cent as they cause no inhibition of radial growth, sporulation and viability while chlorpyriphos 0.05 per cent exhibited high degree of inhibition. Golshan *et al.* (2013) reported that malathion 0.15 per cent inhibited germination of *B. bassiana*. Anis (2014) and Lokesh (2014) reported compatibility of *B. bassiana* (Bb 5) with imidacloprid 0.006 and emamectin benzoate 0.002 per cent, respectively.

### 2.5.2 *M. anisopliae*

Filho *et al.* (2001) observed that the reproductive and vegetative growth of *M. anisopliae* was not affected by thiamethoxam 0.005 per cent, imidacloprid 0.005 per cent or acephate 0.15 per cent. Reduction in radial growth of the fungus to the extent of 60.69, 46.66 and 45.45 per cent by chlorinated hydro carbons, organophospahtes and carbamates was reported by Rachappa *et al.* (2007). Dhar

and Kaur (2009) documented high compatibility of the fungus with acetamiprid 0.003 per cent. The incompatibility of the isolate CG 891 of *M. anisopliae* with fenitrothion and carbofuran was reported by Ferreira and Teresinha (2010). The compatibility of *M. anisopliae* (strain CG 168) with the insecticides, thiamethoxam 0.005 per cent and lambda cyhalothrin 0.003 per cent was documented by Silva *et al.* (2013). Anis (2014) and Lokesh (2014) reported compatibility of *M. anisopliae* (Ma 4) with imidacloprid 0.006 per cent and emamectin benzoate 0.002 per cent, respectively. A decrease in the germination of *M. anisopliae* with increasing concentration of the imidacloprid (0.0005, 0.0025 and 0.005 per cent) and quinalphos (0.005, 0.025 and 0.05 per cent) was recorded by Babu *et al.* (2014).

### 2.5.3 Other Entomopathogenic Fungi

Wenzel *et al.* (2004) documented the compatibility of *V. lecanii*, with imidacloprid 0.005 per cent fungi as there was no inhibition in growth, sporulation and conidial viability. Archana and Ramaswamy (2012) reported that *Paecilomyces fumosoroseus* (Wize) was compatible with malathion, chloropyrifos, deltamethrin and permethrin @ 250, 500 and 1000 ppm. The compatibility of *A. flavus* with temephos (1 ppm) was observed by Bhan *et al.* (2013).

## 2.6 DEVELOPMENT OF PESTICIDE TOLERANT STRAINS AND THEIR MOLECULAR CHARACTERISATION

Different techniques were followed for the development of pesticide tolerant strains of entomopathogenic fungi. Hoy (1986) and Gaugler (1987) suggested artificial selection as a simpler approach for increasing fungicide tolerance of beneficial fungi. Goettel *et al.* (1990) transformed *M. anisopliae* to benomyl tolerant using pBENA3 plasmid. St. Leger *et al.* (1995) used electroporation and biolistic delivery to transform *M. anisopliae* with the plasmids pNOM102 and pBENA3 for beta gluconidase and benomyl resistance. Sudharma (2006) revealed the possibilities of developing improved pesticide tolerant strains of the

entomopathogenic fungus *Fusarium pallidoroseum* (Cooke) Sacc. through artificial selection.

### 2.6.1 Pesticide Tolerant Strain

#### *2.6.1.1 B. bassiana*

Shapiro *et al.* (2002) reported enhanced tolerance of *B. bassiana* to dodine, fenbuconazole and triphenyltin hydroxide through artificial selection. Anis (2014) reported that artificial selection increased tolerance of *B. bassiana*. The increase in tolerance was to the tune of 32 times higher the recommended field dose of imidacloprid, 16 times higher the field dose of carbendazim and carbosulfan and eight times higher the field dose of chlorpyriphos, carbofuran, lambda cyhalothrin, mancozeb and malathion after ten passages through respective poisoned media.

#### *2.6.1.2 Metarhizium sp.*

Shapiro *et al.* (2011) reported increased resistance of *Metarhizium brunneum* Petch. to fenbuconazole and triphenyltin hydroxide by artificial selection. Possibility of developing pesticide tolerance strains of *M. anisopliae* through artificial selection was documented by Anis (2014) and the tolerance level varied with the pesticides in the poisoned media.

### 2.6.2 Molecular Characterisation of Pesticide Tolerant Strains

Molecular characterisation of artificially selected *B. bassiana* and *M. anisopliae*, by serially passing the fungi ten times through poisoned media exhibited polymorphism. The extent of polymorphism was greater for *B. bassiana* (82.3 per cent) compared to *M. anisopliae* (26.58 per cent), Anis (2014).

# *Materials and Methods*

# 3. MATERIALS AND METHODS

The study entitled "Entomopathogenic fungi for the management of insect pests in rice ecosystem" was conducted at College of Agriculture, Vellayani and at Cropping System Research Centre (CSRC), Karamana during 2011-2014. The materials and methods adopted for the investigation are detailed below.

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

### 3.1.1 Isolation of Entomopathogenic Fungi

#### *3.1.1.1 From Mycosed Cadavers*

##### *3.1.1.1.1 Collection of Cadavers*

Monitoring was done at monthly intervals in thirty rice fields having 50 cents each in Anadu block of Thiruvananthapuram district, where rice was cultivated continuously. The period of observation was from November 2011 to February 2012 (puncha), June 2012 to September 2012 (virippu) and November 2012 to February 2013 (puncha) that coincided with the rice cultivation. Two different methods were followed for the collection of mycosed insects. In the first method, dead insects were collected directly from the rice ecosystem. Insect cadavers collected were brought to laboratory for isolation of fungi, if any. In the second method, sweep net collection of insects were taken and live insects were brought to laboratory and observed regularly for the development of symptoms of fungal infection.

##### *3.1.1.1.2 Isolation of Fungi from Cadavers*

The cadavers collected were kept separately in nine cm Petri plates with moistened filter papers for development of fungal growth. Those cadavers with fungal growth were then surface sterilized for one min in 0.1 per cent mercuric chloride and this was followed by three repeated washings in sterile water under aseptic conditions in a laminar air flow chamber (Hareedranath, 1989). Afterwards, the specimens were dried by keeping it in sterilized filter paper for two min. The cadavers were then placed in Petri plates with Potato Dextrose

Agar (PDA) for development of mycelia. After the mycelial development, sub culturing was done for thrice, transferred to PDA slants and purified by hyphal tip method (Cloh, 1999). The fungi thus obtained were maintained in PDA slants under refrigerated conditions for further studies. Virulence of the fungi was maintained by periodically passing them through adult *L. acuta*.

### *3.1.1.2 From Soil*

#### *3.1.1.2.1 Collection of Soil Samples*

Soil samples were collected from rice fields of Vellayani, Karamana and Anadu of Thiruvananthapuram district. From each location 10 samples, each weighing 200 g were randomly collected at a depth of 20 cm using an auger (Hasan *et al.*, 2012).

#### *3.1.1.2.2 Isolation of Fungi Using Insect Bait*

The Galleria bait method developed by Meyling, 2007 with slight modification was used to trap entomopathogenic fungi from soil.

##### *3.1.1.2.2.1 Preparation of Diet for Rearing Wax Moth (Galleria mellonella* L.)

The diet for rearing *G. mellonella* larvae was prepared as suggested by Woodring and Kaya (1988). Corn flour (200 g), atta (200 g) and milk powder (100 g) were weighed and mixed thoroughly in a container. Afterwards, honey (100 ml) and glycerol (150 ml) were mixed homogenously in another container. The honey glycerol mixture was then slowly added to the flour ingredients and mixed well. The medium was then sterilised at 121°C and 1.06 kg $cm^{-2}$ for 20 min. Powdered yeast (100 g) was then added to the medium, after it had attained room temperature 30 ± 2°C. The prepared medium was stored in an air tight container for up to two weeks.

##### *3.1.1.2.2.2 Stock Culture of G.mellonella*

Eggs of *G. mellonella*, obtained from Department of Agrl. Entomology, College of Agriculture,Vellayani were kept in plastic boxes of dimensions 18 cm x 12 cm. The first instar larvae that emerged were fed with the diet on alternate

days until pupation in the rearing containers. The pupae were collected carefully and transferred to plastic boxes of 18 cm x 14 cm for adult emergence. Cotton soaked in 10 per cent honey solution was placed in the bottom of bottles as feed for adults. Folded paper strips (15 cm x 6 cm) were hung inside the bottle containing adults, to facilitate egg laying. Fourth instar larvae obtained from the stock culture were used for trapping fungi from soil.

***3.1.1.2.2.3 Trapping Technique***

The soil samples collected as detailed in 3.1.1.2.1 were air dried to ward off entomopathogenic nematodes likely to be present in it. The dried samples were then moistened with sterile water @ 10 $ml^{-100\ g}$ soil and were taken in medium sized plastic containers of 10 cm x 20 cm. The fourth instar larvae used for trapping entomopathogenic fungi were initially subjected to heat treatment to prevent excessive webbing. For this, they were dipped in hot water at 56°C for one min and afterwards cooled in running water for 30 seconds. The larvae were dried by placing them on a tissue paper and were kept in dark for four h. Subsequently, five larvae were released into each container with 20 g soil sample, and the containers were covered with lids having holes to facilitate aeration. Four replications were maintained for each soil sample. These containers were placed on bricks in a basin with water and covered with towel such that the bottom of the towel got dipped in water in order to maintain sufficient humidity that would favour fungal growth. The containers were examined after 10 days for mycosed larvae. The infected larvae were collected and surface sterilized as described in 3.1.1.1.2. The fungus thus isolated from the cadavers was brought to pure culture in PDA slants.

***3.1.1.2.3 Isolation of Fungi Using Selective Media***

From each soil sample collected from each of the locations, 10 g was weighed out and suspended in 100 ml sterile water. One ml of the soil suspension was transferred to Petri plate and spread plate method (Buck and Cleverdon, 1960) was adopted for the isolation of fungi. The composition of the media used was the same as that of the Veens semiselective media (Veen and Ferron, 1966),

except for the addition of amoxocyllin (0.10 per cent) and the substitution of dodine with Cetyl Trimethyl Ammonium Bromide (CTAB) at a concentration of 600 mg $l^{-1}$ media. Four replications were maintained for each sample. The plates were incubated for 48 h and observed for the development of fungal colonies. Isolated fungi were then brought under pure culture for further studies.

### 3.1.2 Identification of Entomopathogenic Fungi

#### *3.1.2.1 Morphological Identification*

The isolated fungi were grown separately by placing five mm mycelial disc of respective fungus at the center of nine cm Petri plate with PDA at room temperature (30 ± 2°C). These plates were regularly observed for mycelial colour and growth. Slide culturing of the fungus was adopted for the morphological studies following the method of Harris (1986). The shape and size of the conidia was studied using Motic BA 210 compound microscope under 40 X magnification. The size of conidia was computed by measuring the dimensions of 100 conidia and the mean value was worked out.

#### *3.1.2.2 Molecular Identification*

For molecular characterisation, Internal Transcribed Spacer (ITS) sequencing was done with the facilities available at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram. The protocol for DNA barcoding using universal primers of ITS was as follows

##### *3.1.2.2.1 DNA Isolation*

25 mg of the mycelium was homogenized using liquid nitrogen and the powdered mycelium was transferred to a micro centrifuge tube. 400 µl of buffer PL1 was added and vortexed for one min. 10 µl of RNase A solution was added and inverted to mix. The homogenate was incubated at 65°C for 10 min. The lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for two min. The flow through liquid was collected and the filter was discarded. 450 µl of buffer PC was added and mixed well. The solution was transferred to a Nucleospin Plant II column, centrifuged for one min and the flow through liquid

was discarded. 400 µl of buffer PW1 was added to the column, centrifuged at 11000 x g for one min and flow though liquid was discarded. Then 700 µl PW2 was added, centrifuged at 11000 x g and flow through liquid was discarded. Finally 200 µl of PW2 was added and centrifuged at 11000 x g for two min 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 five min. The column was then centrifuged at 11000 x g for one min to elute the DNA. The eluted DNA was stored at 4°C.

***3.1.2.2.2 DNA Quality Check***

The quality of the DNA isolated was checked using agarose gel electrophoresis. One µl of 6X gel-loading buffer (0.25 per cent bromophenol blue, 30 per cent sucrose in TE buffer, pH-8.0) was added to five µl of DNA. The samples were loaded to 0.8 per cent agarose gel prepared in 0.5X Tris-Borate-EDTA (TBE) buffer containing 0.5 µg $ml^{-1}$ 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 trans illuminator (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 Phire Hotstart II DNA polymerase enzyme, 0.1 mg $ml^{-1}$ BSA and 3 per cent DMSO, 0.5M Betaine, 5pM of forward and reverse primers. Primers used were,

| 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).

PCR amplification profile

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

***3.1.2.2.4 Agarose Gel Electrophoresis of PCR Products***

The PCR products were checked in 1.2 per cent agarose gels prepared in 0.5X TBE buffer containing 0.5 µg $ml^{-1}$ ethidium bromide. One µl of 6X loading dye was mixed with five µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about two 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 trans illuminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

***3.1.2.2.5 ExoSAP-IT Treatment***

ExoSAP-IT (GE Healthcare) consisted 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 µl of PCR product was mixed with two µl of ExoSAP-IT and incubated at 37°C for 15 min followed by enzyme inactivation at 80°C for 15 min.

### *3.1.2.2.6 Sequencing Using BigDye Terminator v3.1*

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v 3.1 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 | - | make up to 10µl |

The sequencing PCR temperature profile consisted of a 1st cycle at 96°C for two min followed by 30 cycles at 96°C for 30 sec, 50°C for 40 seconds and 60°C for four min for all the primers.

### *3.1.2.2.7 Post Sequencing PCR Clean Up*

Master mix I was prepared by mixing of 10 µl milli Q and 2 µl 125mM EDTA. 12 µl of master mix I was added to each reaction containing 10 µl of reaction contents and was properly mixed. Master mix II was prepared by mixing 2 µl of 3M sodium acetate pH 4.6 and 50 µl of ethanol per reaction. Master mix II at the rate of 52 µl was added to each reaction. Contents were mixed by inverting. The tubes and incubated at room temperature for 30 min. The tubes were centrifuged at 14,000 rpm for 30 min. The supernatant was decanted and 100 µl of 70 per cent ethanol was added and centrifuged again at 14,000 rpm for 20 min. This was repeated and the pellet was air dried. The cleaned up and air dried product was sequenced in ABI 3500 DNA Analyser.

*3.1.2.2.8 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). The identity of ITS-rDNA conserved region of the isolates was established by performing a similarity search using Basic Local Alignment Search Tool (BLAST) in the National Centre for Biotechnology Information (NCBI) database and the sequences were matched with existing available database for species confirmation. Based on the sequence matching results, the rDNA sequences were submitted in the NCBI database and accession numbers were obtained.

3.2 PATHOGENICITY

Laboratory experiments were conducted to assess the pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin (Bb 5 and Bb 21), *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ma 4) and the indigenous fungi *viz., Aspergillus flavus* Link. (Af-m1), *B. bassiana* (Bb-m2, Bb-m3, Bb-m4 and Bb-m5) and *M. anisopliae* (Ma-m1) against major rice pests *viz., Cnaphalocrocis medinalis* Guen. the rice leaf folder and *Leptocorisa acuta* (Thunb), the rice bug.

**3.2.1 Maintenance of Fungi**

*B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4), the isolates from National Bureau of Agriculturally Important Insects (NBAII) (now renamed as National Bureau of Agricultural Insect Resources NBAIR), Bengaluru and *B. bassiana* (Bb 21) from the Department of Microbiology, College of Agriculture, Vellayani and the indigenous isolates collected during the present investigation *viz., A. flavus* (Af-m1), *B. bassiana* (Bb-m2, Bb-m3, Bb-m4 and Bb-m5) and *M. anisopliae* (Ma-m1) were maintained in PDA slants at a temperature of 30 $\pm$ 2°C. The virulence of the each fungus was maintained by periodically spraying spore suspension of the respective fungus on the adults of *L. acuta* and reisolating fungi from cadavers as described in 3.1.1.1.2.

### 3.2.2 Stock Culture of Insects

The two test insects, *C. medinalis* and *L. acuta* were reared on rice plants kept in rearing cage of 165 cm x 120 cm made of aluminium frame and nylon wire mesh. The cage was provided with removable doors on two opposite sides for placing potted rice plants. The small windows on all four sides aided in the collection of insects. Ant pans were also placed at the base of the cage to avoid entry of ants.

#### *3.2.2.1 Maintenance of Host Plants*

Seeds of rice variety, Uma (Mo 16) were sown in plastic crates (75 cm x 50 cm x 45 cm) and plastic pots (22 cm x 20 cm) filled with clay collected from rice field. Sequential sowing was done for getting plants of proper stage for rearing the two test insects.

#### *3.2.2.2 Maintenance of Test Insects*

##### *3.2.2.2.1 C. medinalis*

The adult moths collected from rice fields were released into insect rearing cage with 60 day old rice plants. Five ml of 10 per cent honey mixed with three drops of vitamin E was provided in small Petri plates as feed. The larvae that emerged from newly laid eggs were provided with fresh rice plants as and when required until pupation. The pupae that were found inside the leaf folds were left undisturbed till adult emergence.

##### *3.2.2.2.2 L. acuta*

Adults of *L. acuta* collected from rice fields were released into insect cage with rice plants having panicles in the milky stage. The nymphs that emerged from newly laid eggs, fed from the panicles and leaves of rice plants that were provided sequentially.

### 3.2.3 Assessment of Pathogenicity

#### *3.2.3.1 Preparation of Spore Suspension*

The spore suspensions were prepared aseptically by pouring 10 ml of sterile water into 14 day old culture of all test fungi except *A. flavus,* for which seven day old culture was used. 10 ml spore suspension thus obtained from each Petri plate was made up to 30 ml and applied topically on the test insects using an atomizer for assessing pathogenicity.

#### *3.2.3.2 Pathogenicity to C. medinalis*

##### *3.2.3.2.1 Eggs*

The eggs were clipped off from the plants and dipped in spore suspension of the respective fungus for one min and placed in moist chamber made by lining Petri plate with sterile, moist filter paper. The eggs dipped in sterile water served as untreated check. The eggs were observed for symptom development for 10 days, if any.

##### *3.2.3.2.2 Larvae*

The third instar larvae collected from the stock culture were kept in refrigerator for two min to reduce their activity. Larvae sprayed with water served as untreated check. After one h, the treated / untreated larvae were transferred to rice plants kept in 250 ml conical flasks containing water and the mouth of the flask were covered with cotton. The larvae were confined to these plants with perforated polythene covers, free end of which was tied to the neck of the conical flasks. The larvae were observed for symptom development for two weeks.

##### *3.2.3.2.3 Pupae*

The pupae collected from leaf rolls were dipped in spore suspension prepared as mentioned in 3.2.3.1 for one min. Subsequently, they were transferred to moist chamber made as in para 3.2.3.2.1. The pupae in the untreated check were dipped in sterile water. They were examined regularly for 10 days for any symptom development.

*3.2.3.2.4 Adult*

The spore suspensions prepared as mentioned in 3.2.3.1 were sprayed on the inner side of glass jars (24 cm x 13.5 cm), adult *C. medinalis* collected from the stock culture using an insect net were released in to the jars. The cotton soaked in 10 per cent honey solution was placed as feed. They were regularly observed for symptom development for eight days.

*3.2.3.3 Pathogenicity to L. acuta*

*3.2.3.3.1 Eggs*

The leaf with egg mass was clipped off from the plant and dipped in fungal spore suspension for one min. Effect of the fungal isolates to the eggs of *L. acuta* was assessed as mentioned under 3.2.3.2.1.

*3.2.3.3.2 Nymphs and Adults*

The third instar nymphs and one day old adults collected from the stock culture were kept in a refrigerator for two min to reduce their activity. Later, they were transferred to a jar and sprayed with spore suspension of the respective fungi. Fresh rice panicles in milky stage were provided as food for the insects in confinement and observed for symptom development for three weeks.

**3.2.4 Single Dose Screening Assay**

For comparing the pathogenicity of the fungal isolates at a single dose, an experiment was carried out in completely randomised design (CRD) with three replications, each replication having 10 insects. The spore concentration used against third instar larvae of *C. medinalis* and third instar nymphs of *L. acuta* was $10^8$ spores $ml^{-1}$ and that evaluated against adults of *L. acuta* was $10^9$ spores $ml^{-1}$ based on the preliminary trials conducted. Application of spore suspension and assessment of pathogenicity was done as in 3.2.3. The treated insects were observed daily until cent per cent mortality was observed in any one of the treatments. Percentage mortality was corrected using Abbot's formula (Abbot, 1925) and the angular transformed values were statistically analysed using ANOVA.

### 3.2.5 Assessment of Pathogenicity to Natural Enemies

Adults of the coccinellids *viz., Coccinella transversalis* (Fab.) and *Micraspis* sp. and the predatory spiders *viz., Tetragnatha* sp. *and Oxyopus* sp. collected from rice fields were kept in plastic bottles of 15 cm x 12 cm separately and provided with aphids as food. The grubs / spiderlings that emerged were transferred to new jars and were provided with aphids as feed till adulthood.

Preparation and application of spore suspension and assessment of pathogenicity was done as mentioned under 3.2.3.1 and 3.2.3.2, respectively.

## 3.3 BIOASSAY

Experiments were carried out to fix $LC_{50}$, $LC_{90}$ and $LT_{50}$ of *B.bassiana* (Bb 5, Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1). Fungal cultures were maintained in PDB.

### 3.3.1 Preparation of Potato Dextrose Broth (PDB)

For the preparation of PDB, 200 g potato was peeled, cubed, boiled and cooked in 500 ml water and sieved through muslin cloth. The potato extract obtained was mixed with 20 g dextrose dissolved in 500 ml water and made up the final volume to one l. 650 ml of the prepared media was then poured to three l fermenter flasks and sterilised at 121°C at 1.06 kg $cm^{-2}$ pressure for 15 min. The sterilized media was inoculated with 30 ml of 10 day old respective fungal culture and incubated for 14 days.

### 3.3.2 Preparation of Spore Concentrations and Application

Spore suspensions were prepared by blending the respective fungal cultures in a mixer for 30 sec and then sieving through muslin cloth. Neubauer's haemocytometer was used for determining the spore concentrations. The spore suspensions were then serially diluted to get five different concentrations of the respective fungi *i.e.*, $10^8$, $10^7$, $10^6$, $10^5$ and $10^4$ spores $ml^{-1}$ for evaluation against third instar larvae of *C. medinalis* and third instar nymphs of *L. acuta* and at $10^9$, $10^8$, $10^7$, $10^6$ and $10^5$ spores $ml^{-1}$ against adults of *L. acuta*, the spore concentrations were fixed based on the preliminary trials. The spore suspension

was applied on test insects as mentioned in 3.2.3.2.2 and 3.2.3.3.2. The experiments were laid out in CRD with three replications and an untreated check. In each replication, 10 test insects were used. The symptoms and mortality of test insects were recorded daily and the percentage mortality was worked out after making necessary corrections using Abbott's formula (Abbott, 1925).

SPSS (Version 16.0) was used to analyse the dose-mortality relationships and $LC_{50}$, $LC_{90}$ and $LT_{50}$ were determined. Fiducial limits and other regression parameters were also worked out.

3.4 FIELD EXPERIMENT

Two field experiments were conducted to assess the efficacy of the fungal pathogens selected from experiments 3.3.2 and 3.3.3, in managing rice pests under field conditions. The experiments were laid out at Cropping System Research Centre (CSRC), Karamana during November 2012 to March 2013 (puncha) and June 2013 to October 2013 (virippu). The crops were raised using seeds of medium duration rice variety Uma (MO 16) obtained from College of Agriculture, Vellayani. Planting, application of fertilizers and other crop husbandry practices such as weeding and irrigation were done as per Package of Practices Recommendations of the Kerala Agricultural University (KAU, 2011) excluding the plant protection measures. The details of the experiment were as follows:

| | | |
|---|---|---|
| Design | : | RBD |
| Plot size | : | 5 m x 2 m |
| Spacing | : | 20 cm x 10 cm |
| Replications | : | 3 |
| Treatments | : | 11 |
| T1 | : | Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ |
| T2 | : | Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ |
| T3 | : | Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ |

T4 : *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$

T5 : *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$

T6 : *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$

T7 : Acephate 75 per cent SP @ 750 g a.i $ha^{-1}$

T8 : Chlorantraniliprole 18.5 per cent SC @ 30 g a.i $ha^{-1}$

T9 : Malathion 50 per cent EC @ 575 g a.i $ha^{-1}$

T10 : Thiamethoxam 25 per cent WG @ 25 g a.i $ha^{-1}$

T11 : Untreated

The treatments were applied on need basis at 95 and 105 days after sowing (DAS) during first and second field trials, respectively.

### 3.4.1 Preparation of Spray Solutions

#### *3.4.1.1 Talc Based Formulations of Fungi*

*B. bassiana* (Bb 5 and Bb 21) and *M. anisopliae* (Ma 4) were cultured in PDB as described under 3.3. Fourteen day old, fungal cultures of the respective fungi, were blended in a mixer for 30 sec and 30 ml of the spore suspension was added to 100 g of talc, taken in separate plastic basins of 45 cm diameter, and mixed thoroughly. This was air dried for 48-72 h so as to reduce the moisture level to less than 10 per cent. Talc based formulation of each of the fungi was taken @ 20 g $l^{-1}$ for preparing the spray fluids and were sieved through muslin cloth of pore size 2 mm to prevent blockage of the spray nozzle.

#### *3.4.1.2 Spore Suspensions*

*B. bassiana* (Bb 5), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1) were cultured in PDB and the spore count in the spore suspensions prepared from 14 day old culture of Bb 5 and Ma 4 and seven day old culture of Af-m1 were estimated using an improved Neubauer's haemocytometer, and the spore concentration was adjusted to $10^{10}$ spores $ml^{-1}$.

*3.4.1.3 Insecticide Solutions*

*3.4.1.3.1 Acephate*

Three g of the commercial pesticide, Acetaf 75 per cent SP was mixed in one and a half litres of water to get the recommended dose of 750 g a.i $ha^{-1}$.

*3.4.1.3.2 Chlorantraniliprole*

Coragen 18.5 per cent SC (0.45 ml) was mixed in one and a half litres of water to get the recommended dose of 30 g a.i $ha^{-1}$.

*3.4.1.3.3 Thiamethoxam*

The recommended dose of 25 g a.i $ha^{-1}$ was prepared by mixing 300 mg of Actara 25 per cent WG in one and a half litres of water .

*3.4.1.3.4 Malathion*

Malathion 50 per cent EC @ 3.45 ml in one and a half litres of water was used to get the recommended dose of 575 g a.i $ha^{-1}$, for treating three plots.

**3.4.2 Application of Spray Fluids**

Spraying was done using a knapsack sprayer, during the evening hours. The quantity of spray fluid used was 500 ml $plot^{-1}$. While spraying, a wind screen was used to avoid drift.

**3.4.3 Assessment of Pest Population and Intensity of Damage**

To assess the population and intensity of damage before and after treatment, pre treatment count and post treatment count at four, seven, 14 and 21 days after treatment (DAT) were taken.

*3.4.3.1 C. medinalis*

*3.4.3.1.1 Population*

From each plot, 10 sweep net collections were made by walking diagonally through the plot and moving the net to and fro with full stretched hand as one sweep. The number of adults in 10 sweeps $plot^{-1}$ was recorded. The number of larvae in 10 randomly selected hills $plot^{-1}$ was also recorded (Smitha, 2004).

*3.4.3.1.2 Intensity of Damage*

Intensity of damage by *C.medinalis* was assessed by computing the percentage of damaged leaves, from the data collected on the number of leaves damaged and total number of leaves present in 10 randomly selected hills plot$^{-1}$ (Sivasundaram *et al.*, 2008).

*3.4.3.2 L. acuta*

*3.4.3.2.1 Population*

From each plot, 10 sweep net collections were made as described in 3.4.2.1.1 and the number of nymphs and adults were recorded. The population was also assessed by counting the number of nymphs and adults present in 10 randomly selected hills plot$^{-1}$. Count from the 10 hills was added to the sweep net count and this was treated as the total population of *L. acuta* in each plot (Smitha, 2004).

*3.4.3.2.2 Intensity of Damage*

The intensity of damage by *L. acuta* was assessed on the basis of the damaged grains. Total number of grains and the number of damaged grains *i.e.*, brownish, discoloured or chaffy grains were recorded from 10 panicles randomly selected from 10 hills and the percentage of damaged grains was computed (Kalita *et al.*, 2009).

**3.4.4 Assessment of Population of Natural Enemies**

Number of hymenopteran parasitoids, insect and spider predators collected in 10 sweeps plot$^{-1}$ was recorded as mentioned in 3.4.3.

**3.4.5 Yield**

Harvesting was done at 120 DAS in the first field trial and128 DAS in the second field trial. The grains from the plots were sun dried, winnowed, weighed and expressed as kg plot$^{-1}$. The straw was dried under sun for 10 days and the weight was expressed as kg plot$^{-1}$.

### 3.4.6 Benefit- Cost Ratio (BCR)

While computing BCR, 25 per cent enhancement in price was provided for the yield of grains obtained from the various treatments with entomopathogenic fungi and also for the yield obtained from the untreated check.

### 3.4.7 Grading of Treatments

Grading of treatments was done based on the overall effect in both the field trials. The parameters considered for grading were population and damage of *C. medinalis* and *L. acuta*, population of natural enemies (21 DAT) and benefit-cost ratio. The treatments were graded on a one to 10 scale. For grading the effect of treatments on pest population and extent of damage, the treatment with the lowest value at 21 DAT was given the highest grade of 10 and for grading the effect on natural enemies and BCR, that treatment with the highest value was given the grade point of 10.

## 3.5 COMPATIBILITY OF FUNGI WITH PESTICIDES

The compatibility of the fungal isolates *B. bassiana* (Bb 5), *B. bassiana* (Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1) with the three insecticides recommended by the Central Insecticides Board and Registration Committee (CIB and RC) for rice pest management was assessed in the laboratory by adopting poisoned food technique (Zentmeyer, 1955). The details of the insecticides used are given below,

| Chemical | Commercial formulation | Concentration (%) |
|---|---|---|
| Acephate | Acetaf 75 per cent SP | 0.075, 0.150 (field dose) and 0.225 |
| Chlorantraniliprole | Coragen 18.5 per cent SC | 0.004, 0.006 (field dose) and 0.008 |
| Thiamethoxam | Actara 25 per cent WG | 0.003, 0.005 (field dose) and 0.008 |

The fungi grown in media without insecticide served as check. The experiment was conducted in CRD with three replications. The growth, spore count, conidial viability and bioefficacy of the fungi grown in poisoned media and that in unpoisoned media was assessed. The data was further expressed as percentage inhibition in growth, spore count and germination (Rajanikanth *et al.*, 2010).

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

where

R = Per cent reduction in growth, spore count, conidial germination

C = Growth, spore count, conidial germination of fungi grown in unpoisoned media

T = Growth, spore count, conidial germination of fungi grown in poisoned media.

### 3.5.1 Assessment of Growth

Insecticides at the required concentrations were incorporated aseptically into the melted sterile PDA, before solidification. The medium was swirled for mixing of the contents. Afterwards, it was poured into sterile Petri plates and allowed to solidify. Circular mycelial discs of five mm diameter were cut from the seven day old culture of respective fungi, by means of sterile cork borer and placed in the centre of each Petri plate. The plates were sealed with parafilm and incubated at room temperature for 14 days. The observations on the growth of fungi were taken at one, three, five, seven, nine, 11 and 14 days after inoculation (DAI).

### 3.5.2 Estimation of Spore Count

Ten ml sterile water was poured into each Petri plate with sporulating fungi and the surface was gently scrubbed with a sterile spatula, the spore suspension

thus collected after necessary dilution was used to estimate the spore count (Rombach *et al.*, 1986b). The spore count was observed on the seventh day for *A. flavus* and fourteenth day for *B. bassiana* (Bb 5 and Bb 21) and *M. anisopliae* (Ma 4), the days that coincided with maximum sporulation.

### 3.5.3 Estimation of Conidial Viability

Ten µl of conidial suspension was poured to a cavity slide with 100 µl poisoned PDA. The unit was kept in Petri plates lined with slightly moistened filter paper to avoid drying up of media. The slides were observed under compound microscope after 48 h to count the number of germinated conidia. A conidium was considered to be germinated when the germ tube that projected from it, was at least twice the diameter of the conidium (Shampa *et al.*, 2009). Three replications were maintained for each treatment and 100 spores (selected randomly and marked by the line tool in Motic BA 210 compound microscope under 40 X magnification) were observed in each replication and the percentage of germination was determined. The fungi in plain PDA served as untreated check.

### 3.5.4 Bioefficacy

The bioefficacy of the fungi cultured in poisoned media was tested on newly emerged adults of *L. acuta*. The test insects were obtained from the insect stock culture maintained (3.2.2.2.2). The spore suspension was prepared as mentioned in 3.5.2 and applied on the test insects as given in 3.2.3.3.2. The experimental design was CRD, with three replications. The insects sprayed with spore suspension from culture grown in media without pesticide served as untreated. Each replication contained 10 insects. Observations on mortality were taken daily and the cumulative percentage mortality at 10 DAT was assessed.

## 3.6 DEVELOPMENT OF PESTICIDE TOLERANT STRAINS AND THEIR MOLECULAR CHARACTERISATION

Laboratory experiments were conducted with a view to develop insecticide tolerant strains of *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4). The insecticides used in the experiment were those mentioned in 3.5.

### 3.6.1 Assessment of Insecticide Tolerance

The method suggested by Shapiro *et al.* (2002) for assessing the fungicide tolerance of *B. bassiana* was adopted with modifications for assessing the insecticide tolerance of the fungi in the present study.

#### *3.6.1.1 Fixing the Highest Tolerable Dose*

In order to find out the highest dose of each insecticide, that can be tolerated by *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4), they were initially grown in poisoned media containing different doses of insecticides as given below.

| Chemical | Dose (%) | |
|---|---|---|
| Acephate | x (field dose) | 0.150 |
| | 2x | 0.300 |
| | 4x | 0.600 |
| | 8x | 1.200 |
| | 10x | 1.500 |
| Chlorantaniliprole | x (field dose) | 0.006 |
| | 2x | 0.012 |
| | 4x | 0.024 |
| | 8x | 0.048 |
| | 10x | 0.060 |
| Thiamethoxam | x (field dose) | 0.005 |

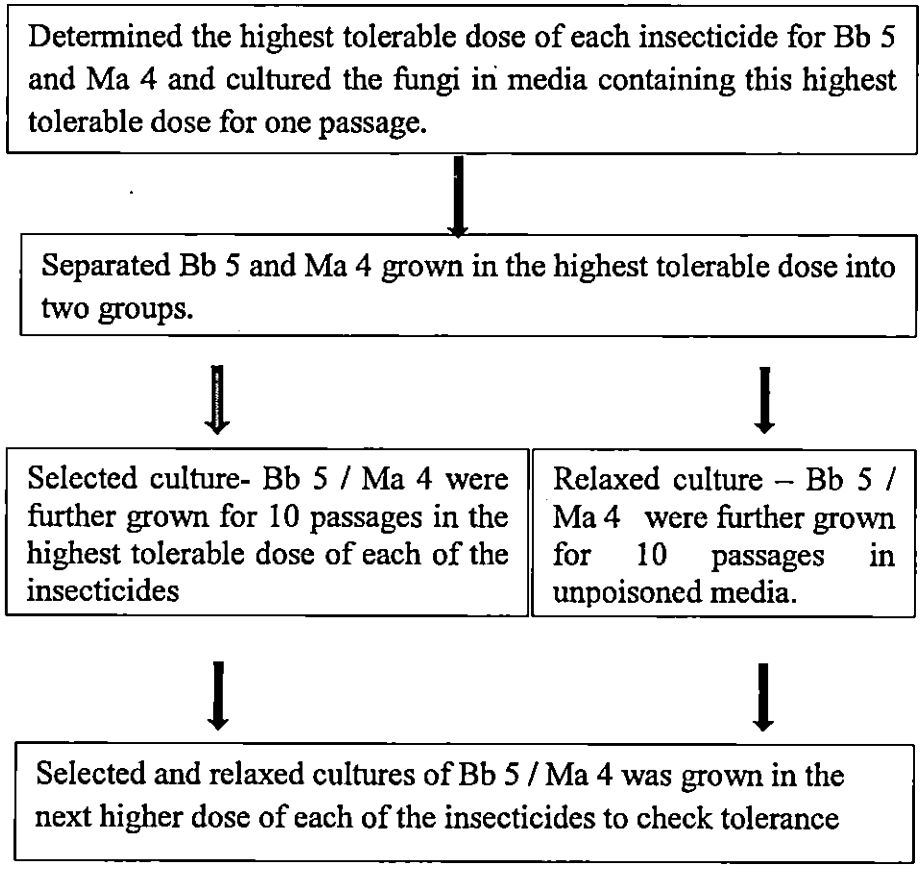
| | | |
|---|---|---|
| | 2x | 0.010 |
| | 4x | 0.020 |
| | 8x | 0.040 |
| | 10x | 0.050 |

As growth of *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) was not observed at 8x and 10x for acephate and 10x for both chlorantraniliprole and thiamethoxam, subsequently they were grown in 4.5x and 5x of acephate and 8.5x and 9x of chlorantraniliprole and thiamethoxam, respectively for determining their tolerance to higher doses of the insecticides. For this, fungal disc were cut and placed in poisoned media as mentioned in 3.5.1. Fungi grown in unpoisoned PDA was maintained as check. Growth of the fungi was measured at 14 DAI.

### *3.6.1.2 Culturing of Fungi for the Development of Insecticide Tolerant Strains*

Both *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) were grown separately in the highest tolerable dose of each of the insecticides *viz.,* acephate, chlorantraniliprole and thiamethoxam for one passage. Afterwards, *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) were separated into two sets. The first set of each of the fungi was further grown in media with the highest tolerable dose of each of the insecticides for 10 passages and this formed the 'selected cultures' *i.e.,* SB-acephate, SB-chlorantraniliprole and SB-thiamethoxam for *B. bassiana* (Bb 5) and SM-acephate, SM-chlorantraniliprole and SM-thiamethoxam for *M. anisopliae* (Ma 4). The second set, termed as the 'relaxed cultures' *i.e.,* RB-acephate, RB-chlorantraniliprole and RB-thiamethoxam for *B. bassiana* (Bb 5) and RM-acephate, RM-chlorantraniliprole and RM-thiamethoxam for *M. anisopliae* (Ma 4) were grown in the highest dose of each of the insecticide for one passage and were further grown in unpoisoned media for 10 passages. Along with these cultures, *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) grown in unpoisoned PDA from the initiation of the experiment formed the untreated check (UB and UM). After the tenth passage, selected, relaxed and untreated cultures of

*B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) were inoculated in PDA containing still higher concentrations of the insecticides *i.e.,* 4.5 x of acephate and 8.5 x of, chlorantraniliprole and thiamethoxam in order to determine their tolerance to these higher concentrations after 10 passages through poisoned / unpoisoned media. The flow chart of the methodology adopted is given below,




The experiment was laid as CRD with three replications. The observations on growth, spore count and bioefficacy of Bb 5 and Ma 4 during each passage were recorded as mentioned in 3.5.1, 3.5.2 and 3.5.4, respectively.

### 3.6.2 Molecular Characterisation

Molecular analysis of the selected, relaxed and untreated cultures of both *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) after the tenth passage, was carried out to analyse the changes in the genetic makeup, if any using RAPD- PCR analysis.

### *3.6.2.1 DNA Isolation*

Seven day old mycelia of selected, relaxed and untreated cultures of *B. bassiana* and *M. anisopliae* were used for DNA isolation. Genomic DNA of *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) of tenth passage from all the seven treatments was extracted from fungal mycelia according to the method described by Pfeifer and Khachatourians (1993) with slight modifications. The seven day old mycelium was filtered through sterile muslin cloth. The excess moisture was removed by keeping the mycelial mat in sterile filter paper for 10 min. Mycelia were homogenized using liquid nitrogen. The powdered mycelia were transferred to two ml microcentrifuge tubes such that half of the tube was filled. Equal volume of pre warmed extraction buffer (0.1M Tris HCl (pH 8.0), 0.1M EDTA, 0.1 M NaCl, 4 per cent SDS) was added to the tube, inverted to mix and was incubated at 60°C for 60 min with occasional mixing. The suspension was extracted twice with phenol: chloroform: isoamyl alcohol (25:24:1) mixture by 20 min centrifugation at 10000 rpm at 20°C. The cleared aqueous phase was transferred to another tube and DNA was precipitated by the addition of $1/10^{th}$ volume of 3 M sodium acetate (pH 5.4) and 2V of 95 per cent ethanol and kept for overnight at 4°C for precipitation. The next day sample was centrifuged at 10000 rpm for 20 min at 4°C. The supernatant was discarded and the pellet was washed twice with 70 per cent ethanol by five min centrifugation at 8000 rpm at 4°C. The supernatant was discarded and the pellet was air dried. The DNA pellet was dissolved in 40 µl of TE buffer (0.01M Tris HCl (pH 8.0), 0.001M EDTA). Quantification of DNA was done using a spectrophotometer followed by analyzing purified DNA on 0.9 per cent agarose gel as explained in 3.1.2.2.4. DNA was diluted in distilled sterile water to a concentration of approximately 50 ng $\mu l^{-1}$.

### *3.6.2.2 Random Amplified Polymorphic DNA (RAPD) Reaction*

Isolated DNA was subjected to RAPD analysis using the following 10 fungal primers from GeNei Pvt. Ltd.

| Sl No. | Primer | Accession number | Sequences |
|---|---|---|---|
| 1 | RFu 1 | AM911695 | CCTGGGCCAG |
| 2 | RFu 2 | AM911696 | CCTGGGCGAG |
| 3 | RFu 3 | AM911697 | CCTGGGCTGG |
| 4 | RFu 4 | AM773320 | CCTGGGCTAT |
| 5 | RFu 5 | AM911698 | CCTGGGCTTG |
| 6 | RFu 6 | AM765822 | CCTGGGCTAC |
| 7 | RFu 7 | AM911699 | CCTGGGCTTA |
| 8 | RFu 8 | AM773321 | CCTGGGTCGA |
| 9 | RFu 9 | AM773779 | CCTGGGTGCA |
| 10 | RFu 10 | AM765832 | CCTGGGTGAC |

Amplification was carried out in a 30 µl reaction mixture containing 22.2 µl distilled sterile water, three µl PCR assay buffer, 1.8 µl dNTPs, 1 µl each of Taq DNA polymerase, primer and DNA template. The reaction was carried out in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). After an initial denaturation of 94°C for five min, samples were subjected to 10 cycles of denaturation (94°C for one min, 35°C for one min, 72°C for 1.5 min), primer annealing (94°C for 45 sec, 37°C for 45 sec), primer extension (72°C for one min) and final extension at 72°C for 10 min. The PCR products were size fractionated on a two per cent agarose gel prepared in TAE buffer stained with ethidium bromide. The molecular standard used was 100 base pair (bp) DNA ladder. DNA fragments were visualized and were captured under UV light using Gel documentation system (Bio-Rad).

### *3.6.2.3 Data Analysis*

The PCR product was scored for the presence (+) or absence (-) of bands. The number of monomorphic bands and polymorphic bands were recorded. Thus, banding pattern of all the 10 primers for the seven samples each of *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) were scored as 1 and 0 in the excel sheet and percentage of polymorphism was worked out.

## 3.7 STATISTICAL ANALYSIS

Data obtained from all the experiments were transformed as required and subjected to analysis of variance (Panse and Sukhatme, 2000).

# *Results*

# 4. RESULTS

## 4.1 ISOLATION AND IDENTIFICATION OF INDIGENOUS STRAINS OF ENTOMOPATHOGENIC FUNGI

### 4.1.1 Isolation of Entomopathogenic Fungi

The details of the five entomopathogenic fungi collected from the three rice growing areas *viz.,* Anadu, Karamana and Vellayani in Thiruvananthapuram district adopting different methods of collection are given in Table 1.

#### *4.1.1.1 From Mycosed Cadavers*

*Aspergillus flavus* Link. (Af-m1) was isolated from the cadaver of rice bug, *Leptocorisa acuta* (Thunb) collected from the rice fields of Anadu.

#### *4.1.1.2 From Soil*

##### *4.1.1.2.1 Using Insect Bait*

One isolate of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ma-m1) and two isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Bb-m2 and Bb-m5) were obtained from soil samples collected from Vellayani, Karamana and Vellayani, respectively.

##### *4.1.1.2.2 Using Selective Media*

Two isolates of *B. bassiana* (Bb-m3 and Bb-m4) were obtained from soil samples collected from Anadu and Karamana, respectively.

Table 1. Details of fungi isolated from different locations in Thiruvananthapuram district

| Sl No. | Isolate code | Isolate | Source and method of collection | Place of collection | Accession number | Reference isolates |
|---|---|---|---|---|---|---|
| 1 | Af-m1 | *Aspergillus flavus* Link. | IC | Anadu | KP 739825 | *A.flavus* PW2961 (KF 562204.1) |
| 2 | Bb-m2 | *Beauveria bassiana* (Balsamo) Vuillemin | Soil (IB) | Karamana | KP 739828 | *B. bassiana* LPSC 1067 (KF 500409.1) |
| 3 | Bb-m3 | *B. bassiana* | Soil (SM) | Anadu | KP 739829 | *B. bassiana* SD 15 (KC 55195.1) |
| 4 | Bb-m4 | *B. bassiana* | Soil (SM) | Karamana | KP 739830 | *B. bassiana* A 64 (KC 461106.1) |
| 5 | Bb-m5 | *B. bassiana* | Soil (IB) | Vellayani | KP 739831 | *B. bassiana* BBPTG (KC 759729.1) |
| 6 | Ma-m1 | *Metarhizium anisopliae* (Metschnikoff) Sorokin | Soil (IB) | Vellayani | KP 739826 | *M. anisopliae* MAGW7 (KF 913494.1) |

IB- Insect bait IC- Insect cadaver SM- Selective media

### 4.1.2 Identification

#### 4.1.2.1 *Morphological Identification*

The morphological characters of the newly collected isolates are given in Table 2.

##### *4.1.2.1.1 A. flavus (Af-m1)*

The fungus produced bright greenish coloured colonies with white margins. The isolate produced globose conidia with a mean diameter of 2.7 µm (Plate 1 A and 2 A). The isolate had a growth of 90 mm on the seventh day after inoculation. Conidia were produced in chains on flask shaped phialides on one layer of metuale on an apical vesicle.

##### *4.1.2.1.2 B. bassiana (Bb-m2)*

The fungus obtained through insect bait method, from the soil sample collected from Karamana, produced white colonies with light pink margin. The isolate had a growth of 75 mm on the fourteenth day after inoculation. The conidia were globose with a mean diameter of 1.4 µm (Plate 1 B and 2 B) and they were produced on clustered conidiogenous cells.

##### *4.1.2.1.3 B. bassiana (Bb-m3)*

The isolate obtained from the soil sample collected from Anadu using selective media produced yellowish white colonies with light pink margin. The isolate had a growth of 55 mm on the fourteenth day after inoculation. The conidia were globose with a mean diameter of 2.1 µm (Plate 1 C and 2 C). Conidiogenous cells were found in groups.

Table 2. Morphological characters of the indigenous fungal isolates

| Sl No. | Isolate | Colony colour | Conidial shape | Conidial size (µm)* | Radial growth (mm) |
|---|---|---|---|---|---|
| 1 | *A.flavus* (Af-m1) | Green with white margins | Globose | 2.7 | 90*** |
| 2 | *B. bassiana* (Bb-m2) | White with pink margins | Globose | 1.4 | 75** |
| 3 | *B. bassiana* (Bb-m3) | Yellowish white with light pink margins | Globose | 2.1 | 55** |
| 4 | *B. bassiana* (Bb-m4) | White | Globose | 2.1 | 55** |
| 5 | *B. bassiana* (Bb-m5) | Off white | Globose | 1.2 | 65** |
| 6 | *M. anisopliae* (Ma-m1) | Dark green with white margin | Cylindrical | 4.62 x 1.50 | 75** |

* Mean of 100 conidia

** 14 days after inoculation

***Seven days after inoculation

*4.1.2.1.4 B. bassiana (Bb-m4)*

The fungus obtained from the soil sample collected from Karamana using selective media produced bright dense white colonies. The isolate had a growth of 55 mm on the fourteenth day after inoculation. The conidia developed on clustered conidiogenous cells were globose with a mean diameter of 2.1 µm (Plate 1 D and 2 D).

*4.1.2.1.5 B. bassiana (Bb-m5)*

The isolate obtained using insect bait method from the soil sample collected from Vellayani produced off white colonies which had powdery appearance. The fungus had growth of 65 mm on the fourteenth day after inoculation. The conidia were globose with mean diameter of 1.2 µm (Plate 1 E and 2 E) and they were formed on clustered conidiogenous cells.

*4.1.2.1.6 M. anisopliae (Ma-m1)*

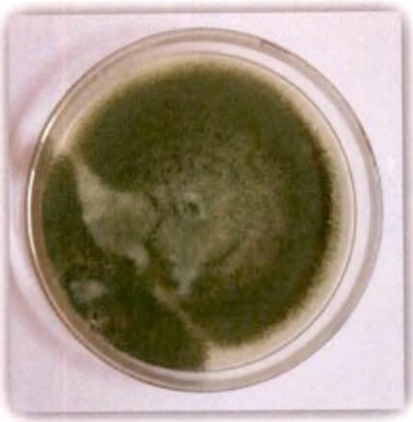
The fungus obtained from the soil sample of Vellayani using insect bait method produced dark green coloured colonies with white margin and produced light yellow pigmentation in the media. The isolate had a growth of 75 mm on the fourteenth day after inoculation. The conidia formed on elongated phialides were cylindrical with a mean length of 4.62 µm and width of 1.50 µm (Plate 1 F and 2 F).

*4.1.2.2 Molecular Identification*

*4.1.2.2.1 A. flavus (Af-m1)*

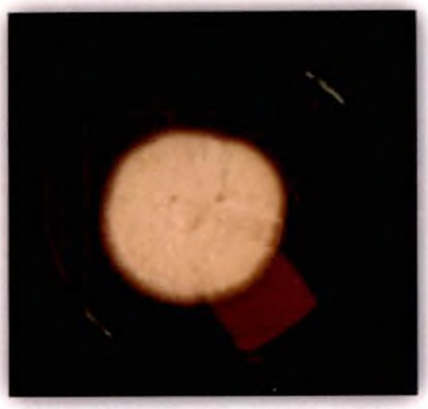
The internal transcribed spacer (ITS) sequencing of the fungus yielded 555 base pair (bp) sequences as given below.

AAGGATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAACCTCCCACC
CGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCG



(A) *A. flavus* (Af-m1)

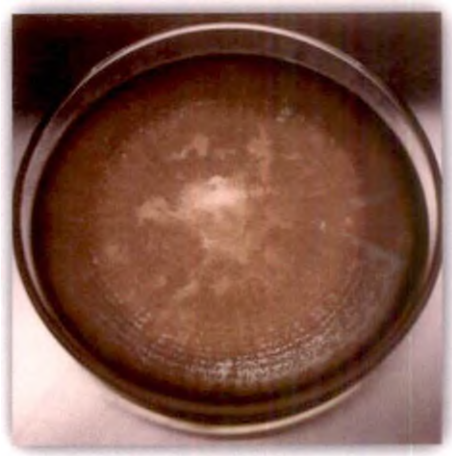
(B) *B. bassiana* (Bb-m2)



(C) *B. bassiana* (Bb-m3)

(D) *B. bassiana* (Bb-m4)

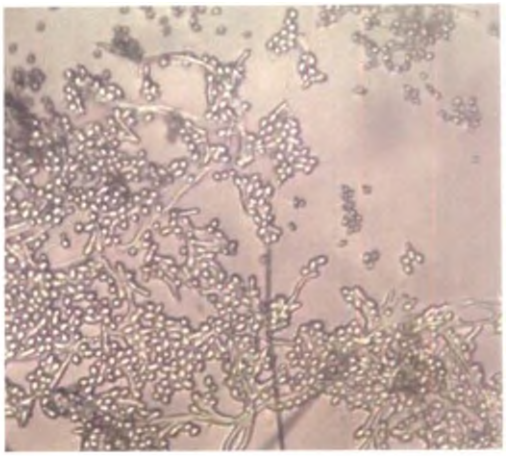
(E) *B. bassiana* (Bb-m5)



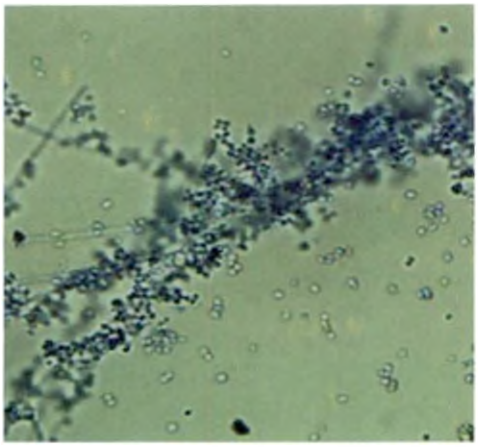
(F) *M. anisopliae* (Ma-m1)

Plate 1. Indigenous fungal isolates in potato dextrose agar

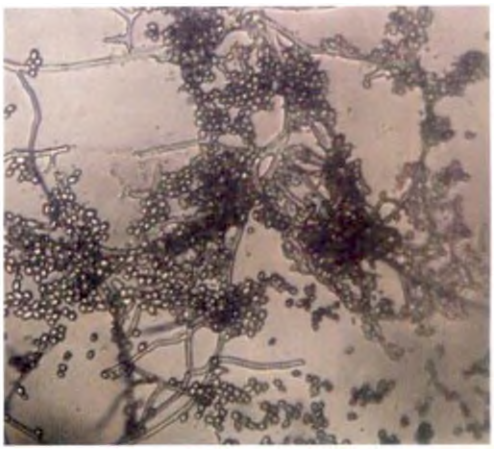
(A) *A. flavus* (Af-m1)



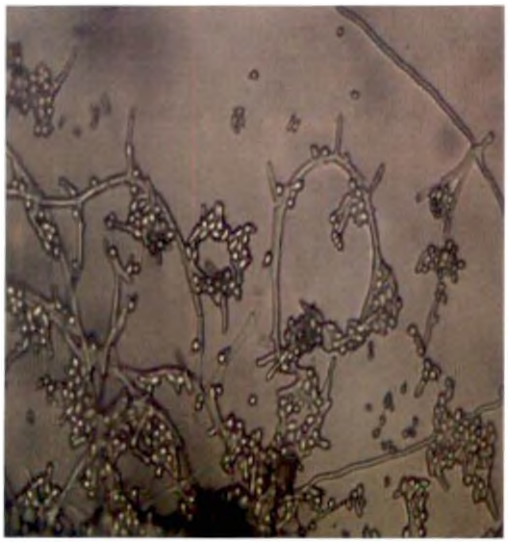
(B) *B. bassiana* (Bb-m2)



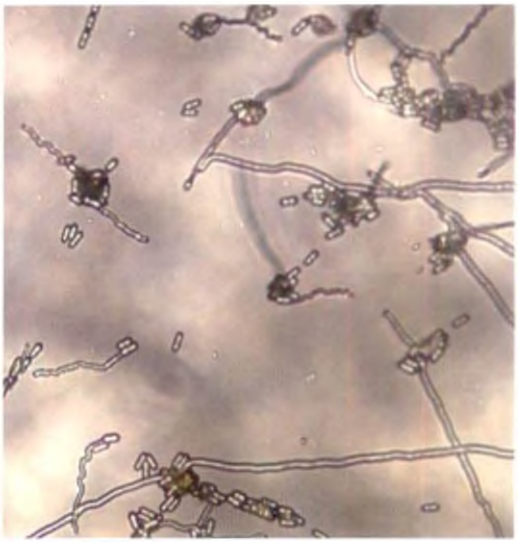
(C) *B. bassiana* (Bb-m3)



(D) *B. bassiana* (Bb-m4)



(E) *B. bassiana* (Bb-m5)



(F) *M. anisopliae* (Ma-m1)

Plate 2. Photomicrographs of conidia

CCGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAA
CTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAA
AACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAG
CGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTGAATCATCGAG
TCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTC
CGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCG
TCCCCTCTCCGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCG
TCCGATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGG
CCGGCGCTTGCCGAACGCAAATCAATCTTTTCCAGGTTGACCTCGGAT
CAGGTAGGGATACCCGCTGAACTTAA

The above sequence when subjected to nucleotide Basic Local Alignment Search Tool (BLAST) analysis showed cent per cent similarity with *A. flavus* strain PW 2961 having accession number KF 562204.1. The accession number assigned by National Centre for Biotechnology Information (NCBI) to this isolate was KP 739825.

***4.1.2.2.1 B. bassiana (Bb-m2)***

The ITS sequencing of the fungus yielded 530 bp sequences as given below.

AGGGATCATTACCGAGTTTTCAACTCCCTAACCCTTCTGTGAACCTACC
TATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACTGGACCA
GCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCTTCTGAAT
ACGCCGCAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCT
CTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
TGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACATTGCG
CCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT
CGACCTCCCCTGGGGGAGGTCGGCGTTGGGGACCGGCAGCACACCGC
CGGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGCGACCTCTGCGTAGT
AATACAGCTCGCACCGGAACCCCGACGCGGCCACGCCGTAAAACACC

CAACTTCTGAACGTTGACCTCGAATCAGGTAGGACTACCCGCTGAACT
TAA

The above sequence on nucleotide BLAST analysis showed cent per cent similarity with *B. bassiana* isolate LPSC1067 having accession numbers KF 500409.1. The accession number assigned by NCBI to this isolate was KP 739828.

***4.1.2.2.3 B. bassiana (Bb-m3)***

The ITS sequencing of the fungus yielded 530 bp sequences as given below.

AGGGATCATTACCGAGTTTTCAACTCCCTAACCCTTCTGTGAACCTACC
TATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACTGGACCA
GCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCTTCTGAAT
ACGCCGCAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCT
CTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
TGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACATTGCG
CCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT
CGACCTCCCCTGGGGGAGGTCGGCGTTGGGGACCGGCAGCACACCGC
CGGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGCGACCTCTGCGTAGT
AATACAGCTCGCACCGGAACCCCGACGCGGCCACGCCGTAAAACACC
CAACTTCTGAACGTTGACCTCGAATCAGGTAGGACTACCCGCTGAACT
TAA.

The above sequence on nucleotide BLAST analysis showed cent per cent similarity with *B. bassiana* isolates SD15 having accession numbers KC55195.1. The accession number assigned by NCBI to this isolate was KP 739829.

***4.1.2.2.4 B. bassiana (Bb-m4)***

The ITS sequencing of the fungus yielded 530 bp sequences as given below.

AGGGATCATTACCGAGTTTTCAACTCCCTAACCCTTCTGTGAACCTACC
TATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACTGGACCA
GCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCTTCTGAAT
ACGCCGCAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCT
CTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
TGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACATTGCG
CCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT
CGACCTCCCCTGGGGGAGGTCGGCGTTGGGGACCGGCAGCACACCGC
CGGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGCGACCTCTGCGTAGT
AATACAGCTCGCACCGGAACCCCGACGCGGCCACGCCGTAAAACACC
CAACTTCTGAACGTTGACCTCGAATCAGGTAGGACTACCCGCTGAACT
TAA.

The above sequence on nucleotide BLAST analysis showed cent per cent similarity with *B. bassiana* isolates A64 having accession numbers KC461106.1. The accession number assigned by NCBI to this isolate was KP 739830.

***4.1.2.2.5 B. bassiana (Bb-m5)***

The ITS sequencing of the fungus yielded 530 bp sequences as given below.

AGGGATCATTACCGAGTTTTCAACTCCCTAACCCTTCTGTGAACCTACC
TATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACTGGACCA
GCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCTTCTGAAT
ACGCCGCAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCT
CTTGGCTCTGGCATCGATGAAGAACGCAGCGAAACGCGATAAGTAAT
GTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACC
CTCGACCTCCCCTTGGGGGGGTCGGCGTTGGGGACCGGCAGCACACCG
CCGGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGCGACCTCTGCGCAG
TAATACAGCTCGCACCGGAACCCCGACGCGGCCACGCCGTAAAACAC

CCAACTTCTGAACGTTGACCTCGAATCAGGTAGGACTACCCGCTGAAC
TTAA.

The above sequence on nucleotide BLAST analysis showed cent per cent similarity with *B. bassiana* isolates BBPTG2 having accession numbers KC 759729.1. The accession number assigned by NCBI to this isolate was KP 739831.

***4.1.2.2.6 M. anisopliae (Ma-m1)***

The ITS sequencing of the fungus yielded 521 bp sequences as given below.

AGGGATCATTACCGAGTTATCCAACTCCCAACCCCTGTGAATTATACC
TTTAATTGTTGCTTCGGCGGGACTTCGCGCCCGCCGGGGACCCAAACC
TTCTGAATTTTTTAATAAGTATCTTCTGAGTGGTTAAAAAAAAAATGA
ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAA
CGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCA
TCGAATCTTTGAACGCACATTGCGCCCGTCAGTATTCTGGCGGGCATG
CCTGTTCGAGCGTCATTACGCCCCTCAAGTCCCCTGTGGACTTGGTGTT
GGGGATCGGCGAGGCTGGTTTTCCAGCACAGCCGTCCCTTAAATTAAT
TGGCGGTCTCGCCGTGGCCCTCCTCTGCGCAGTAGTAAAGCACTCGCA
ACAGGAGCCCGGCGCGGTCCACTGCCGTAAAACCCCCCAACTTTTTAT
AGTTGACCTCGAATCAGGTAGGACTACCCGCTGAACTTAA.

The above sequence when subjected to nucleotide BLAST analysis showed cent per cent similarity with *M. anisopliae* strain MAGW7 having accession number KF 913494.1. The accession number assigned by NCBI to this isolate was KP 739826.

4.2 PATHOGENICITY

The pathogenicity of the fungal isolates from NBAIR *viz.*, *B. bassiana* (Bb 5), *B. bassiana* (Bb 21), *M. anisopliae* (Ma 4) and the six new indigenous isolates,

*A. flavus* (Af-m1), *B. bassiana* (Bb-m2, Bb-m3, Bb-m4 and Bb-m5) and *M. anisopliae* (Ma-m1) was tested against *Cnaphalocrocis medinalis* Guen. and *L. acuta* and it was confirmed through Koch's postulates. The fungi varied in their pathogenicity to insects. All the isolates tested were pathogenic to larvae of *C. medinalis* and nymphs and adults of *L. acuta*. Pathogenicity to eggs was exhibited only by *B. bassiana* (Bb 5). The isolates did not produce any symptom on the pupae and adult of *C. medinalis* (Table 3).

### 4.2.1 Symptoms of Infection

#### *4.2.1.1 B. bassiana (Bb 5)*

##### *4.2.1.1.1 C. medinalis*

The treated eggs did not hatch and turned black in colour. Thin white mycelia appeared on the eggs two days after treatment (Plate 3 A).

In infected larvae, feeding and movement reduced considerably. Healthy larvae produced silken threads to move from one leaf to another, whereas in infected ones silk production was completely arrested. Mortality commenced four days after treatment and white mycelial growth covered cadavers within three days (Plate 3 B).

##### *4.2.1.1.2 L. acuta*

The treated eggs turned darker in colour and did not hatch. Fluffy white mycelial growth appeared on treated eggs three days after treatment (Plate 4 A).

The nymphs of *L. acuta* were normally very active and always found feeding, by inserting their stylets into the grains, but in the infected nymphs there was marked reduction in feeding as well as movement. Generally, the healthy nymphs were found in groups, instead, the diseased ones preferred to stay singly. The feeding, movement and copulation of infected adults were also reduced. Mortality of nymphs and adults of *L. acuta* initiated five days after treatment and

Table 3. Pathogenicity of entomopathogenic fungal isolates to rice pests.

| Fungus | Pest | | | | | | |
|---|---|---|---|---|---|---|---|
| | *C. medinalis* | | | | *L. acuta* | | |
| | E | L | P | A | E | N | A |
| *B. bassiana* (Bb 5) | + | + | - | - | + | + | + |
| *B. bassiana* (Bb 21) | - | + | - | - | - | + | + |
| *M. anisopliae* (Ma 4) | - | + | - | - | - | + | + |
| *A. flavus* (Af-m1) | - | + | - | - | - | + | + |
| *B. bassiana* (Bb-m2) | - | + | - | - | - | + | + |
| *B. bassiana* (Bb-m3) | - | + | - | - | - | + | + |
| *B. bassiana* (Bb-m4) | - | + | - | - | - | + | + |
| *B. bassiana* (Bb-m5) | - | + | - | - | - | + | + |
| *M. anisopliae* (Ma-m1) | - | + | - | - | - | + | + |

A – Adult E – Egg L – Larva N – Nymph P – Pupa

+ Pathogenic – Non pathogenic

white mycelial growth appeared on the cadaver two days after the death (Plate 4 B and 5 A).

#### *4.2.1.2 B. bassiana (Bb 21)*

##### *4.2.1.2.1 C. medinalis*

The symptoms produced on the larvae were similar to that mentioned in 4.2.1.1.1. Mortality occurred four days after treatment and white mycelial growth covered cadaver two days after the death (Plate 3 C).

##### *4.2.1.2.2 L. acuta*

The symptoms produced were similar to that mentioned in 4.2.1.1.2. Mortality occurred five days after treatment and white mycelial growth covered cadaver four days after the death (Plate 4 C and 5 B).

#### *4.2.1.3 M. anisopliae (Ma 4)*

##### *4.2.1.3.1 C. medinalis*

The symptom development was similar to that described under 4.2.1.1.1. White mycelium covered the cadaver three days after the death and the mycelia turned dark green colour within two days (Plate 3 D).

##### *4.2.1.3.2 L. acuta*

The symptoms produced on nymphs and adults were similar to that described in 4.2.1.1.2. White mycelium covered the cadaver three days after the death, but after two days, the mycelium turned dark green in colour (Plate 4 D and 5 C).

#### *4.2.1.4 A. flavus (Af-m1)*

##### *4.2.1.4.1 C. medinalis*

Mortality of larvae occurred on the fourth day after treatment and dark green coloured erect conidiophores appeared on the cadaver of larvae two days after the death (Plate 3 E).

##### *4.2.1.4.2 L. acuta*

Mortality of both nymphs and adults occurred five days after treatment and green coloured mycelia appeared on the cadaver two days after the death (Plate 4 E and 5 D).

#### *4.2.1.5 Isolates of B. bassiana (Bb-m2, Bb-m3, Bb-m4 and Bb-m5)*

##### *4.2.1.5.1 C. medinalis*

The isolates of *B. bassiana* were pathogenic only to the larvae of *C. medinalis* and the symptoms were similar to that described under 4.2.1.1.1 (Plate 3 F, G, H, I).

##### *4.2.1.5.2 L. acuta*

The isolates were pathogenic to the nymphs and adults of *L. acuta* and the symptoms were similar to that described under 4.2.1.1.2 (Plate 4 F, G, H, I and 5 E, F, G, H).

#### *4.2.1.6 M. anisopliae (Ma-m1)*

##### *4.2.1.6.1 C. medinalis*

The infected larvae showed reduction in feeding and movement. Mortality occurred four days after treatment and blackish green coloured mycelial growth appeared on the cadaver three days after the death (Plate 3 J).

*4.2.1.6.2 L. acuta*

The symptoms developed on nymphs and adults of *L. acuta* on treating with *M. anisopliae* (Ma-m1) were similar to that described under 4.2.1.1.2. Blackish green coloured mycelia appeared on the cadavers three days after the death (Plate 4 J and 5 I).

**4.2.2 Single Dose Screening Assay**

*4.2.2.1 C. medinalis*

The data on the mortality of the larvae of *C. medinalis* treated with different fungi, at a spore concentration of $10^8$ spores $ml^{-1}$ are presented in Table 4.

Four days after treatment, both *B. bassiana* (Bb 5) and *B. bassiana* (Bb 21) caused mean mortality to the tune of 33.33 per cent which was on par with that of *M. anisopliae* (Ma 4) (26.67 per cent) and *A. flavus* (Af-m1) (16.67 per cent). The isolates Bb-m2, Bb-m3, Bb-m4 and Ma-m1 caused mean mortality ranging from 3.33 per cent to 10.00 per cent and were on par. The isolate Bb-m5 did not cause any mortality.

*B. bassiana* (Bb 5) caused 76.67 per cent mean mortality on the sixth DAT and was statistically on par with that of *B. bassiana* (Bb 21) (73.33 per cent). *M. anisopliae* (Ma4) produced mean mortality of 50 per cent, which was statistically superior to that of *A. flavus* (Af-m1) (30.00 per cent). The isolate Bb-m2 produced 20.00 per cent mean mortality and was statistically on par with the mean mortality by Bb-m3 and Ma-m1 (16.67 per cent) each. The indigenous isolates Bb-m4 and Bb-m5 collected from soil caused only 6.67 per cent mortality.

On the eighth DAT, cent per cent mean mortality achieved by *B. bassiana* (Bb 5) was statistically superior to all other isolates. This was followed by *B. bassiana* (Bb 21) with 90.00 per cent mortality. *M. anisopliae* (Ma 4)

(A) *B. bassiana* (Bb5) infected egg

(B) *B. bassiana* (Bb5) infected larva

(C) *B. bassiana* (Bb21) infected larva

(D) *M. anisopliae* (Ma4) infected larva

(E) *A. flavus* (Af-m1) infected larva

(F) *B. bassiana* (Bb-m2) infected larva

Plate 3. Symptoms of infection by fungal isolates on *C. medinalis*

(G) *B. bassiana* (Bb-m3) infected larva

(H) *B. bassiana* (Bb-m4) infected larva

(I) *B. bassiana* (Bb-m5) infected larva

(J) *M. anisopliae* (Ma-m1) infected larva

Plate 3. Symptoms of infection by fungal isolates on *C. medinalis* (Contd.)

(A) *B. bassiana* (Bb5) infected egg

(B) *B. bassiana* (Bb5) infected nymph

(C) *B. bassiana* (Bb21) infected nymph

(D) *M. anisopliae* (Ma4) infected nymph

(E) *A. flavus* (Af-m1) infected nymph

Plate 4. Symptoms of infection by fungal isolates on *L. acuta*

(F) *B. bassiana* (Bb-m2) infected nymph

(G) *B. bassiana* (Bb-m3) infected nymph

(H) *B. bassiana* (Bb-m4) infected nymph

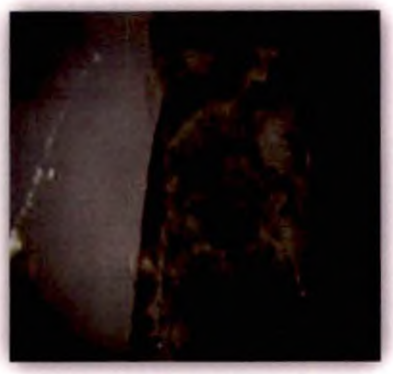
(I) *B. bassiana* (Bb-m5) infected nymph

(J) *M. anisopliae* (Ma-m1) infected nymph

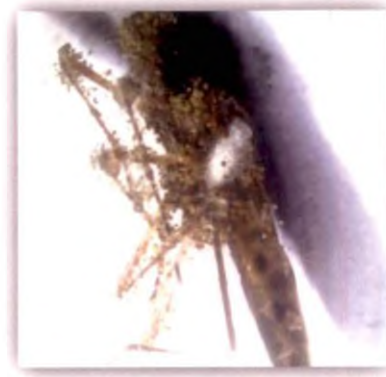
Plate 4. Symptoms of infection by fungal isolates on *L. acuta* (Contd.)

(A) *B. bassiana* (Bb5) infected adult

(B) *B. bassiana* (Bb21) infected adult



(C) *M. anisopliae* (Ma4) infected adult



(D) *A. flavus* (Af-m1) infected adult

(E) *B. bassiana* (Bb-m2) infected adult

Plate 5. Symptoms of infection by fungal isolates on *L. acuta* adult

(F) *B. bassiana* (Bb-m3) infected adult

(G) *B. bassiana* (Bb-m4) infected adult

(H) *B. bassiana* (Bb-m5) infected adult

(I) *M. anisopliae* (Ma-m1) infected adult

Plate 5. Symptoms of infection by fungal isolates on *L. acuta* adult (Contd.)

(73.33 per cent) and *A. flavus* (Af-m1) (66.67 per cent) were statistically on par. The isolate, Bb-m3 which caused 33.33 per cent mean mortality was on par with Bb-m2 (30.00 per cent) and Ma-m1 (23.33 per cent). The performance of Bb-m4 and Bb-m5 was inferior and caused only 16.67 and 13.33 per cent mortality, respectively.

From the observations taken at different intervals after treatment, it was seen that *B. bassiana* (Bb 5) was the most virulent isolate.

### *4.2.2.2 L.acuta*

#### *4.2.2.2.1 Nymphs*

The data on the mortality of the third instar nymphs of *L. acuta* treated with different fungi at spore concentration of $10^8$ spores $ml^{-1}$ are presented in Table 5.

At five DAT, *M. anisopliae* (Ma 4) caused mean mortality to the tune of 43.33 per cent which was on par with the mortality by *A. flavus* (Af-m1) (33.33 per cent), *B. bassiana* (Bb 21) (26.67 per cent) and *B. bassiana* (Bb 5) (20.00 per cent). *B. bassiana* (Bb-m2) caused 6.67 per cent mortality and it was statistically similar to the mortality caused by Bb 5 (20.00 per cent). *M. anisopliae* (Ma-m1) and *B. bassiana* (Bb-m3) caused 3.33 per cent mortality each whereas no mortality was caused by the isolates *B. bassiana* (Bb-m4 and Bb-m5) five days after treatment.

On the seventh DAT, *B. bassiana* (Bb 5) recorded 53.33 per cent mortality which was on par with the mortality caused by *M. anisopliae* (Ma 4) (50.00 per cent), *B. bassiana* (Bb 21) (40.00 per cent) and *A. flavus* (Af-m1) (40.00 per cent). *B. bassiana* (Bb-m2 and Bb-m3) were less pathogenic each with 16.67 per cent mortality and these were on par with *M. anisopliae* (Ma-m1) (13.33 per cent) and *B. bassiana* (Bb-m4 and Bb-m5) (10.00 per cent).

Table 4. Pathogenicity of fungal isolates to third instar larvae of *C. medinalis*

| Treatments @ $10^8$ spores $ml^{-1}$ | Mean mortality (%) at different DAT | | |
|---|---|---|---|
| | 4 | 6 | 8 |
| *B. bassiana* (Bb 5) | 33.33 (35.01) | 76.67 (61.22) | 100.00 (89.09) |
| *B. bassiana* (Bb 21) | 33.33 (35.01) | 73.33 (59.00) | 90.00 (74.70) |
| *M. anisopliae* (Ma 4) | 26.67 (30.99) | 50.00 (45.00) | 73.33 (59.00) |
| *A. flavus* (Af-m1) | 16.67 (23.86) | 30.00 (33.21) | 66.67 (54.78) |
| *B. bassiana* (Bb-m2) | 10.00 (18.44) | 20.00 (26.57) | 30.00 (33.21) |
| *B. bassiana* (Bb-m3) | 6.67 (12.59) | 16.67 (23.86) | 33.33 (35.22) |
| *B. bassiana* (Bb-m4) | 3.33 (6.75) | 6.67 (12.59) | 16.67 (23.86) |
| *B. bassiana* (Bb-m5) | 0.00 (0.91) | 6.67 (12.59) | 13.33 (21.15) |
| *M. anisopliae* (Ma-m1) | 3.33 (6.75) | 16.67 (23.86) | 23.33 (28.29) |
| CD (0.05) | (12.142) | (9.541) | (7.136) |

Mean of three replications
10 insects $replication^{-1}$
Figures in parentheses are angular transformed values.
DAT- Days after treatment.

*M. anisopliae* (Ma 4) and *B. bassiana* (Bb 21) that recorded 73.33 per cent mortality each was on par with *B. bassiana* (Bb 5) (70.00 per cent) and *A. flavus* (Af-m1) (60.00 per cent) nine days after treatment. The effect of *B. bassiana* (Bb-m2), *B. bassiana* (Bb-m3) (23.33 per cent), *M. anisopliae* (Ma-m1) (20.00 per cent) and *B. bassiana* (Bb-m4 and Bb-m5) (13.33 per cent) were statistically on par.

Cent per cent mortality was obtained in the treatment with *M. anisopliae* (Ma 4) on the tenth day after treatment, and it was on par with *B. bassiana* (Bb 5) that caused 90.00 per cent mortality. *B. bassiana* (Bb 21) and *A. flavus* (Af-m1) each of which caused 83.33 per cent mortality were statistically on par with *B. bassiana* (Bb 5). The mortality caused by the isolates *B. bassiana* (Bb-m3), (Bb-m2) (Ma-m1) (Bb-m5) (Bb-m4) ranged from 16.67 to 33.33 per cent only.

***4.2.2.2.2 Adults***

The data on the mortality of the adult *L. acuta* treated with different fungi at a spore concentration of $10^9$ spores $ml^{-1}$ are presented in Table 6.

Five days after treatment, *M. anisopliae* (Ma 4) caused mortality to the tune of 40.00 per cent and was on par with the mortality by *B. bassiana* (Bb 5) (23.33 per cent) and *B. bassiana* (Bb 21) (20.00 per cent). *A. flavus* that caused 16.67 per cent mortality was on par with *B. bassiana* (Bb 5 and Bb 21). The effect of the isolates, Bb-m2, Bb-m3 and Bb-m5 was the same (3.33 per cent). No mortality of adult *L. acuta* was seen in the treatments with Bb-m4 and Ma-m1.

On seven DAT, *M. anisopliae* (Ma 4) recorded 53.33 per cent mortality which was on par with that of *B. bassiana* (Bb 21) 43.33 per cent. *A. flavus* (Af-m1) and *B. bassiana* (Bb 5) caused 33.33 per cent mortality and it was on par with *B. bassiana* (Bb 21). The isolates Bb-m2 and Bb-m3 caused 13.33 per cent and 10.00 per cent mortality, respectively and were on par with *M. anisopliae* (Ma-m1) (6.67 per cent). The isolates Bb-m4 and Bb-m5 caused mean mortality of only 3.33 per cent, and showed statistical similarity with Ma-m1.

Table 5. Pathogenicity of fungal isolates to third instar nymphs of *L. acuta*

| Treatments @ $10^8$ spores $ml^{-1}$ | Mean mortality (%) at different DAT | | | |
|---|---|---|---|---|
| | 5 | 7 | 9 | 10 |
| *B. bassiana* (Bb 5) | 20.00 (26.57) | 53.33 (46.92) | 70.00 (56.99) | 90.00 (74.69) |
| *B. bassiana* (Bb 21) | 26.67 (30.79) | 40.00 (38.86) | 73.33 (59.70) | 83.33 (70.48) |
| *M. anisopliae* (Ma 4) | 43.33 (39.99) | 50.00 (44.71) | 73.33 (60.00) | 100.00 (89.09) |
| *A. flavus* (Af-m1) | 33.33 (35.01) | 40.00 (38.86) | 60.00 (51.14) | 83.33 (69.77) |
| *B. bassiana* (Bb-m2) | 6.67 (12.59) | 16.67 (23.86) | 23.33 (28.78) | 30.00 (33.00) |
| *B. bassiana* (Bb-m3) | 3.33 (6.75) | 16.67 (23.86) | 23.33 (28.07) | 33.33 (35.22) |
| *B. bassiana* (Bb-m4) | 0.00 (0.91) | 10.00 (18.44) | 13.33 (21.14) | 16.67 (23.86) |
| *B. bassiana* (Bb-m5) | 0.00 (0.91) | 10.00 (18.44) | 13.33 (21.15) | 20.00 (26.07) |
| *M. anisopliae* (Ma-m1) | 3.33 (6.75) | 13.33 (21.15) | 20.00 (26.07) | 23.33 (28.78) |
| CD (0.05) | (15.792) | (14.310) | (15.235) | (17.854) |

Mean of three replications
10 insects $replication^{-1}$
Figures in parentheses are angular transformed values.
DAT- Days after treatment.

No significant difference in the effect of *B. bassiana* (Bb 21), *M. anisopliae* (Ma 4) *B. bassiana* (Bb 5) and *A. flavus* (Af-ml) was observed on the nine DAT, the mortality recorded was 76.67, 66.67, 63.33 and 56.67 per cent, respectively. The effect of isolates, Bb-m2, Bb-m3, Bb-m4, Bb-m5 and Ma-ml was statistically on par and it ranged from 10.00 to 16.67 per cent only.

Cent per cent mortality by *M. anisopliae* (Ma 4) was observed at 10 DAT, and it was significantly superior to all other treatments. *B. bassiana* (Bb 21) recorded 80.00 per cent mortality which was statistically on par with *B. bassiana* (Bb 5) (76.67 per cent) and *A. flavus* (Af-ml) (70.00 per cent). The isolates, Bb-m2, Bb-m3, Bb-m4, Bb-m5 and Ma-ml recorded mortality ranging from 13.33 to 20.00 per cent and were on par.

### 4.2.3 Assessment of Pathogenicity to Natural Enemies

None of the grubs / adults of the coccinellids, *Coccinella transversalis* (Fab.) and *Micraspis* sp. and spiderlings / adult of the spiders *Tetragnatha* sp. and *Oxyopus* sp. were found dead on treatment with spore suspension of fourteen day old cultures of isolates *viz., B. bassiana* (Bb 5 and Bb 21), *M. anisopliae* (Ma 4), and *A. flavus* (Af-ml), *B. bassiana* (Bb-m2, Bb-m3, Bb- m4 and Bb-m5) and *M. anisopliae* (Ma-ml).

## 4.3 BIOASSAY

The data on dose-mortality responses *i.e* the effect of different concentrations of the isolates of *B. bassiana* (Bb 5 and Bb 21), *M. anisopliae* (Ma 4), and *A. flavus* (Af-ml) on the third instar larvae of *C. medinalis*, third instar nymphs and one day old adults of *L. acuta* are presented in Tables 7 to 18.

Table 6. Pathogenicity of fungal isolates to adults of *L. acuta*.

| Treatments @ $10^9$ spores $ml^{-1}$ | Mean mortality (%) at different DAT | | | |
|---|---|---|---|---|
| | 5 | 7 | 9 | 10 |
| *B. bassiana* (Bb 5) | 23.33 (28.78) | 33.33 (35.22) | 63.33 (52.78) | 76.67 (61.71) |
| *B. bassiana* (Bb 21) | 20.00 (26.57) | 43.33 (41.15) | 76.67 (61.71) | 80.00 (63.93) |
| *M. anisopliae* (Ma 4) | 40.00 (39.23) | 53.33 (46.92) | 66.67 (54.78) | 100.00 (89.09) |
| *A. flavus* (Af-m1) | 16.67 (20.23) | 33.33 (35.22) | 56.67 (48.93) | 70.00 (56.99) |
| *B. bassiana* (Bb-m2) | 3.33 (6.75) | 13.33 (21.15) | 16.67 (23.86) | 16.67 (23.86) |
| *B. bassiana* (Bb-m3) | 3.33 (6.75) | 10.00 (18.44) | 13.33 (21.15) | 20.00 (26.57) |
| *B. bassiana* (Bb-m4) | 0.00 (0.91) | 3.33 (6.75) | 10.00 (15.30) | 20.00 (26.57) |
| *B. bassiana* (Bb-m5) | 3.33 (6.75) | 3.33 (6.75) | 10.00 (15.30) | 13.33 (21.15) |
| *M. anisopliae* (Ma-m1) | 0.00 (0.91) | 6.67 (12.59) | 13.33 (21.15) | 20.00 (26.57) |
| CD (0.05) | (14.158) | (11.083) | (13.450) | (8.321) |

Mean of three replications
10 insects $replication^{-1}$
Figures in parentheses are angular transformed values.
DAT- Days after treatment.

### 4.3.1 *B. bassiana* (Bb 5)

#### *4.3.1.1 C. medinalis*

The mortality of larvae of *C. medinalis* treated with the spore concentrations, $10^4$ to $10^8$ spores $ml^{-1}$ of *B. bassiana* (Bb 5) ranged from 5.00 to 30.00 per cent at four DAT, (Table 7). At six and eight DAT, at the same concentrations, the mortality ranged from 10.00 to 52.50 and 12.50 to 92.50 per cent, respectively. Ten days after treatment, cent per cent mortality was observed at the highest concentration of $10^8$ spores $ml^{-1}$.

The minimum time required to bring about fifty per cent mortality at a concentration of $10^8$ spores $ml^{-1}$ was 5.38 days. As the concentration reduced to $10^7$, $10^6$, $10^5$, and $10^4$, the $LT_{50}$ recorded were 6.80, 8.21, 9.15 and 13.27 days, respectively.

As spore concentration varied, the mortality also varied. To bring about fifty per cent mortality at four DAT, a spore concentration of $1.70 \times 10^8$ spores $ml^{-1}$ was required whereas concentration of $0.87 \times 10^8$, $0.20 \times 10^8$ and $0.002 \times 10^8$ spores $ml^{-1}$ were sufficient to bring about fifty per cent mortality at sixth, eighth and tenth DAT. The $LC_{90}$ values at four, six, eight and ten DAT were $3.50 \times 10^8$, $2.59 \times 10^8$, $0.86 \times 10^8$ and $0.10 \times 10^8$ spores $ml^{-1}$, respectively.

#### *4.3.1.2 L. acuta*

##### *4.3.1.2.1 Nymphs*

The mortality of *L. acuta* nymphs ranged from 7.50 to 35.00 per cent on five DAT, at concentration ranging from $10^4$ to $10^8$ spores $ml^{-1}$. An increase in mortality was noted in the subsequent observations taken at seventh and ninth DAT, the mortality ranged from 10.00 to 65.00 per cent and 10.00 to 85.00 per cent, respectively. At 10 DAT, cent per cent mortality was obtained for the concentration of $10^8$ spores $ml^{-1}$ (Table 8).

Table 7. Dose-mortality responses of third instar larvae of *C. medinalis* treated with *B. bassiana* (Bb 5)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | |
| $10^8$ | 30.00 | 52.50 | 92.50 | 100.00 | 5.38 |
| $10^7$ | 20.00 | 40.00 | 60.00 | 87.50 | 6.80 |
| $10^6$ | 12.50 | 37.50 | 40.00 | 70.00 | 8.21 |
| $10^5$ | 10.00 | 20.00 | 37.50 | 60.00 | 9.15 |
| $10^4$ | 5.00 | 10.00 | 12.50 | 30.00 | 13.27 |

Probit analysis

| DAT | $LC_{50}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$ ($10^8$ spores $ml^{-1}$) | $LC_{90}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{90}$ ($10^8$ spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 4 | 1.70 | 1.53 - 1.88 | 3.50 | 3.16 - 3.59 | 3.629 | Y=1.213+0.134x |
| 6 | 0.87 | 0.70 - 1.04 | 2.59 | 2.26 - 2.67 | 11.387 | Y=0.648+0.110x |
| 8 | 0.20 | 0.13 - 0.26 | 0.86 | 0.74 - 0.89 | 14.559 | Y=0.387+0.105x |
| 10 | 0.002 | 0.001 - 0.008 | 0.10 | 0.08 - 0.11 | 11.625 | Y=0.028+0.119x |

DAT- Days after treatment

A concentration of $10^8$ spores $ml^{-1}$ resulted in fifty per cent mortality in the shortest period of 6.02 days. As the concentration reduced to $10^7$, $10^6$, $10^5$ and $10^4$ spores $ml^{-1}$, the $LT_{50}$ increased and were 6.66, 7.77, 12.57 and 14.07 days, respectively.

To attain fifty per cent mortality on five DAT, spore concentration required was $1.63 \times 10^8$ spores $ml^{-1}$. While a concentration of $0.46 \times 10^8$, $0.05 \times 10^8$ and $0.03 \times 10^8$ spores $ml^{-1}$ were enough to attain fifty per cent mortality, in a longer period of seven, nine and ten day after treatment. The corresponding $LC_{90}$ values were $3.92 \times 10^8$, $1.99 \times 10^8$, $1.17 \times 10^8$ and $0.15 \times 10^8$ spores $ml^{-1}$.

*4.3.1.2.2 Adult*

Differences in mortality were observed when adult *L. acuta* was treated with different spore concentrations of *B. bassiana* (Bb 5) (Table 9). As the spore concentration increased, the mortality of adult *L. acuta* also increased. The mortality ranged from 2.50 to 25.00 per cent, 12.50 to 55.00 per cent and 17.50 to 80.00 per cent at five, seven, nine DAT, respectively for concentrations ranging from $10^5$ to $10^9$ spores $ml^{-1}$. On 10 DAT, 97.50 per cent mortality was recorded at the highest concentration of $10^9$ spores $ml^{-1}$.

$LT_{50}$ values recorded against adult *L. acuta* treated with *B. bassiana* (Bb 5), at concentrations of $10^9$, $10^8$, $10^7$, $10^6$ and $10^5$ spores $ml^{-1}$ were 6.65, 7.39, 8.41, 11.14 and 13.04 days, respectively.

A spore concentration of $2.08 \times 10^{10}$ spores $ml^{-1}$ brought about $LC_{50}$ at five days after treatment. An inverse relationship between spore concentration and duration was observed. The $LC_{50}$ values recorded at seven, nine and 10 DAT were $0.84 \times 10^{10}$, $0.25 \times 10^{10}$ and $0.18 \times 10^{10}$ spores $ml^{-1}$, respectively. The corresponding $LC_{90}$ values were $4.23 \times 10^{10}$, $2.18 \times 10^{10}$, $1.31 \times 10^{10}$ and $0.51 \times 10^{10}$ spores $ml^{-1}$, respectively.

Table 8. Dose-mortality responses of third instar nymphs of *L. acuta* treated with *B. bassiana* (Bb 5)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 5 | 7 | 9 | 10 | |
| $10^8$ | 35.00 | 65.00 | 85.00 | 100.00 | 6.02 |
| $10^7$ | 30.00 | 60.00 | 75.00 | 75.00 | 6.66 |
| $10^6$ | 22.50 | 47.50 | 60.00 | 70.00 | 7.77 |
| $10^5$ | 15.00 | 27.50 | 30.00 | 37.50 | 12.57 |
| $10^4$ | 7.50 | 10.00 | 10.00 | 10.00 | 14.07 |

Probit analysis

| DAT | $LC_{50}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$ ($10^8$ spores $ml^{-1}$) | $LC_{90}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{90}$ ($10^8$spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 5 | 1.63 | 1.40 - 1.86 | 3.92 | 3.48 - 4.02 | 6.562 | Y=0.914+0.119x |
| 7 | 0.46 | 0.32 - 0.62 | 1.99 | 1.70 - 2.06 | 22.856 | Y=0.392+0.105x |
| 9 | 0.05 | 0.03 - 0.58 | 1.17 | 0.93 - 1.22 | 14.597 | Y=0.012+0.119x |
| 10 | 0.03 | 0.02 - 0.04 | 0.15 | 0.12 - 0.16 | 26.502 | Y=0.343+0.122x |

DAT- Days after treatment

Table 9. Dose-mortality responses of adults of *L. acuta* treated with *B. bassiana* (Bb 5)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 5 | 7 | 9 | 10 | |
| $10^9$ | 25.00 | 55.00 | 80.00 | 97.50 | 6.65 |
| $10^8$ | 20.00 | 32.50 | 77.50 | 90.00 | 7.39 |
| $10^7$ | 12.50 | 22.50 | 60.00 | 75.00 | 8.41 |
| $10^6$ | 10.00 | 20.00 | 30.00 | 42.50 | 11.14 |
| $10^5$ | 2.50 | 12.50 | 17.50 | 25.00 | 13.04 |

Probit analysis

| DAT | $LC_{50}$ ($10^{10}$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$ ($10^{10}$ spores $ml^{-1}$) | $LC_{90}$ ($10^{10}$ spores $ml^{-1}$) | Fiducial limit for $LC_{90}$ ($10^{10}$ spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 5 | 2.08 | 1.87 - 2.29 | 4.23 | 3.81 - 4.32 | 5.525 | Y=1.243+0.136x |
| 7 | 0.84 | 7.14 - 9.75 | 2.18 | 1.92 - 2.24 | 3.479 | Y=0.813+0.115x |
| 9 | 0.25 | 0.13 - 0.36 | 1.31 | 1.09 - 1.37 | 32.322 | Y=0.144+0.103x |
| 10 | 0.18 | 0.11 - 0.24 | 0.51 | 0.40 - 0.54 | 36.279 | Y=0.128+0.104x |

DAT- Days after treatment

### 4.3.2 *B. bassiana* (Bb 21)

#### *4.3.2.1 C. medinalis*

The mortality of larvae of *C. medinalis* ranged from 15.00 to 35.00 per cent at four DAT for varying concentrations from $10^4$ to $10^8$ spores $ml^{-1}$ (Table 10) and it increased from 22.50 to 65.00 per cent, from 30.00 to 82.50 and 37.50 to 100 per cent, respectively in the subsequent observations on six, eight and 10 DAT.

The shortest $LT_{50}$ of 5.08 days was achieved on treating with the $10^8$ spores $ml^{-1}$. For the concentrations, $10^7$, $10^6$, $10^5$, and $10^4$ spores $ml^{-1}$, the $LT_{50}$ values were 6.93, 8.27, 9.01 and 11.19 days, respectively.

The $LC_{50}$ values recorded against *C. medinalis* were 1.54 x $10^8$ spores $ml^{-1}$, 0.57 x $10^8$, 0.19 x $10^8$ and 0.01 x $10^8$ spores $ml^{-1}$ at fourth, sixth, eighth and tenth day after treatment, respectively. The corresponding $LC_{90}$ values were 3.40 x $10^8$, 1.86 x $10^8$, 1.27 x $10^8$ and 0.14 x $10^8$spores $ml^{-1}$.

#### *4.3.2.2 L. acuta*

##### *4.3.2.2.1 Nymphs*

Nymphs treated with varying spore concentrations of *B. bassiana* (Bb 21) showed differences in mortality (Table 11). The mortality ranged from 10.00 to 20.00 per cent, 20.00 to 40.00 per cent and 20.00 to 82.50 per cent on five, seven and nine DAT. 95.00 per cent mortality was noted on 10 DAT, for concentration of $10^8$ spores $ml^{-1}$.

At $10^8$ spores $ml^{-1}$ of *B. bassiana* (Bb 21), 7.08 days was needed to obtain fifty per cent mortality. As the concentration reduced to $10^7$, $10^6$, $10^5$, and $10^4$ spores $ml^{-1}$, still longer period of 7.72, 8.59, 10.31 and 16.55 days, respectively was required.

Table 10. Dose-mortality responses of third instar larvae of *C. medinalis* treated with *B. bassiana* (Bb 21)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | |
| $10^8$ | 35.00 | 65.00 | 82.50 | 100.00 | 5.08 |
| $10^7$ | 20.00 | 47.50 | 52.50 | 82.50 | 6.93 |
| $10^6$ | 17.50 | 25.00 | 47.50 | 67.50 | 8.27 |
| $10^5$ | 17.50 | 25.00 | 40.00 | 60.00 | 9.01 |
| $10^4$ | 15.00 | 22.50 | 30.00 | 37.50 | 11.19 |

Probit analysis

| DAT | $LC_{50}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$ ($10^8$spores $ml^{-1}$) | $LC_{90}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{90}$ ($10^8$ spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 4 | 1.54 | 1.36 - 1.72 | 3.40 | 3.05 - 3.49 | 3.923 | Y=1.061+0.125x |
| 6 | 0.57 | 0.44 - 0.69 | 1.86 | 1.62 - 1.92 | 5.705 | Y=0.564+0.108x |
| 8 | 0.19 | 0.08 - 0.29 | 1.27 | 1.06 - 1.32 | 3.385 | Y=0.227+0.103x |
| 10 | 0.01 | 0.005 - 0.03 | 0.14 | 0.11 - 0.16 | 6.565 | Y=0.084+0.119x |

DAT- Days after treatment

A minimum period of five days was essential to attain fifty mortality at a concentration of 3.55 x $10^8$ spores $ml^{-1}$. The $LC_{50}$ values ranged from 1.69 x $10^8$ to 0.09 x $10^8$ spores $ml^{-1}$ during seven to ten day after treatment. The corresponding $LC_{90}$ values ranged from 7.55 x $10^8$ to 0.67 x $10^8$ spores $ml^{-1}$ from the fifth to tenth day after treatment.

#### *4.3.2.2.2 Adult*

The mortality ranged from 22.50 to 95.00 per cent from five to 10 DAT for concentrations ranging from $10^5$ to $10^9$ spores $ml^{-1}$ of *B. bassiana* (Bb 21) (Table 12).

To achieve fifty percent mortality in 6.92 days, higher concentration of $10^9$ spores $ml^{-1}$ was required. When treated with $10^8$, $10^7$,$10^6$ and $10^5$ spores $ml^{-1}$, the $LT_{50}$ recorded were 7.02, 10.27, 14.07 and 14.87 days, respectively.

The $LC_{50}$ values recorded for the five, seven, nine and 10 DAT were 1.08 x $10^{10}$, 0.94 x $10^{10}$, 0.33 x $10^{10}$ and 0.15 x $10^{10}$ spores $ml^{-1}$. The corresponding $LC_{90}$ values were 4.11 x $10^{10}$, 6.12 x $10^{10}$, .1.84 x $10^{10}$ and 0.69 x $10^{10}$ spores $ml^{-1}$.

### 4.3.3 *M. anisopliae* (Ma 4)

#### *4.3.3.1 C. medinalis*

The mortality percentage ranged from 5.00 to cent per cent during the period from fourth to tenth day after treatment with spore concentrations ranging from $10^4$ to $10^8$ spores $ml^{-1}$ of *M .anisopliae* against *C. medinalis* (Table 13).

$LT_{50}$ recorded was 4.69 days for the concentration of $10^8$ spores $ml^{-1}$ while the values were 5.79, 8.92, 11.75 and 17.04 days, for concentrations $10^7$, $10^6$, $10^5$, and $10^4$ spores $ml^{-1}$, respectively.

Table 11. Dose-mortality responses of third instar nymphs of *L. acuta* treated with *B. bassiana* (Bb 21)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 5 | 7 | 9 | 10 | |
| $10^8$ | 20.00 | 40.00 | 82.50 | 95.00 | 7.08 |
| $10^7$ | 20.00 | 30.00 | 65.00 | 87.50 | 7.72 |
| $10^6$ | 12.50 | 30.00 | 52.50 | 70.00 | 8.59 |
| $10^5$ | 10.00 | 30.00 | 42.50 | 42.50 | 10.31 |
| $10^4$ | 10.00 | 20.00 | 20.00 | 25.00 | 16.55 |

Probit analysis

| DAT | $LC_{50}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$ ($10^8$spores $ml^{-1}$) | $LC_{90}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{90}$($10^8$ spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 5 | 3.55 | 3.16 - 3.94 | 7.55 | 6.78 - 7.73 | 2.043 | Y=1.138+0.130x |
| 7 | 1.69 | 1.34 - 2.03 | 5.23 | 4.55 - 5.39 | 1.402 | Y=0.069+0.109x |
| 9 | 0.15 | 0.04 - 0.25 | 1.24 | 1.03 - 1.29 | 14.853 | Y=0.170+0.103x |
| 10 | 0.09 | 0.02 - 0.16 | 0.67 | 0.53 - 0.70 | 31.792 | Y=0.092+0.103x |

DAT- Days after treatment

Table 12. Dose-mortality responses of adults of *L. acuta* treated with *B. bassiana* (Bb 21)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 5 | 7 | 9 | 10 | |
| $10^9$ | 47.50 | 50.00 | 72.50 | 95.00 | 6.92 |
| $10^8$ | 45.00 | 47.50 | 62.50 | 85.00 | 7.02 |
| $10^7$ | 35.00 | 45.00 | 47.50 | 47.50 | 10.27 |
| $10^6$ | 30.00 | 37.50 | 40.00 | 40.00 | 14.07 |
| $10^5$ | 22.50 | 35.00 | 35.00 | 35.00 | 14.87 |

Probit analysis

| DAT | $LC_{50}$ ($10^{10}$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$ ($10^{10}$ spores $ml^{-1}$) | $LC_{90}$ ($10^{10}$ spores $ml^{-1}$) | Fiducial limit for $LC_{90}$ ($10^{10}$ spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 5 | 1.08 | 0.78 - 1.38 | 4.11 | 3.53 - 4.25 | 4.212 | Y=0.456+0.106x |
| 7 | 0.94 | 0.43 - 1.45 | 6.12 | 5.13 - 6.36 | 1.564 | Y=0.232+0.103x |
| 9 | 0.33 | 0.16 - 0.49 | 1.84 | 1.52 - 1.92 | 5.493 | Y=0.125+0.102x |
| 10 | 0.15 | 0.08 -0.22 | 0.69 | 0.56 - 0.72 | 18.237 | Y=0.022+0.103x |

DAT- Days after treatment

The $LC_{50}$ values on six, eight and 10 DAT were 1.02 x $10^8$, 0.76 x $10^8$, 0.16 x $10^8$ and 0.02 x $10^8$ spores $ml^{-1}$. The $LC_{90}$ values ranged from 2.25 x $10^8$ to 0.10 x $10^8$ spores $ml^{-1}$ during the same period of observation.

### *4.3.3.2 L. acuta*

#### *4.3.3.2.1 Nymphs*

The mortality ranged from 10.00 to 50.00, 25.00 to 70.00, 27.50 to 92.50 and 30.00 to 100 per cent, at $10^4$, $10^5$, $10^6$, $10^7$ and $10^8$ spores $ml^{-1}$ on five, seven, nine and 10 DAT (Table 14).

$LT_{50}$ recorded was 5.26 days at the concentration of $10^8$ spores $ml^{-1}$. The $LT_{50}$ values increased to 5.75, 7.94, 11.24 and 13.48 days as concentration reduced to $10^7$, $10^6$, $10^5$and $10^4$ spores $ml^{-1}$, respectively.

To get fifty per cent mortality of nymphs of *L. acuta* within five days after treatment, a concentration of 0.95 x $10^8$ spores $ml^{-1}$ of *M. anisopliae* (Ma 4) was required. Spore concentrations of 0.26 x $10^8$, 0.24 x $10^8$ and 0.01 x $10^8$ spores $ml^{-1}$ took longer period to bring fifty per cent mortality. $LC_{90}$ values of *M. anisopliae* (Ma 4) against *L. acuta* nymphs ranged between 2.96 x $10^8$ to 0.09 x $10^8$ spores $ml^{-1}$ during the period from five to 10 DAT.

#### *4.3.3.2.2 Adult*

*M. anisopliae* (Ma 4) at $10^5$ to $10^9$ spores $ml^{-1}$ brought about mortality ranging from 2.50 to 30.00, 10.00 to 47.50 and 12.50 to 72.50 and 27.50 to 95.00 per cent at five, seven, nine and 10 DAT, respectively in adult *L. acuta* (Table 15).

The $LT_{50}$ value on treating with $10^9$ spores $ml^{-1}$ was 7.13 days. As the concentration reduced to $10^8$, $10^7$,$10^6$ and $10^5$ spores $ml^{-1}$, the $LT_{50}$ increased to 8.41, 9.40, 10.59 and 12.88 days, respectively.

Table 13. Dose-mortality responses of third instar larvae of *C. medinalis* treated with *M . anisopliae* (Ma 4)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | |
| $10^8$ | 47.50 | 55.00 | 97.50 | 100.00 | 4.69 |
| $10^7$ | 32.50 | 52.50 | 70.00 | 87.50 | 5.79 |
| $10^6$ | 20.00 | 30.00 | 40.00 | 60.00 | 8.92 |
| $10^5$ | 10.00 | 22.50 | 25.00 | 40.00 | 11.75 |
| $10^4$ | 5.00 | 10.00 | 12.50 | 12.50 | 17.04 |

Probit analysis

| DAT | $LC_{50}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$ ($10^8$spores $ml^{-1}$) | $LC_{90}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{90}$ ($10^8$ spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 4 | 1.02 | 0.89 - 1.14 | 2.25 | 2.01 - 2.30 | 15.230 | Y=1.061+0.125x |
| 6 | 0.76 | 0.60 - 0.92 | 2.39 | 2.08 - 2.47 | 16.344 | Y=0.061+0.109x |
| 8 | 0.16 | 0.11 - 0.20 | 0.62 | 0.53 - 0.64 | 21.798 | Y=0.436+0.106x |
| 10 | 0.02 | 0.01 - 0.03 | 0.10 | 0.08 - 0.12 | 15.324 | Y=0.399+0.122x |

DAT- Days after treatment

Table 14. Dose-mortality responses of third instar nymphs of *L. acuta* treated with *M. anisopliae* (Ma 4)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 5 | 7 | 9 | 10 | |
| $10^8$ | 50.00 | 70.00 | 92.50 | 100.00 | 5.26 |
| $10^7$ | 40.00 | 65.00 | 90.00 | 90.00 | 5.75 |
| $10^6$ | 40.00 | 47.50 | 52.50 | 57.50 | 7.94 |
| $10^5$ | 22.50 | 35.00 | 37.50 | 45.00 | 11.24 |
| $10^4$ | 10.00 | 25.00 | 27.50 | 30.00 | 13.48 |

Probit analysis

| DAT | $LC_{50}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$ ($10^8$spores $ml^{-1}$) | $LC_{90}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{90}$($10^8$ spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 5 | 0.95 | 0.75 - 1.15 | 2.96 | 2.58 - 3.06 | 11.821 | Y=0.607+0.109x |
| 7 | 0.26 | 0.10 - 0.42 | 1.88 | 1.57 - 1.96 | 12.498 | Y=0.209+.103x |
| 9 | 0.24 | 0.16 - 0.32 | 0.78 | 0.64 - 0.82 | 29.345 | Y=0.020+0.103x |
| 10 | 0.01 | 0.005 - 0.02 | 0.09 | 0.08 - 0.10 | 3.942 | Y=0.213+0.120x |

DAT- Days after treatment

To get fifty per cent mortality within the shortest period of five days after treatment with *M. anisopliae* (Ma 4), a concentration of 1.53 x $10^{10}$ spores $ml^{-1}$ was required. The $LC_{50}$ values were 1.05 x $10^{10}$, 0.39 x $10^{10}$ and 0.13 x $10^{10}$ spores $ml^{-1}$ at seven, nine and ten days after treatment and the $LC_{90}$ values were 2.85 x $10^{10}$, 2.39 x $10^{10}$, 1.59 x $10^{10}$ and 0.69 x $10^{10}$ spores $ml^{-1}$, respectively at five, seven, nine and ten days after treatment.

### 4.3.4 *A. flavus* (Af- ml)

#### *4.3.4.1 C. medinalis*

When the third instar larvae of *C. medinalis* were treated with Af-ml, the mortality varied from 0.00 to 30.00, 10.00 to 62.50 and 22.50 to 90.00 per cent for concentrations ranging from $10^4$ to $10^8$ spores $ml^{-1}$ at six, eight and 10 DAT (Table 16).

While 7.39 days was enough to attain fifty per cent mortality at a concentration of $10^8$ spores $ml^{-1}$, a much longer period of 12.51days was required at the lowest concentration of $10^4$ spores $ml^{-1}$ of *A. flavus* (Af- ml) evaluated.

The $LC_{50}$ and the $LC_{90}$ values recorded at six, eight and 10 DAT were 1.66 x $10^8$, 0.65 x $10^8$ and 0.17 x $10^8$ spores $ml^{-1}$ and 3.37 x$10^8$, 1.81 x $10^8$ and 0.93 x $10^8$ spores $ml^{-1}$, respectively.

#### *4.3.4.2 L. acuta*

##### *4.3.4.2.1 Nymphs*

Spore concentrations of *A. flavus*, ranging from $10^4$ to $10^8$ spores $ml^{-1}$ caused mortality from 0.00 to 27.50 and 0.00 to 35.00 per cent, respectively on five and seven DAT (Table 17). Subsequently, on 10 DAT, mortality increased from 12.50 to 72.50 per cent for the same concentrations.

Table 15. Dose-mortality responses of adults of *L. acuta* treated with *M. anisopliae* (Ma 4)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 5 | 7 | 9 | 10 | |
| $10^9$ | 30.00 | 47.50 | 72.50 | 95.00 | 7.13 |
| $10^8$ | 12.50 | 25.00 | 52.50 | 80.00 | 8.41 |
| $10^7$ | 7.50 | 20.00 | 42.5 | 60.00 | 9.40 |
| $10^6$ | 7.50 | 12.50 | 32.50 | 45.00 | 10.59 |
| $10^5$ | 2.50 | 10.00 | 12.50 | 27.50 | 12.88 |

Probit analysis

| DAT | $LC_{50}$ ($10^{10}$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$ ($10^{10}$ spores $ml^{-1}$) | $LC_{90}$ ($10^{10}$ spores $ml^{-1}$) | Fiducial limit for $LC_{90}$ ($10^{10}$ spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 5 | 1.53 | 1.40 - 1.66 | 2.85 | 2.60 - 2.91 | 2.154 | Y=1.477+0.153x |
| 7 | 1.05 | 0.91 - 1.18 | 2.39 | 2.14 - 2.46 | 3.020 | Y=0.995+0.122x |
| 9 | 0.39 | 0.28 - 0.52 | 1.59 | 1.37 - 1.65 | 13.169 | Y=0.427+0.105x |
| 10 | 0.13 | 0.06 - 0.20 | 0.69 | 0.56 - 0.77 | 18.678 | Y=0.015+0.103x |

DAT- Days after treatment

Table 16. Dose-mortality responses of third instar larvae of *C. medinalis* treated with *A. flavus* (Af-m1)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | |
| $10^8$ | 0.00 | 30.00 | 62.50 | 90.00 | 7.39 |
| $10^7$ | 0.00 | 20.00 | 50.00 | 70.00 | 8.07 |
| $10^6$ | 0.00 | 15.00 | 22.50 | 47.50 | 10.14 |
| $10^5$ | 0.00 | 10.00 | 17.50 | 25.00 | 11.71 |
| $10^4$ | 0.00 | 0.00 | 10.00 | 22.50 | 12.51 |

Probit analysis

| DAT | $LC_{50}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$ ($10^8$ spores $ml^{-1}$) | $LC_{90}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{90}$ ($10^8$ spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 6 | 1.66 | 1.49 - 1.83 | 3.37 | 3.04 - 3.45 | 7.925 | Y=1.248+0.136x |
| 8 | 0.65 | 0.54 - 0.77 | 1.81 | 1.59 - 1.86 | 15.766 | Y=0.727+0.112x |
| 10 | 0.17 | 0.09 - 0.24 | 0.93 | 0.78 - 0.96 | 18.791 | Y=0.286+0.104x |

DAT- Days after treatment

$LT_{50}$ of 8.22 days observed in the higher concentration of $10^8$ spores $ml^{-1}$ of *A. flavus*, increased from 9.23 to 15.51 days as the concentration reduced from $10^7$ to $10^4$ spores $ml^{-1}$, respectively.

To get fifty per cent mortality within five days after treatment, spore concentration of 1.74 x $10^8$ spores $ml^{-1}$ was required. The $LC_{50}$ and $LC_{90}$ values recorded on the tenth day after treatment were 0.45 x $10^8$ spores $ml^{-1}$ and 1.48 x $10^8$ spores $ml^{-1}$, respectively.

***4.3.4.2.2 Adult***

The mortality percentage ranged from 2.50 to 87.50 per cent for concentrations ranging from $10^5$ to $10^9$ spores $ml^{-1}$ on five to 10 DAT with *A. flavus* (Af-m1) (Table 18).

A higher concentration of $10^9$ spores $ml^{-1}$ was required to achieve fifty per cent mortality within a shorter period of 7.96 days and as the concentration reduced to $10^8$, $10^7$,$10^6$ and $10^5$ spores $ml^{-1}$ , the $LT_{50}$ recorded increased to 8.97, 12.23, 14.76 and 15.37days, respectively.

The $LC_{50}$ values of *A. flavus* (Af-m1) ranged from 4.84 x $10^{10}$ to 0.26 x $10^{10}$ spores $ml^{-1}$ and $LC_{90}$ values ranged from 8.72 x $10^{10}$ to 1.03 x $10^{10}$ spores $ml^{-1}$ at different concentrations evaluated against adult *L. acuta*.

## 4.4 FIELD EXPERIMENT

The results of two field experiments conducted to evaluate the effectiveness of fungal pathogens and chemical pesticides in the management of *C. medinalis* and *L. acuta* are presented in Tables 19 to 36.

Table 17. Dose-mortality responses of third instar nymphs of *L. acuta* treated with *A. flavus* (Af-m1)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 5 | 7 | 9 | 10 | |
| $10^8$ | 27.50 | 35.00 | 50.00 | 72.50 | 8.22 |
| $10^7$ | 25.00 | 30.00 | 45.00 | 60.00 | 9.23 |
| $10^6$ | 17.50 | 27.50 | 27.50 | 32.50 | 11.24 |
| $10^5$ | 0.00 | 0.00 | 17.50 | 17.50 | 13.48 |
| $10^4$ | 0.00 | 0.00 | 10.00 | 12.50 | 15.51 |

Probit analysis

| DAT | $LC_{50}$ ($10^8$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$($10^8$ spores $ml^{-1}$) | $LC_{90}$ ($10^8$spores $ml^{-1}$) | Fiducial limit for $LC_{90}$($10^8$ spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 5 | 1.74 | 1.75 - 1.91 | 3.46 | 3.13 - 3.54 | 19.023 | Y=1.292+0.139x |
| 7 | 1.44 | 1.27 - 1.60 | 3.10 | 2.78 - 3.18 | 25.910 | Y=1.107+0.128x |
| 9 | 0.93 | 0.77 - 1.09 | 2.61 | 2.29 - 2.69 | 12.700 | Y=0.713+0.112x |
| 10 | 0.45 | 0.35 - 0.55 | 1.48 | 1.28 - 1.53 | 21.103 | Y=0.564+0.108x |

DAT- Days after treatment

Table 18. Dose-mortality responses of adults of *L. acuta* treated with *A. flavus* (Af-m1)

| Concentration (spores $ml^{-1}$) | Cumulative per cent mortality at different days after treatment | | | | $LT_{50}$ (days) |
|---|---|---|---|---|---|
| | 5 | 7 | 9 | 10 | |
| $10^9$ | 10.00 | 47.50 | 62.50 | 87.50 | 7.96 |
| $10^8$ | 7.50 | 35.00 | 40.00 | 67.50 | 8.97 |
| $10^7$ | 7.50 | 15.00 | 17.50 | 37.50 | 12.33 |
| $10^6$ | 5.00 | 12.50 | 17.50 | 20.00 | 14.76 |
| $10^5$ | 2.50 | 5.00 | 15.00 | 15.00 | 15.37 |

Probit analysis

| DAT | $LC_{50}$ ($10^{10}$ spores $ml^{-1}$) | Fiducial limit for $LC_{50}$ ($10^{10}$ spores $ml^{-1}$) | $LC_{90}$ ($10^{10}$ spores $ml^{-1}$) | Fiducial limit for $LC_{90}$ ($10^{10}$ spores $ml^{-1}$) | $X^2$ | Regression |
|---|---|---|---|---|---|---|
| 5 | 4.84 | 4.46 - 5.22 | 8.72 | 7.98 - 8.89 | 1.206 | Y=1.601+0.166x |
| 7 | 1.02 | 0.89 - 1.14 | 2.31 | 2.06 - 2.37 | 11.450 | Y=1.008+0.122x |
| 9 | 0.69 | 0.58 - 0.79 | 1.79 | 1.58 - 1.84 | 6.728 | Y=0.804+0.114x |
| 10 | 0.26 | 0.19 - 0.34 | 1.03 | 0.86 - 1.04 | 22.811 | Y=0.457+0.106x |

DAT- Days after treatment

### 4.4.1 First Field Trial

#### *4.4.1.1 C. medinalis*

##### *4.4.1.1.1 Population*

###### *4.4.1.1.1.1 Larval Population*

The data on the larval population of *C. medinalis* assessed on the basis of the number of larvae present in ten hills plot$^{-1}$ are presented in Table 19.

The pretreatment population of larvae of *C. medinalis*, in various plots was statistically similar. On the fourth day after spraying, all the treatments showed significant reduction in the population when compared to untreated (22.33). Among the fungal pathogens, the lowest population was observed in plots treated with *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ (12.33) which was statistically on par with *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ (13.33), talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (13.67), talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (14.00), talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ (15.00) and *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ (15.00). Chlorantraniliprole @ 30 g a.i ha$^{-1}$ treated plots had the lowest mean population (2.00) and it was significantly superior to all other treatments. Thiamethoxam @ 25 g a.i ha$^{-1}$ (4.00) and acephate @ 750 g a.i ha$^{-1}$ (4.67) was statistically on par. Malathion @ 575 g a.i ha$^{-1}$ treated plots (6.00) was statistically similar with acephate @ 750 g a.i ha$^{-1}$. In this initial observation, the larval population in plots treated with entomopathogens was significantly higher than that treated with chemical pesticides.

At seven DAT also, all the treatments showed significant reduction in the population than untreated (18.67). *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ treated plots had the lowest mean population of 7.67 among the fungal treatments and was statistically on par with talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (9.67), acephate @ 750 g a.i ha$^{-1}$ (5.67), chlorantraniliprole @ 30 g a.i ha$^{-1}$ (6.00), malathion @ 575 g a.i ha$^{-1}$ (7.33) and thiamethoxam @ 25 g a.i ha$^{-1}$ (8.00).

*M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ treated plots had mean population count of 10.67 and was statistically similar to thiamethoxam, talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (11.67), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (12.00) and *A. flavus* (Af-m1) @ $10^{10}$spores $ml^{-1}$ (12.67).

Significant reduction in the population of *C. medinalis* larvae in all the treatments was evident at 14 DAT also, when compared to that of the untreated (22.67). *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ treated plots had the lowest mean population of 7.33, among the fungal treatments, which was on par with the insecticides, chlorantraniliprole @ 30 g a.i $ha^{-1}$ (6.00), acephate @ 750 g a.i $ha^{-1}$ (6.33), thiamethoxam @ 25g a.i $ha^{-1}$ (7.67), malathion @ 575 g a.i $ha^{-1}$ (7.67), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (11.67), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (12.00), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (13.00), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (13.00) and talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (13.67) were found to be statistically on par.

At 21 DAT, the trend was similar to that of the previous days, with significant difference in the population of *C. medinalis* present in untreated (19.33) and other treated plots. Among the fungal treatments, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ had the lowest mean population of 8.00 $ten^{-1}$ hills $plot^{-1}$ which was on par with talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (10.33), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (11.00), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (11.00), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (11.00) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (11.33). Among the insecticides, chlorantraniliprole @ 30 g a.i $ha^{-1}$ recorded the lowest population of 5.67 and this was on par with thiamethoxam @ 25 g a.i $ha^{-1}$ (6.00), acephate @ 750 g a.i $ha^{-1}$ (7.00) and malathion @ 575 g a.i $ha^{-1}$ (8.00). The effect of *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ was on par with all the insecticides while that of talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ was on par with acephate @ 750 g a.i $ha^{-1}$ and malathion @ 575 g a.i $ha^{-1}$.

Table 19. Effect of treatments on the population of *C. medinalis* larvae (Field trial-I)

| Treatments | Mean number of larva $10^{-1}$ hills $plot^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 19.00 (4.36) | 13.67 (3.69) | 9.67 (3.09) | 11.67 (3.41) | 10.33 (3.21) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 16.67 (4.08) | 14.00 (3.73) | 12.00 (3.46) | 12.00 (3.46) | 11.00 (3.31) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 17.00 (4.12) | 15.00 (3.87) | 11.67 (3.41) | 13.67 (3.68) | 11.00 (3.29) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ | 17.33 (4.16) | 13.33 (3.65) | 7.67 (2.76) | 7.33 (2.69) | 8.00 (2.83) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 16.00 (3.99) | 15.00 (3.87) | 10.67 (3.26) | 13.00 (3.60) | 11.00 (3.31) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 18.00 (4.24) | 12.33 (3.51) | 12.67 (3.56) | 13.00 (3.58) | 11.33 (3.36) |
| Acephate @ 750 g a.i $ha^{-1}$ | 17.00 (4.12) | 4.67 (2.14) | 5.67 (2.37) | 6.33 (2.51) | 7.00 (2.62) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 16.67 (4.08) | 2.00 (1.38) | 6.00 (2.44) | 6.00 (2.44) | 5.67 (2.38) |
| Malathion @ 575 g a.i $ha^{-1}$ | 18.00 (4.24) | 6.00 (2.44) | 7.33 (2.69) | 7.67 (2.76) | 8.00 (2.83) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 16.33 (4.04) | 4.00 (1.99) | 8.00 (2.81) | 7.67 (2.76) | 6.00 (2.43) |
| Untreated | 19.33 (4.39) | 22.33 (4.73) | 18.67 (4.32) | 22.67 (4.76) | 19.33 (4.36) |
| CD (0.05) | NS | (0.411) | (0.447) | (0.508) | (0.617) |

Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

*4.4.1.1.1.2 Adult Population*

The data on the adult population of *C. medinalis* assessed on the basis of the number of adult present in ten sweeps plot$^{-1}$ are presented in Table 20.

There was no significant difference in the pretreatment population of *C. medinalis* adults in various plots. No significant difference was observed in the population of adults in untreated (13.00) and entomopathogen treated plots on the fourth day after spraying. The mean number of adults present in *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ (11.67), *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ (11.67), talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (12.00), *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ (12.00), talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ (13.00), talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (13.33) were statistically on par. The lowest population was recorded in chlorantraniliprole @ 30 g a.i ha$^{-1}$ treated plots (3.00) which was on par with acephate @ 750 g a.i ha$^{-1}$ (4.00) and thiamethoxam @ 25 g a.i ha$^{-1}$ (4.00). Malathion @ 575 g a.i ha$^{-1}$ treated plots harboured mean population of 6.00 and was statistically inferior to other insecticides evaluated.

At seven DAT, all the treatments showed significant reduction in the population than untreated (16.00). *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ treated plots had mean population of 8.00 and it was significantly lower than that in the other fungal treated plots and was on par with malathion @ 575 g a.i ha$^{-1}$ treated plots (7.00). Talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (10.33), talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ (11.00), talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (11.33) and *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ (11.00) were statistically on par. *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ treated plots had mean population of 13.00 and it was on par with other fungal treatments except *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ and talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$. Chlorantraniliprole @ 30 g a.i ha$^{-1}$ treated plots recorded mean population count of 5.33 and was on par with acephate @ 750 g a.i ha$^{-1}$ (6.00) and thiamethoxam @ 25 g a.i ha$^{-1}$(6.00). Malathion @ 575 g a.i ha$^{-1}$ (7.00) was on par with other chemicals except chlorantraniliprole @ 30 g a.i ha$^{-1}$.

There was significant difference in the adult population in treated and untreated (16.33) at 14 DAT. Among the fungal treatments, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ treated plots had the lowest mean population of 8.00 adults $ten^{-1}$ sweeps $plot^{-1}$, which was statistically on par with the effect of talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (9.00), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (9.00), acephate @ 750 g a.i $ha^{-1}$ (6.67) and malathion @ 575 g a.i $ha^{-1}$(7.00). *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (10.00), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (10.67) and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (10.67) were statistically similar. The mean population in thiamethoxam @ 25 g a.i $ha^{-1}$, chlorantraniliprole @ 30 g a.i $ha^{-1}$, acephate @ 750 g a.i $ha^{-1}$ and malathion @ 575 g a.i $ha^{-1}$ treated plots was 6.00, 6.00, 6.67 and 7.00, respectively and were on par.

The effect of the fungal pathogens as well as insecticides continued to 21 DAT also, with significant difference in adult population in the treated and untreated plots (13.67). The lowest population of 8.00 was recorded in talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ and *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ which was on par with *A. flavus* (Af-m1) @ $10^{10}$spores $ml^{-1}$ (8.33), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (8.67), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (9.33) and talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (9.67). Among the insecticides, the lowest mean population of 6.00 in thiamethoxam @ 25 g a.i $ha^{-1}$ treated plots was statistically on par with chlorantraniliprole @ 30 g a.i $ha^{-1}$ (6.33), acephate @ 750 g a.i $ha^{-1}$ (7.00) and malathion @ 575 g a.i $ha^{-1}$(7.33). Talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ and *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ were on par with all chemicals tested while *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ was on par with all chemicals except thiamethoxam. Talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ were on par with acephate @ 750 g a.i $ha^{-1}$and malathion @ 575 g a.i $ha^{-1}$.

Table 20. Effect of treatments on the population of *C. medinalis* adult (Field trial-I)

| Treatments | Mean number of adult $10^{-1}$ sweeps $plot^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 13.67 (3.69) | 12.00 (3.46) | 10.33 (3.21) | 9.00 (2.99) | 8.00 (2.82) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 13.67 (3.69) | 13.33 (3.65) | 11.33 (3.37) | 10.67 (3.26) | 9.67 (3.11) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 13.33 (3.64) | 13.00 (3.60) | 11.00 (3.31) | 9.00 (2.99) | 8.67 (2.94) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ | 14.00 (3.74) | 11.67 (3.41) | 8.00 (2.83) | 8.00 (2.83) | 8.00 (2.82) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 13.67 (3.69) | 11.67 (3.42) | 11.00 (3.31) | 10.67 (3.27) | 9.33 (3.05) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 12.67 (3.55) | 12.00 (3.46) | 13.00 (3.60) | 10.00 (3.16) | 8.33 (2.87) |
| Acephate @ 750 g a.i $ha^{-1}$ | 14.67 (3.83) | 4.00 (1.99) | 6.00 (2.44) | 6.67 (2.58) | 7.00 (2.63) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 16.00 (3.99) | 3.00 (1.72) | 5.33 (2.29) | 6.00 (2.44) | 6.33 (2.52) |
| Malathion @ 575 g a.i $ha^{-1}$ | 12.67 (3.55) | 6.00 (2.44) | 7.00 (2.64) | 7.00 (2.64) | 7.33 (2.69) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 13.33 (3.65) | 4.00 (1.99) | 6.00 (2.44) | 6.00 (2.44) | 6.00 (2.44) |
| Untreated | 14.67 (3.83) | 13.00 (3.60) | 16.00 (3.99) | 16.33 (4.04) | 13.67 (3.69) |
| CD (0.05) | NS | (0.372) | (0.338) | (0.276) | (0.425) |

Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

*4.4.1.1.2 Extent of Damage*

The data on the effect of treatments on the extent of damage by *C. medinalis*, assessed in terms of the percentage of damaged leaves in ten hills plot$^{-1}$ are presented in Table 21.

There was no significant difference in the leaf damage in the pretreatment observations. On the fourth DAT, *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ treated plots had the lowest leaf damage of 3.67 per cent and it was on par with *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ (3.80) and talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (4.07). These three fungal treatments were significantly superior to untreated plots (5.07 per cent). However, there was no significant difference in the percentage of leaves damaged in talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ (4.20), *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ (4.27) talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (4.53) treated and untreated plots. Malathion @ 575 g a.i ha$^{-1}$ had the lowest leaf damage of 1.13 per cent which was statistically on par with acephate @ 750 g a.i ha$^{-1}$ (1.20), thiamethoxam @ 25 g a.i ha$^{-1}$ (1.20) and chlorantraniliprole @ 30 g a.i ha$^{-1}$ (1.40).

There was significant difference in the percentage of leaf damaged in treated and untreated plots (8.53) at seven DAT. *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ treated plots showed the lowest leaf damage of 2.60 per cent among the different fungal treatments and were on par with acephate @ 750 g a.i ha$^{-1}$ (2.27), chlorantraniliprole @ 30 g a.i ha$^{-1}$ (2.40) and thiamethoxam @ 25 g a.i ha$^{-1}$ (2.67). *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ (3.47) was on par with talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (3.53), talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (3.67), talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ (3.87), *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ (4.47) and thiamethoxam @ 25 g a.i ha$^{-1}$. All the fungal treatments, except *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ were statistically on par with malathion @ 575 g a.i ha$^{-1}$ (3.60).

At 14 DAT, all the treatments recorded significantly lower leaf damage than the untreated (9.00). The lowest leaf damage was recorded in *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (2.13) treated plots and it was on par with acephate @ 750 g a.i $ha^{-1}$ (2.60) and chlorantraniliprole @ 30 g a.i $ha^{-1}$ (2.73) treated plots. Talc based *B. bassiana* (Bb 5 and Bb 21) @ 20 g $l^{-1}$ (3.07) was on par with *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (3.67), thiamethoxam @ 25 g a.i $ha^{-1}$ (3.07) and chlorantraniliprole @ 30 g a.i $ha^{-1}$. Talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (4.40) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (4.53) were statistically similar to that in malathion @ 575 g a.i $ha^{-1}$ (3.87 per cent).

All the treatments recorded significantly lower leaf damage than that in the untreated plots (7.73) at 21 DAT also. *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (1.87) treated plots that recorded lowest leaf damage was on par with acephate @ 750 g a.i $ha^{-1}$ (2.27). The effect of talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (2.80), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (2.87) and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (3.07) were statistically similar to thiamethoxam @ 25 g a.i $ha^{-1}$ (2.60) and malathion @ 575 g a.i $ha^{-1}$ (3.13). *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ treated plots recorded 3.60 per cent leaf damage and was on par with talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (3.73) and malathion @ 575 g a.i $ha^{-1}$. Chlorantraniliprole @ 30 g a.i $ha^{-1}$ treated plots showed leaf damage to the tune of 2.33 per cent and was on par with acephate @ 750 g a.i $ha^{-1}$ and thiamethoxam 25 g a.i $ha^{-1}$.

### *4.4.1.2 L. acuta*

#### *4.4.1.2.1 Population*

The population of *L. acuta* assessed in terms of the total number of nymphs and adults present in ten sweeps $plot^{-1}$ and also that present in ten hills $plot^{-1}$ are presented in Table 22.

The pretreatment population of *L. acuta* was non significant. On the fourth DAT, all treatments except talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (37.00)

Table 21. Effect of treatments on the extent of damage by *C. medinalis* (Field trial-I)

| Treatments | Mean damaged leaves $10^{-1}$ hills plot $^{-1}$ (%) | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 4.33 (2.15) | 4.07 (2.02) | 3.67 (1.92) | 3.07 (1.75) | 2.80 (1.67) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 4.60 (2.14) | 4.53 (2.12) | 3.53 (1.87) | 3.07 (1.74) | 2.87 (1.69) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 4.27 (2.02) | 4.20 (2.04) | 3.87 (1.97) | 4.40 (2.09) | 3.73 (1.93) |
| *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$ | 4.60 (2.14) | 3.80 (1.95) | 2.60 (1.61) | 2.13 (1.46) | 1.87 (1.37) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 4.27 (2.07) | 3.67 (1.91) | 3.47 (1.86) | 3.67 (1.91) | 3.07 (1.75) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 4.93 (2.21) | 4.27 (2.07) | 4.47 (2.10) | 4.53 (2.13) | 3.60 (1.89) |
| Acephate @ 750 g a.i $ha^{-1}$ | 5.13 (2.26) | 1.20 (1.09) | 2.27 (1.50) | 2.60 (1.61) | 2.27 (1.51) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 4.07 (2.01) | 1.40 (1.18) | 2.40 (1.54) | 2.73 (1.65) | 2.33 (1.53) |
| Malathion @ 575 g a.i $ha^{-1}$ | 4.53 (2.13) | 1.13 (1.06) | 3.60 (1.88) | 3.87 (1.96) | 3.13 (1.77) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 4.40 (2.09) | 1.20 (1.09) | 2.67 (1.63) | 3.07 (1.75) | 2.60 (1.61) |
| Untreated | 5.13 (2.26) | 5.07 (2.25) | 8.53 (2.92) | 9.00 (3.00) | 7.73 (2.78) |
| CD (0.05) | NS | (0.215) | (0.241) | (0.189) | (0.141) |

Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

caused significant reduction in the population of rice bug compared to untreated (42.33). Among the fungal treatments, *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ treated plots recorded the lowest mean population of 25.67 $ten^{-1}$ hills and $ten^{-1}$ sweeps $plot^{-1}$ and was on par with *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (26.67), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (26.67) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (28.00). Talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ treated plots showed mean population of 32.67 $ten^{-1}$ hills and $ten^{-1}$ sweeps $plot^{-1}$ and was statistically similar to all the fungal treatments except *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$. The insecticide treated plots recorded significantly lower population than the entomopathogenic fungi treated plots. Among the chemicals, chlorantraniliprole @ 30 g a.i. $ha^{-1}$, recorded the lowest mean population of 4.33 $ten^{-1}$ hills and $ten^{-1}$ sweeps $plot^{-1}$, which was statistically similar to that in thiamethoxam @ 25 g a.i $ha^{-1}$ (6.67) and acephate @ 750 g a.i $ha^{-1}$ (7.00). In malathion @ 575 g a.i $ha^{-1}$ treated plots, mean population count of 8.00 $ten^{-1}$ hills and $ten^{-1}$ sweeps $plot^{-1}$ was recorded and was on par with that of acephate @ 750 g a.i $ha^{-1}$and thiamethoxam @ 25 g a.i $ha^{-1}$.

At seven DAT, the trend was similar with that of the previous observation with significantly lower population in all the treated plots except talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ treated plots (32.00) compared to untreated plots (39.00). The mean population of 23.67 $plot^{-1}$ in *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ treated plots harboured the lowest among the fungal treatments. The mean population $plot^{-1}$ in talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (24.33), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (26.00), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (25.33) were statistically similar to *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$. Talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ treated plots had mean population of 30.67 $ten^{-1}$ hills and $ten^{-1}$ sweeps $plot^{-1}$ which was statistically similar to the other fungal treatments, except *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$. The insecticides evaluated were significantly superior to the fungal treatments, with the lowest mean population of 7.00 $plot^{-1}$ in chlorantraniliprole @ 30 g a.i. $ha^{-1}$, and this was on par with malathion @ 575 g a.i $ha^{-1}$ (9.67) and thiamethoxam @

25 g a.i. $ha^{-1}$ (10.00). Acephate @ 750 g a.i $ha^{-1}$ treated plots, had population count of 10.67, which was statistically similar to malathion @ 575 g ai $ha^{-1}$ and thiamethoxam @ 25 g a.i $ha^{-1}$.

There was significant difference in the population in treated and untreated plots (38.00) at 14 DAT also. *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ treated plots had the lowest mean population of 20.67 $ten^{-1}$ sweeps $plot^{-1}$, which was statistically on par with talc based *M. anisopliae* (Ma 4) @ 20g $l^{-1}$ (22.00), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (22.00) and *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (22.67). Talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ and talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ treated plots had mean population of 26.00 and 26.33 $ten^{-1}$ hills and $ten^{-1}$ sweeps $plot^{-1}$, respectively and were on par. The chemical treated plots recorded significantly lower population than fungal treated plots. Chlorantraniliprole @ 30 g a.i. $ha^{-1}$ treated plots had the lowest mean population $plot^{-1}$(12.00). Acephate @ 750 g a.i $ha^{-1}$, malathion @ 575 g a.i $ha^{-1}$ and thiamethoxam @ 25 g a.i $ha^{-1}$ treated plots had mean population of 14.00, 15.00 and 15.67 $ten^{-1}$ sweeps $plot^{-1}$, respectively and were statistically on par.

The trend in population of *L. acuta* at 21 DAT was similar to that in the earlier observations, with significant difference in the population in treated and untreated plots (36.33). Among the fungal treatments, *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ treated plots had the lowest mean population count of 17.00 $ten^{-1}$ hills and $ten^{-1}$ sweeps $plot^{-1}$, which was on par with that in the talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (17.33), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (18.67), thiamethoxam @ 25 g a.i $ha^{-1}$ (15.33) and malathion 575 g a.i $ha^{-1}$ (17.67). *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (23.00), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (24.33) and talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (24.67) were statistically on par. Chlorantraniliprole @ 30 g a.i. $ha^{-1}$ treated plots had the lowest mean population of 12.00 $ten^{-1}$ hills and $ten^{-1}$ sweeps $plot^{-1}$ and were statistically similar to acephate @ 750 g a.i $ha^{-1}$ (14.00). Thiamethoxam @ 25 g a.i $ha^{-1}$ (15.33) was on par with acephate @ 750 g a.i $ha^{-1}$ (14.00) and malathion @ 575 g a.i $ha^{-1}$ (17.67).

Table 22. Effect of treatments on the population of *L.acuta* (Field trial-I)

| Treatments | Mean number of *L. acuta* (10 sweeps + 10 hills)$^{-1}$ plot$^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ | 36.00 (5.99) | 32.67 (5.68) | 30.67 (5.53) | 26.33 (5.13) | 24.33 (4.93) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ | 37.00 (6.07) | 37.00 (6.08) | 32.00 (5.65) | 26.00 (5.09) | 24.67 (4.97) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ | 40.00 (6.05) | 26.67 (5.16) | 24.33 (4.93) | 22.00 (4.69) | 17.33 (4.16) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ | 32.33 (5.66) | 26.67 (5.16) | 25.33 (5.03) | 22.67 (4.76) | 18.67 (4.32) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ | 37.33 (6.11) | 25.67 (5.07) | 23.67 (4.86) | 20.67 (4.55) | 17.00 (4.12) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ | 32.67 (5.71) | 28.00 (5.29) | 26.00 (5.07) | 22.00 (4.69) | 23.00 (4.79) |
| Acephate @ 750 g a.i ha$^{-1}$ | 33.67 (5.78) | 7.00 (2.63) | 10.67 (3.26) | 14.00 (3.74) | 14.00 (3.74) |
| Chlorantraniliprole @ 30 g a.i ha$^{-1}$ | 32.33 (5.66) | 4.33 (2.08) | 7.00 (2.63) | 12.00 (3.46) | 12.00 (3.46) |
| Malathion @ 575 g a.i ha$^{-1}$ | 36.00 (5.99) | 8.00 (2.83) | 9.67 (3.09) | 15.00 (3.87) | 17.67 (4.19) |
| Thiamethoxam @ 25 g a.i ha$^{-1}$ | 34.33 (5.86) | 6.67 (2.58) | 10.00 (3.15) | 15.67 (3.96) | 15.33 (3.91) |
| Untreated | 37.67 (6.13) | 42.33 (6.50) | 39.00 (6.25) | 38.00 (6.16) | 36.33 (6.03) |
| CD (0.05) | NS | (0.610) | (0.598) | (0.258) | (0.321) |

Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

*4.4.1.2.2 Extent of Damage*

The data on the effect of treatments on the extent of damage by *L. acuta*, assessed in terms of the percentage of damaged grains in ten hills plot$^{-1}$ are presented in Table 23.

Pretreatment observations showed no significant difference in the extent of damage by rice bug. On four DAT, there was significant difference between treated and untreated plots (3.40). *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ treated plots had the lowest damage of 2.07 per cent ten$^{-1}$ hills plot$^{-1}$ and was on par with talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ (2.20) and *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ (2.30). Talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (2.80), *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ (2.83) and talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (2.87) were statistically similar. The insecticide treated plots had significantly lower grain damage than fungal treated plots. Among the different chemicals evaluated, chlorantraniliprole @ 30 g a.i. ha$^{-1}$ treated plots recorded the lowest grain damage (0.10) and was on par with acephate @ 750 g a.i ha$^{-1}$ (0.23). Plots treated with thiamethoxam @ 25 g a.i ha$^{-1}$ and malathion @ 575 g a.i ha$^{-1}$ showed damage to the extent of 0.37 and 0.40 per cent, respectively and were on par with acephate @ 750 g a.i ha$^{-1}$.

The trend in the extent of damage noticed at seven DAT was similar to that of the previous observation, with significant difference in treated and untreated plots (2.67). *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ treated plots had the lowest mean damage of 1.70 ten$^{-1}$ hills plot$^{-1}$ which was significantly lower than all other fungal treatments. All the other fungal treatments were statistically similar and the mean damage percentage ranged between 2.00 and 2.23. Chlorantraniliprole @ 30 g a.i ha$^{-1}$ treated plots had the lowest mean damage of 0.57 per cent among all the treatments, and was statistically similar to acephate @ 750 g a.i ha$^{-1}$ (0.73) and thiamethoxam @ 25 g a.i ha$^{-1}$ (0.73). Malathion @ 575 g a.i ha$^{-1}$ treated plots indicated a mean damage of 0.93 per cent which was on par with acephate @ 750 g ai ha$^{-1}$ and thiamethoxam @ 25 g a.i.ha$^{-1}$.

As in the earlier observations, at 14 DAT also, there was significant difference in the damage caused by *L. acuta* between treated and untreated plots (2.07). *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ maintained the superiority with the lowest mean damage of 0.77 per cent $ten^{-1}$ hills $plot^{-1}$, which was on par with the insecticides, chlorantraniliprole @ 30 g a.i $ha^{-1}$, acephate @ 750 g a.i $ha^{-1}$ and thiamethoxam 25 g a.i $ha^{-1}$, which had mean grain damage of 0.83, 0.90 and 1.00 per cent $ten^{-1}$ hills $plot^{-1}$, respectively. *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ treated plots had the mean damage of 1.10 per cent $ten^{-1}$ hills $plot^{-1}$ and was statistically on par with talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (1.17) and talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (1.37). *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ was statistically on par with insecticides *viz.*, acephate @ 750 g a.i $ha^{-1}$, thiamethoxam 25 g a.i $ha^{-1}$ and malathion @ 575 g a.i $ha^{-1}$ (1.13 per cent). *A. flavus* (Af-ml) @ $10^{10}$ spores $ml^{-1}$ (1.43) and talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (1.47) were on par with talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$.

Significant difference in the percentage of grain damaged in the treated and untreated plots (1.87) was evident at 21 DAT too. Among the fungal treatments, the performance of *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ was the best with only 0.73 per cent damage, however it was on par with the insecticides, chlorantraniliprole @ 30 g a.i $ha^{-1}$ (0.63 per cent $ten^{-1}$ hills $plot^{-1}$) and acephate 750 g a.i $ha^{-1}$ (0.83 per cent $ten^{-1}$ hills $plot^{-1}$). *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ and talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ treated plots recorded mean grain damage of 1.00 and 1.03 per cent $ten^{-1}$ hills $plot^{-1}$, respectively and was statistically similar to that in malathion @ 575 g a.i $ha^{-1}$ (1.00) and thiamethoxam @ 25 g a.i $ha^{-1}$ (1.07). Talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (1.30), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (1.37) and *A. flavus* (Af-ml) @ $10^{10}$ spores $ml^{-1}$ (1.47) were statistically similar.

Table 23. Effect of treatments on the extent of damage by *L. acuta* (Field trial-I)

| Treatments | Mean damaged grains $10^{-1}$ hills $plot^{-1}$ (%) | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 3.20 (2.03) | 2.80 (1.95) | 2.13 (1.77) | 1.47 (1.57) | 1.37 (1.54) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 4.47 (2.34) | 2.87 (1.97) | 2.23 (1.79) | 1.37 (1.54) | 1.30 (1.52) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 3.53 (2.12) | 2.20 (1.79) | 2.07 (1.75) | 1.17 (1.47) | 1.03 (1.43) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ | 3.37 (2.08) | 2.30 (1.82) | 2.00 (1.73) | 1.10 (1.45) | 1.00 (1.41) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 3.77 (2.18) | 2.07 (1.75) | 1.70 (1.64) | 0.77 (1.33) | 0.73 (1.32) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 3.60 (2.29) | 2.83 (1.96) | 2.13 (1.77) | 1.43 (1.56) | 1.47 (1.57) |
| Acephate @ 750 g a.i $ha^{-1}$ | 4.30 (2.21) | 0.23 (1.11) | 0.73 (1.32) | 0.90 (1.37) | 0.83 (1.35) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 4.40 (2.32) | 0.10 (1.05) | 0.57 (1.25) | 0.83 (1.35) | 0.63 (1.28) |
| Malathion @ 575 g a.i $ha^{-1}$ | 3.60 (2.14) | 0.40 (1.18) | 0.93 (1.39) | 1.13 (1.46) | 1.00 (1.41) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 3.90 (2.14) | 0.37 (1.17) | 0.73 (1.32) | 1.00 (1.41) | 1.07 (1.44) |
| Untreated | 4.00 (2.24) | 3.40 (2.09) | 2.67 (1.91) | 2.07 (1.75) | 1.87 (1.69) |
| CD (0.05) | NS | (0.091) | (0.084) | (0.094) | (0.085) |

Mean of three replications
Figures in parentheses are $\sqrt{x+1}$ transformed values.
DAT- Days after treatment
NS- Non significant

### *4.4.1.3 Natural Enemies*

#### *4.4.1.3.1 Parasitoids*

The population of hymenopteran parasitoids assessed in terms of the number of parasitoids present in ten sweeps plot$^{-1}$ are presented in Table 24.

No significant difference in the pretreatment observation on the mean population of hymenopteran parasitoids was seen. On four DAT, *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$, talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$, *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ recorded mean population of 3.67 ten$^{-1}$ sweeps plot$^{-1}$ each and was on par with that in the talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (3.00), talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (3.00), *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ (2.67) and untreated plots (3.67). Among the insecticides, chlorantraniliprole @ 30 g a.i. ha$^{-1}$ (3.00) was statistically on par with all the fungal treatments and untreated. Thiamethoxam @ 25 g a.i ha$^{-1}$, acephate @ 750 g a.i ha$^{-1}$ and malathion @ 575 g a.i ha$^{-1}$ harboured significantly lower population of 1.00, 0.67 and 0.33 ten$^{-1}$ sweeps plot$^{-1}$, respectively and were on par.

The highest mean population of 3.67 ten$^{-1}$ sweeps plot$^{-1}$ observed in *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$, *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$, talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ and *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ on the seventh day after treatment, was statistically on par with talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (3.33), talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (3.00) and untreated plot (3.67). Chlorantraniliprole @ 30 g a.i ha$^{-1}$ treated plots also had mean population of 3.67 ten$^{-1}$ sweeps plot$^{-1}$ and was on par with that in the fungal treated and untreated plots, whereas thiamethoxam @ 25 g a.i ha$^{-1}$, acephate @ 750 g a.i ha$^{-1}$ and malathion @ 575 g a.i ha$^{-1}$ harboured significantly lower mean population of 1.33, 1.00 and 0.33 ten$^{-1}$ sweeps plot$^{-1}$, respectively and were on par.

At 14 DAT also, the trend in hymenopteran parasitoid population was similar to that in the previous observations. *M. anisopliae* (Ma 4) @ $10^{10}$ spores

$ml^{-1}$ treated and untreated plot had the highest mean population of 4.33 $ten^{-1}$ sweeps $plot^{-1}$ each and it was statistically on par with other fungal treatments *viz.*, talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (4.00), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (3.00), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (3.33), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$(3.33) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (3.33). Plots treated Chlorantraniliprole @ 30 g a.i $ha^{-1}$ also had higher mean population of 4.00 $ten^{-1}$ sweeps $plot^{-1}$ and was on par with all the fungal treated and untreated plots as observed earlier. Acephate @ 750 g a.i $ha^{-1}$(1.67), thiamethoxam @ 25 g a.i $ha^{-1}$(1.33) and malathion @ 575 g a.i $ha^{-1}$ (1.33) were on par with talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$, talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ and *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$.

The prolonged safety of the fungal pathogens was evident from the observations at 21 DAT. *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ treated plots contained highest mean population of 4.33 $ten^{-1}$ sweeps $plot^{-1}$ and was statistically on par with all other treatments except acephate @ 750 g a.i $ha^{-1}$ (1.33), thiamethoxam @ 25 g a.i $ha^{-1}$ (1.67) and malathion @ 575 g a.i $ha^{-1}$ (1.33). The mean population count in *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (3.67), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (4.00), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$(3.67), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (3.67), chlorantraniliprole @ 30 g a.i $ha^{-1}$ (4.00) were statistically on par.



***4.4.1.3.2 Predators***

***4.4.1.3.2.1 Insects***

The population of insect predators assessed in terms of the number of insect predators present in ten sweeps $plot^{-1}$ are presented in Table 25.

Pretreatment observations showed no significant difference in the mean population of insect predators in various plots. On four DAT, *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (14.33), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$(14.00), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$(13.33), talc based *B. bassiana* (Bb 5) @ 20

Table 24. Effect of treatments on the population of hymenopteran parasitoids (Field trial-I)

| Treatments | Mean number of parasitoids* $10^{-1}$ sweeps $plot^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 2.67 (1.87) | 3.00 (1.99) | 3.33 (2.07) | 3.00 (1.99) | 3.67 (2.14) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 1.33 (1.47) | 3.00 (1.99) | 3.00 (1.99) | 3.33 (2.07) | 4.00 (2.23) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 3.33 (2.07) | 3.67 (2.14) | 3.67 (2.14) | 4.00 (2.23) | 3.67 (2.14) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ | 2.33 (1.82) | 3.67 (2.14) | 3.67 (2.14) | 3.33 (2.07) | 3.67 (2.14) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 1.67 (1.63) | 2.67 (1.90) | 3.67 (2.14) | 4.33 (2.28) | 4.33 (2.28) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 2.67 (1.90) | 3.67 (2.14) | 3.67 (2.14) | 3.33 (2.07) | 4.33 (2.28) |
| Acephate @ 750 g a.i $ha^{-1}$ | 3.00 (1.99) | 0.67 (1.28) | 1.00 (1.38) | 1.67 (1.58) | 1.33 (1.49) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 2.33 (1.82) | 3.00 (1.99) | 3.67 (2.14) | 4.00 (2.23) | 4.00 (2.23) |
| Malathion @ 575 g a.i $ha^{-1}$ | 1.67 (1.58) | 0.33 (1.14) | 0.33 (1.14) | 1.33 (1.49) | 1.33 (1.49) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 1.67 (1.58) | 1.00 (1.41) | 1.33 (1.49) | 1.33 (1.49) | 1.67 (1.58) |
| Untreated | 3.33 (2.07) | 3.67 (2.14) | 3.67 (2.14) | 4.33 (2.30) | 4.33 (2.30) |
| CD (0.05) | NS | (0.382) | (0.547) | (0.600) | (0.551) |

*- Braconids, Chalcids, Ichneumonids.
Mean of three replications
Figures in parentheses are $\sqrt{x+1}$ transformed values.
DAT- Days after treatment
NS- Non significant

g $l^{-1}$ (13.00), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$(12.67) was on par with that in the untreated plots (15.00). Among the insecticides evaluated, chlorantraniliprole @ 30 g a.i. $ha^{-1}$ (12.33) was on par with all fungal treatments and untreated plots. Thiamethoxam @ 25 g a.i $ha^{-1}$, acephate @ 750 g a.i $ha^{-1}$ and malathion @ 575 g a.i $ha^{-1}$ had significantly lower mean population of 1.33, 1.33 and 1.67 $ten^{-1}$ sweeps $plot^{-1}$, respectively and were on par.

The highest mean population of 13.67 $ten^{-1}$ sweeps $plot^{-1}$ observed in *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ was statistically on par with *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$(13.33), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (13.33), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (13.00), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (13.00), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (12.33) and untreated plot (14.67) on seven DAT. As in the previous observations, chlorantraniliprole @ 30 g a.i $ha^{-1}$ treated plots had statistically similar population (12.67) with fungal and untreated plots, whereas acephate, malathion @ 575 g a.i $ha^{-1}$, acephate @ 750 g a.i $ha^{-1}$ and thiamethoxam @ 25 g a.i $ha^{-1}$ showed significantly lower mean population of 2.67, 3.33 and 5.67 $ten^{-1}$ sweeps $plot^{-1}$, respectively and were on par.

At 14 DAT, plots treated with talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ treated and untreated plot recorded the highest mean population of 14.33 $ten^{-1}$ sweeps $plot^{-1}$ however it was statistically on par with other fungal treatments *viz.*, *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$(13.67), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$(13.67), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (13.67), talc based *B. bassiana* (Bb 5 @ 20 g $l^{-1}$ (13.33), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (12.67). The insecticide chlorantraniliprole @ 30 g a.i $ha^{-1}$ treated plots also had a higher mean population of 12.67 $ten^{-1}$ sweeps $plot^{-1}$ and were on par with the population of insect predators in the fungal treatments and untreated plot. Acephate @ 750 g a.i $ha^{-1}$(5.33), malathion @ 575 g a.i $ha^{-1}$ (5.67) and thiamethoxam @ 25 g a.i $ha^{-1}$ (6.00) were statistically on par with each other.

Talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ recorded the highest mean population of 14.67 $ten^{-1}$ sweeps $plot^{-1}$ and was on par with all other treatments except acephate @ 750 g a.i $ha^{-1}$ (6.33), thiamethoxam @ 25 g a.i $ha^{-1}$ (7.33) and malathion @ 575 g a.i $ha^{-1}$ (6.67) at 21 DAT. The mean population count in *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (14.33), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (14.33), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$(14.00), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (13.67), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$(13.67) and chlorantraniliprole @ 30 g a.i $ha^{-1}$ (13.33) were on par.

*4.4.1.3.2.2 Spiders*

The population of spider predators assessed in terms of the number of spider predators present in ten sweeps $plot^{-1}$ are presented in Table 26.

There was no significant difference in the pretreatment observations on the population of spiders. On four DAT, among the treatments, *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ treated plots had the highest mean population of 15.67 $ten^{-1}$ sweeps $plot^{-1}$, and was statistically on par with all fungal treated and untreated plots (16.00). The mean spider population in plots treated with talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$, talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$, talc based *B bassiana* (Bb 5) @ 20 g $l^{-1}$, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ were 14.67, 14.33, 13.33, 13.33 and 11.67, respectively and were on par. Among the insecticide treated plots, chlorantraniliprole 30 g a.i $ha^{-1}$ recorded mean population of 13.33 $ten^{-1}$ sweeps $plot^{-1}$ and was statistically on par with untreated and fungal treated plots. Other chemical treated plots *viz.*, malathion @ 575 g a.i $ha^{-1}$, acephate 750 g a.i $ha^{-1}$ and thiamethoxam 25 g a.i $ha^{-1}$ recorded significantly lower mean population of 5.33, 4.33 and 3.67 $ten^{-1}$ sweeps $plot^{-1}$, respectively.

At seven DAT, among the fungal treated plots, talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ had the highest mean population of 14.00 $ten^{-1}$ sweeps $plot^{-1}$, which was statistically on par with *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (12.67), talc

Table 25. Effect of treatments on the population of insect predators (Field trial-I)

| Treatments | Mean number of insect predators* $10^{-1}$ sweeps $plot^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 14.00 (3.86) | 13.00 (3.74) | 12.33 (3.64) | 13.33 (3.79) | 14.33 (3.92) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 14.00 (3.86) | 12.00 (3.59) | 13.33 (3.79) | 12.67 (3.69) | 14.67 (3.96) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 13.00 (3.74) | 13.33 (3.79) | 13.00 (3.74) | 14.33 (3.91) | 13.67 (3.82) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ | 13.33 (3.79) | 14.00 (3.86) | 13.33 (3.79) | 13.67 (3.81) | 14.33 (3.91) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 12.67 (3.69) | 14.33 (3.91) | 13.67 (3.81) | 13.67 (3.81) | 14.00 (3.86) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 12.33 (3.65) | 12.67 (3.69) | 13.00 (3.74) | 13.67 (3.81) | 13.67 (3.81) |
| Acephate @ 750 g a.i $ha^{-1}$ | 13.00 (3.74) | 1.33 (1.49) | 2.67 (1.91) | 5.33 (2.49) | 6.33 (2.70) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 13.00 (3.74) | 12.33 (3.65) | 12.67 (3.69) | 12.67 (3.69) | 13.33 (3.79) |
| Malathion @ 575 g a.i $ha^{-1}$ | 12.00 (3.59) | 1.67 (1.61) | 3.33 (2.03) | 5.67 (2.58) | 6.67 (2.77) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 13.33 (3.79) | 1.33 (1.49) | 5.67 (2.58) | 6.00 (2.63) | 7.33 (2.87) |
| Untreated | 14.00 (3.86) | 15.00 (3.99) | 14.67 (3.94) | 14.33 (3.91) | 14.67 (3.94) |
| CD (0.05) | NS | (0.538) | (0.457) | (0.451) | (0.402) |

*- *Coccinella* sp. *Micraspis* sp., Reduvid bug, Damsel flies, Dragon flies.
Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

based *B bassiana* (Bb 5) @ 20 g $l^{-1}$(12.67), *A. flavus* (Af-m1) @ $10^{10}$spores $ml^{-1}$ (12.33), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (11.67), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (11.00), chlorantraniliprole 30 g a.i $ha^{-1}$(11.67) and untreated plots (15.00). The chemicals, malathion @ 575 g a.i $ha^{-1}$, acephate 750 g a.i $ha^{-1}$ and thiamethoxam 25 g a.i $ha^{-1}$ had significantly lower population than fungal treatments, the values being 6.67, 7.33 and 8.67 $ten^{-1}$ sweeps $plot^{-1}$, respectively.

The mean population of spiders was the highest in the talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ treated plots at 14 DAT, which was statistically on par with the population present in all the other plots except those treated with acephate 750 g a.i $ha^{-1}$and thiamethoxam 25 g a.i $ha^{-1}$. In plots treated with talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$, *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$, talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$, *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ and *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$, the mean population recorded was 12.67, 12.33, 11.67, 11.33 and 10.67, respectively. The plots treated with chlorantraniliprole 30 g a.i $ha^{-1}$ and malathion @ 575 g a.i $ha^{-1}$ had mean population of 11.33 and 10.00 $ten^{-1}$ sweeps $plot^{-1}$, respectively while thiamethoxam 25 g a.i $ha^{-1}$ and acephate 750 g a.i $ha^{-1}$ recorded only 8.00 and 7.33 $ten^{-1}$ sweeps $plot^{-1}$, respectively.

At 21 DAT, talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ applied plots had the highest mean population of 14.00 $ten^{-1}$ sweeps $plot^{-1}$ and was on par with that in *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (13.67), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (13.33), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (13.00), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (13.00), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$(11.67) chlorantraniliprole 30 g a.i $ha^{-1}$ (12.67) treated and untreated plots (14.00). Significantly lower mean population of 9.67, 8.67 and 8.67 $ten^{-1}$ sweeps $plot^{-1}$, was recorded in malathion 575 g a.i $ha^{-1}$, acephate 750 g a.i $ha^{-1}$ and thiamethoxam 25 g a.i $ha^{-1}$ treated plots, respectively.

Table 26. Effect of treatments on the population of spiders (Field trial-I)

| Treatments | Mean number of spiders* $10^{-1}$ sweeps $plot^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 17.00 (4.12) | 13.33 (3.65) | 12.67 (3.54) | 11.67 (3.42) | 13.33 (3.65) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 15.33 (3.88) | 14.67 (3.81) | 14.00 (3.74) | 12.67 (3.54) | 14.00 (3.74) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 15.67 (3.96) | 14.33 (3.78) | 11.67 (3.41) | 12.67 (3.54) | 13.00 (3.59) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ | 15.67 (3.96) | 13.33 (3.65) | 11.00 (3.31) | 10.67 (3.26) | 13.00 (3.59) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 17.00 (4.12) | 15.67 (3.95) | 12.67 (3.55) | 12.33 (3.51) | 13.67 (3.69) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 16.33 (4.04) | 11.67 (3.42) | 12.33 (3.51) | 11.33 (3.63) | 11.67 (3.41) |
| Acephate @ 750 g a.i $ha^{-1}$ | 15.67 (3.95) | 4.33 (2.08) | 7.33 (2.71) | 7.33 (2.71) | 8.67 (2.94) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 15.67 (3.96) | 13.33 (3.65) | 11.67 (3.42) | 11.33 (3.63) | 12.67 (3.55) |
| Malathion @ 575 g a.i $ha^{-1}$ | 15.00 (3.86) | 5.33 (2.31) | 6.67 (2.57) | 10.00 (3.15) | 9.67 (3.09) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 15.33 (3.91) | 3.67 (1.90) | 8.67 (2.94) | 8.00 (2.83) | 8.67 (2.94) |
| Untreated | 18.67 (4.32) | 16.00 (3.98) | 15.00 (3.86) | 13.67 (3.69) | 14.00 (3.74) |
| CD (0.05) | NS | (0.551) | (0.350) | (0.414) | (0.390) |

*- *Tetragnatha* sp., *Argiope* sp. *Oxyopus* sp.

Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

*4.4.1.4 Yield*

*4.4.1.4.1 Grain*

The data on the yield from the different treatments and untreated are presented in Table 27. Among the different fungal treated plots, the highest mean grain yield was obtained from *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (3.60 kg) and was on par with the yield from the plots treated with *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (3.40 kg), acephate @ 750 g a.i $ha^{-1}$ (3.77 kg), malathion @ 575 g a.i $ha^{-1}$ (3.70 kg) and thiamethoxam 25 g a.i $ha^{-1}$ (3.53 kg). Talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ and talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ treated plots recorded mean grain yield of 3.13 and 3.00 kg $plot^{-1}$, respectively and was statistically on par. Talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$treated plots showed statistical similarity with mean grain yield of 2.63 and 2.50 kg $plot^{-1}$, respectively. Yield from the plot treated with the insecticide, chlorantraniliprole @ 30 g a.i $ha^{-1}$ was the highest (4.07 kg) and was statistically superior to other treatments. The mean yield in the untreated plot (2.07 kg) was significantly lower than that recorded from all the treated plots.

*4.4.1.4.2 Straw*

There was significant difference between the treatments and untreated, in the yield of straw and it ranged from 3.15 to 6.00 kg $plot^{-1}$. Among the fungal treatments, the highest straw yield was recorded in *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (5.27 kg) and it was on par with *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (5.16 kg), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (5.10 kg), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (4.97 kg) and malathion @ 575 g a.i $ha^{-1}$ (5.00 kg). The yield from plots treated with chlorantraniliprole @ 30 g a.i $ha^{-1}$ that had a mean straw yield of (5.95 kg) was statistically on par with acephate @ 750 g a.i $ha^{-1}$ (5.57 kg) and thiamethoxam 25 g a.i.$ha^{-1}$ (5.75 kg) treated plots. Talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ treated plots showed statistically similar mean straw yield of 4.10 kg $plot^{-1}$ each. The

mean yield in the untreated plot, 3.15 kg plot$^{-1}$ was significantly lower than all the treated plots.

#### *4.4.1.5 Benefit-Cost Ratio (BCR)*

The highest BCR of 1.65 was recorded for the treatment with *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$, and it was followed by the treatments with chlorantraniliprole @ 30 g a.i ha$^{-1}$ (1.58), *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ (1.55), acephate @ 750 g a.i. ha$^{-1}$ (1.48), malathion @ 575 g ai ha$^{-1}$ (1.46), talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (1.44), thiamethoxam 25 g a.i.ha$^{-1}$ (1.39), talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ (1.38), talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (1.20) and *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ (1.12).

### 4.4.2 Second Field Trial

#### *4.4.2.1 C. medinalis*

#### *4.4.2.1.1 Population*

#### *4.4.2.1.1.1 Larval Population*

The pretreatment population of larvae of *C. medinalis* was non significant (Table 28). In the initial observation on the fourth DAT, all fungal treated plots, except *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ (10.67) was on par with untreated plot (20.67). The population in treatments being 16.67, 15.33, 14.67, 13.33 and 12.67 in talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$, talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$, talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$, *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ and *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$, respectively. The population in plots treated with *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ (10.67) and *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ (12.67) were on par with that in malathion @ 575 g ai ha$^{-1}$(6.67). In all the insecticide treated plots, excepting malathion @ 575 g a.i ha$^{-1}$, the population was significantly lower than that in the fungal treated plots. Among the insecticide treated plots, chlorantraniliprole @ 30

Table 27. Grain and straw yield from the different treatments and benefit-cost ratio (BCR) (Field trial-I)

| Treatments | Grain yield (kg plot$^{-1}$) | Straw yield (kg plot$^{-1}$) | BCR |
|---|---|---|---|
| Talc based formulation of *B. bassiana* (Bb 5) @ 20g l$^{-1}$ | 3.13 | 5.10 | 1.44 |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ | 2.63 | 4.10 | 1.20 |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20g l$^{-1}$ | 3.00 | 4.97 | 1.38 |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ | 3.60 | 5.27 | 1.65 |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ | 3.40 | 5.16 | 1.55 |
| *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ | 2.50 | 4.10 | 1.12 |
| Acephate @ 750 g a.i ha$^{-1}$ | 3.77 | 6.00 | 1.48 |
| Chlorantraniliprole @ 30 g a.i ha$^{-1}$ | 4.07 | 5.94 | 1.58 |
| Malathion @ 575 g a.i ha$^{-1}$ | 3.70 | 5.00 | 1.46 |
| Thiamethoxam @ 25 g a.i ha$^{-1}$ | 3.53 | 5.75 | 1.39 |
| Untreated | 2.07 | 3.15 | - |
| CD (0.05) | (0.266) | (0.378) | - |

g a.i $ha^{-1}$ that recorded the lowest mean population of 3.33, was on par with acephate @ 750 g a.i $ha^{-1}$ (4.00), thiamethoxam @ 25 g a.i $ha^{-1}$(4.00) and malathion @ 575 g a.i $ha^{-1}$ (6.67).

On seven DAT, there was significant difference in the mean population of *C. medinalis* larvae, between treated and untreated plots (19.00). As noted in the first field trial, the performance of *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ was the best among the entomopathogens. The mean population of *C. medinalis* larvae recorded in this treatment was 7.00 and it was on par with that in thiamethoxam @ 25 g a.i $ha^{-1}$ (8.00), malathion @ 575 g a.i $ha^{-1}$ (6.00) and acephate @ 750 g a.i $ha^{-1}$ (5.00). Talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (9.00), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (11.00), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (11.00), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (11.33) and*A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (11.67) were statistically on par. The effect of the chemicals also followed the same trend as in the first field trial, with chlorantraniliprole @ 30 g a.i $ha^{-1}$ having the lowest mean population of 3.33 $ten^{-1}$ hills $plot^{-1}$.

The trend in larval population observed at 14 DAT was similar to that noted in the previous observation. The population recorded were 8.00, 9.33, 9.67, 5.67, 7.00, 7.67 and 9.00 in plots treated with *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$, *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$, talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$, chlorantraniliprole @ 30 g a.i $ha^{-1}$, thiamethoxam @ 25 g a.i $ha^{-1}$, acephate @ 750 g a.i $ha^{-1}$and malathion @ 575 g a.i $ha^{-1}$, respectively and were on par. The effect of talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (10.33) was on par with *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (11.00) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (15.33).

Significant difference was observed between treated and untreated (23.00) plots in the mean larval population recorded at 21 DAT also and the trend was comparable to that of the previous observations. *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ and talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ treated plots recorded the lowest mean population of 10.33 $ten^{-1}$ hills $plot^{-1}$ each, and was statistically on par

with plots treated with *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (11.00), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (11.33), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (11.67), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (15.67), chlorantraniliprole @ 30 g a.i $ha^{-1}$ (8.00), acephate @ 750 g a.i $ha^{-1}$ (8.67), thiamethoxam @ 25 g a.i $ha^{-1}$ (9.00) and malathion @ 575 g a.i $ha^{-1}$ (11.33). All fungal treatments, except *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$, was on par with chlorantraniliprole @ 30 g a.i $ha^{-1}$, acephate @ 750 g a.i $ha^{-1}$ and thiamethoxam @ 25 g a.i $ha^{-1}$.

***4.4.2.1.1.2 Adult Population***

Pretreatment observation on the population of adult *C. medinalis* in the different plots did not show any significant variation (Table 29). On four DAT, there was significant difference between the treatments and untreated plots (15.00) except that in talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (13.67) and talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (12.00). *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ recorded the lowest mean population of 10.33 and was statistically on par with talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (11.33), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$(11.67) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (11.67). Immediate effect of the insecticides was evident from the significantly lower population, with chlorantraniliprole @ 30 g a.i $ha^{-1}$ recording the lowest mean population of 3.00 $ten^{-1}$ sweeps $plot^{-1}$, that was closely followed by malathion @ 575 g a.i $ha^{-1}$ (4.67), acephate @ 750 g a.i $ha^{-1}$ (5.33) and thiamethoxam @ 25 g a.i $ha^{-1}$ (6.00).

The effect of treatments continued in the observations taken at seven DAT also. Among the fungal treatments, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ treated plots recorded the lowest mean population of 7.33 $ten^{-1}$ sweeps $plot^{-1}$ which was statistically on par with that in acephate @ 750 g a.i $ha^{-1}$ (5.67) and malathion @ 575 g a.i $ha^{-1}$ (6.33). Talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (10.67), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$(11.00), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (11.67), talc based (Bb 21) @ 20 g $l^{-1}$(12.33) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (12.67) were statistically on par. Chlorantraniliprole @ 30 g a.i $ha^{-1}$ and thiamethoxam @ 25 g a.i $ha^{-1}$ showed the lowest mean population of 4.67

Table 28. Effect of treatments on the population of *C. medinalis* larvae (Field trial-II)

| Treatments | Mean number of larva $10^{-1}$ hills $plot^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 15.33 (3.91) | 15.33 (3.91) | 9.00 (2.99) | 10.33 (3.21) | 11.67 (3.41) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 15.00 (3.87) | 16.67 (4.04) | 11.00 (3.31) | 9.67 (3.00) | 11.33 (3.32) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 15.33 (3.91) | 14.67 (4.23) | 11.33 (3.36) | 9.33 (3.01) | 10.33 (3.19) |
| *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$ | 15.00 (3.87) | 12.67 (3.54) | 7.00 (2.64) | 8.00 (2.83) | 10.33 (3.21) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 15.67 (3.96) | 10.67 (3.14) | 11.00 (3.29) | 11.00 (3.29) | 11.00 (3.29) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 16.00 (3.99) | 13.33 (3.64) | 11.67 (3.37) | 15.33 (3.91) | 15.67 (3.96) |
| Acephate @ 750 g a.i $ha^{-1}$ | 15.00 (3.87) | 4.00 (1.89) | 5.00 (2.23) | 7.67 (2.76) | 8.67 (2.88) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 16.33 (4.04) | 3.33 (1.76) | 3.33 (1.79) | 5.67 (2.37) | 8.00 (2.81) |
| Malathion @ 575 g a.i $ha^{-1}$ | 17.00 (4.12) | 6.67 (2.55) | 6.00 (2.43) | 9.00 (3.00) | 11.33 (3.37) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 16.00 (3.99) | 4.00 (1.95) | 8.00 (2.83) | 7.00 (2.61) | 9.00 (2.99) |
| Untreated | 17.00 (4.12) | 20.67 (4.52) | 19.00 (4.36) | 24.67 (4.96) | 23.00 (4.78) |
| CD (0.05) | NS | (1.080) | (0.624) | (0.805) | (0.761) |

Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

ten$^{-1}$ sweeps plot$^{-1}$ each, and was significantly lower than that in all the fungal treatments.

The trend in the adult population was similar at 14 DAT too, with the lowest mean population of 8.00 ten$^{-1}$ sweeps plot$^{-1}$ in *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ treated plots, which was statistically similar to talc based *B. bassiana* (Bb 5) @ 20g l$^{-1}$ (9.00), thiamethoxam @ 25 g a.i ha$^{-1}$ (6.00), chlorantraniliprole @ 30 g a.i ha$^{-1}$ (6.00) and malathion @ 575 g a.i ha$^{-1}$ (7.00). Talc based *B. bassiana* (Bb 5) @ 20g l$^{-1}$ (9.00) was statistically on par with all fungal treatments and malathion @ 575 g a.i ha$^{-1}$. Talc based *M. anisopliae* (Ma 4) @ 20g l$^{-1}$ (9.67), talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (10.00), *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$(10.00) and *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ (10.00) were statistically on par. Among the insecticides, acephate @ 750 g a.i ha$^{-1}$ showed the lowest mean population of 5.67 ten$^{-1}$ sweeps plot$^{-1}$ and it was on par with other chemicals too.

At 21 DAT also, plots that received various treatments harboured significantly lower population of *C. medinalis* adult than that in the untreated plots (16.67). The lowest mean population ten$^{-1}$ sweeps plot$^{-1}$ was observed in talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (8.33) which was on par with talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ (10.00), *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ (10.00), *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ (10.33), talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (10.67), *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ (10.67), acephate @ 750 g a.i ha$^{-1}$ (7.33), thiamethoxam @ 25 g a.i ha$^{-1}$ (7.67), chlorantraniliprole @ 30 g a.i ha$^{-1}$ (8.33) and malathion @ 575 g a.i ha$^{-1}$ (9.33). All the fungal treatments except talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ and *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ were on par with acephate @ 750 g a.i ha$^{-1}$.

#### *4.4.2.1.2 Extent of Damage*

Extent of damage by *C. medinalis* in the pretreatment observations did not vary significantly in all the treatments (Table 30). At four DAT, the mean

Table 29. Effect of treatments on the population of *C. medinalis* adult (Field trial-II)

| Treatments | Mean number of adult $10^{-1}$ sweeps $plot^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 13.00 (3.59) | 12.00 (3.46) | 11.00 (3.31) | 9.00 (2.99) | 8.33 (2.85) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 12.33 (3.51) | 11.33 (3.36) | 12.33 (3.51) | 10.00 (3.15) | 10.67 (3.25) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 13.67 (3.69) | 13.67 (3.69) | 10.67 (3.26) | 9.67 (3.10) | 10.00 (3.16) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ | 14.00 (3.73) | 11.67 (3.41) | 7.33 (2.69) | 8.00 (2.83) | 10.00 (3.15) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 13.33 (3.64) | 10.33 (3.21) | 11.67 (3.41) | 10.00 (3.16) | 10.33 (3.19) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 14.00 (3.74) | 11.67 (3.42) | 12.67 (3.56) | 10.00 (3.16) | 10.67 (3.27) |
| Acephate @ 750 g a.i $ha^{-1}$ | 15.67 (3.95) | 5.33 (2.29) | 5.67 (2.37) | 5.67 (2.37) | 7.33 (2.70) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 15.00 (3.86) | 3.00 (1.72) | 4.67 (2.14) | 6.00 (2.44) | 8.33 (2.88) |
| Malathion @ 575 g a.i $ha^{-1}$ | 12.67 (3.55) | 4.67 (2.14) | 6.33 (2.51) | 7.00 (2.64) | 9.33 (3.05) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 14.00 (3.74) | 6.00 (2.44) | 4.67 (2.14) | 6.00 (2.44) | 7.67 (2.76) |
| Untreated | 13.00 (3.59) | 15.00 (3.87) | 16.33 (4.04) | 16.33 (4.04) | 16.67 (4.07) |
| CD (0.05) | NS | (0.428) | (0.494) | (0.393) | (0.498) |

Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

percentage leaf damage in all treatments was significantly lower than that in the untreated plots (6.33). The lowest damage of 4.07 per cent observed in *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ was on par with that in *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (4.27) and talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (4.53). This was followed by the damage recorded in talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (5.07) , talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (5.20) and *A. flavus* (Af-m1) @ $10^{10}$spores $ml^{-1}$ (5.33). The insecticide treated plots had significantly lower damage than fungal treated plots, with chlorantraniliprole @ 30 g a.i $ha^{-1}$ recording the lowest mean damage of 1.20 per cent. All the other insecticides treatments, acephate @ 750 g a.i $ha^{-1}$ (1.87), malathion @ 575 g a.i $ha^{-1}$ (1.87) and thiamethoxam 25 g a.i $ha^{-1}$ (1.67) were on par.

Significant superiority of the treatments over untreated (9.87) was evident at seven DAT also. The treatment with *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ that recorded the lowest mean damage of 2.80 per cent was on par with thiamethoxam @ 25 g a.i $ha^{-1}$ (2.73). *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ and talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ treated plots recorded 3.73 per cent mean leaf damage each and was on par with malathion 575 g a.i $ha^{-1}$ (3.47) treated plots. For the other treatments the order of effectiveness was, talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$, talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ with the mean values of 4.33, 4.33 and 4.67, respectively. The lowest damage was in acephate @ 750 g a.i $ha^{-1}$ treated plots (2.13) and was statistically on par with chlorantraniliprole @ 30 g a.i $ha^{-1}$ treated plots (2.27).

The effect of treatments was similar at 14 DAT also, with significant difference between treated and untreated plots (9.47). *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ that recorded the lowest leaf damage of 2.47 per cent was followed by acephate @ 750 g a.i $ha^{-1}$ (2.53) and chlorantraniliprole @ 30 g a.i $ha^{-1}$ (2.87). Talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$and talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ with mean values of 3.47 and 3.67 per cent, respectively were on par with thiamethoxam @ 25 g a.i $ha^{-1}$ (3.20). The effect of talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (3.67) and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (4.20) came on par

with that of malathion @ 575 g a.i $ha^{-1}$ (3.87). Talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ had statistical similarity, each having 4.60 per cent leaf damage. Among the insecticides, acephate @ 750 g a.i $ha^{-1}$ recorded the lowest leaf damage of 2.53 per cent and was on par with chlorantraniliprole @ 30 g a.i $ha^{-1}$.

As in the earlier three observations, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ treated plots recorded the lowest leaf damage (2.93) at 21 DAT. However, this treatment was on par with acephate @ 750 g a.i $ha^{-1}$ (2.93), chlorantraniliprole @ 30 g a.i $ha^{-1}$ (3.27) and thiamethoxam @ 25 g a.i $ha^{-1}$ (3.33). Both talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (3.60) and *B. bassiana* (Bb 21) @ 20 g $l^{-1}$(3.73) were on par with chlorantraniliprole @ 30 g a.i $ha^{-1}$ and thiamethoxam @ 25 g a.i $ha^{-1}$. Talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$, *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ recorded leaf damage of 4.27, 4.20 and 4.60 per cent, respectively and was on par with the effect of malathion @ 575 g a.i $ha^{-1}$ (4.20).

### *4.4.2.2 L. acuta*

#### *4.4.2.2.1 Population*

The pretreatment variation in the population of *L. acuta* in the treated and the untreated plots was not significant (Table 31). At four DAT, the fungal treated and untreated plots (44.00) did not show any significant difference. However, the immediate effect of the insecticides was clear from the significantly lower population of *L. acuta* recorded from this plots. The lowest mean population recorded in chlorantraniliprole @ 30 g a.i $ha^{-1}$ (4.67) was on par with thiamethoxam @ 25 g a.i $ha^{-1}$ (6.00), acephate @ 750 g a.i $ha^{-1}$ (6.33) and malathion 50 @ 575 g a.i $ha^{-1}$(7.67).

Significant difference in the mean population of *L. acuta* between treated and untreated plots (38.67) was observed on seven DAT also.. Among the fungal treated plots, *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ contained the lowest mean

Table 30. Effect of treatments on the extent of damage by *C. medinalis* (Field trial-II)

| Treatments | Mean damaged leaves $10^{-1}$ hills plot$^{-1}$ (%) | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ | 8.77 (2.96) | 4.53 (2.13) | 4.33 (2.08) | 3.67 (1.91) | 3.60 (1.89) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ | 8.93 (2.99) | 5.20 (2.28) | 3.73 (1.93) | 3.47 (1.86) | 3.73 (1.93) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ | 7.93 (2.97) | 5.07 (2.24) | 4.33 (2.08) | 4.60 (2.14) | 4.27 (2.06) |
| *B. bassiana* (Bb 5) @$10^{10}$ spores ml$^{-1}$ | 9.73 (3.12) | 4.07 (2.02) | 2.80 (1.67) | 2.47 (1.57) | 2.93 (1.71) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ | 8.87 (2.98) | 4.27 (2.06) | 3.73 (1.93) | 4.20 (2.05) | 4.20 (2.05) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ | 8.87 (2.97) | 5.33 (2.31) | 4.67 (2.16) | 4.60 (2.14) | 4.60 (2.14) |
| Acephate @ 750 g a.i ha$^{-1}$ | 9.33 (3.05) | 1.87 (1.37) | 2.13 (1.46) | 2.53 (1.59) | 2.93 (1.71) |
| Chlorantraniliprole @ 30 g a.i ha$^{-1}$ | 9.13 (3.02) | 1.20 (1.09) | 2.27 (1.50) | 2.87 (1.69) | 3.27 (1.81) |
| Malathion @ 575 g a.i ha$^{-1}$ | 8.87 (2.98) | 1.87 (1.37) | 3.47 (1.86) | 3.87 (1.97) | 4.20 (2.05) |
| Thiamethoxam @ 25 g a.i ha$^{-1}$ | 8.87 (2.97) | 1.67 (1.29) | 2.73 (1.65) | 3.20 (1.79) | 3.33 (1.82) |
| Untreated | 10.13 (3.18) | 6.33 (2.52) | 9.87 (3.14) | 9.47 (3.08) | 8.73 (2.95) |
| CD (0.05) | NS | (0.170) | (0.136) | (0.145) | (0.140) |

Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

population of 24.67 ten$^{-1}$ hills and ten$^{-1}$ sweeps plot$^{-1}$, which was on par with that in the talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ (25.00), *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ (26.33) and talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (29.00). The mean population of 30.67 and 32.33 in *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ and talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ treated plots were on par. The insecticide, chlorantraniliprole @ 30 g a.i ha$^{-1}$ recorded significantly lower mean population of 6.33 ten$^{-1}$ hills and ten$^{-1}$ sweeps plot$^{-1}$ and was followed by thiamethoxam @ 25 g a.i ha$^{-1}$ (9.00), acephate @ 750 g a.i ha$^{-1}$ (9.67) and malathion @ 575 g a.i ha$^{-1}$ (11.33), which showed superiority over fungal treatments in the initial observations.

The trend of significant population reduction in all the treatments when compared to untreated plots (37.67) was evident at 14 DAT too. Among the entomopathogenic fungi, *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ indicated the least population of 18.67 ten$^{-1}$ hills and ten$^{-1}$ sweeps plot$^{-1}$ and it was on par with malathion @ 575 g a.i ha$^{-1}$ (16.00). The population of *L. acuta* in plots treated with *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ (22.67), *M. anisopliae* (Ma 4) in talc @ 20 g l$^{-1}$ (22.67), *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ (23.00), talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (25.67), talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (25.67) were similar. The lowest mean population of 11.00 ten$^{-1}$ hills and ten$^{-1}$ sweeps plot$^{-1}$ recorded in chlorantraniliprole @ 30 g a.i ha$^{-1}$ treated plots was significantly lower than that in the other plots. Acephate @ 750 g a.i ha$^{-1}$ and thiamethoxam @ 25 g a.i ha$^{-1}$ treated plots had mean population of 13.33 and 15.33 ten$^{-1}$ hills and ten$^{-1}$ sweeps plot$^{-1}$, respectively and were statistically on par.

At 21 DAT, the trend was similar to that in the earlier observations, with significant difference in the mean population in treated and untreated plots (35.67). Among the fungal treatments, *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ recorded the lowest mean population of 16.33 ten$^{-1}$ hills and ten$^{-1}$ sweeps plot$^{-1}$ and was on par with acephate @ 750 g a.i ha$^{-1}$ (14.33), thiamethoxam @ 25 g a.i ha$^{-1}$ (14.67) and malathion @ 575 g a.i ha$^{-1}$ (15.67). Talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ (17.33) was on par with *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$

(18.33) and malathion @ 575 g a.i $ha^{-1}$. The effect of *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (23.67) was on par with both talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (24.00) and *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (24.33). The least mean population of 12.67 observed in chlorantraniliprole @ 30 g a.i $ha^{-1}$ carried and was on par with acephate @ 750 g a.i $ha^{-1}$ (14.33). The effectiveness of the other two insecticides, thiamethoxam 25 WG @ 25 g a.i $ha^{-1}$ (14.67) and malathion @ 575 g a.i $ha^{-1}$ (15.67) were on par.

#### *4.4.2.2.2 Extent of Damage*

Pretreatment observations on the grain damage by *L. acuta* in the different plots did not vary significantly (Table 32). At four DAT, all the treatments except talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (2.77) and *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (3.00) showed significantly lower grain damage than untreated plots (3.13). The extent of damage observed in plots treated with *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (2.13) and talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (2.27) were on par while *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (2.50) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (2.60) was similar. The insecticide treated plots recorded significantly lower grain damage than that in the fungal treatments. Acephate @ 750 g a.i $ha^{-1}$ treated plots recorded the lowest mean grain damage (0.20) which was on par with that in chlorantraniliprole @ 30 g a.i $ha^{-1}$ (0.23) and this was followed by thiamethoxam @ 25 g a.i $ha^{-1}$ (0.40) and malathion @ 575 g a.i $ha^{-1}$ (0.50).

All the treatments recorded significantly lower grain damage than that in the untreated plots (2.70) at seven DAT. *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ with the lowest grain damage (1.83) among the fungal treatments was on par with *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (1.90), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (1.97), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (2.10), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (2.13) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (2.13). The insecticide treated plots showed significantly lower grain damage than the fungi treated plots with the lowest grain damage in chlorantraniliprole @

Table 31. Effect of treatments on the population of *L.acuta* (Field trial-II)

| Treatments | Mean number of *L. acuta* (10 sweeps + 10 hills)$^{-1}$ plot$^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ | 32.33 (5.69) | 43.67 (6.61) | 32.33 (5.68) | 25.67 (5.07) | 24.00 (4.89) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ | 34.67 (5.89) | 42.33 (6.49) | 29.00 (5.38) | 25.67 (5.07) | 24.33 (4.93) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ | 32.67 (5.71) | 37.67 (6.12) | 25.00 (5.00) | 22.67 (4.76) | 17.33 (4.16) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ | 31.67 (5.63) | 37.33 (6.06) | 26.33 (5.13) | 23.00 (4.79) | 18.33 (4.28) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ | 34.33 (5.86) | 36.33 (6.00) | 24.67 (4.97) | 18.67 (4.31) | 16.33 (4.04) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ | 33.33 (5.77) | 37.33 (6.07) | 30.67 (5.53) | 22.67 (4.76) | 23.67 (4.87) |
| Acephate 75% SP @ 750 g a.i ha$^{-1}$ | 33.67 (5.80) | 6.33 (2.51) | 9.67 (3.11) | 13.33 (3.65) | 14.33 (3.79) |
| Chlorantraniliprole 18.5% SC @ 30 g a.i ha$^{-1}$ | 31.67 (5.62) | 4.67 (2.15) | 6.33 (2.49) | 11.00 (3.31) | 12.67 (3.56) |
| Malathion 50 EC @ 575 g a.i ha$^{-1}$ | 33.33 (5.77) | 7.67 (2.76) | 11.33 (3.35) | 16.00 (3.99) | 15.67 (3.95) |
| Thiamethoxam 25% WG @ 25 g a.i ha$^{-1}$ | 33.00 (5.74) | 6.00 (2.47) | 9.00 (2.99) | 15.33 (3.91) | 14.67 (3.83) |
| Untreated | 32.60 (5.71) | 44.00 (6.63) | 38.67 (6.23) | 37.67 (6.14) | 35.67 (5.97) |
| CD (0.05) | NS | (0.675) | (0.481) | (0.328) | (0.257) |

Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

30 g a.i $ha^{-1}$ (0.60) and it was followed by acephate @ 750 g a.i $ha^{-1}$ (0.67) and thiamethoxam @ 25 g a.i $ha^{-1}$ (0.70).

The trend was similar to earlier observations with significant difference in the treated and untreated plots (2.13) at 14 DAT also. *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ recorded lowest mean grain damage of 0.70 per cent and was on par with chlorantraniliprole @ 30 g a.i $ha^{-1}$ (0.70) and acephate @ 750 g a.i $ha^{-1}$ (0.80). The per cent grain damage in talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (1.10) was on par with *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (1.20), malathion @ 575 g ai $ha^{-1}$ (1.00) and thiamethoxam @ 25 g a.i $ha^{-1}$ (1.10). Talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$, *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$, and talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ recorded mean grain damage of 1.40, 1.40 and 1.47 per cent, respectively and were on par.

There was significant difference in the percentage of grain damaged between treated and untreated plots (1.87) at 21 DAT as in the earlier observations. The mean grain damage of 0.73 per cent observed in *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ was the lowest damage in the fungal treatments and was statistically on par with that noted in chlorantraniliprole @ 30 g a.i $ha^{-1}$ (0.70) and acephate @ 750 g a.i $ha^{-1}$ (0.90). Talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (1.00) and *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (1.13) were on par with acephate @ 750 g a.i $ha^{-1}$ (0.90), thiamethoxam @ 25 g a.i $ha^{-1}$ (1.00) and malathion @ 575 g a.i $ha^{-1}$ (1.03) in their effectiveness. The mean grain damage of 1.30, 1.30 and 1.47 per cent $ten^{-1}$ hills $plot^{-1}$ recorded in talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$, talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ were on par.

### *4.4.2.3 Natural Enemies*

#### *4.4.2.3.1 Parasitoids*

No significant difference in the pretreatment observations was seen with respect to the population of hymenopteran parasitoids (Table 33). At four DAT,

Table 32. Effect of treatments on the extent of damage by *L. acuta* (Field trial-II)

| Treatments | Mean damaged grains $10^{-1}$ hills $plot^{-1}$ (%) | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 4.07 (2.25) | 2.77 (1.94) | 2.10 (1.76) | 1.40 (1.55) | 1.30 (1.52) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 4.33 (2.31) | 3.00 (1.99) | 2.13 (1.77) | 1.47 (1.57) | 1.30 (1.52) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 4.13 (2.26) | 2.27 (1.77) | 1.97 (1.72) | 1.10 (1.45) | 1.00 (1.41) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ | 3.33 (2.08) | 2.50 (1.87) | 1.90 (1.70) | 1.20 (1.48) | 1.13 (1.46) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 4.03 (2.24) | 2.13 (1.81) | 1.83 (1.68) | 0.70 (1.30) | 0.73 (1.32) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 4.10 (2.26) | 2.60 (1.89) | 2.13 (1.77) | 1.40 (1.55) | 1.47 (1.57) |
| Acephate @ 750 g a.i $ha^{-1}$ | 4.57 (2.36) | 0.20 (1.09) | 0.67 (1.29) | 0.80 (1.34) | 0.90 (1.38) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 4.43 (2.33) | 0.23 (1.11) | 0.60 (1.26) | 0.70 (1.30) | 0.70 (1.30) |
| Malathion @ 575 g a.i $ha^{-1}$ | 3.23 (2.06) | 0.50 (1.22) | 1.07 (1.44) | 1.00 (1.41) | 1.03 (1.43) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 3.90 (2.21) | 0.40 (1.18) | 0.70 (1.30) | 1.10 (1.45) | 1.00 (1.41) |
| Untreated | 3.87 (2.21) | 3.13 (2.03) | 2.70 (1.92) | 2.13 (1.77) | 1.87 (1.69) |
| CD (0.05) | NS | (0.079) | (0.092) | (0.056) | (0.100) |

Mean of three replications
Figures in parentheses are $\sqrt{x+1}$ transformed values.
DAT- Days after treatment
NS- Non significant

the favourability of fungal treatments was evident from the observations. *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ that recorded the highest mean population (2.67) was on par with all other fungal treatments, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (2.33), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (2.00), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (2.33), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (2.33). It was also seen that the insecticide, chlorantraniliprole @ 30 g a.i $ha^{-1}$ equally supported the population of parasitoids, the value in this treatment being 2.33. The insecticides, thiamethoxam @ 25 g a.i $ha^{-1}$, acephate @ 750 g a.i $ha^{-1}$ and malathion @ 575 g a.i $ha^{-1}$ treated plots recorded significantly lower mean population of 0.67, 0.33 and 0.33 $ten^{-1}$ sweeps $plot^{-1}$, respectively and were on par.

On seven DAT, all the fungal treatments showed statistically similar mean population of parasitoids with that in the untreated plots (3.33). Talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (2.67), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (2.67 ), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (2.67), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (3.00), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (2.67) and *A. flavus* (Af-m1) @ $10^{10}$spores $ml^{-1}$ (2.67) were statistically on par. Among the insecticides, only chlorantraniliprole @ 30 g a.i $ha^{-1}$ (3.33) was on par with fungal treatments and the untreated plot as in earlier observations while thiamethoxam @ 25 g a.i $ha^{-1}$, acephate @ 750 g a.i $ha^{-1}$ and malathion @ 575 g a.i $ha^{-1}$ had significantly lower mean population of 0.67, 0.33 and 0.67 $ten^{-1}$ sweeps $plot^{-1}$, respectively and were on par.

At 14 DAT also, the trend was similar, with statistical similarity in the mean population between the fungal treated and untreated plots. The highest population recorded in *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$(3.00) was followed by that in talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (2.67), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (3.00), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (3.33), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (2.67) and talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (2.67) which were statistically similar. Chlorantraniliprole @ 30 g a.i $ha^{-1}$ continued to maintain the highest population of parasitoids among the insecticides

(3.33) while the population in thiamethoxam @ 25 g a.i $ha^{-1}$, malathion @ 575 g a.i $ha^{-1}$ and acephate @ 750 g a.i $ha^{-1}$ were less (1.00, 0.67, 0.67), respectively.

At 21 DAT, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ treated plots had the highest mean population of 3.67 $ten^{-1}$ sweeps $plot^{-1}$ and was on par with talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$(3.33), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (3.33), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (3.33), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (3.33), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (3.33), chlorantraniliprole @ 30 g a.i $ha^{-1}$ (3.33) and untreated plots (3.67). Significantly lower mean population was recorded in Thiamethoxam @ 25 g a.i $ha^{-1}$ (1.00), malathion @ 575 g a.i $ha^{-1}$ (1.00) and acephate @ 750 g a.i $ha^{-1}$ (0.67) treated plots.

***4.4.2.3.2 Predators***

***4.4.2.3.2.1 Insects***

There was no significant difference in the pretreatment population of insect predators (Table 34). As noted in the case of parasitoids all the fungal treatments and the insecticide, chlorantraniliprole @ 30 g a.i $ha^{-1}$ supported higher population of insect predators, compared to other insecticides evaluated. On four DAT, the population was 12.33, 12.33, 12.00, 11.00, 10.67, 10.33 and 11.33 $ten^{-1}$ sweeps $plot^{-1}$ in *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$, *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$, *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$, talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$, talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$, talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ and chlorantraniliprole @ 30 g a.i $ha^{-1}$, respectively and these treatments were on par with the untreated plots (12.67). The mean population of insect predators in acephate @ 750 g a.i $ha^{-1}$ (3.67), malathion @ 575 g a.i $ha^{-1}$ (3.00) and thiamethoxam @ 25 g a.i $ha^{-1}$ (2.67) were on par and significantly lower than the other treatments.

On seven DAT also, a similar trend was seen. The mean population in *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ was the highest with 13.67 $ten^{-1}$ sweeps

Table 33. Effect of treatments on the population of hymenopteran parasitoids (Field trial-II)

| Treatments | Mean number of parasitoids* $10^{-1}$ sweeps $plot^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 1.67 (1.58) | 2.33 (1.82) | 2.67 (1.87) | 2.67 (1.87) | 3.33 (2.07) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 2.33 (1.82) | 2.00 (1.72) | 2.67 (1.87) | 2.67 (1.87) | 3.33 (2.07) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 2.00 (1.72) | 2.33 (1.82) | 3.00 (1.99) | 3.00 (1.99) | 3.33 (2.07) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ | 1.67 (1.58) | 2.33 (1.82) | 2.67 (1.87) | 3.00 (1.99) | 3.67 (2.14) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 2.00 (1.72) | 2.67 (1.87) | 2.67 (1.87) | 3.00 (1.99) | 3.33 (2.07) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 0.67 (1.28) | 2.00 (1.72) | 2.67 (1.87) | 3.33 (2.07) | 3.33 (2.07) |
| Acephate @ 750 g a.i $ha^{-1}$ | 2.33 (1.82) | 0.33 (1.14) | 0.33 (1.14) | 0.67 (1.28) | 0.67 (1.28) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 1.00 (1.41) | 2.33 (1.82) | 3.33 (2.07) | 3.33 (2.07) | 3.33 (2.07) |
| Malathion @ 575 g a.i $ha^{-1}$ | 1.33 (1.49) | 0.33 (1.14) | 0.67 (1.28) | 0.67 (1.28) | 1.00 (1.41) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 1.67 (1.58) | 0.67 (1.28) | 0.67 (1.28) | 1.00 (1.41) | 1.00 (1.41) |
| Untreated | 1.33 (1.49) | 2.67 (1.87) | 3.33 (2.07) | 3.33 (2.07) | 3.67 (2.14) |
| CD (0.05) | NS | (0.435) | (0.409) | (0.315) | (0.439) |

*- Braconids, Chalcids, Ichneumonids
Mean of three replications
Figures in parentheses are $\sqrt{x+1}$ transformed values.
DAT- Days after treatment
NS- Non significant

plot$^{-1}$ and was on par with all other fungal and untreated plots. The mean population in talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$, *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$, *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$, talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ and talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ were 13.33, 12.67, 12.67,12.33 and 11.33 ten$^{-1}$ sweeps plot$^{-1}$, respectively. Chlorantraniliprole @ 30 g a.i ha$^{-1}$ had the highest mean population (12.00) among the insecticides and was on par with all fungal treated and untreated plots (13.67). The plots treated with thiamethoxam 25 WG @ 25 g a.i ha$^{-1}$ (4.67), acephate 750 g a.i ha$^{-1}$ (4.33) and malathion @ 575 g a.i ha$^{-1}$ (4.00) were on par and significantly lower than all the other treatments.

At 14 DAT, the effect of treatments on the population of predators was as in the earlier observations. The highest mean population of 14.67 in *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ was on par with talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ (14.67), talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ (14.33), *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ (13.67), *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ (13.67), talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ (13.33), chlorantraniliprole @ 30 g a.i ha$^{-1}$ (12.67) and untreated plots (14.67). The insecticides, thiamethoxam @ 25 g a.i ha$^{-1}$, acephate @ 750 g a.i ha$^{-1}$ and malathion @ 575 g ai ha$^{-1}$ recorded significantly lower mean population of 5.67, 4.67 and 4.67, respectively.

The impact of the treatments on the population of insect predators was the same at 21 DAT also. The talc based *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ treated plots had the highest mean population of 15.00 and was statistically similar with all other fungal treatments, chlorantraniliprole @ 30 g a.i ha$^{-1}$ (13.33) and untreated plots (15.33). *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$, *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ and talc based *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ had mean population of 14.33 each while talc based *B. bassiana* (Bb 5) @ 20 g l$^{-1}$ and *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ treated plots had mean population of 13.67 each. Significantly lower population of 7.67, 7.33 and 6.33 was recorded in

thiamethoxam @ 25 g a.i $ha^{-1}$, acephate @ 750 g a.i $ha^{-1}$ and malathion @ 575 g a.i $ha^{-1}$ treated plots, respectively.

*4.4.2.3.2.2 Spiders*

There was no significant difference in the pretreatment observations on the mean population of spiders (Table 35). At four DAT, *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ treated plots had the highest mean population of 14.67 and was on par with talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (14.00), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (13.00), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (13.00), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (12.67), *A. flavus* (Af-m1) @ $10^{10}$spores $ml^{-1}$ (11.67), chlorantraniliprole @ 30 g a.i $ha^{-1}$ (13.67) and untreated plots (14.67). The other chemical treated plots *viz.,* thiamethoxam @ 25 g a.i $ha^{-1}$, malathion @ 575 g a.i $ha^{-1}$ and acephate @ 750 g a.i $ha^{-1}$ had significantly lower mean population of 8.67, 8.67 and 8.00 $ten^{-1}$ sweeps $plot^{-1}$, respectively.

On seven DAT also the trend was similar and among the fungal treatments, talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ favoured the highest mean population of 14.00 and was statistically similar to that in *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (13.33), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (12.67), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (12.67), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (12.00), chlorantraniliprole @ 30g ai $ha^{-1}$(11.33) and untreated plots (14.67). Among the insecticides, thiamethoxam @ 25 g a.i $ha^{-1}$ had mean population of 9.00 and was on par with *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$, acephate @ 750 g a.i $ha^{-1}$ (8.00) and malathion @ 575 g a.i $ha^{-1}$ (7.67).

The highest mean population of 14.33 was recorded in talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ at 14 DAT, and it was on par with talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (14.00), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (13.67), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (13.33), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (12.33), chlorantraniliprole @ 30g a.i $ha^{-1}$ (12.00) and untreated plots (14.67). *A. flavus* (Af-m1) @ $10^{10}$spores $ml^{-1}$ contained mean

Table 34. Effect of treatments on the population of insect predators (Field trial-II)

| Treatments | Mean number of insect predators* $10^{-1}$ sweeps $plot^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 14.67 (3.94) | 10.67 (3.27) | 12.33 (3.51) | 13.33 (3.79) | 13.67 (3.81) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 13.33 (3.79) | 10.33 (3.19) | 13.33 (3.79) | 14.67 (3.94) | 15.00 (3.87) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 12.33 (3.64) | 11.00 (3.31) | 11.33 (3.36) | 14.33 (3.92) | 14.33 (3.92) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ | 14.33 (3.77) | 12.33 (3.51) | 12.67 (3.69) | 13.67 (3.81) | 14.33 (3.92) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 13.00 (3.58) | 12.33 (3.51) | 13.67 (3.81) | 14.67 (3.94) | 14.33 (3.92) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 12.67 (3.69) | 12.00 (3.46) | 12.67 (3.69) | 13.67 (3.81) | 13.67 (3.81) |
| Acephate @ 750 g a.i $ha^{-1}$ | 13.33 (3.79) | 3.67 (1.90) | 4.33 (2.08) | 4.67 (2.15) | 7.33 (2.70) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 14.33 (3.92) | 11.33 (3.37) | 12.00 (3.46) | 12.67 (3.69) | 13.33 (3.79) |
| Malathion @ 575 g a.i $ha^{-1}$ | 16.00 (3.98) | 3.00 (1.73) | 4.00 (1.99) | 4.67 (2.09) | 6.33 (2.46) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 14.00 (3.70) | 2.67 (1.63) | 4.67 (2.16) | 5.67 (3.38) | 7.67 (2.77) |
| Untreated | 14.33 (3.92) | 12.67 (3.55) | 13.67 (3.69) | 14.67 (3.94) | 15.33 (3.91) |
| CD (0.05) | NS | (0.405) | (0.371) | (0.439) | (0.428) |

*- *Coccinella* sp. *Micraspis* sp., Reduvid bug, Damsel flies, Dragon flies.
Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

population of 10.33 and was statistically similar to malathion @ 575 g a.i $ha^{-1}$ and acephate @ 750 g a.i $ha^{-1}$ which had mean population count of 11.00 and 10.00, respectively. Thiamethoxam @ 25 g a.i $ha^{-1}$ had significantly lower mean population of 6.33 $ten^{-1}$ sweeps $plot^{-1}$only.

At 21 DAT, the highest mean population of 14.67 $ten^{-1}$ sweeps $plot^{-1}$ in talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ was on par with talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$(14.67), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (14.33), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (13.67), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (13.67), *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (12.67), chlorantraniliprole @ 30 g a.i $ha^{-1}$ (14.00) and untreated plots (15.67). Malathion @ 575 g ai $ha^{-1}$ had 10.67 $ten^{-1}$ sweeps $plot^{-1}$, and was statistically on par with *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$, *A. flavus* (Af-m1) @ $10^{10}$spores $ml^{-1}$, acephate @ 750 g a.i $ha^{-1}$ (9.33) and thiamethoxam @ 25 g a.i $ha^{-1}$ (9.00).

### *4.4.2.4 Yield*

#### *4.4.2.4.1 Grain*

The highest mean grain yield of 3.67 kg $plot^{-1}$ was recorded in *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ treated plots and was on par with that in *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (3.52 kg), *M. anisopliae* (Ma 4) in talc @ 20 g $l^{-1}$ (3.25 kg) and talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (3.25 kg) (Table 36). Talc based *B. bassiana* (Bb 21) @ 20 $gl^{-1}$ (3.08 kg) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$(3.07 kg) were statistically similar to all other fungal treatments except *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$. The mean grain yield from *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ was on par with that in chlorantraniliprole @ 30 g a.i $ha^{-1}$(4.00 kg), acephate @ 750 g a.i $ha^{-1}$ (3.83 kg), malathion @ 575 g ai $ha^{-1}$ (3.67 kg) and thiamethoxam @ 25 g a.i.$ha^{-1}$ (3.67 kg). The mean grain yield from untreated plot (2.13 kg) was significantly lower than the other plots.

Table 35. Effect of treatments on the population of spiders (Field trial-II)

| Treatments | Mean number of spiders* $10^{-1}$ sweeps $plot^{-1}$ | | | | |
|---|---|---|---|---|---|
| | Precount | 4 DAT | 7 DAT | 14 DAT | 21 DAT |
| Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ | 17.33 (4.16) | 13.00 (3.59) | 12.00 (3.44) | 14.00 (3.74) | 14.33 (3.78) |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ | 15.33 (3.90) | 12.67 (3.56) | 14.00 (3.74) | 14.33 (3.78) | 14.67 (3.83) |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ | 14.00 (3.74) | 14.00 (3.74) | 12.67 (3.56) | 13.67 (3.69) | 14.67 (3.83) |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ | 15.00 (3.86) | 13.00 (3.59) | 10.67 (3.27) | 13.33 (3.64) | 13.67 (3.69) |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ | 13.33 (3.64) | 14.67 (3.83) | 13.33 (3.64) | 12.33 (3.51) | 13.67 (3.69) |
| *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ | 16.00 (3.99) | 11.67 (3.42) | 12.67 (3.56) | 10.33 (3.21) | 12.67 (3.56) |
| Acephate @ 750 g a.i $ha^{-1}$ | 14.33 (3.78) | 8.00 (2.83) | 8.00 (2.83) | 10.00 (3.15) | 9.33 (3.03) |
| Chlorantraniliprole @ 30 g a.i $ha^{-1}$ | 14.00 (3.74) | 13.67 (3.69) | 11.33 (3.36) | 12.00 (3.46) | 14.00 (3.74) |
| Malathion @ 575 g a.i $ha^{-1}$ | 15.67 (3.93) | 8.67 (2.90) | 7.67 (2.77) | 11.00 (3.31) | 10.67 (3.26) |
| Thiamethoxam @ 25 g a.i $ha^{-1}$ | 14.33 (3.75) | 8.67 (2.94) | 9.00 (3.00) | 6.33 (2.51) | 9.00 (2.99) |
| Untreated | 16.67 (4.07) | 14.67 (3.82) | 14.67 (3.82) | 14.67 (3.82) | 15.67 (3.96) |
| CD (0.05) | NS | (0.424) | (0.371) | (0.439) | (0.428) |

*- *Tetragnatha* sp., *Argiope* sp. *Oxyopus* sp.
Mean of three replications
Figures in parentheses are $\sqrt{x}$ transformed values.
DAT- Days after treatment
NS- Non significant

*4.4.2.4.2 Straw*

There was significant difference in the yield of straw in the treated and untreated plots (Table 36). The highest straw yield was obtained from plots treated with *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (5.40 kg) which was statistically on par with that in *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (5.25 kg), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (5.03 kg), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (5.00 kg), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (4.90 kg) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (4.90 kg). The yield from plots treated with chlorantraniliprole @ 30 g a.i $ha^{-1}$ treated plots (5.90 kg) was on par with acephate @ 750 g a.i $ha^{-1}$ (5.70 kg), thiamethoxam @ 25 g a.i $ha^{-1}$ (5.70 kg) and malathion @ 575 g a.i $ha^{-1}$ (5.40 kg). *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ and *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ was statistically similar to all insecticide treatments. The mean straw yield obtained from the untreated plot (3.16 kg) was significantly lower than all treatment plots.

*4.4.2.5 Benefit-Cost Ratio (BCR)*

The highest BCR of 1.63 was recorded for the treatment with *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ and this was followed by the treatments *viz., B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$(1.56), chlorantraniliprole @ 30 g a.i $ha^{-1}$ (1.50), acephate @ 750 g a.i $ha^{-1}$ (1.46), talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ (1.45), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (1.45), thiamethoxam 25 g a.i $ha^{-1}$ (1.39) and malathion @ 575 g a.i $ha^{-1}$(1.39), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (1.38) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (1.35).

**4.4.3 Grading of Treatments**

Grading from one to ten on the basis of the population and damage of *C. medinalis* and *L. acuta*, population of natural enemies and benefit-cost ratio of treatments in both the field trials (Table 37), showed that the grade was the

Table 36. Grain and straw yield from the different treatments and benefit-cost ratio (BCR) (Field trial-II)

| Treatments | Grain yield (kg plot$^{-1}$) | Straw yield (kg plot$^{-1}$) | BCR |
|---|---|---|---|
| Talc based formulation of *B. bassiana* (Bb 5) @ 20g l$^{-1}$ | 3.25 | 5.03 | 1.45 |
| Talc based formulation of *B. bassiana* (Bb 21) @ 20 g l$^{-1}$ | 3.08 | 5.00 | 1.38 |
| Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g l$^{-1}$ | 3.25 | 4.90 | 1.45 |
| *B. bassiana* (Bb 5) @ $10^{10}$ spores ml$^{-1}$ | 3.52 | 5.25 | 1.56 |
| *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$ | 3.67 | 5.40 | 1.63 |
| *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$ | 3.07 | 4.90 | 1.35 |
| Acephate @ 750 g a.i ha$^{-1}$ | 3.83 | 5.70 | 1.46 |
| Chlorantraniliprole @ 30 g a.i ha$^{-1}$ | 4.00 | 5.90 | 1.50 |
| Malathion @ 575 g a.i ha$^{-1}$ | 3.67 | 5.40 | 1.39 |
| Thiamethoxam @ 25 g a.i ha$^{-1}$ | 3.67 | 5.70 | 1.39 |
| Untreated | 2.13 | 3.16 | - |
| CD (0.05) | (0.500) | (0.834) | - |

highest for chlorantraniliprole @ 30 g a.i $ha^{-1}$ and it was followed by *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ > acephate @ 750 g a.i $ha^{-1}$ > *M. anisopliae* @ $10^{10}$ spores $ml^{-1}$ (Ma 4) > thiamethoxam @ 25 g a.i $ha^{-1}$ > talc based *B. bassiana* (Bb 5) = talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ > malathion @ 575 g a.i $ha^{-1}$ > talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ > *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$.

## 4.5. COMPATABILITY OF FUNGI WITH PESTICIDES

The entomopathogenic fungi, *B. bassiana* (Bb 5), *B. bassiana* (Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1) were tested for their compatibility with three insecticides *viz.*, acephate, chlorantraniliprole and thiamethoxam at three different concentrations. The data on the mean growth, spore count, conidial viability and bioefficacy are presented in Tables 38 to 45.

### *4.5.1 B. bassiana* (Bb 5)

#### *4.5.1.1 Growth*

The growth of *B. bassiana* (Bb 5) was not at measurable level till five days after inoculation. At five DAI, the mean growth of fungus in media poisoned with chlorantraniliprole 0.004 per cent (1.67 cm), chlorantraniliprole 0.006 per cent (1.63 cm) and thiamethoxam 0.003 per cent (1.63 cm) was on par with that in the unpoisoned media (1.73 cm) (Table 38). No significant difference in the mean growth of *B. bassiana* (Bb 5) in chlorantraniliprole 0.004, 0.006 and 0.008 per cent was observed, the values being 1.67, 1.63 and 1.57 cm, respectively and the growth was on par with that in thiamethoxam 0.003 (1.63 cm), 0.005 per cent (1.53 cm) and 0.008 per cent (1.43 cm). The mean growth of 1.40 and 1.33 cm in acephate 0.075, 0.150 per cent was on par while the lowest mean growth observed in media with acephate 0.225 per cent (1.20 cm) was significantly lower than the other treatments (Plate 6 A).

Table 37. Grading of different treatments in the field trials based on the population and damage of pests, population of natural enemies and benefit-cost ratio.

| Treatments | *C. medinalis* larval population | | *C. medinalis* adult population | | *C. medinalis* damage | | *L. acuta* population | | *L. acuta* damage | | Parasitoids | | Insect predators | | Spider predators | | Benefit-cost ratio. | | Total grade points | Grade |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I trial | II trial | I trial | II trial | I trial | II trial | I trial | II trial | I trial | II trial | I trial | II trial | I trial | II trial | I trial | II trial | I trial | II trial | | |
| T1 | 6 | 4 | 6 | 8 | 6 | 7 | 2 | 2 | 3 | 4 | 8 | 9 | 9 | 8 | 8 | 9 | 5 | 6 | 110 | 6 |
| T2 | 5 | 5 | 2 | 4 | 5 | 6 | 1 | 1 | 4 | 4 | 8 | 10 | 9 | 9 | 7 | 7 | 2 | 4 | 93 | 8 |
| T3 | 5 | 7 | 4 | 6 | 1 | 4 | 6 | 5 | 6 | 7 | 8 | 9 | 7 | 9 | 7 | 10 | 3 | 6 | 110 | 6 |
| T4 | 7 | 7 | 6 | 6 | 10 | 10 | 4 | 4 | 7 | 5 | 9 | 9 | 10 | 10 | 10 | 10 | 10 | 9 | 143 | 2 |
| T5 | 5 | 6 | 3 | 5 | 4 | 5 | 7 | 6 | 9 | 9 | 10 | 9 | 8 | 9 | 9 | 7 | 8 | 10 | 129 | 4 |
| T6 | 4 | 3 | 5 | 4 | 3 | 3 | 3 | 3 | 2 | 3 | 10 | 9 | 7 | 8 | 5 | 6 | 1 | 3 | 82 | 9 |
| T7 | 8 | 9 | 8 | 10 | 9 | 10 | 9 | 9 | 8 | 8 | 6 | 7 | 3 | 5 | 3 | 4 | 7 | 7 | 130 | 3 |
| T8 | 10 | 10 | 9 | 8 | 8 | 9 | 10 | 10 | 10 | 10 | 9 | 9 | 6 | 7 | 6 | 8 | 9 | 8 | 156 | 1 |
| T9 | 7 | 5 | 7 | 7 | 2 | 5 | 5 | 7 | 7 | 6 | 6 | 8 | 4 | 4 | 4 | 5 | 6 | 5 | 100 | 7 |
| T10 | 9 | 8 | 10 | 9 | 7 | 8 | 8 | 8 | 5 | 7 | 7 | 8 | 5 | 6 | 3 | 3 | 4 | 5 | 120 | 5 |

T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$
T3 - Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$
T4 - *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$

T5 - *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$
T6 - *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$
T7 - Acephate @ 750 g a.i $ha^{-1}$
T8 - Chlorantraniliprole @ 30 g a.i $ha^{-1}$
T9 - Malathion @ 575 g a.i $ha^{-1}$
T10 - Thiamethoxam @ 25 g a.i $ha^{-1}$

The growth of fungus in unpoisoned media (3.23) was superior at seven DAI, however a mean growth of 2.37, 2.20 and 2.30 cm in chlorantraniliprole 0.004, 0.006 per cent and thiamethoxam 0.003 per cent, respectively was observed and were on par. In the higher doses of these insecticides, *i.e.,* thiamethoxam 0.005, 0.008 per cent and chlorantraniliprole 0.008 per cent the mean growth was significantly lower (2.17, 2.07 and 2.07 cm), respectively which were on par. The lower dose of acephate 0.075 per cent supported significantly higher growth of 1.80 cm than its higher doses of 0.150 and 0.225 per cent, the growth being, 1.60 and 1.53 cm, respectively which were on par.

At nine DAI, the highest mean growth in chlorantraniliprole 0.004 per cent mixed media was 2.90 cm and the growth was significantly higher than that in the higher doses, 0.006 (2.47 cm) and 0.008 per cent (2.23 cm). This was followed by thiamethoxam 0.003 per cent that supported mean growth of 2.47 cm and was on par with that in 0.005 (2.33 cm) and 0.008 per cent (2.27 cm). Acephate 0.075 and 0.150 per cent poisoned media had mean growth of 2.07 and 1.90 cm, respectively and were statistically superior to the growth in its higher dose of 0.225 per cent which supported only 1.70 cm. The highest mean growth of *B. bassiana* (Bb 5) in the unpoisoned media (3.60 cm) was significantly superior to all other treatments.

The mean growth of 3.57 and 3.17 cm in media poisoned with chlorantraniliprole 0.004 and 0.006 per cent ranked top at 11 DAI and these were on par. The higher dose of chlorantraniliprole 0.008 per cent poisoned media supported mean growth of 3.07 cm and was on par with the growth in the lower doses of thiamethoxam 0.003 and 0.005 per cent (2.83 and 2.67 cm), respectively. The thiamethoxam 0.008 mixed media supported mean growth of 2.40 cm. The growth of *B. bassiana* (Bb 5) in acephate 0.075 and 0.150 per cent mixed media was 2.53 cm and 2.50 cm, respectively and was superior to that in its higher dose of 0.225 per cent (2.13 cm). The growth in unpoisoned media (4.43 cm) as in earlier observations was significantly superior to all other treatments.

The mean growth in chlorantraniliprole 0.004 and 0.006 per cent added media were 3.60 and 3.53 cm, respectively and were on par with that in the thiamethoxam 0.003 per cent (3.43 cm) at 14 DAI. Chlorantraniliprole 0.008, thiamethoxam 0.003 and 0.005 per cent supported statistically similar mean growth of 3.33, 3.43 cm and 3.27 cm, respectively. Acephate 0.075 and 0.150 per cent favoured mean growth of 2.97 and 2.87 cm, respectively and was on par with that in thiamethoxam 0.008 per cent (3.07 cm). The lowest mean growth of 2.63 cm in acephate 0.225 per cent was significantly lower than that in the other treatments. The mean growth of fungi in unpoisoned media was 5.20 cm and it was significantly superior to all other treatments.

### *4.5.1.2 Spore Count*

The media with the highest dose of thiamethoxam 0.008 supported the highest spore count of $5.00 \times 10^9$ spores $ml^{-1}$ at 14 DAI and it was on par with that in the highest dose of chlorantraniliprole 0.008 per cent ($4.90 \times 10^9$) (Table 39). This was followed by thiamethoxam 0.005 per cent ($4.50 \times 10^9$) and was on par with that recorded in acephate 0.225 ($4.27 \times 10^9$) and thiamethoxam 0.003 per cent ($4.23 \times 10^9$). Acephate 0.075, 0.150 and chlorantraniliprole 0.006 per cent produced spore count of $4.07 \times 10^9$, $4.13 \times 10^9$, $4.03 \times 10^9$ spores $ml^{-1}$, respectively and were on par. The lowest dose of chlorantraniliprole at 0.004 per cent recorded spore count of $3.73 \times 10^9$ spores $ml^{-1}$ and it was on par with that in the lowest dose of acephate 0.075 per cent. The unpoisoned media had the highest mean spore count of $5.47 \times 10^9$ spore $ml^{-1}$ and was statistically superior to other treatments.

### *4.5.1.3 Conidial Viability*

The germination of *B. bassiana* (Bb 5) was found unaffected even when mixed in the highest doses of chlorantraniliprole 0.008 and thiamethoxam 0.008 per cent (81.33 and 78.67), respectively which were on par with that in the unpoisoned media (85.33) (Table 39). Thiamethoxam 0.005 and

Table 38. Growth of *B. bassiana* (Bb 5) in poisoned media

| Treatments | Mean growth (cm) | | | | |
|---|---|---|---|---|---|
| | 5 DAI | 7 DAI | 9 DAI | 11 DAI | 14 DAI |
| Acephate 0.075 % | 1.40 (1.18) | 1.80 (1.34) | 2.07 (1.44) | 2.53 (1.59) | 2.97 (1.72) |
| Acephate 0.150 % | 1.33 (1.15) | 1.60 (1.26) | 1.90 (1.38) | 2.50 (1.58) | 2.87 (1.69) |
| Acephate 0.225 % | 1.20 (1.09) | 1.53 (1.24) | 1.70 (1.30) | 2.13 (1.46) | 2.63 (1.62) |
| Chlorantraniliprole 0.004 % | 1.67 (1.29) | 2.37 (1.54) | 2.90 (1.70) | 3.57 (1.89) | 3.60 (1.89) |
| Chlorantraniliprole 0.006 % | 1.63 (1.28) | 2.20 (1.48) | 2.47 (1.57) | 3.17 (1.78) | 3.53 (1.88) |
| Chlorantraniliprole 0.008 % | 1.57 (1.25) | 2.07 (1.44) | 2.23 (1.49) | 3.07 (1.75) | 3.33 (1.83) |
| Thiamethoxam 0.003 % | 1.63 (1.28) | 2.30 (1.52) | 2.47 (1.57) | 2.83 (1.68) | 3.43 (1.85) |
| Thiamethoxam 0.005 % | 1.53 (1.24) | 2.17 (1.47) | 2.33 (1.53) | 2.67 (1.64) | 3.27 (1.81) |
| Thiamethoxam 0.008 % | 1.43 (1.97) | 2.07 (1.44) | 2.27 (1.51) | 2.40 (1.55) | 3.07 (1.75) |
| Unpoisoned | 1.73 (1.32) | 3.23 (1.79) | 3.60 (1.89) | 4.43 (2.10) | 5.20 (2.28) |
| CD (0.05) | (0.062) | (0.065) | (0.072) | (0.122) | (0.068) |

Figures in parentheses are $\sqrt{x}$ transformed values
Mean of three replications
DAI- Days after inoculation

chlorantraniliprole 0.006 per cent supported germination of 77.33 per cent each and were on par with that in the lower doses of these insecticides, the value being 76.00 per cent each. These treatments were also on par with 0.150 and 0.225 per cent of acephate (70.67 and 76.00) respectively. In media treated with 0.075 per cent of acephate, germination was only 69.33 per cent, but it was on par with that in its higher doses.

#### *4.5.1.4 Bioefficacy*

Bioefficacy, assessed in terms of the cumulative per cent mortality against adult rice bug (Table 39) showed that there was no significant difference in the bioefficacy of *B. bassiana* (Bb 5) that were grown in media poisoned with different insecticides and that grown in unpoisoned media (86.67 per cent). The highest mean mortality of 93.33 per cent was observed when sprayed with spore suspensions prepared from 14 day old culture of *B. bassiana* (Bb 5) grown in chlorantaniliporle 0.008 per cent, this was followed by 86.67 per cent mortality shown by its lower concentration of 0.006 and acephate 0.225 per cent. While the fungus grown in chlorantraniliprole 0.004, thiamethoxam 0.005 and 0.008, acephate 0.150 per cent showed 83.33 per cent mortality, that grown in the lower doses of thiamethoxam and acephate caused mortality of 80.00 per cent.

### *4.5.2 B. bassiana* (Bb 21)

#### *4.5.2.1 Growth*

At five DAI, there was no significant difference in the mean growth of *B. bassiana* (Bb 21) grown in media with varying concentrations of chlorantraniliprole and thiamethoxam, the values being 1.67 cm in all doses of chlorantraniliprole and 1.63, 1.63 and 1.53 cm in media poisoned with thiamethoxam 0.003, 0.005 and 0.008 per cent, respectively (Table 40). The mean growth in media mixed with acephate 0.150 per cent was 1.40 cm and it was on par with the growth of 1.47 and 1.27 cm in its lower and higher doses of 0.075 and 0.225 per cent, respectively. The unpoisoned media supported the highest

Table 39. Spore count, germination and bioefficacy of *B. bassiana* (Bb 5) in poisoned media

| Treatments | Spore count at 14 DAI ($10^9$ spores $ml^{-1}$) | Germination (%) | Mortality of *L. acuta* at 10 DAT (%) |
|---|---|---|---|
| Acephate 0.075 % | 4.07 (2.02) | 69.33 (8.33) | 80.00 (8.94) |
| Acephate 0.150 % | 4.13 (2.03) | 70.67 (8.39) | 83.33 (9.13) |
| Acephate 0.225 % | 4.27 (2.07) | 76.00 (8.72) | 86.67 (9.31) |
| Chlorantraniliprole 0.004 % | 3.73 (1.93) | 76.00 (8.72) | 83.33 (9.13) |
| Chlorantraniliprole 0.006 % | 4.03 (2.01) | 77.33 (8.79) | 86.67 (9.31) |
| Chlorantraniliprole 0.008 % | 4.90 (2.21) | 81.33 (9.02) | 93.33 (9.61) |
| Thiamethoxam 0.003 % | 4.23 (2.06) | 76.00 (8.72) | 80.00 (8.94) |
| Thiamethoxam 0.005 % | 4.50 (2.12) | 77.33 (8.79) | 83.33 (9.13) |
| Thiamethoxam 0.008 % | 5.00 (2.24) | 78.67 (8.87) | 83.33 (9.13) |
| Unpoisoned | 5.47 (2.34) | 85.33 (9.24) | 86.67 (9.31) |
| CD (0.05) | (0.085) | (0.425) | NS |

Figures in parentheses are $\sqrt{x}$ transformed values
Mean of three replications
DAI- Days after inoculation, DAT- Days after treatment
NS- Non significant

mean growth of 1.83 cm and it showed significant superiority over the different poisoned media (Plate 6 B).

At seven DAI, the highest mean growth of 2.47 cm observed in media poisoned with the lower dose of chlorantraniliprole 0.004 per cent was on par with that in its 0.006 and 0.008 per cent (2.33 and 2.27cm) and also with 2.40, 2.33 cm recorded in thiametoxam 0.003 and 0.005 per cent, respectively. The growth in the higher dose of thiamethoxam (2.20 cm) was on par with that in the higher dose of chlorantaniliprole. Acephate 0.075 per cent mixed media supported mean growth of 1.87 cm and it showed significant superiority to the growth in its higher doses of 0.150 and 0.225 per cent, which supported only 1.60 and 1.57 cm, respectively. The mean growth of fungi in unpoisoned media was 3.40 cm and was significantly superior to other treatments.

At nine DAI, the growth of *B. bassiana* (Bb 21) in chlorantraniliprole 0.004 per cent (3.00 cm) was statistically superior to the other treatments. The next higher growth was seen in the lower dose of thiamethoxam 0.003 per cent (2.70 cm) and it was on par with that in thiamethoxam 0.005 and chlorantraniliprole 0.006 per cent, the values being 2.57 and 2.67 cm, respectively. The higher doses of chlorantraniliprole and thiamethoxam mixed media favoured growth of 2.50 and 2.47 cm, respectively and were on par. As in the earlier observations, acephate 0.225 per cent mixed media recorded the lowest mean growth of 1.83 cm and it was significantly lower than in 0.075 and 0.150 per cent of acephate (2.27 and 2.07 cm), respectively. The maximum growth of 3.67 cm of *B. bassiana* (Bb 21) observed in the unpoisoned media was statistically superior to other treatments.

At 11 DAI the highest growth of *B. bassiana* (Bb 21) in chlorantraniliprole 0.004 per cent mixed media (3.60 cm) was on par with that in its 0.006 (3.47 cm) and 0.008 per cent (3.33 cm). The lower dose thiamethoxam 0.003 per cent had growth of 3.03 cm, and it was on par with that in the 0.005 and 0.008 per cent of thiamethoxam (2.87 and 2.63 cm) , respectively and also with

that in the higher dose of chlorantraniliprole. The growth in media poisoned with acephate 0.075, 0.150 and 0.225 per cent was 2.70, 2.60 and 2.37 cm, respectively and was on par with that in the higher doses of thiamethoxam. The highest mean growth of 4.63 cm in unpoisoned media was statistically superior to other treatments.

At 14 DAI also, the mean growth observed in media poisoned with the lower dose of chlorantraniliprole 0.004 per cent (3.80 cm) was on par with that recorded in its 0.006 per cent (3.73 cm) and lower dose of thiamethoxam 0.003 per cent (3.73 cm). Chlorantraniliprole 0.008 per cent supported mean growth of 3.57 cm and was on par with that in the 0.003 and 0.005 per cent of thiamethoxam (3.73 and 3.53 cm), respectively. As in the previous observation, the mean growth in thiamethoxam 0.008, acephate 0.075 and 0.150 per cent were on par, the values being 3.30, 3.23 and 3.17 cm, respectively. The higher dose of acephate 0.225 per cent, recorded significantly lower mean growth of 2.93 cm. The unpoisoned media had growth of 5.43 cm and it was significantly superior to other treatments.

### *4.5.2.2 Spore Count*

The spore count determined on the fourteenth day after inoculation (Table 41) revealed that the media mixed with the higher concentration of thiamethoxam 0.008 per cent supported the maximum spore count of 4.77 x $10^9$ spores $ml^{-1}$ of *B. bassiana* (Bb 21) and it was on par with that in the highest concentration of chlorantraniliprole 0.008 per cent (4.53 x $10^9$) and unpoisoned media (5.00 x $10^9$). This treatments was followed by thiamethoxam 0.005, acephate 0.225, 0.150 and chlorantraniliprole 0.006 per cent that favoured spore count of 4.20 x $10^9$, 4.17 x $10^9$ ,4.03 x $10^9$ and 4.03 x $10^9$ spores $ml^{-1}$, respectively. The lower doses of acephate and chlorantraniliprole produced 3.90 x $10^9$ and 3.43 x $10^9$ spores $ml^{-1}$, respectively.

Table 40. Growth of *B. bassiana* (Bb 21) in poisoned media

| Treatments | Mean growth (cm) | | | | |
|---|---|---|---|---|---|
| | 5 DAI | 7 DAI | 9 DAI | 11 DAI | 14 DAI |
| Acephate 0.075 % | 1.47 (1.21) | 1.87 (1.37) | 2.27 (1.51) | 2.70 (1.64) | 3.23 (1.79) |
| Acephate 0.150 % | 1.40 (1.18) | 1.60 (1.26) | 2.07 (1.44) | 2.60 (1.61) | 3.17 (1.78) |
| Acephate 0.225 % | 1.27 (1.13) | 1.57 (1.25) | 1.83 (1.35) | 2.37 (1.54) | 2.93 (1.71) |
| Chlorantraniliprole 0.004 % | 1.67 (1.29) | 2.47 (1.57) | 3.00 (1.73) | 3.60 (1.93) | 3.80 (1.95) |
| Chlorantraniliprole 0.006 % | 1.67 (1.29) | 2.33 (1.53) | 2.67 (1.63) | 3.47 (1.86) | 3.73 (1.93) |
| Chlorantraniliprole 0.008 % | 1.67 (1.29) | 2.27 (1.51) | 2.50 (1.58) | 3.33 (1.82) | 3.57 (1.89) |
| Thiamethoxam 0.003 % | 1.63 (1.28) | 2.40 (1.55) | 2.70 (1.64) | 3.03 (1.74) | 3.73 (1.93) |
| Thiamethoxam 0.005 % | 1.63 (1.28) | 2.33 (1.53) | 2.57 (1.60) | 2.87 (1.69) | 3.53 (1.88) |
| Thiamethoxam 0.008 % | 1.53 (1.24) | 2.20 (1.48) | 2.47 (1.57) | 2.63 (1.62) | 3.30 (1.82) |
| Unpoisoned | 1.83 (1.35) | 3.40 (1.84) | 3.67 (1.92) | 4.63 (2.15) | 5.43 (2.33) |
| CD (0.05) | (0.061) | (0.0510 | (0.0570 | (0.117) | (0.057) |

Figures in parentheses are $\sqrt{x}$ transformed values
Mean of three replications
DAI- Days after inoculation

### *4.5.2.3 Conidial Viability*

Maximum germination (86.33 per cent) of *B. bassiana* (Bb 21) was seen in the highest concentration of thiamethoxam and chlorantraniliprole 0.008 per cent and it was on par with that observed in unpoisoned media (86.00) and thiamethoxam 0.005 per cent (84.67) (Table 41). The germination percentage in media poisoned with chlorantraniliprole 0.006 and the higher dose of acephate 0.225 were 83.67 and 82.67 per cent, respectively and were on par. The lower doses of chlorantraniliprole and thiamethoxam supported germination of 80.67 and 80.33 per cent, respectively and these were on par with that in acephate 0.150 per cent (80.00). In acephate 0.075 per cent supplemented media significantly lower conidial germination of 75.33 only was recorded.

### *4.5.2.4 Bioefficacy*

There was no significant difference in the bioefficacy of *B. bassiana* (Bb 21) grown in different poisoned and unpoisoned media (86.67) (Table 41). The highest mean mortality of 93.33 per cent recorded by *B. bassiana* (Bb 21) grown in chlorantraniliprole 0.008 per cent was followed by 86.67 per cent by the fungus in acephate 0.225 and chlorantraniliprole 0.006 per cent. Mortality of *L. acuta* to the tune of 83.33 per cent was observed in treatments with *B. bassiana* (Bb 21) grown in acephate 0.150, chlorantraniliprole 0.004 and thiamethoxam 0.005 per cent while that grown in acephate 0.075 and thiamethoxam 0.003 per cent caused 80.00 per cent mortality.

### *4.5.3 M. anisopliae* (Ma 4)

### *4.5.3.1 Growth*

At five DAI, the mean growth of *M. anisopliae* (Ma 4) grown in chlorantraniliprole 0.004 (1.50 cm) was on par with that in the unpoisoned media (1.63 cm). Chlorantraniliprole 0.006 and 0.008 per cent mixed media supported mean growth of 1.47 and 1.43 cm, respectively, and were on par with the growth

Table 41. Spore count, germination and bioefficacy of *B. bassiana* (Bb 21) in poisoned media

| Treatments | Spore count at 14 DAI ( $10^9$ spores $ml^{-1}$) | Germination (%) | Mortality of *L. acuta* at 10 DAT (%) |
|---|---|---|---|
| Acephate 0.075 % | 3.90 (1.98) | 75.33 (8.68) | 80.00 (8.94) |
| Acephate 0.150 % | 4.03 (2.01) | 80.00 (8.94) | 83.33 (9.13) |
| Acephate 0.225 % | 4.17 (2.04) | 82.67 (9.09) | 86.67 (9.31) |
| Chlorantraniliprole 0.004 % | 3.43 (1.85) | 80.67 (8.98) | 83.33 (9.13) |
| Chlorantraniliprole 0.006 % | 4.03 (2.01) | 83.67 (9.15) | 86.67 (9.31) |
| Chlorantraniliprole 0.008 % | 4.53 (2.13) | 86.33 (9.29) | 93.33 (9.61) |
| Thiamethoxam 0.003 % | 3.97 (1.99) | 80.33 (8.96) | 80.00 (8.94) |
| Thiamethoxam 0.005 % | 4.20 (2.05) | 84.67 (9.20) | 83.33 (9.13) |
| Thiamethoxam 0.008 % | 4.77 (2.18) | 86.33 (9.29) | 83.33 (9.13) |
| Unpoisoned | 5.00 (2.34) | 86.00 (9.27) | 86.67 (9.31) |
| CD (0.05) | (0.058) | (0.091) | NS |

Figures in parentheses are $\sqrt{x}$ transformed values
Mean of three replications
DAI- Days after inoculation, DAT- Days after treatment
NS- Non significant

in the lower dose of thiamethoxam 0.003 per cent (1.40 cm) (Table 42). The growth of 1.37 cm seen in media mixed with acephate 0.075 per cent was on par with that in the higher doses of thiamethoxam 0.005 and 0.008 per cent (1.30 and 1.20 cm), respectively. Acephate 0.150 and 0.225 per cent supported mean growth of 1.17 and 1.13 cm, respectively and were on par (Plate 6 C).

On the seventh day after inoculation of *M. anisopliae* (Ma 4), the mean growth in media with thiamethoxam 0.003 and chlorantraniliprole 0.004 per cent was 1.80 and 1.77 cm, respectively and these were on par with that in the unpoisoned media (1.90 cm). Chlorantraniliprole 0.006 and thiamethoxam 0.005 per cent favoured growth of 1.73 and 1.63 cm, respectively and were on par. The growth of fungus in media supplemented with lower dose of acephate 0.075 per cent (1.57 cm) was on par with that in the higher doses of chlorantraniliprole and thiamethoxam 0.008 per cent (1.53 cm). The higher doses of acephate 0.150 and 0.225 per cent that showed significantly lower growth of 1.40 and 1.33 cm, respectively and were on par.

At nine DAI, chlorantraniliprole 0.004 per cent poisoned media supported the highest mean growth of 3.30 cm and it was on par with that in the unpoisoned media (3.43 cm). The next higher growth of 3.23 and 3.10 cm observed in chlorantraniliprole 0.006 and 0.008 per cent mixed media were on par. Thiamethoxam 0.003, 0.005 per cent supplemented media had statistically similar growth of 2.83, 2.67 cm respectively, while its higher dose supported growth of 2.40 cm only. Significantly lower growth of 1.77, 1.67 and 1.53 cm was seen in acephate 0.075, 0.150 and 0.225 per cent, respectively, the lower doses being on par.

At 11 DAI, the mean growth of *M. anisopliae* (Ma 4) in 0.004 and 0.006 per cent of chlorantraniliprole (3.73 and 3.70 cm) was on par with that in the unpoisoned media (3.83 cm). The higher dose of chlorantraniliprole 0.008 per cent favoured growth of 3.53 cm and was statistically on par with the growth in its lower doses and thiamethoxam 0.003 per cent (3.33 cm). The higher doses of

thiamethoxam 0.005 and 0.008 per cent mixed media had growth of 3.13 and 3.07 cm respectively, and were on par. Acephate 0.075, 0.150 and 0.225 per cent poisoned media resulted in significantly lower growth of 2.37, 2.10 and 2.07 cm respectively, the growth in the higher doses were on par.

At 14 DAI, media mixed with lower doses of chlorantraniliprole 0.004 and 0.006 and thiamethoxam 0.003 per cent supported growth of 5.20, 5.07 cm and 5.13 cm, respectively and were on par with that grown in the unpoisoned media (5.43 cm). The mean growth of 4.97 cm observed in the higher dose of chlorantraniliprole 0.008 per cent was statistically similar to that in its lower doses and that of thiamethoxam 0.005 per cent (4.87 cm). Similar to the previous observations, significantly lower mean growth was observed in media poisoned with acephate 0.075, 0.150 and 0.225 per cent, the values being 2.73, 2.47 and 2.37 cm, respectively.

### *4.5.3.2 Spore Count*

The spore count of 5.63 x $10^9$ spores $ml^{-1}$ of *M. anisopliae* (Ma 4) recorded in media with chlorantraniliprole 0.008 per cent at 14 DAI was significantly superior to other treatments (Table 43). The fungus grown in chlorantaniliprole 0.006 and thiamethoxam 0.008 per cent produced spore count of 5.37 x $10^9$ spores $ml^{-1}$ each, and these were followed by that grown in thiamethoxam 0.005 (5.17 x $10^9$) and chlorantraniliprole 0.004 (5.10 x $10^9$). In 0.003 and 0.225 per cent concentrations of thiamethoxam and acephate, spore count recorded was 4.90 x $10^9$ and 4.77 x $10^9$ spores $ml^{-1}$, respectively and were on par. Significantly lower spore count of 4.20 x $10^9$, 4.56 x $10^9$ spores $ml^{-1}$ was recorded in acephate 0.075, 0.150 per cent mixed media, respectively. The unpoisoned media supported significantly higher spore count of 5.83 x $10^9$ spores $ml^{-1}$.

Table 42. Growth of *M. anisopliae* (Ma 4) in poisoned media

| Treatments | Mean growth (cm) | | | | |
|---|---|---|---|---|---|
| | 5 DAI | 7 DAI | 9 DAI | 11 DAI | 14 DAI |
| Acephate 0.075 % | 1.37 (1.17) | 1.57 (1.25) | 1.77 (1.33) | 2.37 (1.54) | 2.73 (1.65) |
| Acephate 0.150 % | 1.17 (1.08) | 1.40 (1.18) | 1.67 (1.29) | 2.10 (1.45) | 2.47 (1.57) |
| Acephate 0.225 % | 1.13 (1.06) | 1.33 (1.16) | 1.53 (1.24) | 2.07 (1.44) | 2.37 (1.54) |
| Chlorantraniliprole 0.004 % | 1.50 (1.22) | 1.77 (1.33) | 3.30 (1.82) | 3.73 (1.93) | 5.20 (2.28) |
| Chlorantraniliprole 0.006 % | 1.47 (1.21) | 1.73 (1.32) | 3.23 (1.79) | 3.70 (1.92) | 5.07 (2.25) |
| Chlorantraniliprole 0.008 % | 1.43 (1.19) | 1.53 (1.24) | 3.10 (1.76) | 3.53 (1.88) | 4.97 (2.23) |
| Thiamethoxam 0.003 % | 1.40 (1.18) | 1.80 (1.34) | 2.83 (1.68) | 3.33 (1.83) | 5.13 (2.27) |
| Thiamethoxam 0.005 % | 1.30 (1.14) | 1.63 (1.28) | 2.67 (1.63) | 3.13 (1.77) | 4.87 (2.21) |
| Thiamethoxam 0.008 % | 1.20 (1.09) | 1.53 (1.24) | 2.40 (1.55) | 3.07 (1.75) | 4.40 (2.09) |
| Unpoisoned | 1.63 (1.28) | 1.90 (1.38) | 3.43 (1.85) | 3.83 (1.96) | 5.43 (2.33) |
| CD (0.05) | (0.057) | (0.053) | (0.058) | (0.055) | (0.099) |

Figures in parentheses are $\sqrt{x}$ transformed values
Mean of three replications
DAI- Days after inoculation

*4.5.3.3 Conidial Viability*

The highest germination of 86.67 per cent of *M. anisopliae* (Ma 4) was observed in media with 0.008 per cent of thiamethoxam and was on par with that in chlorantraniliprole 0.008 (86.33) and 0.006 (83.33), acephate 0.225 (83.33), thiamethoxam 0.005 per cent (83.00) and in the unpoisoned media (84.00) (Table 43). The mean germination percentage of 80.67 in acephate 0.150 mixed media was on par with that in thiamethoxam 0.003 (80.33). Lower germination percentage of 79.67 and 76.67 was noted in chlorantraniliprole 0.004 and acephate 0.075 mixed media were on par.

*4.5.2.4 Bioefficacy*

There was no significant difference in the bioefficacy of *M. anisopliae* (Ma 4) grown in different insecticide mixed and unpoisoned media (Table 43). The highest mean mortality of 93.33 per cent was recorded for *M. anisopliae* (Ma 4) grown in chlorantraniliprole 0.008, thiamethoxam 0.005, 0.008 per cent and unpoisoned media. Fungus cultured in media containing chlorantraniliprole 0.004 and 0.006 per cent resulted in 90.00 per cent mortality of *L. acuta* which was closely followed by 86.67 mortality for *M. anisopliae* (Ma 4) grown in thaimethoxam 0.003 per cent mixed media. *M. anisopliae* (Ma 4) grown in acephate 0.075, 0.150 and 0.225 per cent mixed media caused mortality of 80.00, 83.33 and 83.33 per cent, respectively.

*4.5.4 A. flavus* **(Af ml)**

*4.5.4.1 Growth*

At three DAI the highest mean growth of *A. flavus* (Af-ml) was seen in media mixed with chlorantraniliprole 0.004 (2.40 cm) and it was on par with that in thiamethoxam 0.003 per cent (2.33 cm) and unpoisoned media (2.53 cm) (Table 44). The higher doses of chlorantraniliprole 0.006 and 0.008 per cent supported statistically similar growth of 2.27 and 2.23 cm, respectively while that

Table 43. Spore count, germination and bioefficacy of *M. anisopliae* (Ma 4) in poisoned media

| Treatments | Spore count at 14 DAI ($10^9$ Spores $ml^{-1}$) | Germination (%) | Mortality of *L. acuta* at 10 DAT (%) |
|---|---|---|---|
| Acephate 0.075 % | 4.20 (2.05) | 76.67 (8.76) | 80.00 (8.93) |
| Acephate 0.150 % | 4.56 (2.14) | 80.67 (8.98) | 83.33 (9.11) |
| Acephate 0.225 % | 4.77 (2.18) | 83.33 (9.13) | 83.33 (9.11) |
| Chlorantraniliprole 0.004 % | 5.10 (2.26) | 79.67 (8.93) | 90.00 (9.49) |
| Chlorantraniliprole 0.006 % | 5.37 (2.32) | 83.33 (9.13) | 90.00 (9.49) |
| Chlorantraniliprole 0.008 % | 5.63 (2.37) | 86.33 (9.29) | 93.33 (9.66) |
| Thiamethoxam 0.003 % | 4.90 (2.21) | 80.33 (8.96) | 86.67 (9.65) |
| Thiamethoxam 0.005 % | 5.17 (2.27) | 83.00 (9.11) | 93.33 (9.66) |
| Thiamethoxam 0.008 % | 5.37 (2.32) | 86.67 (9.31) | 93.33 (9.66) |
| Unpoisoned | 5.83 (2.42) | 84.00 (9.17) | 93.33 (9.66) |
| CD (0.05) | (0.037) | (0.201) | NS |

Figures in parentheses are $\sqrt{x}$ transformed values
Mean of three replications
DAI- Days after inoculation
DAT- Days after treatment
NS- Non significant

in thiamethoxam 0.005 and 0.008 per cent were 2.13 and 2.07 cm. Statistically similar mean growth of 1.90, 1.83 and 1.80 cm in acephate 0.075, 0.150 and 0.225 per cent mixed media were significantly lower than that of the other treatments. The growth in unpoisoned media was 2.53 cm and it was significantly superior to other treatments (Plate 6 D).

The highest mean growth of 2.80 cm in media with chlorantraniliprole 0.004 and thiamethoxam 0.003 per cent was on par with that in the higher doses of chlorantraniliprole 0.006 and 0.008 per cent (2.73 and 2.70 cm), respectively. These were followed by the growth of 2.67 cm each of the fungus grown in 0.005 and 0.008 per cent thiamethoxam poisoned media. Media with acephate 0.075 and 0.150 per cent supported statistically similar mean l growth of 2.50 and 2.40 cm, respectively. Significantly lower growth of 2.27 cm was observed in acephate 0.225 per cent. The unpoisoned media favoured significantly higher mean growth of 3.00 cm.

On the seventh day after inoculation, the highest growth of 4.90 cm in media poisoned with thiamethoxam 0.003 per cent was significantly superior to other treatments. This was followed by 4.77 cm growth in chlorantraniliprole 0.004 and thiamethoxam 0.005 and 0.008 per cent. Acephate 0.075 per cent treated media supported growth of 4.73 cm and was on par with that in 0.006 per cent chlorantraniliprole (4.63 cm) while acephate 0.150 and chlorantraniliprole 0.008 per cent added media recorded 4.60 and 4.57 cm, respectively. Significantly lower mean growth of 4.47 cm in 0.225 per cent acephate was recorded as in the earlier observation. The highest mean growth of 8.40 cm in the unpoisoned media was significantly superior to poisoned media.

### *4.5.4.2 Spore Count*

The highest spore count of 3.53 x $10^9$ spores $ml^{-1}$ of *A. flavus* (Af-ml) noted in media poisoned with chlorantraniliprole 0.008 per cent spores $ml^{-1}$ was on par with that in the unpoisoned media (3.63 x $10^9$) (Table 45). The spore count

Table 44. Growth of *A. flavus* (Af-m1) in poisoned media

| Treatments | Mean growth (cm) | | |
|---|---|---|---|
| | 3 DAI | 5 DAI | 7 DAI |
| Acephate 0.075 % | 1.90 (1.38) | 2.50 (1.58) | 4.73 (2.17) |
| Acephate 0.150 % | 1.83 (1.35) | 2.40 (1.55) | 4.60 (2.14) |
| Acephate 0.225 % | 1.80 (1.34) | 2.27 (1.51) | 4.47 (2.11) |
| Chlorantraniliprole 0.004 % | 2.40 (1.55) | 2.80 (1.67) | 4.77 (2.18) |
| Chlorantraniliprole 0.006 % | 2.27 (1.51) | 2.73 (1.65) | 4.63 (2.15) |
| Chlorantraniliprole 0.008 % | 2.23 (1.49) | 2.70 (1.64) | 4.57 (2.13) |
| Thiamethoxam 0.003 % | 2.33 (1.53) | 2.80 (1.67) | 4.90 (2.21) |
| Thiamethoxam 0.005 % | 2.13 (1.46) | 2.67 (1.63) | 4.77 (2.18) |
| Thiamethoxam 0.008 % | 2.07 (1.44) | 2.67 (1.63) | 4.77 (2.18) |
| Unpoisoned | 2.53 (1.59) | 3.00 (1.73) | 8.40 (2.89) |
| CD (0.05) | (0.039) | (0.037) | (0.022) |

Figures in parentheses are $\sqrt{x}$ transformed values
Mean of three replications
DAI- Days after inoculation

in chlorantraniliprole 0.006, 0.004 and thiamethoxam 0.008 per cent was 3.37 x $10^9$, 3.33 x $10^9$ and 3.33 x $10^9$ spores $ml^{-1}$, respectively and were on par. This was followed by spore count of 3.20 x $10^9$ and 3.13 x $10^9$ spores $ml^{-1}$ produced by *A. flavus* (Af-ml) in thiamethoxam 0.005 and 0.003 per cent mixed media, respectively. This fungus when grown in acephate 0.150 and 0.225 per cent mixed media produced spores to the tune of 2.87 x $10^9$ and 3.00 x $10^9$ spores $ml^{-1}$, respectively and were on par. A significantly lower sporulation (2.60 x $10^9$ spores ml) had shown by the fungus grown in acephate 0.075 per cent mixed media.

### *4.5.4.3 Conidial Viability*

There was no significant difference in the viability of *A. flavus* (Af-ml) grown in various poisoned and unpoisoned media (Table 45). The highest germination percentage observed in unpoisoned media (88.00) was closely followed by that of the conidia in chlorantraniliprole 0.008, 0.006 and 0.004 per cent poisoned media which showed 84.00, 84.00 and 82.67 per cent germination, respectively. In thiamethoxam 0.003, 0.005 and 0.008 per cent mixed media, the viability was 80.00, 81.33 and 82.67 per cent, respectively while the conidia in acephate 0.075, 0.150 and 0.225 per cent recorded 80.00 per cent each.

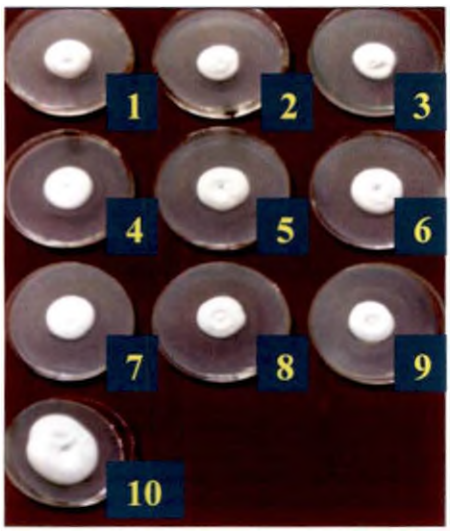
### *4.5.2.4 Bioefficacy*

There was no significant difference in the bioefficacy of *A. flavus* (Af-ml) grown in different poisoned and unpoisoned media (83.33) (Table 45). The highest mean mortality of 93.33 per cent induced by the fungus grown in chlorantraniliprole 0.008 per cent mixed media was followed by that in acephate 0.225 and chlorantraniliprole 0.006 per cent which recorded 86.67 per cent mortality each. The mortality of *L. acuta* caused by the fungus grown in acephate 0.150, thiamethoxam 0.005 and 0.008 per cent were 83.33 per cent each while that in 0.075 acephate and 0.003 thiamethoxam were 80.00 per cent each.

Table 45. Spore count, germination and bioefficacy of *A. flavus* (Afm1) in poisoned media

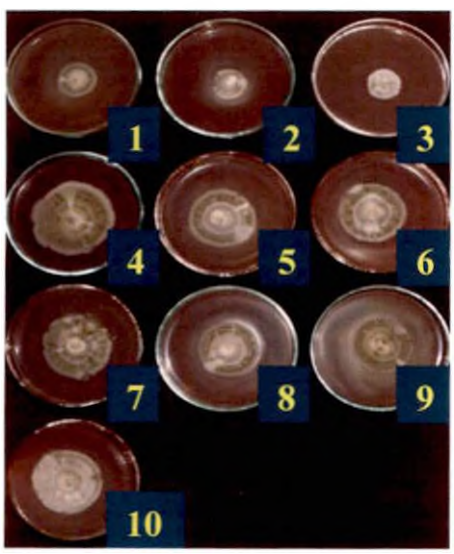
| Treatments | Spore count at 7 DAI ($10^9$ spores $ml^{-1}$) | Germination (%) | Mortality of *L. acuta* at 10 DAT (%) |
|---|---|---|---|
| Acephate 0.075 % | 2.60 (1.61) | 80.00 (8.94) | 80.00 (8.94) |
| Acephate 0.150 % | 2.87 (1.69) | 80.00 (8.94) | 83.33 (9.13) |
| Acephate 0.225 % | 3.00 (1.73) | 80.00 (8.94) | 86.67 (9.31) |
| Chlorantraniliprole 0.004 % | 3.33 (1.83) | 82.67 (9.09) | 83.33 (9.13) |
| Chlorantraniliprole 0.006 % | 3.37 (1.84) | 84.00 (9.16) | 86.67 (9.31) |
| Chlorantraniliprole 0.008 % | 3.53 (1.88) | 84.00 (9.16) | 93.33 (9.61) |
| Thiamethoxam 0.003 % | 3.13 (1.77) | 80.00 (8.94) | 80.00 (8.94) |
| Thiamethoxam 0.005 % | 3.20 (1.79) | 81.33 (9.02) | 83.33 (9.13) |
| Thiamethoxam 0.008 % | 3.33 (1.83) | 82.67 (9.09) | 83.33 (9.13) |
| Unpoisoned | 3.63 (1.91) | 88.00 (9.24) | 83.33 (9.13) |
| CD (0.05) | (0.042) | NS | NS |

Figures in parentheses are $\sqrt{x}$ transformed values
Mean of three replications
DAI- Days after inoculation
DAT- Days after treatment
NS- Non significant

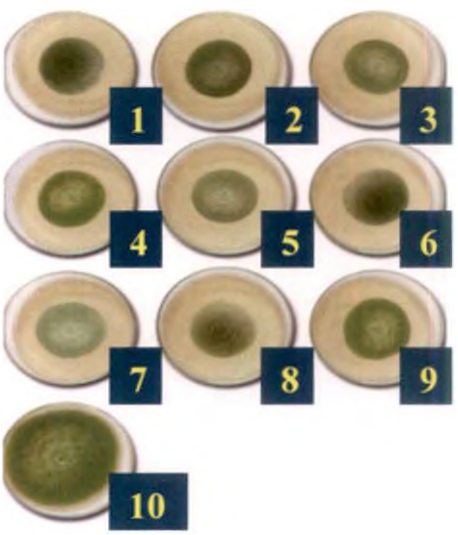



(A) *B.bassiana* (Bb5)


(B) *B.bassiana* (Bb21)




(C) *M.anisopliae* (Ma4)




(D) *A. flavus* (Af-m1)

1 - Acephate 0.075 %
2 - Acephate 0.150 %
3 - Acephate 0.225 %
4 - Chlorantraniliprole 0.004 %
5 - Chlorantraniliprole 0.006 %
6 - Chlorantraniliprole 0.008 %
7 - Thiamethoxam 0.003 %
8 - Thiamethoxam 0.005 %
9 - Thiamethoxam 0.008 %
10 - Unpoisoned

Plate 6. Growth of fungi in poisoned media

## 4.6 DEVELOPMENT OF PESTICIDE TOLERANT STRAINS AND THEIR MOLECULAR CHARACTERISATION

### 4.6.1 Assessment of Pesticide Tolerance

#### *4.6.1.1 Fixing the Highest Tolerable Dose*

For fixing the highest tolerable dose of *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) they were grown in varying doses of insecticides *i.e.,* x, 2x, 4x, 4.5x and 5x of acephate and x, 2x, 4x, 8x, 8.5x and 9x of chlorantraniliprole and thiamethoxam, x being the field dose. The results on the growth of fungi are presented in Table 46.

*B. bassiana* (Bb 5) had mean growth of 3.13, 2.47 and 1.63 cm in media poisoned with 0.150 (x), 0.300 (2x) and 0.600 (4x) per cent of acephate whereas *M. anisopliae* (Ma 4) had growth of 3.27, 2.53 and 2.07 cm, respectively. At 0.675 (4.5x) and 0.750 (5x) per cent concentration of acephate in the media, no growth was observed for both the fungi. In media containing chlorantraniliprole 0.006 (x), 0.012 (2x), 0.024 (4x) and 0.048 (8x) per cent, *B. bassiana* (Bb 5) had mean growth of 4.23 cm, 3.97 cm, 3.47cm and 2.07 cm, respectively. Corresponding mean growth of *M. anisopliae* (Ma 4) was 4.33 cm, 4.03 cm, 3.50 cm and 2.20, respectively. While in media mixed with thiamethoxam at 0.005 (x), 0.010 (2x), 0.020 (4x) and 0.040 (8x) percent, *B. bassiana* (Bb 5) had mean growth of 4.07 cm, 3.90 cm, 3.63 cm and 2.23 cm whereas *M. anisopliae* (Ma 4) had growth of 4.20, 4.03, 3.77 and 2.37 cm respectively. Both the fungi had no growth at 0.051 (8.5 x) and 0.054 per cent (9x) of chlorantraniliprole and 0.043 (8.5x) and 0.045per cent (9x) of thiamethoxam. Based on these results, 0.60 per cent (4x) of acephate and 0.048 per cent (8x) of chlorantraniliprole and 0.040 per cent (8x) of thiamethoxam were fixed as the highest tolerable doses of *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4).

Table 46. Growth of *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) in poisoned media

| Treatments | Growth at 14 DAI (cm) | |
|---|---|---|
| | *B. bassiana* (Bb 5) | *M. anisopliae* (Ma 4) |
| Acephate 0.150 % (x) | 3.13 | 3.27 |
| Acephate 0.300 % (2x) | 2.47 | 2.53 |
| Acephate 0.600 % (4x) | 1.63 | 2.07 |
| Acephate 0.675 % (4.5x) | 0.00 | 0.00 |
| Acephate 0.750 % (5x) | 0.00 | 0.00 |
| Chlorantraniliprole 0.006 % (x) | 4.23 | 4.33 |
| Chlorantraniliprole 0.012 % (2x) | 3.97 | 4.03 |
| Chlorantraniliprole 0.024 % (4x) | 3.47 | 3.50 |
| Chlorantraniliprole 0.048 % (8x) | 2.07 | 2.20 |
| Chlorantraniliprole 0.051 % (8.5x) | 0.00 | 0.00 |
| Chlorantraniliprole 0.054 % (9x) | 0.00 | 0.00 |
| Thiamethoxam 0.005 % (x) | 4.07 | 4.20 |
| Thiamethoxam 0.010 % (2x) | 3.90 | 4.03 |
| Thiamethoxam 0.020 % (4x) | 3.63 | 3.77 |
| Thiamethoxam 0.040 % (8x) | 2.23 | 2.37 |
| Thiamethoxam 0.043 % (8.5x) | 0.00 | 0.00 |
| Thiamethoxam 0.045 % (9x) | 0.00 | 0.00 |
| Unpoisoned | 4.50 | 4.70 |

Mean of three replications.

### *4.6.1.2 Culturing of Fungi for Development of Insecticide Tolerant Strains*

*B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) grown in the highest tolerable doses of the respective insecticides were separated into two, as selected (SB-acephate, SB- chlorantraniliprole, SB-thiamethoxam for *B. bassiana* (Bb 5) and SM-acephate, SM- chlorantraniliprole, SM-thiamethoxam for *M. anisopliae*, Ma 4) and relaxed (RB-acephate, RB- chlorantraniliprole, RB-thiamethoxam for *B. bassiana* (Bb 5) and RM-acephate, RM- chlorantraniliprole, RM-thiamethoxam for *M. anisopliae* Ma 4). The selected fungi were further passed through poisoned media containing the highest tolerable dose of the respective insecticides for ten passages while the relaxed fungi were grown for ten passages in unpoisoned media. *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) grown in unpoisoned PDA from the initiation of the experiment formed the untreated check,UB and UM for *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4), respectively. The growth, spore count and bioefficacy of both the selected and relaxed cultures of *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) in each passage are presented in Tables 47 to 52.

#### *4.6.1.2.1 B. bassiana (Bb 5)*

##### *4.6.1.2.1.1 Growth*

Growth of selected relaxed and untreated cultures of *B. bassiana* (Bb 5) is presented in Table 47.

During the first passage, the growth of relaxed cultures, RB-thiamethoxam, RB-chlorantraniliprole and RB-acephate were 5.07, 5.03 and 5.03 cm and the growth was significantly superior to that of their corresponding selected cultures (SB-thiamethoxam, SB-chlorantraniliprole, SB-acephate), the values being 2.37, 2.13 and 1.47 cm, respectively. The growth of all the relaxed fungi was on par whereas the growth of selected fungi showed variation; the growth of SB-acephate was significantly lower than that of the SB-thiamethoxam

and SB-chlorantraniliprole. The growth of untreated check (UB) was statistically similar to that in the relaxed cultures (5.10 cm).

The trend was similar in the second passage too. The growth of RB-thiamethoxam and RB-chlorantraniliprole were 5.07 cm each while the growth of SB-thiamethoxam and SB-chlorantraniliprole were only 2.50 and 2.57 cm, respectively. RB-acephate and SB-acephate had grown to the extent of 5.00 and 1.93 cm, respectively. As in the first passage, the growth of all the relaxed cultures was on par while the growth of SB-acephate was significantly lower than that of SB-thiamethoxam and SB-chlorantraniliprole. Untreated (UB) had a growth 5.20 cm and was on par with that of the relaxed cultures.

During the third passage also, significant difference in the mean growth of the selected and relaxed cultures were seen. The growth of RB-thiamethoxam (4.97 cm) was significantly superior to that of SB-thiamethoxam (2.40 cm). Similarly, RB-acephate and RB-chlorantraniliprole had growth of 4.83 and 4.43 cm, respectively and showed significant superiority over the growth of SB-acephate and SB-chlorantraniliprole (2.03 and 3.10 cm). Among the selected cultures, the growth of SB-chlorantraniliprole was significantly superior to that of SB-acephate and SB-thiamethoxam while that of the relaxed cultures was on par. The untreated fungus (UB) had growth of 5.17 cm and was statistically on par with the growth in RB-thiamethoxam and RB-acephate.

Growth in the fourth passage followed the same trend as in the previous one. RB-acephate, RB-thiamethoxam and RB-chlorantraniliprole had a growth of 4.83, 4.87 and 4.37 cm, respectively while that of SB-acephate, SB-thiamethoxam and SB-chlorantranilirpole was only 2.47, 2.87 and 3.07 cm, respectively. The higher growth in SB-chlorantranilirpole was on par with that in the SB-thiamethoxam, but significantly higher than that of SB-acephate. Growth of untreated fungus (UB) was 5.30 cm and it was on par with the growth in RB-thiamethoxam and RB-acephate.

During the fifth passage also, the growth of the relaxed and selected cultures of *B. bassiana* (Bb 5) was significantly different. The growth of RB-acephate (4.83 cm), RB-thiamethoxam (4.63 cm) and RB-chlorantraniliprole (4.30 cm) was higher than that of the corresponding selected cultures, the values being 2.93, 3.33 and 2.97 cm, respectively. It was also seen that the growth of all the selected cultures was on par and the untreated check (UB) that had mean growth of 5.37 cm was on par with RB-acephate.

In the sixth, seventh and eighth passages also the growth of the relaxed and selected cultures showed significant differences, but it was seen that the growth of the relaxed cultures followed a decreasing trend while that of the selected cultures showed an increasing trend in all these three passages. RB-acephate had 4.80, 4.57 and 4.53 cm, respectively in the sixth, seventh and eighth while the corresponding values of SB-acephate were 2.63, 3.00 and 3.20 cm. The mean growth of RB-chlorantraniliprole was 4.73, 4.30 and 4.13 and that of SB-chlorantraniliprole was 2.93, 3.33 and 3.57 cm, respectively for the three passages. The growth of RB-thiamethoxam was to the tune of 4.67, 4.43, 4.37 whereas that of SB-thiamethoxam was only 3.03, 3.57 and 4.00 cm, respectively. In the sixth passage, the growth of all the selected cultures was on par while in the seventh and eighth passages, SB-thiamethoxam had higher growth which was on par with the growth of SB-chlorantraniliprole and it also showed significant superiority over SB-acephate. The growth of untreated (UB) was 4.47, 4.47 and 4.43 cm, respectively for the three passages and was on par with that of the relaxed cultures.

During the ninth passage, SB-thiamethoxam attained a growth of 4.57 cm and was even significantly superior to RB-thiamethoxam (4.13 cm). SB-chlorantraniliprole and RB-chlorantraniliprole attained statistical similarity with a mean growth of 3.97 and 4.00 cm, respectively. RB-acephate had growth to the extent of 4.13 cm and was significantly superior to SB-acephate (3.57 cm). SB-thiamethoxam had significant superiority over SB-chlorantraniliprole and SB-acephate with respect to the growth while the growth of all the relaxed cultures

was on par. Untreated check (UB) had growth of 4.37 cm which was on par with that of the relaxed cultures.

At the tenth passage, SB-thiamethoxam had significantly superior growth of 5.43 cm than RB-thiamethoxam (4.00 cm) and it was significantly higher than that of untreated check (UB) (4.33 cm). There was no significant difference in the growth between SB-chlorantraniliprole (4.23) and RB-chlorantraniliprole (3.87 cm) and also between SB-acephate (4.03 cm) and RB-acephate (4.03 cm). As in the ninth passage, SB-thiamethoxam had significant superiority over the other two.

*4.6.1.2.1.2 Spore Count*

The sporulation of the selected, relaxed and untreated cultures during the ten passages is shown in Table 48.

The spore count of selected cultures of *B. bassiana* (Bb 5) was significantly superior to that in the relaxed cultures in all the passages. In the first passage, SB-chlorantraniliprole produced 3.30 x $10^9$ spores $ml^{-1}$ while RB-chlorantraniliprole had only 2.47 x $10^9$ spores $ml^{-1}$. SB-thiamethoxam and RB-thiamethoxam had spore production to the tune of 3.20 x $10^9$ and 2.63 x $10^9$ spores $ml^{-1}$, respectively while in SB-acephate and RB-acephate it was still lower, 2.87 x $10^9$ and 1.93 x $10^9$ spores $ml^{-1}$, respectively. SB-chlorantraniliprole and SB-thiamethoxam had significantly higher spore count than SB-acephate and same trend was observed in the relaxed cultures too. A higher spore count of 5.13 x $10^9$ spore $ml^{-1}$ observed for untreated fungus (UB) was significantly superior to all others.

The trend in the second, third and fourth passages were similar and SB-chlorantraniliprole had spore production of 3.37 x $10^9$ spores $ml^{-1}$ in each of these passages while the relaxed cultures had 2.53, 2.63 and 2.57 x $10^9$ spores $ml^{-1}$, respectively. Similarly, SB-thiamethoxam and SB-acephate favoured spore production of 3.17, 3.27 and 3.27 x $10^9$ spores $ml^{-1}$ and 2.83 x $10^9$, 2.77 x $10^9$ and

Table 47. Growth of selected, relaxed and untreated cultures of *B. bassiana* (Bb 5) during ten passages

| Treatments | Growth (cm) at 14 DAI | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X |
| SB-acephate | 1.47 (1.21) | 1.93 (1.39) | 2.03 (1.42) | 2.47 (1.57) | 2.93 (1.71) | 2.63 (1.62) | 3.00 (1.73) | 3.20 (1.79) | 3.57 (1.89) | 4.03 (2.01) |
| SB-chlorantraniliprole | 2.13 (1.46) | 2.57 (1.60) | 3.10 (1.76) | 3.07 (1.75) | 3.33 (1.82) | 2.93 (1.71) | 3.33 (1.82) | 3.57 (1.89) | 3.97 (1.99) | 4.23 (2.06) |
| SB-thiamethoxam | 2.37 (1.54) | 2.50 (1.58) | 2.40 (1.55) | 2.87 (1.69) | 2.97 (1.72) | 3.03 (1.74) | 3.57 (1.89) | 4.00 (2.00) | 4.57 (2.14) | 5.43 (2.33) |
| RB-acephate | 5.03 (2.24) | 5.00 (2.24) | 4.83 (2.20) | 4.83 (2.20) | 4.83 (2.20) | 4.80 (2.19) | 4.57 (2.14) | 4.53 (2.13) | 4.13 (2.03) | 4.03 (2.01) |
| RB-chlorantraniliprole | 5.03 (2.24) | 5.07 (2.25) | 4.43 (2.10) | 4.37 (2.09) | 4.30 (2.07) | 4.73 (2.18) | 4.30 (2.07) | 4.13 (2.03) | 4.00 (2.00) | 3.87 (1.97) |
| RB-thiamethoxam | 5.07 (2.25) | 5.07 (2.25) | 4.97 (2.23) | 4.87 (2.21) | 4.63 (2.15) | 4.67 (2.16) | 4.43 (2.10) | 4.37 (2.09) | 4.13 (2.03) | 4.00 (2.00) |
| Untreated (UB) | 5.10 (2.26) | 5.20 (2.28) | 5.17 (2.27) | 5.30 (2.30) | 5.37 (2.32) | 4.47 (2.11) | 4.47 (2.11) | 4.43 (2.10) | 4.37 (2.09) | 4.33 (2.08) |
| CD (0.05) | (0.124) | (0.101) | (0.147) | (0.133) | (0.144) | (0.134) | (0.134) | (0.129) | (0.104) | (0.118) |

Figures in parentheses are $\sqrt{x}$ transformed values.
Mean of three replications
DAI- Days after inoculation

2.77 x $10^9$ spores $ml^{-1}$, respectively, while their relaxed cultures had only 2.43, 2.43 and 2.40 x $10^9$ spores $ml^{-1}$ and 2.07, 2.27 and 2.40 x $10^9$ spore $ml^{-1}$, respectively. Significant superiority of SB-chlorantraniliprole over SB-thiamethoxam and SB-acephate was seen in the second passage while in the third and the fourth it was on par with that of SB-thiamethoxam. The highest mean spore count of 5.37 x $10^9$, 5.10 x $10^9$ and 5.13 x $10^9$ spores $ml^{-1}$ in the second, third and fourth subcultures, respectively was observed for untreated check (UB).

In the fifth passage, SB-chlorantraniliprole, SB-thiamethoxam and SB-acephate had spore production of 3.17 x $10^9$, 3.13 x $10^9$, 2.67 x $10^9$ spores $ml^{-1}$, respectively and RB-chlorantraniliprole, RB-thiamethoxam and RB-acephate had 2.37 x $10^9$, 2.40 x $10^9$ and 2.40 x $10^9$ spores $ml^{-1}$, respectively while the untreated (UB) produced the highest mean spore count of 5 x $10^9$ spores $ml^{-1}$ and was statistically superior to the other treatments. SB-chlorantraniliprole and SB-thiamethoxam had significantly higher spore count than SB-acephate while that of all the relaxed cultures were on par.

In the sixth passage, SB-chlorantraniliprole and RB-chlorantraniliprole had spore production of 3.07 and 2.13 x $10^9$ spores $ml^{-1}$, respectively while that in SB-acephate and SB-thiamethoxam were 2.53 and 3.17 x $10^9$ spores $ml^{-1}$ and that in RB-acephate and RB-thiamethoxam was 2.03 and 2.20 x $10^9$ spores $ml^{-1}$, respectively. As in the earlier passage SB-thiamethoxam and SB-chlorantraniliprole had significantly higher spore count than SB-acephate while all the relaxed cultures were on par. The spore production of untreated fungus (UB) reduced substantially and it was significantly lower (0.49 x $10^9$ spores $ml^{-1}$) than selected and relaxed cultures.

From the seventh passage onwards, spore count of all the relaxed cultures showed substantial reduction. SB-chlorantraniliprole in the seventh, eighth, ninth and tenth passages yielded spores to the extent of 3.17, 3.20. 3.33 and 3.27 x $10^9$ spores $ml^{-1}$, respectively while that in relaxed cultures were 0.15, 0.02, 0.02 and 0.02 x $10^9$ spores $ml^{-1}$ only. Similarly, higher sporulation of 3.20, 3.40, 3.27, 3.17

x $10^9$ spores $ml^{-1}$ was observed in SB-thiamethoxam, while the sporulation in RB-thiamethoxam was only 0.15, 0.02, 0.02 and 0.02 x $10^9$ spores $ml^{-1}$. SB-acephate and RB-acephate produced spores in a similar way, the values being 2.60, 2.57, 2.77, 2.70 x $10^9$ spores $ml^{-1}$ for SB-acephate and 0.21, 0.02, 0.02 and 0.01 x $10^9$ spores $ml^{-1}$, respectively for RB-acephate. From the seventh to tenth passages, SB-thiamethoxam and SB-chlorantraniliprole had significantly higher spore count than SB-acephate while all the relaxed cultures had statistically similar spore count. In these observations also, the spore production was the least in the untreated check (UB) and the corresponding values were 0.48, 0.02, 0.02 and 0.01 x $10^9$ spores $ml^{-1}$.

***4.6.1.2.1.3 Bioefficacy***

Bioefficacy of selected, relaxed and untreated *B. bassisana* (Bb 5) against *L. acuta* are presented in Table 49. There was no significant difference in the bioefficacy of selected, relaxed and untreated cultures for the passages from one to five. From the first to fifth passage SB-chlorantraniliprole showed mortality of 83.33 per cent each while RB-chlorantraniliprole caused mortality of *L. acuta* that ranged from 70.00 to 76.67 per cent during these passages. The percentage mortality by SB-thiamethoxam and RB-thiamethoxam varied between 86.67 to 80.00 per cent and 70.00 to 76.67 per cent, respectively in the five passages. SB-acephate and RB-acephate produced mortality of 76.67 per cent each and 73.33 to 76.67 per cent, respectively. The untreated check (UB) caused mortality that ranged from 76.67 to 83.33 per cent during the five passages.

In the sixth passage, bioefficacy of the selected and relaxed fungi of each insecticide was on par. SB-thiamethoxam and RB-thiamethoxam caused mortality of 80.00 and 66.67 per cent, respectively. Similarly, SB-chlorantraniliprole and SB-acephate produced 76.67 per cent mortality each, which was on par with the mortality by RB-chlorantraniliprole and RB-acephate (66.67 and 63.33 per cent), respectively. Untreated (UB) recorded significantly lower mortality of 46.67 per cent only.

Table 48. Spore count of selected, relaxed and untreated cultures of *B. bassiana* (Bb 5) during ten passages

| Treatments | Spore count ($10^9$ spores $ml^{-1}$) at 14 DAI | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X |
| SB-acephate | 2.87 (1.69) | 2.83 (1.68) | 2.77 (1.66) | 2.77 (1.66) | 2.67 (1.63) | 2.53 (1.59) | 2.60 (1.61) | 2.57 (1.60) | 2.77 (1.66) | 2.70 (1.64) |
| SB-chlorantraniliprole | 3.30 (1.82) | 3.37 (1.84) | 3.37 (1.84) | 3.37 (1.84) | 3.17 (1.78) | 3.07 (1.75) | 3.17 (1.78) | 3.20 (1.79) | 3.33 (1.83) | 3.27 (1.81) |
| SB-thiamethoxam | 3.20 (1.79) | 3.17 (1.78) | 3.27 (1.81) | 3.27 (1.81) | 3.13 (1.77) | 3.17 (1.78) | 3.20 (1.79) | 3.40 (1.84) | 3.27 (1.81) | 3.17 (1.78) |
| RB-acephate | 1.93 (1.39) | 2.07 (1.44) | 2.27 (1.51) | 2.40 (1.55) | 2.40 (1.55) | 2.03 (1.43) | 0.21 (0.46) | 0.02 (0.14) | 0.02 (0.14) | 0.01 (0.07) |
| RB-chlorantraniliprole | 2.47 (1.57) | 2.53 (1.59) | 2.63 (1.62) | 2.57 (1.60) | 2.37 (1.54) | 2.13 (1.46) | 0.15 (0.39) | 0.02 (0.14) | 0.02 (0.14) | 0.02 (0.14) |
| RB-thiamethoxam | 2.63 (1.62) | 2.43 (1.56) | 2.43 (1.56) | 2.40 (1.55) | 2.40 (1.55) | 2.20 (1.48) | 0.15 (0.39) | 0.02 (0.14) | 0.02 (0.14) | 0.02 (0.14) |
| Untreated (UB) | 5.13 (2.27) | 5.37 (2.32) | 5.10 (2.26) | 5.13 (2.27) | 5.00 (2.24) | 0.49 (0.70) | 0.48 (0.69) | 0.02 (0.14) | 0.02 (0.14) | 0.01 (0.07) |
| CD (0.05) | (0.051) | (0.056) | (0.062) | (0.072) | (0.063) | (0.065) | (0.048) | (0.035) | (0.045) | (0.033) |

Figures in parentheses are $\sqrt{x}$ transformed values.
Mean of three replications
DAI- Days after inoculation

From the seventh to tenth passages, bioefficacy of selected and relaxed fungi showed significant differences, the selected ones showed superior performance. SB-thiamethoxam caused mortality of 80.00, 76.67, 73.33 and 70.00 per cent, respectively in the seventh, eighth, ninth and tenth passages while RB-thiamethoxam caused only 60.00, 56.67, 46.67 and 46.67 per cent mortality. SB-acephate caused 70.00 per cent mortality in all these passages, while SB-chlorantraniliprole caused mortality varying from 73.33 to 70.00 per cent. RB-acephate and RB-chlorantraniliprole caused 53.33 to 43.33 and 60.00 to 46.67 per cent, respectively. The mortality caused by the selected fungal cultures was on par during these passages and same was with the case of relaxed cultures too. During the passages from seventh to tenth, untreated fungus caused mortality from 43.33 to 30.00 per cent only.

#### *4.6.1.2.2 M. anisopliae (Ma 4)*

##### *4.6.1.2.2.1 Growth*

The growth of selected, relaxed and untreated *M. anisopliae* (Ma 4) during the ten passages is given in Table 50.

From the first to seventh passage, the growth of relaxed and selected fungus varied significantly. The growth of RM-thiamethoxam during these passages was 5.37, 5.47, 5.53, 5.50, 5.63, 5.57, 5.53 cm and that of SM-thiamethoxam was 2.53, 2.80, 3.07, 3.10, 3.63, 3.90 and 4.03 cm, respectively. RM-chlorantraniliprole and SM-chlorantraniliprole had growth of 5.27, 5.37, 5.37, 5.30, 5.43, 5.27, 5.37 cm and 2.47, 2.73, 2.93, 3.30, 3.20, 3.80, 4.07 cm, respectively. RM-acephate had growth of 3.70, 4.03, 4.17, 4.50, 4.87, 4.77 and 5.10 while SM- acephate had 2.33, 2.53, 2.80, 3.13, 2.47, 3.37 and 3.63 cm, respectively. All the selected cultures were on par from the first to fourth passages, whereas in the fifth, sixth and seventh the growth of SM-chlorantraniliprole and SM- thiamethoxam was on par and significantly superior to that of SM-acephate. The untreated fungus (UM) attained growth of 5.33, 5.43,

Table 49. Bioefficacy of selected, relaxed and untreated cultures of *B. bassiana* (Bb 5) against *L. acuta*

| Treatments | Number of passages in poisoned / unpoisoned media | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X |
| | Mortality (%) of *L. acuta* at 10 DAT | | | | | | | | | |
| SB-acephate | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 70.00 (8.35) | 70.00 (8.35) | 70.00 (8.35) | 70.00 (8.35) |
| SB-chlorantraniliprole | 83.33 (9.13) | 83.33 (9.13) | 83.33 (9.13) | 83.33 (9.13) | 83.33 (9.13) | 76.67 (8.75) | 73.33 (8.56) | 70.00 (8.35) | 70.00 (8.35) | 70.00 (8.35) |
| SB-thiamethoxam | 86.67 (9.31) | 83.33 (9.13) | 83.33 (9.13) | 83.33 (9.13) | 80.00 (8.94) | 80.00 (8.94) | 80.00 (8.94) | 76.67 (8.75) | 73.33 (8.56) | 70.00 (8.35) |
| RB-acephate | 76.67 (8.75) | 73.33 (8.56) | 76.67 (8.75) | 76.67 (8.75) | 73.33 (8.56) | 63.33 (7.95) | 53.33 (7.29) | 53.33 (7.29) | 46.67 (6.82) | 43.33 (6.57) |
| RB-chlorantraniliprole | 73.33 (8.56) | 73.33 (8.56) | 76.67 (8.75) | 70.00 (8.35) | 76.67 (8.75) | 66.67 (8.16) | 60.00 (7.75) | 56.67 (7.50) | 46.67 (6.82) | 46.67 (6.82) |
| RB-thiamethoxam | 73.33 (8.56) | 76.67 (8.75) | 76.67 (8.75) | 70.00 (8.35) | 76.67 (8.75) | 66.67 (8.16) | 60.00 (7.75) | 56.67 (7.50) | 46.67 (6.82) | 46.67 (6.82) |
| Untreated (UB) | 80.00 (8.94) | 83.33 (9.13) | 83.33 (9.13) | 80.00 (8.93) | 76.67 (8.75) | 46.67 (6.82) | 43.33 (6.57) | 40.00 (6.33) | 30.00 (5.43) | 30.00 (5.48) |
| CD (0.05) | NS | NS | NS | NS | NS | (0.889) | (0.641) | (0.665) | (0.909) | (0.879) |

Figures in parentheses are $\sqrt{x}$ transformed values.
Mean of three replications DAI- Days after inoculation, DAT- Days after treatment
NS-Non significant

5.37, 5.33, 5.43, 5.10 and 4.87 cm in the passages from one to seven and was on par with relaxed chlorantraniliprole and thiamethoxam till the fifth passage.

In the eighth passage, the growth of the selected cultures was same as that in the previous passages. RM- chlorantraniliprole and SM-chlorantraniliprole had growth of 4.83 and 4.63 cm, respectively and were on par. However, the growth in the relaxed and selected thiamethoxam and acephate cultures were significantly different, the values being 5.03, 4.53 cm and 4.43, 3.93 cm, respectively. The growth of untreated fungus was 4.77 cm and was on par with that of the relaxed chlorantraniliprole and thiamethoxam.

During the ninth passage, the growth of relaxed and selected cultures of acephate and chlorantraniliprole was on par. RM-acephate and SM-acephate had growth of 4.23 and 4.17 cm, respectively while RM-chlorantraniliprole and SM-chlorantraniliprole had growth of 4.73 and 4.50 cm, respectively. The growth of RM-thiamethoxam (4.93 cm) was statistically superior to that in SM-thiamethoxam (4.27 cm). The growth of SM-chlorantraniliprole was significantly superior to that of SM-thiamethoxam and SM-acephate and the growth of 4.53 cm of untreated fungus (UM) was on par with that of the relaxed chlorantraniliprole and thiamethoxam.

The growth of relaxed and selected fungi during the tenth passage was significantly different. The growth of RM-chlorantraniliprole and SM-chlorantraniliprole were 4.53 and 4.27 cm, respectively, while that of thiamethoxam and acephate were 4.53 and 3.90 cm and 3.07 and 4.00 cm, respectively. The growth of SM- chlorantraniliprole was superior to the other two in the tenth passage also. The growth of RM-acephate was significantly lower than that of RM-chlorantraniliprole and RM-thiamethoxam in all the ten passages. The untreated fungus (UM) attained growth of 4.33 cm and was on par with the growth of the relaxed chlorantraniliprole and thiamethoxam.

Table 50. Growth of selected, relaxed and untreated cultures of *M. anisopliae* (Ma 4) during ten passages

| Treatments | Growth (cm) at 14 DAI | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X |
| SM-acephate | 2.33 (1.53) | 2.53 (1.59) | 2.80 (1.67) | 3.13 (1.77) | 2.47 (1.57) | 3.37 (1.83) | 3.63 (1.91) | 3.93 (1.98) | 4.17 (2.04) | 4.00 (2.00) |
| SM-chlorantraniliprole | 2.47 (1.57) | 2.73 (1.65) | 2.93 (1.71) | 3.30 (1.82) | 3.20 (1.79) | 3.80 (1.95) | 4.07 (2.02) | 4.63 (2.15) | 4.50 (2.12) | 4.27 (2.07) |
| SM-thiamethoxam | 2.53 (1.59) | 2.80 (1.67) | 3.07 (1.75) | 3.10 (1.76) | 3.63 (1.90) | 3.90 (1.97) | 4.03 (2.01) | 4.53 (2.13) | 4.27 (2.06) | 3.90 (1.97) |
| RM-acephate | 3.70 (1.92) | 4.03 (2.01) | 4.17 (2.04) | 4.50 (2.12) | 4.87 (2.21) | 4.77 (2.18) | 5.10 (2.26) | 4.43 (2.11) | 4.23 (2.06) | 3.07 (1.75) |
| RM-chlorantraniliprole | 5.27 (2.29) | 5.37 (2.32) | 5.37 (2.32) | 5.30 (2.30) | 5.43 (2.33) | 5.27 (2.29) | 5.37 (2.32) | 4.83 (2.19) | 4.73 (2.18) | 4.53 (2.13) |
| RM-thiamethoxam | 5.37 (2.32) | 5.47 (2.34) | 5.53 (2.35) | 5.50 (2.35) | 5.63 (2.37) | 5.57 (2.36) | 5.53 (2.35) | 5.03 (2.24) | 4.93 (2.22) | 4.53 (2.13) |
| Untreated (UM) | 5.33 (2.31) | 5.43 (2.33) | 5.37 (2.32) | 5.33 (2.31) | 5.43 (2.33) | 5.10 (2.26) | 4.87 (2.21) | 4.77 (2.18) | 4.53 (2.13) | 4.33 (2.08) |
| CD (0.05) | (0.117) | (0.130) | (0.096) | (0.106) | (0.101) | (0.069) | (0.081) | (0.085) | (0.069) | (0.051) |

Figures in parentheses are $\sqrt{x}$ transformed values.
Mean of three replications
DAI- Days after inoculation

*4.6.1.2.2.2 Spore Count*

The spore count of selected, relaxed and untreated *M. anisopliae* (Ma 4) during the passages from one to ten is given in Table 51. From the first to fifth passage, there was significant difference in the spore count of selected, relaxed and untreated fungus. All the selected cultures had significantly higher spore count compared to the relaxed cultures. SM-thiamethoxam, SM-chlorantraniliprole and SM-acephate had spore count of 3.50, 3.43, 3.07 x $10^9$ spores $ml^{-1}$ in the first, 3.43, 3.50 and 3.17 x $10^9$ spores $ml^{-1}$in the second, 3.30, 3.47, 3.23 x $10^9$ spores $ml^{-1}$ in the third, 3.07, 3.17, 3.33 x $10^9$ spores $ml^{-1}$ in the fourth and 3.50, 3.37, 3.10 x $10^9$ spores $ml^{-1}$ in the fifth whereas the corresponding values in relaxed cultures were 2.07, 2.13, 2.17, 2.07, 1.87, 2.07, 2.00, 2.07, 2.07, 2.27, 2.60, 2.53, 2.03, 1.97 and 1.87 x $10^9$ spores $ml^{-1}$. In the first and second passages, SM-chlorantraniliprole and SM-thiamethoxam were on par. Significantly lower spore count was seen in SM-acephate had than SM-chlorantraniliprole in both the passages. However, it was on par with SM-thiamethoxam in the second passage. In the third and the fourth passages all the selected cultures were on par and in the fifth passage, SM-acephate showed significant reduction than the other two. The untreated fungus (UM) had significantly higher spore count of 5.00, 5.07, 5.10, 5.20 and 5.00 x $10^9$ spores $ml^{-1}$, respectively during the passages from one to five.

In the sixth passage, spore count of selected cultures had significant superiority over relaxed cultures. The spore count of SM-chlorantraniliprole, SM- thiamethoxam and SM-acephate were 3.37, 3.27 and 3.13 x $10^9$ spores $ml^{-1}$ while that of the relaxed cultures were 1.67, 1.97 and 1.80 x $10^9$ spores $ml^{-1}$, respectively. The untreated fungus (UM) showed drastic reduction in the spore count, the value being 0.01 x $10^9$ spores $ml^{-1}$.

The selected cultures showed significant superiority over relaxed and untreated fungus with respect to spore production in seventh to tenth passages. The spore count in SM- chlorantraniliprole during the respective passages from

seven to ten was 3.43, 3.30, 3.40 and 3.47 x $10^9$ spores $ml^{-1}$ while that of RM-chlorantraniliprole was 0.16, 0.15, 0.01 and 0.01 x $10^9$ spores $ml^{-1}$, respectively. SM-thiamethoxam and SM-acephate had spore count of 3.37, 3.43, 3.47, 3.57 x $10^9$ spores $ml^{-1}$and 3.03, 3.13, 3.20 and 3.37 x $10^9$ spores $ml^{-1}$, respectively while RM-thiamethoxam and RM-acephate produced 0.13, 0.17, 0.02, 0.01 x $10^9$ spores $ml^{-1}$ and 0.17, 0.16, 0.02 and 0.01 x $10^9$ spores $ml^{-1}$, respectively. The spore count of untreated fungus was only 0.01 x $10^9$ spores $ml^{-1}$ each. In all the passages from sixth to tenth, spore count of all selected cultures was statistically on par. The spore count of all relaxed cultures showed statistical similarity in most of the passages except for fourth and sixth passages, during which RM-thiamethoxam had significantly higher spore count than RM-acephate and RM-chlorantraniliprole.

***4.6.1.2.2.3 Bioefficacy***

Bioefficacy of selected, relaxed and untreated *M. anisopliae* (Ma 4) against *L. acuta* are presented in Table 52. There was no significant difference in the bioefficacy of selected, relaxed and untreated cultures for the passages from one to five.

During the passages from one to five, the mortality of *L. acuta* caused by SM-acephate ranged from 76.67 to 83.33 per cent while that caused by RM-acephate ranged from 73.33 to 76.67 per cent. The percentage mortality by SM-thiamethoxam and SM-chlorantraniliprole was 76.67 per cent each while that by RM-thiamethoxam and RM-chlorantraniliprole varied between 70.00 to 76.67 per cent in the five passages. The untreated fungus (UM) caused mortality that varied from 80.00 to 86.67 per cent during the five passages.

In the sixth and seventh passages, there was significant difference in the mortality by SM-acephate and RM-acephate (76.67, 63.33 and 76.67, 56.67 per cent), respectively whereas the mortality by SM-thiamethoxam and RM-thiamethoxam was on par, the values being 73.33 and 60.00 per cent, each. The

Table 51. Spore count of selected, relaxed and untreated cultures of *M. anisopliae* (Ma 4) in poisoned media during ten passages

| Treatments | Spore count ($10^9$ spores $ml^{-1}$) at 14 DAI | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X |
| SM-acephate | 3.07 (1.75) | 3.17 (1.78) | 3.23 (1.79) | 3.33 (1.83) | 3.10 (1.76) | 3.13 (1.77) | 3.03 (1.74) | 3.13 (1.77) | 3.20 (1.79) | 3.37 (1.84) |
| SM-chlorantraniliprole | 3.43 (1.85) | 3.50 (1.87) | 3.47 (1.86) | 3.17 (1.78) | 3.37 (1.84) | 3.37 (1.84) | 3.43 (1.85) | 3.30 (1.82) | 3.40 (1.84) | 3.47 (1.86) |
| SM-thiamethoxam | 3.50 (1.87) | 3.43 (1.85) | 3.30 (1.82) | 3.07 (1.75) | 3.50 (1.87) | 3.27 (1.81) | 3.37 (1.83) | 3.43 (1.85) | 3.47 (1.86) | 3.57 (1.88) |
| RM-acephate | 2.17 (1.47) | 2.07 (1.44) | 2.07 (1.44) | 2.53 (1.59) | 1.87 (1.37) | 1.80 (1.34) | 0.17 (0.41) | 0.16 (0.40) | 0.02 (0.14) | 0.01 (0.07) |
| RM-chlorantraniliprole | 2.13 (1.46) | 1.87 (1.37) | 2.07 (1.44) | 2.60 (1.61) | 1.97 (1.40) | 1.67 (1.29) | 0.16 (0.39) | 0.15 (0.39) | 0.01 (0.07) | 0.01 (0.07) |
| RM-thiamethoxam | 2.07 (1.44) | 2.07 (1.44) | 2.00 (1.42) | 2.27 (1.51) | 2.03 (1.43) | 1.97 (1.40) | 0.13 (0.36) | 0.17 (0.42) | 0.02 (0.14) | 0.01 (0.07) |
| Untreated (UM) | 5.00 (2.24) | 5.07 (2.25) | 5.10 (2.26) | 5.20 (2.28) | 5.00 (2.24) | 0.01 (0.07) | 0.01 (0.07) | 0.01 (0.07) | 0.01 (0.07) | 0.01 (0.07) |
| CD (0.05) | (0.067) | (0.076) | (0.076) | (0.060) | (0.047) | (0.036) | (0.048) | (0.034) | (0.021) | (0.032) |

Figures in parentheses are $\sqrt{x}$ transformed values.
Mean of three replications
DAI- Days after inoculation

effect of SM-chlorantraniliprole and RM-chlorantraniliprole in the sixth passage was on par, the mortality being 76.67 and 63.33 per cent, respectively while in the seventh passage, SM-chlorantraniliprole produced significantly higher mortality of 76.67 per cent over RM-chlorantraniliprole (53.33 per cent). The per cent mortality by untreated fungus (UM) was only 43.33 and 40.00 per cent.

In the passages from eighth to tenth, selected fungal cultures produced significantly higher mortality than the relaxed and untreated cultures. The mortality by SM-acephate during these passages was 73.33 each while that of RM-acephate was only 43.33 each in the eighth and ninth and 36.67 per cent in the tenth. SM-chlorantraniliprole caused 76.67 per cent mortality in the eighth and 73.33 per cent each in the next two passages whereas RM-chlorantraniliprole caused only 53.33, 36.67 and 33.33 per cent mortality. In SM-thiamethoxam, mortality of *L. acuta* was in the tune of 73.33 in the eighth and 70.00 each in ninth and tenth passages and RM-thiamethoxam caused only 36.67, 33.33 and 26.67 per cent mortality, respectively. The untreated fungus (UM) caused only 30.00 and 26.67 per cent mortality. There was no significant difference in the mortality between various selected cultures and also between various relaxed cultures during the passages from sixth to tenth.

### *4.6.1.3 Response of selected, relaxed and untreated cultures of B. bassiana (Bb 5) and M. anisopliae (Ma 4) after ten passages through poisoned media.*

After ten passages through poisoned / unpoisoned media, the selected, relaxed and untreated *B. bassiana* (Bb 5) / *M. anisopliae* (Ma 4) were further grown in media containing doses of insecticides higher than the maximum tolerable dose *i.e.,* 4.5 x for acephate and 8.5 x for both chlorantraniliprole and thiamethoxam. The growth of the selected, relaxed and untreated fungi in these higher concentrations is presented in Table 53.

On culturing selected, relaxed and untreated *B. bassiana* (Bb 5) in media containing 0.043 per cent (8.5x), 0.051 per cent (8.5x) and 0.675 per cent (4.5x)

Table 52. Bioefficacy of selected, relaxed and untreated cultures of *M. anisopliae* (Ma 4) against *L. acuta*

| Treatments | Number of passages in poisoned / unpoisoned media | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | X |
| | Mortality (%) of *L. acuta* at 10 DAT | | | | | | | | | |
| SM-acephate | 80.00 (8.94) | 83.33 (9.13) | 83.33 (9.13) | 80.00 (8.94) | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 73.33 (8.56) | 73.33 (8.56) | 73.33 (8.56) |
| SM-chlorantraniliprole | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 73.33 (8.56) | 73.33 (8.56) |
| SM-thiamethoxam | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 76.67 (8.75) | 73.33 (8.56) | 73.33 (8.56) | 73.33 (8.56) | 70.00 (8.37) | 70.00 (8.37) |
| RM-acephate | 73.33 (8.56) | 73.33 (8.56) | 76.67 (8.75) | 76.67 (8.75) | 73.33 (8.56) | 63.33 (7.95) | 56.67 (7.52) | 43.33 (6.54) | 43.33 (6.54) | 36.67 (6.04) |
| RM-chlorantraniliprole | 76.67 (8.75) | 76.67 (8.75) | 70.00 (8.37) | 73.33 (8.56) | 73.33 (8.56) | 63.33 (7.95) | 53.33 (7.27) | 53.33 (7.29) | 36.67 (6.04) | 33.33 (5.76) |
| RM-thiamethoxam | 73.33 (8.56) | 73.33 (8.56) | 73.33 (8.56) | 76.67 (8.75) | 70.00 (8.37) | 60.00 (7.73) | 60.00 (7.73) | 36.67 (6.04) | 33.33 (5.76) | 26.67 (5.14) |
| Untreated (UM) | 86.67 (9.31) | 86.67 (9.31) | 83.33 (9.13) | 83.33 (9.13) | 80.00 (8.94) | 43.33 (6.57) | 40.00 (6.33) | 30.00 (5.48) | 26.67 (5.14) | 26.67 (5.14) |
| CD (0.05) | NS | NS | NS | NS | NS | (0.724) | (0.832) | (0.632) | (0.980) | (0.999) |

Figures in parentheses are $\sqrt{x}$ transformed values.
Mean of three replications
DAI- Days after inoculation, DAT- Days after treatment , NS-Non significant

of thiamethoxam, chlorantraniliprole and acephate, respectively, all the selected cultures of *B. bassiana* (Bb 5) (SB- thiamethoxam, SB- chlorantraniliprole, and SB-acephate) could grow but there was no growth for the three relaxed (RB-acephate, RB-chlorantraniliprole and RB-thiamethoxam) and untreated (UB). The mean growth observed for the selected cultures (SB- thiamethoxam, SB-chlorantraniliprole, and SB-acephate) at 14 DAI was 1.50, 1.47 and 1.43 cm, respectively and was statistically similar.

Correspondingly, all the selected cultures of *M. anisopliae* (Ma 4) (SM-thiamethoxam, SM-chlorantraniliprole, and SM-acephate) could also grow in these higher concentrations and the growth noted was 1.83, 1.73 and 1.67 cm, respectively and they were significantly different. As observed in *B. bassiana* (Bb 5), all the three relaxed (RM-acephate, RM-chlorantraniliprole and RM-thiamethoxam) and untreated cultures (UM) of *M. anisopliae* (Ma 4) could not grow in media containing higher concentrations of these insecticides (Table 53).

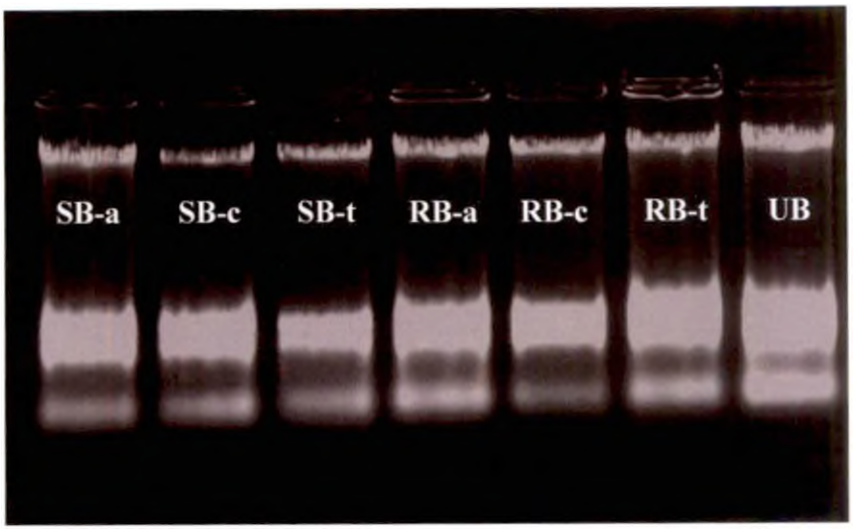
### 4.6.2 Molecular Characterisation

#### *4.6.2.1 DNA Isolation*

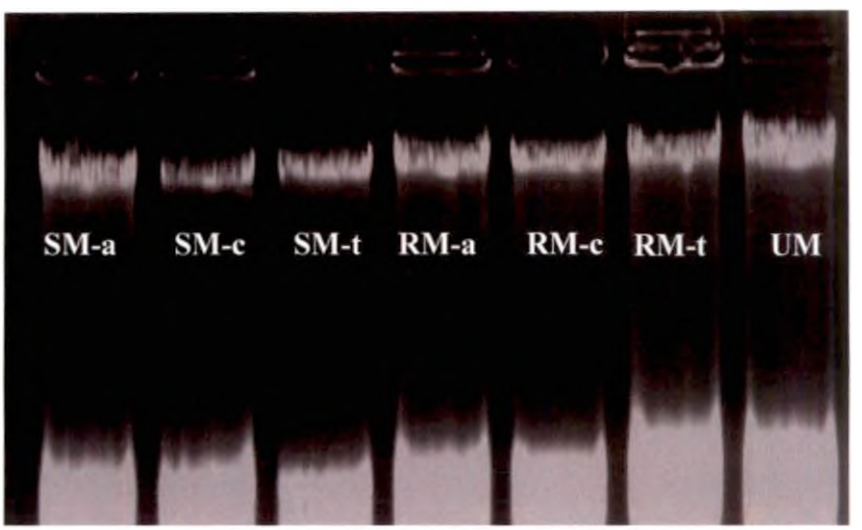
The mycelium of selected cultures of *B. bassiana* (Bb 5) (SB-acephate, SB-chlorantraniliprole and SB-thiamethoxam) and of *M. anisopliae* (Ma 4) (SM-acephate, SM-chlorantraniliprole and SM-thiamethoxam), relaxed cultures of *B. bassiana* (Bb 5) (RB-acephate, RB-chlorantraniliprole and RB-thiamethoxam) and of *M. anisopliae* (RM-acephate, RM-chlorantraniliprole and RM-thiamethoxam) and untreated cultures (UB and UM) yielded good quality DNA (Plate 7 A and B).

#### *4.6.2.2 Random Amplified Polymorphic DNA (RAPD) Reaction*

The number of bands developed per amplification was primer dependant for both the fungi and it varied from six to 18 and five to 18 in *B. bassiana* (Bb 5)




(A) *B. bassiana* (Bb5)




(B) *M. anisopliae* (Ma4)

SB-a: (Selected *B. bassiana*-acephate)
SB-c: (Selected *B. bassiana*-chloratraniliprole
SB-t: (Selected *B. bassiana*-thiamethoxam)
RB-a: (Relaxed *B. bassiana*-acephate)
RB-c: (Relaxed *B. bassiana*-chloratraniliprole)
RB-t: (Relaxed *B. bassiana*-thiamethoxam)
UB: (Untreated *B. bassiana*)

SM-a: (Selected *M. anisopliae*-acephate)
SM-c: (Selected *M. anisopliae*-chloratraniliprole)
SM-t: (Selected *M. anisopliae*-thiamethoxam)
RM-a: (Relaxed *M. anisopliae*-acephate)
RM-c: (Relaxed *M. anisopliae*-chloratraniliprole)
RM-t: (Relaxed *M. anisopliae*-thiamethoxam)
UM: (Untreated *M. anisopliae*)

Plate 7. Agarose gel electrophoresis profile of DNA of selected, relaxed and untreated cultures of *B. bassiana* (Bb5) and *M. anisopliae* (Ma4)

Table 53. Growth of selected, relaxed and untreated *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) in media containing insecticides higher than the maximum tolerable dose

| Treatments | Growth (cm) at 14 DAI | |
|---|---|---|
| | *B. bassiana* (Bb 5) | *M. anisopliae* (Ma 4) |
| Acephate **(S)** 0.675 % (4.5x) | 1.43 (1.56) | 1.67 (1.63) |
| Acephate **(R)** 0.675 % (4.5x) | 0.00 (1.00) | 0.00 (1.00) |
| Chlorantraniliprole **(S)** 0.051 % (8.5x) | 1.47 (1.57) | 1.73 (1.65) |
| Chlorantraniliprole **(R)** 0.051 % (8.5x) | 0.00 (1.00) | 0.00 (1.00) |
| Thiamethoxam **(S)** 0.043 % (8.5x) | 1.50 (1.58) | 1.83 (1.68) |
| Thiamethoxam **(R)** 0.043 % (8.5x) | 0.00 (1.00) | 0.00 (1.00) |
| Acephate 0.675 % (4.5x) (Untreated) | 0.00 (1.00) | 0.00 (1.00) |
| Chlorantraniliprole 0.051 % (8.5x) (Untreated) | 0.00 (1.00) | 0.00 (1.00) |
| Thiamethoxam 0.043 % (8.5x) (Untreated) | 0.00 (1.00) | 0.00 (1.00) |
| CD (0.05) | (0.044) | (0.017) |

Figures in parentheses are $\sqrt{x}$ transformed values.
Mean of three replications
DAI- Days after inoculation
S- Selected
R- Relaxed

and *M. anisopliae* (Ma 4), respectively. The results of the molecular analysis are given in Tables 54 and 55.

#### *4.6.2.2.1 B. bassiana (Bb 5)*

All the primers excepting RFu 4 produced only monomorphic bands. The primer RFu 1 produced 18 monomorphic bands while RFu-2, 3, 4, 5, 6, 7, 8, 9 and 10 produced 13, 13, 14, 17, 14, 10, 9, 6 and 8 monomorphic bands, respectively. The primer RFu 4 produced two specific bands of 800 and 500 bp for SB-chlorantraniliprole and exhibited 12.5 per cent polymorphism. A total of 124 bands were scored using these RFu primers of which 122 were monomorphic and only two were polymorphic. Polymorphism in *B. bassiana* (Bb 5) was only 1.61 per cent (Plate 8 A to J).

#### *4.6.2.2.2 M. anisopliae (Ma 4)*

A total of 107 bands were scored using the RFu primers 1 to 10 when the selected, relaxed and untreated cultures of *M. anisopliae* (Ma 4) were subjected to PCR. RFu 1 to 10 yielded 13, 12, 13, 18, 14, 12, 5, 7, 7 and 6 monomorphic bands, respectively. No polymorphism was induced by the insecticides in *M. anisopliae* (Ma 4) (Plate 9 A to J).

Table 54. DNA fingerprinting of selected, relaxed and untreated cultures of *B. bassiana* (Bb 5)

| Primer | Total no: of bands | | | | | | | No: of monomorphic bands | | | | | | | No: of polymorphic bands | | | | | | | Polym-orphism (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cultures | | | | | | | Cultures | | | | | | | Cultures | | | | | | | |
| | SB-a | SB-c | SB-t | RB-a | RB-c | RB-t | UB | SB-a | SB-c | SB-t | RB-a | RB-c | RB-t | UB | SB-a | SB-c | SB-t | RB-a | RB-c | RB-t | UB | |
| RFu-1 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-2 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-3 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-4 | 14 | 16 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 12.5 |
| RFu-5 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-6 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-7 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-8 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-9 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-10 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 122 | 124 | 122 | 122 | 122 | 122 | 122 | 122 | 122 | 122 | 122 | 122 | 122 | 122 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1.61 |

SB-a: Selected *B. bassiana* (Bb 5) -acephate
SB-c: Selected *B. bassiana* (Bb 5) -chlorantraniliprole
SB-t: Selected *B. bassiana* (Bb 5) -thiamethoxam
RB-a: Relaxed *B. bassiana* (Bb 5)-acephate
RB-c: Relaxed *B. bassiana* (Bb 5)-chlorantraniliprole
RB-t: Relaxed *B. bassiana* (Bb 5)-thiamethoxam
UB: Untreated *B. bassiana* (Bb 5)

Table 55. DNA fingerprinting of selected, relaxed and untreated cultures of *M. anisopliae* (Ma 4)

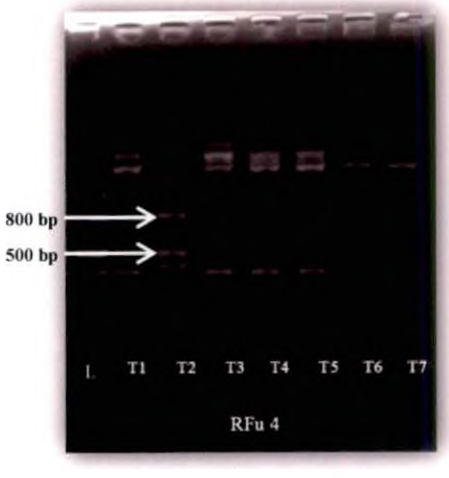
| Primer | Total no: of bands | | | | | | | No: of monomorphic bands | | | | | | | No: of polymorphic bands | | | | | | | Polym-orphism (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cultures | | | | | | | Cultures | | | | | | | Cultures | | | | | | | |
| | SM-a | SM-c | SM-t | RM-a | RM-c | RM-t | UM | SM-a | SM-c | SM-t | RM-a | RM-c | RM-t | UM | SM-a | SM-c | SM-t | RM-a | RM-c | RM-t | UM | |
| RFu-1 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-2 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-3 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-4 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-5 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-6 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-7 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-8 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-9 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RFu-10 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 107 | 107 | 107 | 107 | 107 | 107 | 107 | 107 | 107 | 107 | 107 | 107 | 107 | 107 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

SB-a: Selected *M. anisopliae* (Ma 4)-acephate
SB-c: Selected *M. anisopliae* (Ma 4)-chlorantraniliprole
SB-t: Selected *M. anisopliae* (Ma 4) -thiamethoxam
RB-a: Relaxed *M. anisopliae* (Ma 4)-acephate
RB-c: Relaxed *M. anisopliae* (Ma 4)-chlorantraniliprole
RB-t: Relaxed *M. anisopliae* (Ma 4)-thiamethoxam
UM: Untreated *M. anisopliae* (Ma 4)

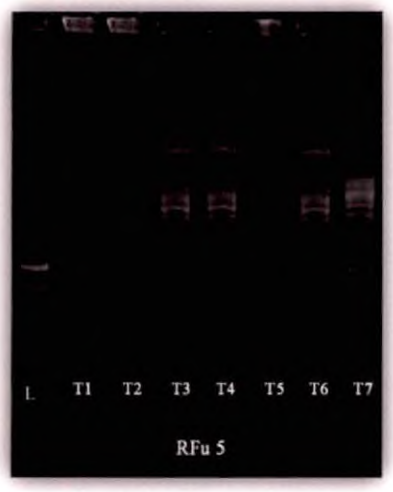

(A) RFu-1


(B) RFu-2


(C) RFu-3




(D) RFu-4



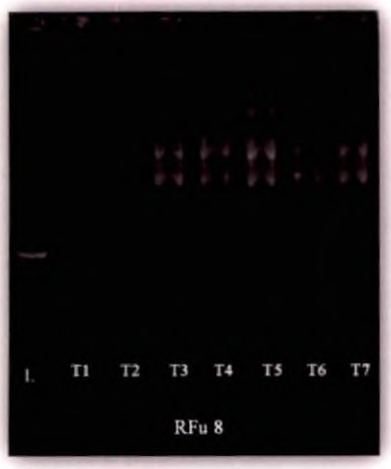

(E) RFu-5

L: (100 bp DNA ladder)
T1: (Selected *B. bassiana* - acephate)
T2: (Selected *B. bassiana* - chloratraniliprole)
T3: (Selected *B. bassiana* - thiamethoxam)
T4: (Relaxed *B. bassiana* - acephate)
T5: (Relaxed *B. bassiana* - chloratraniliprole)
T6: (Relaxed *B. bassiana* - thiamethoxam)
T7: (Untreated *B. bassiana*)

Plate 8. RAPD profile of *B. bassiana* (Bb 5)


(F) RFu-6


(G) RFu-7



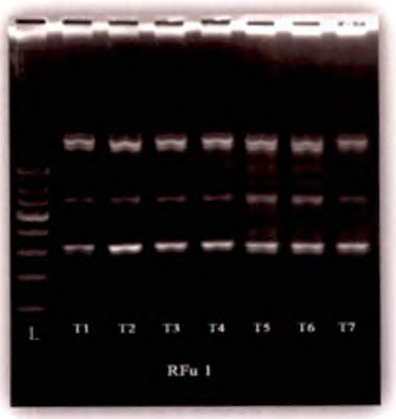

(H) RFu-8


(I) RFu-9

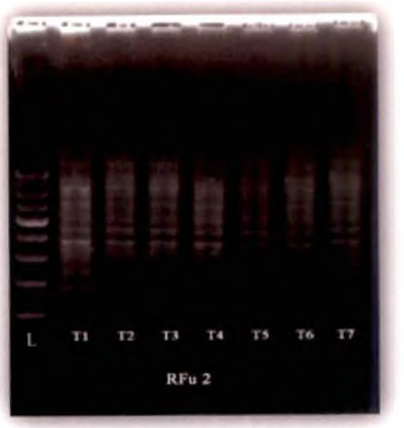

(J) RFu-10

L: (100 bp DNA ladder)
T1: (Selected *B. bassiana* - acephate)
T2: (Selected *B. bassiana* - chloratraniliprole)
T3: (Selected *B. bassiana* - thiamethoxam)
T4: (Relaxed *B. bassiana* - acephate)
T5: (Relaxed *B. bassiana* - chloratraniliprole)
T6: (Relaxed *B. bassiana* - thiamethoxam)
T7: (Untreated *B. bassiana*)

Plate 8. RAPD profile of *B. bassiana* (Bb 5) Contd.



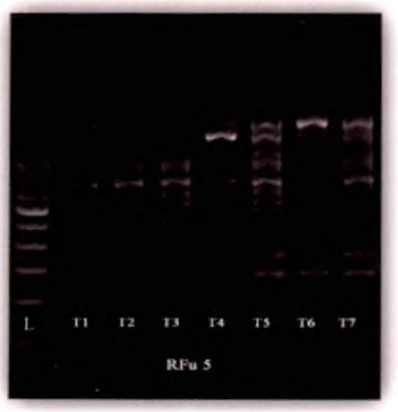

(A) RFu-1




(B) RFu-2


(C) RFu-3

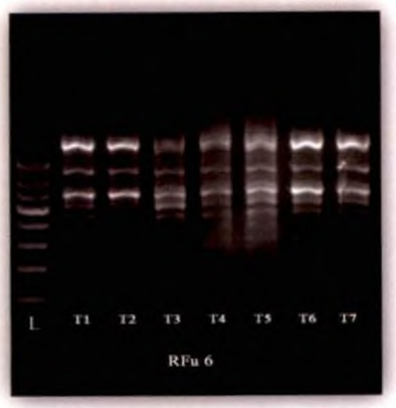

(D) RFu-4



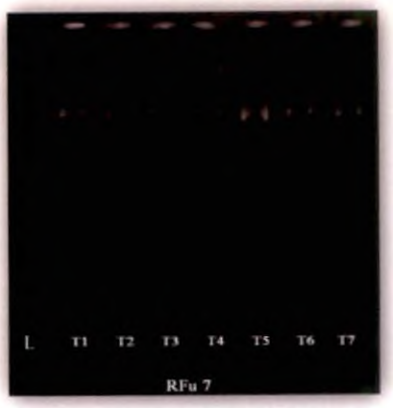

(E) RFu-5

L: (100 bp DNA ladder)
T1: (Selected *M. anisopliae* - acephate)
T2: (Selected *M. anisopliae* - chloratraniliprole)
T3: (Selected *M. anisopliae* - thiamethoxam)
T4: (Relaxed *M. anisopliae* - acephate)
T5: (Relaxed *M. anisopliae* - chloratraniliprole)
T6: (Relaxed *M. anisopliae* - thiamethoxam)
T7: (Untreated *M. anisopliae*)

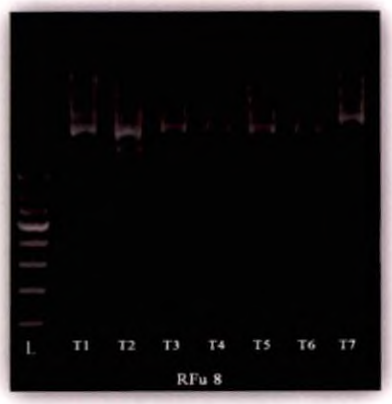
Plate 9. RAPD profile of *M. anisopliae* (Ma 4)



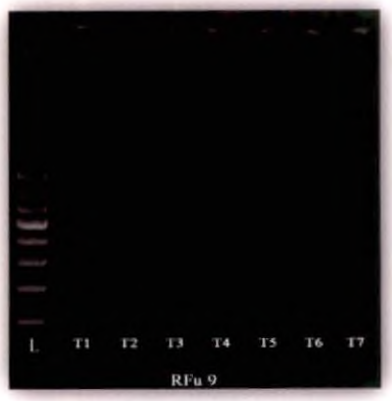

(F) RFu-6




(G) RFu-7




(H) RFu-8




(I) RFu-9


(J) RFu-10

L: (100 bp DNA ladder)
T1: (Selected *M. anisopliae* - acephate)
T2: (Selected *M. anisopliae* - chloratraniliprole)
T3: (Selected *M. anisopliae* - thiamethoxam)
T4: (Relaxed *M. anisopliae* - acephate)
T5: (Relaxed *M. anisopliae* - chloratraniliprole)
T6: (Relaxed *M. anisopliae* - thiamethoxam)
T7: (Untreated *M. anisopliae*)

Plate 9. RAPD profile of *M. anisopliae* Contd.

# *Discussion*

# 5. DISCUSSION

Pest management strategy is undergoing a sea change in recent years in Kerala, as elsewhere, following the awareness on the adversities of chemical pesticides. Of late, the Government has even given its nod for declaring Kerala as an Organic State and actions are under way. Now, for the management of insect pests that limit crop production, biocontrol is much emphasized, based on the knowledge garnered from Mother Nature that, a balance of life exists and that balance is maintained by natural regulatory factors - the bioagents, comprising of parasitoids, predators and pathogens.

The insect disease causing microbial pathogens have already established their role as biocontrol agents. Among the insect pathogenic microbes, entomopathogenic fungi particularly *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin that have wide host range (Khan *et al.*, 1993, Wraight *et al.*, 1998, Thorne and Lord, 2004 and Quesada-Moraga *et al.*, 2006) are in the cutting edge. None the less, it is also known that the so called *B. bassiana*, *M. anisopliae* and *Lecanicillium lecanii* (Zimmermann) Zare and Gams are assemblages of many species or isolates that vary in their infectivity to different insects (Ignoffo and Garcia, 1985; St. Leger *et al.*, 1992 and Steenberg and Humber, 1999). At this juncture, when such pathogens are publicised in pest management programmes the researchers need to quickly move beyond rhetoric, to develop clear pest management strategies employing these microbes for which detailed investigations on their isolates, pathogenicity, effective field doses and field efficacy are vital.

It is worth remembering the fact that chemical insecticides are also requisites for containing crop pests, but as the last option for use during exigencies and that too, with safer ones. It is also to be borne in mind that Integrated Pest Management (IPM) that employs an array of techniques including cultural methods such as host plant resistance, mechanical, biological and chemical methods is a viable strategy even at

this point of time, when Kerala is heading for an Organic State. In an IPM strategy, compatibility of the component tools need to be addressed especially that of the bioagents with chemical pesticides. A better exploitation of the bioagents in IPM could be achieved with pesticide tolerant strains of the bioagents, if developed. Attempts made to genetically improve natural enemies have produced pesticide tolerant strains for several species of parasitoids and predators of insects and mites (Croft, 1990). Multiple insecticide tolerant strains of the parasitoid, *Trichogramma* spp. (Charles *et al.*, 2011), predatory mite, *Metaseiulus (=Typhlodromus) occidentalis* (Nesbitt) (Hoy, 1986) and fungicide tolerant strain of the fungi, *B. bassiana* and *Metarhizium brunneum* Petch have been developed (Shapiro *et al.*, 2002; 2011). Nonetheless, information pertaining to pesticide tolerant strains of entomopathogenic fungi is meagre.

Rice, the staple food of Keralites is grown in an area of 1.97 lakh ha in the State (FIB, 2015); quite often with high load of chemical pesticides to tackle the insect pests that substantially reduce yield. Uma (Mo16), a popular rice variety grown in sixty per cent of the paddy fields in Kerala (Anon., 2012) is tolerant to brown pant hopper, *Nilaparvata lugens* Stal. and gall midge, *Orseolia oryzae* Wood-Mason, but it is known that the leaf roller, *Cnaphalocrocis medinalis* Guen. and the rice bug, *Leptocorisa acuta* (Thunb) are important insect pests in this variety and for the control of which farmers still rely on conventional synthetic chemicals. The basic concept of IPM is to use a tolerant variety that has built in mechanism to minimize the pest damage and also to use other management tools to reduce the population of the competent insect intruders. Though parasitoids *viz., Trichogramma chilonis* Ishii and *Trichogramma japonicum* Ashmead are popular for managing the leaf roller and stem borer infestation, respectively in Kerala, the use of entomopathogenic fungi is virtually nil. Considering the aforesaid facets of the entomopathogenic fungi and rice pest management in Kerala, a project entitled "Entomopathogenic fungi for the management of insect pests in rice ecosystem" was undertaken with the objectives:

Isolation and identification of indigenous fungi, assessment of their pathogenicity to rice pests, determination of their $LC_{50}$, $LC_{90}$ and $LT_{50}$ values through bioassay, field evaluation, assessment of the compatibility with insecticides and to develop pesticide tolerant strains of the fungi.

Different methods were adopted for the isolation of entomopathogenic fungi by researchers. Many of them have followed the technique of isolation from infected cadavers and they brought to light the existence of many new isolates of entomopathogenic fungi *viz.*, *Fusarium pallidoroseum* (Cook) Sacc.(Hareendranath, 1989), *B. bassiana* isolate, ITCC 6063 (Jiji *et al.*, 2006), *Beauveria brongniartii* Saccardo (Anis, 2014 ), *Isaria javanica* Friedrichs and Bali (Lokesh, 2014) infecting *Aphis craccivora* Koch, *Dacus cucurbitae* Coquillett, *Metriona circumdata* H. and *Bemisia tabaci* Gennadius, respectively in Kerala. Ambethgar *et al.* (2007) reported *B. bassiana* isolates BbCmKKL 1100 and BbCmCBE 1100 from *C. medinalis*, BbLaCBE 0201 from *L. acuta* and *M. anisopliae* isolate MaHbCBE 1200 from *Hieroglyphus banian* Fab. Rachappa *et al.* (2007) and Sivasundaram *et al.* (2008) isolated *B. bassiana* infecting different insects from other parts of India.

The technique of isolation of entomopathogenic fungi from soil either adopting dilution plate technique with selective media (Strasser *et al.*, 1996; Goettel and Inglis, 1997; Hu and St. Leger, 2002; Ibrahim *et al.*, 2011 and Hasan *et al.*, 2012 ) or bait method using various insects *viz., Curculio caryae* Horn (David *et al.*, 2003), larvae of *Galleria mellonella* L. (David *et al.*, 2003; Neuman and Shields, 2004 and Revathi *et al.*, 2011), *C. medinalis* (Sivasundaram *et al.*, 2008) and *Tenebrio molitor* (L.) (Sergio *et al.*, 2010) are in practice.

From the monitoring conducted in thirty rice fields of farmers in Thiruvananthapuram district, during the cropping seasons from November 2011 to February 2012, June 2012 to September 2012 and November 2012 to February 2013 , one fungus, *Aspergillus flavus* Link. infecting the rice bug, *L. acuta* was collected.

From soil, four isolates of *B. bassiana* and one isolate of *M. anisopliae* were also collected. It has been reported that, soil is the major inoculum of many hyphomycetous fungi (Steenberg *et al.*, 1995 and Meyling 2007). Many researchers have reported that soil harbour the genera *Beauveria, Isaria, Metarhizium* and *Tolypocladium* (Keller *et al.*, 2003 and Tkaczuk *et al.*, 2014).

The medium originally described by Strasser *et al.* (1996) was used by several researchers to isolate fungi from soil (Enkerli *et al.*, 2004 and Kessler *et al.*, 2004). Many scientists suggested the use of Veen's semiselective medium to isolate entomopathogenic fungi from soil (Goettel and Inglis, 1997; Hu and St. Leger, 2002; and Ibrahim *et al.*, 2011). In different laboratories, modifications have also been made to optimise isolation methods based on experience (Meyling, 2007). A variety of fungicides and antibiotics have been used in selective media to isolate entomopathogenic fungi from soil (Wraight *et al.*, 2007). Better isolation of fungi with incorporation of Cetyl Trimethyl Ammonium Bromide (CTAB) in oat meal agar was reported by Posadas *et al.* (2012).

In the present study, Veens semiselective media (Veen and Ferron, 1966) was modified, by substituting dodine with CTAB and adding amoxocyllin (3.1.1.2.3). This modified media was found successful in isolating *B. bassiana* from the soil samples and two *B. bassiana* isolates were obtained using this medium.

Using *G. mellonella* larva as trap, two isolates of *B. bassiana* and one isolate of *M. anisopliae* were collected.

After isolation of fungi from mycosed cadaver / soil, their pathogenicity was tested against *C. medinalis* and *L. acuta* and these isolates were found pathogenic to both the insects.

Ignoffo and Garcia (1985) stated that, in nature the microbes undergo selection, recombination and mutation depending upon the ecological situations and

this influences their genetic makeup. The most common entomopathogenic genera of hypomycetous fungi, including *Aspergillus, Beauveria, Hirsutella, Metarhizium, Nomuraea, Paecilomyces, Tolypocladium* and *Verticillium* are defined by their characteristic conidiogenesis (Barron, 1968; Samson *et al.,* 1988 and Humber, 1997). However, placement of hyphomycetous fungi within the formed genera based on conidiogenesis does not necessarily reflect phylogenetic groupings and, the application of molecular methods is shedding new light on generic and species concepts within this group of entomopathogenic fungi (Butt *et al.*, 2001).

Other researchers (Zare *et al.,* 2000; Gams and Zare, 2001 and Sung *et al.*, 2001) avowed ITS sequences for grouping of entomopathogenic isolates of *Verticillium lecanii* Zimmerman, under the genus *Lecanicillium* with four distinct species. The internal transcribed spacers of the ribosomal DNA (rDNA-ITS) sequencing have been successfully employed to assess the genetic variability of *Beauveria* spp. (Coates *et al.*, 2002; Gaitan *et al.*, 2002; Muro *et al.*, 2003; 2005 and Wada *et al.*, 2003). The insufficiency of classical taxonomic and morphological characters for revealing the differences among species of fungus was pointed out by Ramanujam *et al.* (2011. Against this backdrop, after morphological identification of the fungi, molecular characterization was done for further identity. DNA barcoding of the fungi was carried out at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram using universal ITS primers (ITS-1F and ITS-4R).

The fungus isolated from *L. acuta* was identified as *Aspergillus flavus* Link. The bioefficacy of *A. flavus* to *L. acuta* is reported for the first time. The fungus as an entomopathogen of *Odoiporus longicollis* Oliver (Padmanaban *et al.,* 2002), *Dysdercus cingulatus* Fab. (Selvaraj *et al.*, 2002), *C. medinalis* (Ambethgar *et al* ., 2007), *Nephopteryx eugraphella* Ragonot (Ghirtlahre *et al.,* 2014) and *Helopeltis antonii* Signoret (Pasaru *et al.*, 2014) was reported earlier.

The sequence of *A. flavus* isolate was deposited in NCBI (National Centre for Biotechnology Information) and the accession number assigned by NCBI was KP 739825. It was having cent per cent similarity with the *A. flavus* strain PW 2961 (KF 562204.1) deposited in NCBI.

Knowledge on the genetics of fungi has much relevance. Exploitation of *A. flavus* in pest management programmes is meager as the fungus was known to cause diseases such as aspergillosis and superficial infection in human beings and also due to aflatoxin and cyclopiazonic acid (Hedayathi *et al.*, 2007; Richard, 2008 and Abbas *et al.*, 2011). However, the research findings of Atehnkeng *et al.* (2008) expose the beneficial aspects of the fungus. The study reveals the importance of strain variations and its implications. According to the author, there are atoxigenic strains of *A. flavus* that can be even exploited for reducing the aflatoxin content produced by toxigenic strains of *A. flavus* contaminating maize. In United States of America, aflatoxin contamination is checked by the biological use of AflaGuard (granular formulation of dry spores of atoxigenic isolates of *A. flavus*), a product registered by Environmental Protection Agency of United States Department of Agriculture (Dorner, 2004; Cotty 2006 and Cotty *et al.*, 2008). The effects of strain variations of *A. flavus* were reinforced by the findings of Abbas *et al.* (2011) who documented the double issues of the fungus. The co-existence of *A. flavus* strains in varying environments and the ability of the atoxigenic strains to compete effectively for the same ecological niche were highlighted. In the light of this information, studies are essential to fix the toxigenic or atoxigenic status of the present isolate of *A. flavus* for adoption for pest management.

Early in 1983, Howarth emphasized *a priori* the need to conserve the indigenous entomopathogens to overcome the possibilities of risks with introduced species such as irreversibility of alien introductions, host switching to innocuous native beneficial insects etc. Many others (Shah and Pell, 2003; Meyling and Eilenberg, 2007) have opined that isolation of indigenous strains of

entomopathogenic fungi is essential as they are often potent candidates for conservation biological control. The aforesaid observations and findings underscore the relevance of isolation of indigenous fungi and further their molecular identification for tapping their potential in biological programmes with less risk.

Five isolates of fungi were collected from soil, among them four were isolates of *B. bassiana* ( Bb- m2, Bb- m3, Bb- m4 and Bb- m5) and the other one was an isolate of *M. anisopliae* (Ma- m1). The accession numbers of isolates Bb- m2, Bb-m3, Bb-m4 and Bb-m5 obtained from NCBI were KP 739828, KP 739829, KP 739830 and KP 739831, respectively and they were cent per cent similar to the isolates *B. bassiana* LPSC1067 (KF500409.1), strain SD15 (KC55195.1), strain A64 (KC461106.1), BBPTG2 (KC759729.1), respectively deposited in NCBI. *M. anisopliae* (Ma-m1) with accession number KP739826 had cent per cent similarity with *M. anisopliae* strain MAGW7 (KF913494.1).

With respect to specificity, the entomopathogenic fungi vary greatly between genera, within genera, among species as well as within species (Shahid *et al.*, 2012 and Hemasree, 2013). It follows that appraisement of their pathogenicity to insects is indispensable for promoting them as biocontrol agents.

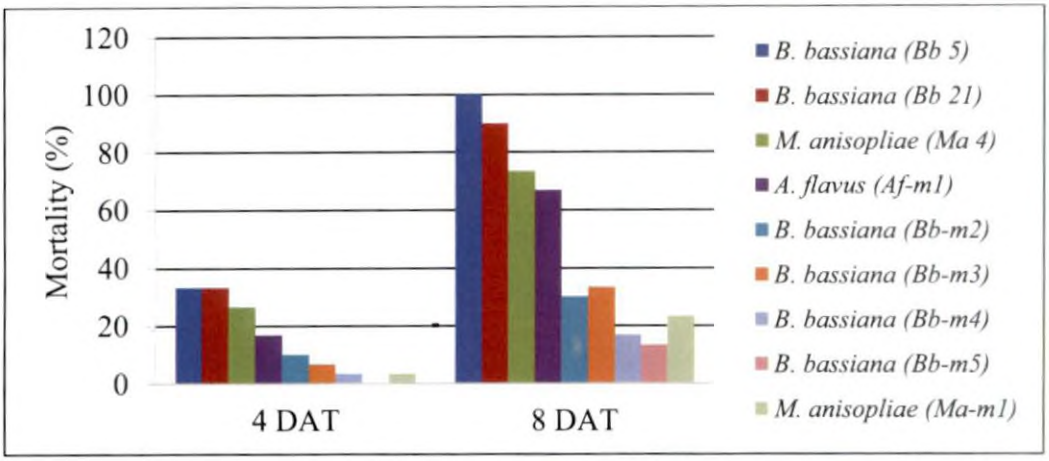
The pathogenicity studies conducted by applying spore suspension from seven day old culture of *A. flavus* produced symptoms of infection on *C. medinalis* larvae and nymphs and adults of *L. acuta*. The infected insects were lethargic and showed reduction in feeding and movement. Another observation was that, the infected nymphs of *L. acuta* remained secluded unlike the healthy ones which were seen gregariously. In healthy adults, mating period lasted for more than thirty minutes, while in the diseased ones it did not last for more than five minutes. The mortality of *C. medinalis* initiated from the fourth day after treatment and that of the nymphs and adults of *L. acuta* on the fifth day after treatment. Rayati and Widayat (1996) were of the opinion that infection of entomopathogenic fungi could lead to death in insects

within three to 10 days. Conidiophores of white and yellowish green colour covered the cadaver in two days after the death (Plate 3E). Similar observations were recorded by Pasaru *et al.* (2014) in *A. flavus* infected *Helopeltis* sp.

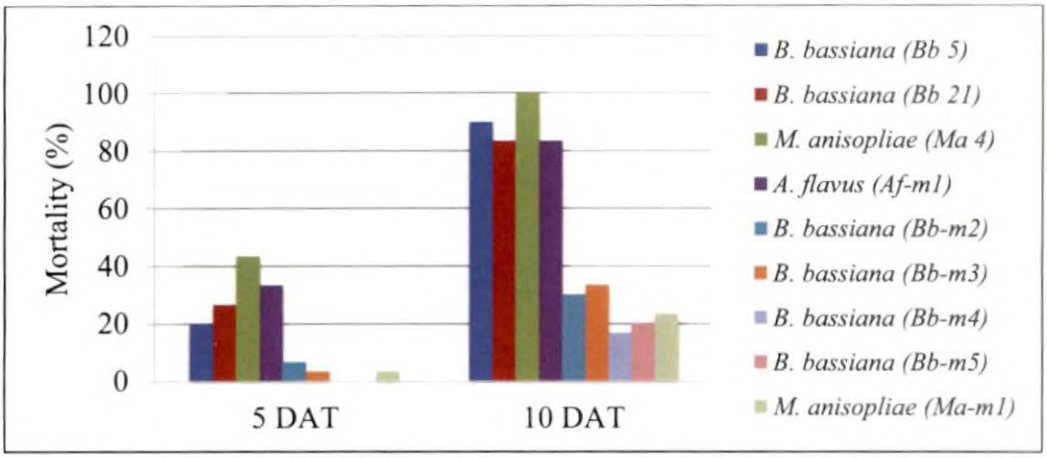
All the *B. bassiana* isolates, Bb 5 and Bb 21 and the newly isolated Bb-m2, Bb-m3, Bb-m4 and Bb-m5 produced symptoms on larvae of *C. medinalis* and nymphs and adults of *L. acuta* which were similar to that in *A. flavus* except the mycelial colour which was white in all *B. bassiana* isolates (Plate 3). Not much difference in symptoms of infection of *M. anisopiae* (Ma 4 and Ma-m1) infected *C. medinalis* larvae and nymphs and adults of *L. acuta* were seen. The fungal growth over the cadaver appeared in three days after the death and was initially white which later changed to dark green in the case of Ma 4 isolate and blackish green in Ma-m1 isolate (Plate 3). During infections of entomopathogenic fungi, symptoms such as altered mating (Noma and Strickler, 2000), behavioural fever *i.e.,* the infected insects changing its body temperature by basking in the sun or using warm surfaces (Blanford and Thomas, 2001), selection of upper surfaces for their positioning (Anis, 2014) and reduced coordination and feeding (Gul *et al.*, 2014) were also recorded.

Of all the isolates tested, only *B. bassiana* isolate (Bb 5) caused mortality of eggs of *C. medinalis* (Plate 3) and *L. acuta* and none of them were pathogenic to pupal and adult stages of *C. medinalis* (Plate 4). Abdel-Baky *et al.* (1998); Long *et al.* (1998) and Al-Deghairi, (2008) observed that eggs were more resistant to fungal infections than other life stages of insects. None of the fungal isolates tested were pathogenic to pupal and adult stages of *C. medinalis*. Inglis *et al.* (2001) and Sahagun *et al.* (2005) have stated that the virulence of fungal biocontrol agents usually differs with different biological stages of insect pests. Asi *et al.* (2013) also reported similar observation of less susceptibility of pupal stage of *Spodoptera litura* Fab. compared to the other stages.

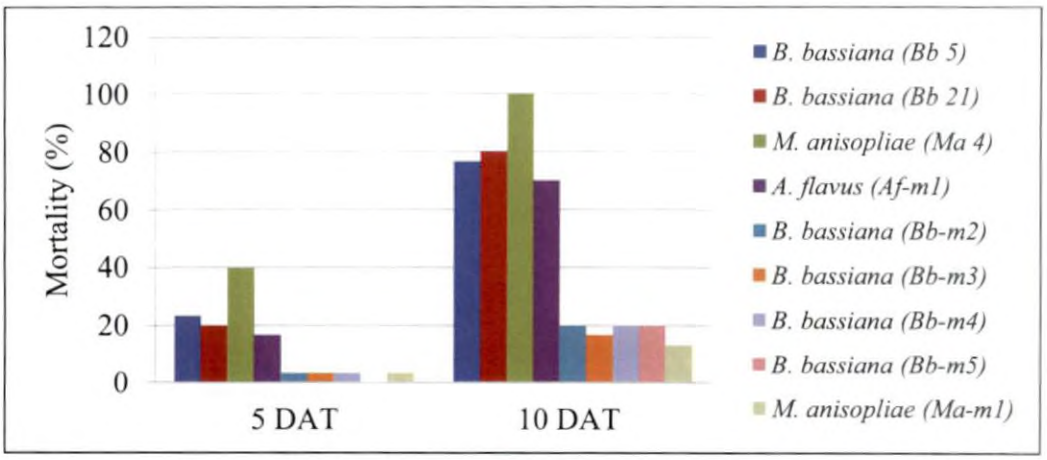
A comparison of the pathogenicity of the fungi, assessed at a spore concentration of $10^8$ spores $ml^{-1}$ against larvae of *C. medinalis* and nymphs of *L. acuta* and at $10^9$ spores $ml^{-1}$ against adults of *L. acuta* showed that the pathogenicity of the different fungi varied significantly, the mortality ranged from 13.33 to cent per cent against larvae of *C. medinalis*, 16.67 to cent per cent against nymphs and 13.33 to cent per cent against adults of *L. acuta*. Superior performance of the NBAII isolates of *B. bassiana* (Bb 5), *M. anisopliae* (Ma 4) and the indigenous isolates, *B. bassiana* (Bb 21) and *A. flavus* (Af-m1) were noted compared to the newly collected indigenous isolates of *B. bassiana* (Bb-m2, Bb-m3, Bb-m4 and Bb-m5) and *M. anisopliae* (Ma-m1) from soil. This was evident from the mortality rates, from the initial observations and over the time exhibited in the larvae of *C. medinalis* (Fig.1A) and also in the nymphs and adults of *L. acuta* (Fig.1B and 1C). The mortality of *C. medinalis* larvae ranged from 33.33 to cent per cent in *B. bassiana* (Bb 5) and it was followed by 33.33 to 90.00 per cent in *B. bassiana* (Bb 21). The mortality caused by *M. anisopliae* (Ma 4) ranged from 43.33 to cent per cent against *L. acuta* nymphs at $10^8$ spores $ml^{-1}$ and 40.00 to cent per cent against adult *L. acuta* at $10^9$ spores $ml^{-1}$. The new indigenous isolates of *B. bassiana* from soil produced only 3.33 to 33.33 percentage mortality in larvae of *C. medinalis* and in the nymphs of *L. acuta*. The mortality was still lower in the adults of *L. acuta* (3.33 to 20.00 per cent). The indigenous soil isolate of *M. anisopliae* (Ma-m1) caused mortality of only 3.33 to 23.33 per cent in larvae of *C. medinalis* and nymphs of *L. acuta* and 3.33 to 13.33 per cent only against adults of *L. acuta*. From the cumulative percentage mortality it was seen that *B. bassiana* (Bb 5) was the most effective one against *C. medinalis* larva whereas *M. anisopliae* (Ma 4) was the most effective one against nymphs and adults of *L. acuta*. In the present study, since a single dose was used for evaluating the pathogenicity of the isolates, it can be concluded that the enhanced performance of the NBAII isolates *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) is due to the inherent character of the isolates. Lokesh (2014) opined that the pathogenicity of fungi varied with pests. In the studies conducted by Carneiro *et al.*




(A) *C. medinalis* larvae




(B) *L. acuta* nymph



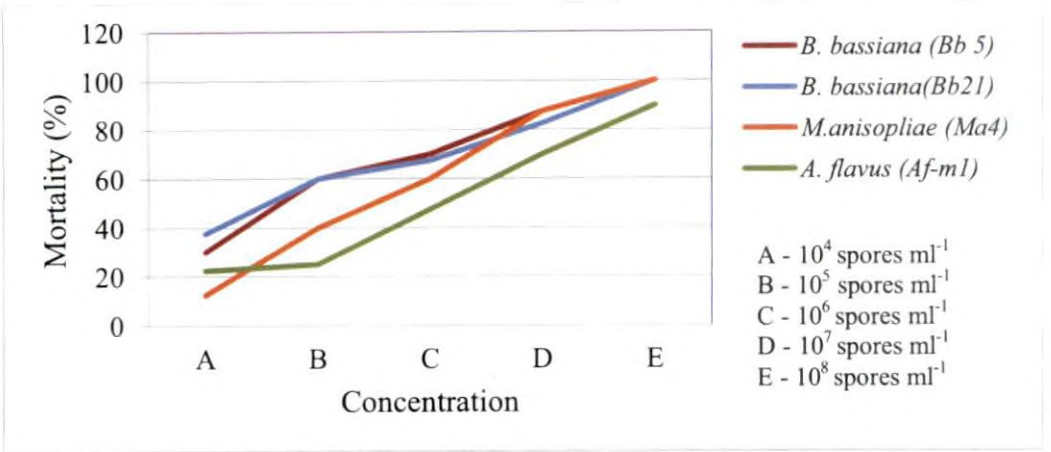

(C) *L. acuta* adult

Fig.1 Pathogenicity of fungal isolates to rice pests.

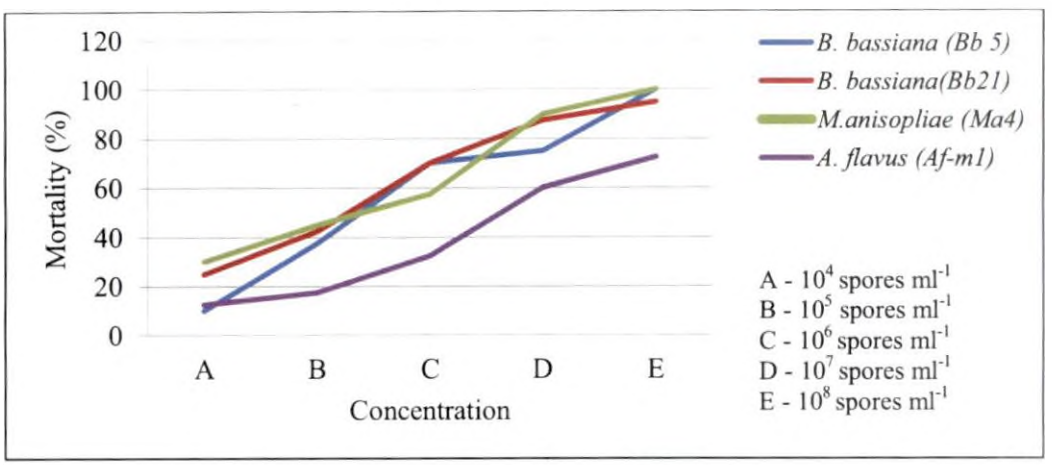
(2008) and Sivasundaram *et al.* (2008), the variations in pathogenicity of the isolates of *B. bassiana* was seen from zero to cent per cent and from 26.67 to 73.33 per cent when evaluated against the fall army worm, *Spodoptera frugiperda* Smith and against *C. medinalis,* respectively. The present research results are also supported by the findings of Lopes *et al.* (2013) who reported low pathogenicity of indigenous isolates of *B. bassiana* and *M. anisopliae*, in the adults of *Cosmopolites sordidus* Germ.. The results necessitate the selection of the right pathogen for effective pest management.

According to Butt *et al.* (2001), dose-mortality studies determine the minimum amount of inoculum required to cause disease in the test insect and also indicate the time the bioagents will take to have an impact on the target organisms. An overview of the bioassay results in the present study revealed a concentration dependent mortality of the test insects (Fig.2. A, B, C). Irrespective of the fungi evaluated, at higher spore concentrations higher mortality was achieved. This trend in mortality was exhibited by all the fungi tested against the larvae of *C. medinalis* as well as against the nymphs and adult of *L. acuta*. Dhuyo and Soomro (2008) observed dose dependent mortality of larva of *Scirpophaga incertulas* (Walker) when treated with *B. bassiana*. Similarly, Herlinda *et al.* (2008) recorded higher mortality in nymphs of rice bug, *Leptocorisa oratorius* F. with increase in concentrations of spores of both *B. bassiana* and *M. anisopliae*. From the studies of Sivasundaram *et al.* (2008) also, a concentration dependent mortality was seen, the mortality of *C. medinalis* reported being 26.67 and 76.67 per cent at $10^2$ and $10^8$ spores $ml^{-1}$ of *B. bassiana*, respectively. The results of bioassay studies conducted by Anis (2014) against coleopteran pests and Lokesh (2014) against sucking pests of chilli are in corroboration with the present findings.

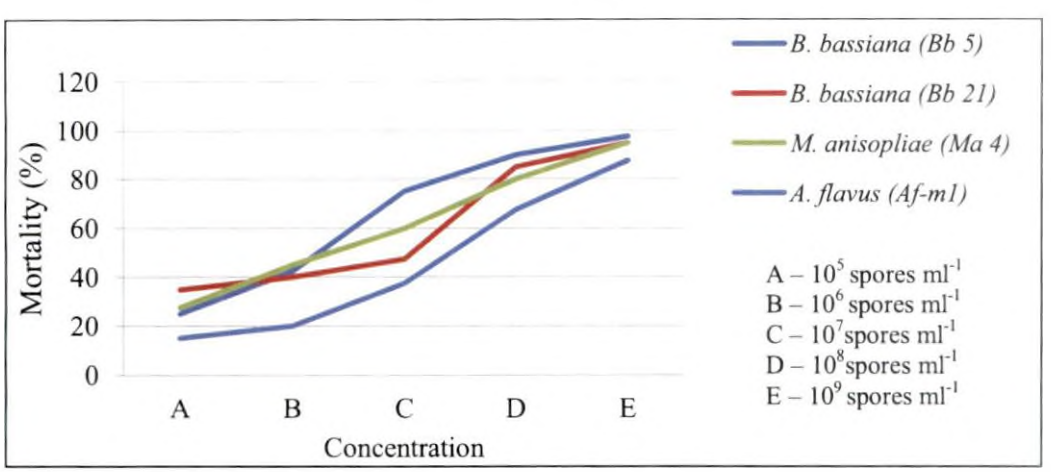
The concentrations required for the pathogens, varied from $1.02 \times 10^8$ to $1.70 \times 10^8$ spores $ml^{-1}$ to bring fifty per cent mortality in the third instar larvae of *C. medinalis* within the shortest period of four days. The studies conducted by Ambethgar *et al.* (2007) also revealed that the dose required to bring fifty per cent




(A) *C. medinalis* larvae




(B) *L. acuta* nymphs




(C) *L. acuta* adults

Fig. 2 Dose - mortality relationships of fungal pathogens and insects

mortality to the third instar larvae of *C. medinalis* varied with fungal isolates. The $LC_{50}$ for the *B. bassiana* isolate, BbCmKKL1100 was observed as 2.80 x $10^3$ spores $ml^{-1}$ in their studies. Sivasundaram *et al.* (2008) further recorded 3.40 x $10^4$ spores $ml^{-1}$ as the $LC_{50}$ value of *B. bassiana* (B2) against *C. medinalis* larvae, which were lower, in comparison with the $LC_{50}$ noted in the present study, this variations can be attributed to the differences in the virulence of the isolates. Kirubakaran *et al.* (2013) reported $LC_{50}$ value of *B. bassiana* MTCC7690 and *M. anisopliae* MTCC4104 as 9.09 x $10^4$ and 6.08 x $10^5$ spores $ml^{-1}$ against *C. medinalis* larvae.

The $LC_{50}$ to bring fifty per cent mortality in the nymphs of *L. acuta* within the shortest period of five days varied from 0.95 x $10^8$ to 3.55 x $10^8$ spores $ml^{-1}$, while it varied from 1.08 x $10^{10}$ to 4.84 x $10^{10}$ against the adults. Among the different fungi evaluated, the lowest value was recorded for *M. anisopliae* (Ma 4).

An analysis of the $LT_{50}$ values showed that, to bring about fifty per cent mortality @ $10^8$ spores $ml^{-1}$, *M. anisopliae* (Ma 4) required only 4.69 days, compared to 5.38, 5.08 and 7.39 days for *B. bassiana* (Bb 5, Bb 21) and *A. flavus* (Af-m1), respectively against the larvae of *C. medinalis*. More or less similar results were obtained by Ambethgar *et al.* (2007) who recorded $LT_{50}$ values ranging from 5.22 to 6.00 days for the various isolates of *B. bassiana* evaluated at a concentration of $10^9$ spores $ml^{-1}$.

Slightly longer period was required to bring fifty per cent mortality in the nymphs and adults of *L. acuta*. The $LT_{50}$ values were 5.26, 6.02, 7.08, and 8.22 days at $10^8$ spores $ml^{-1}$ for *M. anisopliae* (Ma 4), *B. bassiana* (Bb 5, Bb 21) and *A. flavus* (Af-m1) against nymphs of *L. acuta* and the corresponding values in the case of adults were 6.65, 6.92, 7.13 and 7.96 days for these fungal pathogens at spore concentration of $10^9$ spores $ml^{-1}$. Herlinda *et al.* (2008) reported 3.52 days and 5.75 days as $LT_{50}$ of KBC isolate of *B. bassiana* and Mtm isolate *M. anisopliae*, respectively against nymphs of another species of rice bug, *L. oratorius*. Kirubakaran

*et al.* (2013) recorded a shorter period of 4.64 and 4.88 days as $LT_{50}$ values of *B. bassiana* MTCC7690 and *M. anisopliae* MTCC4104 against *C. medinalis* larvae. A comparison of $LT_{50}$ showed that, the mortality in *C. medinalis* larvae was quicker when compared to *L. acuta* for all the isolates tested. It may be noted that the mortality in these periods was achieved at a concentration of $10^8$ for the nymphs of *L. acuta* while a higher concentration of $10^9$ spores $ml^{-1}$ was required for the adults.

The $LC_{90}$ values were observed to range from 2.25 to 3.50 x $10^8$ spores $ml^{-1}$ for the fungal pathogens evaluated against *C. medinalis*, while the values ranged from 2.96 to 7.55 x $10^8$ spores $ml^{-1}$ for *L. acuta* nymphs and 2.85 to 8.72 x$10^{10}$ spores $ml^{-1}$ for adults. It is seen from the results that, the immature stages of both the insects required only lower concentration compared to the adults of *L. acuta* and hence, the $LC_{90}$ value for the adult of *L. acuta* needs to be fixed as the field doses which are 4.23 x $10^{10}$, 4.11 x $10^{10}$, 2.85 x $10^{10}$ and 8.72 x $10^{10}$ for *B. bassiana* (Bb 5), (Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1), respectively.

As the studies on the pathogenicity and the bioassay of fungal pathogens were done with insects reared in the laboratory and the conditions prevailing in the fields are quite different from that prevailing in the laboratory, field evaluation of the fungal pathogens are absolutely essential before embracing these fungal pathogens as biocontrol agents. Padmaja and Kaur (2001) have stated that, the temperature and relative humidity prevailing in the field conditions are critical for fungal infection on insects. There are only few reports on the evaluation of microbial pathogens against rice pests in India (Rao and Singh, 2003; Singh *et al.*, 2008 and Katti, 2013). Therefore, two field experiments were conducted at Cropping System Research Centre, Karamana during November 2012 to March 2013 (puncha) and June 2013 to October 2013 (virippu) using the variety, Uma (Mo16) which was grown in 60 per cent of the paddy fields in Kerala.

Though, a relatively insect tolerant variety, Uma is susceptible to the infestation of the leaf roller, *C. medinalis* and the rice bug, *L. acuta*. *C. medinalis* larvae feed on the leaves by scraping the green matter, resulting in reduced photosynthesis and subsequent yield reduction (Fraenkel and Fallil, 1981). The pest has been recorded to cause 63 to 80 per cent yield loss in rice (Rajendran *et al*, 1986 and Murugesan and Chelliah, 1987). It was reported to cause five to 10 per cent yield loss, but sometimes up to 60 per cent (Pathak and Khan, 1994). The pest as an important production constraint of rice in South Asia and other parts of the world was reported by Dale (1994). *C. medinalis* was reported as a major pest of rice in Kerala by Nair (1978) and Lekha (2003).

The nymphs and adults of *L. acuta*, suck milk from the developing rice grains, in the early stage of grain formation. Gupta *et al.* (1993) reported 2.5 to 6.21 per cent damage and 1.72 to 5.23 per cent damage during wet and dry seasons, respectively. The quality of damaged grains reduced and lowered the market value. Moreover, the damaged grains retained the buggy odour even after cooking (Dale, 1994). Panda and Rath (2003) reported 25 to 51 per cent yield loss due to rice bug infestation. As the rice variety, Uma is susceptible to *C. medinalis* and *L. acuta*, their management is inevitable.

The treatments in the field experiments comprising of the spore suspensions and talc based formulations of the fungi *viz.*, *B. bassiana* ( Bb 5 and Bb 21), *M. anisopliae* (Ma 4), *A. flavus* (Af-m1) and the four insecticides *viz.*, acephate @ 750 g a.i $ha^{-1}$, chlorantraniliprole @ 30 g a.i $ha^{-1}$, malathion @ 575 g a.i $ha^{-1}$ and thiamethoxam @ 25 g a.i $ha^{-1}$ were applied on need basis, 95 DAS in the first trial and 105 DAS in the second trial. The effect was evaluated in terms of the population of *C. medinalis* and *L. acuta*, the extent of damage caused by these pests, yield and population of natural enemies.

In the first field trial, from the observations taken at different intervals, the best treatment among the fungal pathogens for reducing the larval population of *C. medinalis* was found as *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ which recorded a population reduction of 40.30 and 58.61 per cent over untreated on the fourth day and on the twenty first day after treatment, respectively (Fig. 3A). The corresponding reduction in the larval population was 38.78 to 46.56 in talc based *B. bassiana* (Bb 5) @ 20g $l^{-1}$, 37.30 to 43.09 in talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$, 32.83 to 43.09 in talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ respectively. Though the initial percentage reduction was higher (44.78) in *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$, its performance was inferior, subsequently. There are only very few reports on the field evaluation of fungal pathogens against *C. medinalis*. On evaluation of *M. anisopliae* @ $10^{8}$ spores $ml^{-1}$ against *C. medinalis,* Padmaja and Kaur (2001) recorded 60 to 70 per cent mortality of the pest. Shahid *et al.* (2003) observed significant reduction in rice leaf folder incidence by applying *M. anisopliae* @ 250 g $acre^{-1}$.

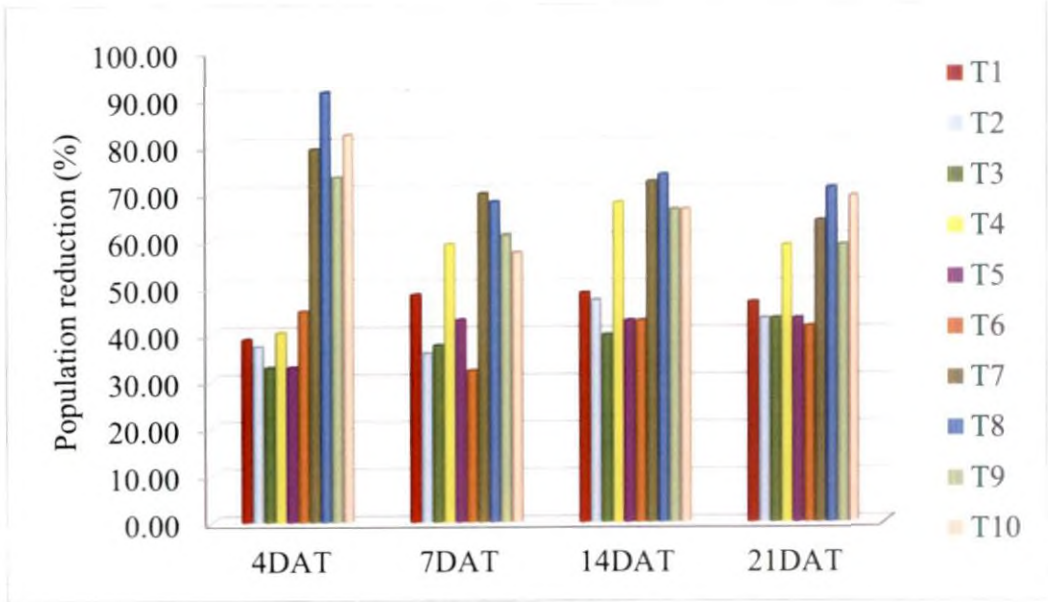
Among the insecticides evaluated, maximum reduction in larval population of *C. medinalis* (91.04 per cent) over the untreated was noted in chlorantraniliprole @ 30 g a.i $ha^{-1}$ and it was followed by thiamethoxam @ 25 g a.i $ha^{-1}$ (82.09), acephate @ 750 g a.i $ha^{-1}$ (79.09) and malathion @ 575 g a.i $ha^{-1}$ (73.13), respectively (Fig. 3A). Though, the initial percentage reduction in the larval population of *C. medinalis* in the treatments with insecticides was significantly higher than that noted in fungal pathogens, over the period of observation it was seen that the effect of the chemical insecticides decreased while that of the fungal treatments increased and on the twenty first day, the effect of *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ was on par with all chemicals. The effect of talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ was on par with acephate @ 750 g a.i $ha^{-1}$ and malathion @ 575 g a.i $ha^{-1}$ on the twenty first day after application of treatments. The present result reflects the beneficial character of the fungal pathogens *i.e.,* extended insect management, which can be accounted to their self perpetuating nature and sustained effect. Similar observations on the

performance of entomopathogenic fungi was recorded by Rombach *et al.* (1986a); Nghiep *et al.* (1999); Li *et al.* (2012); and Reddy *et al.* (2013).

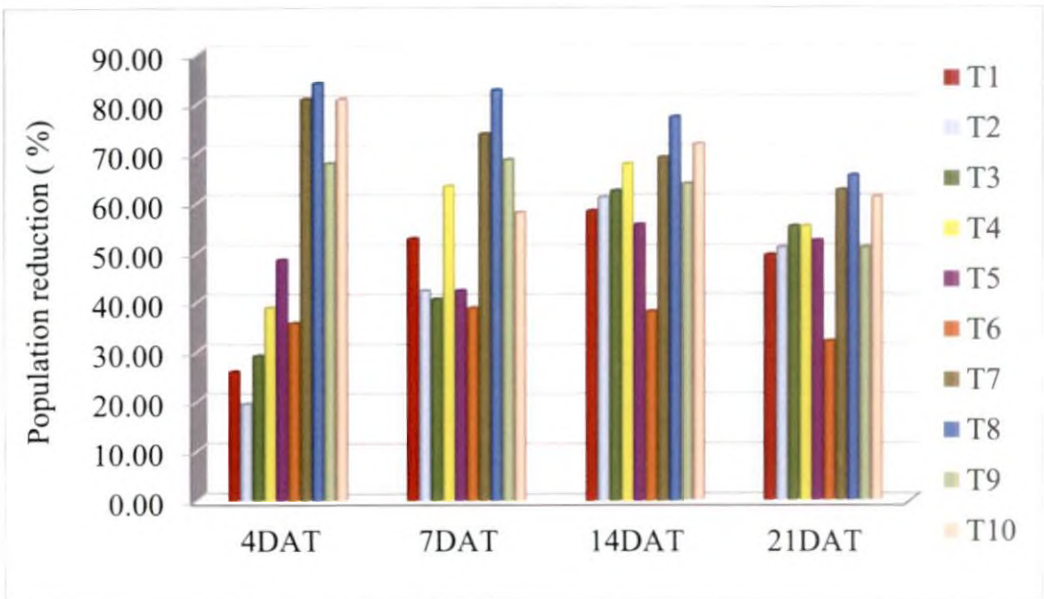
In the second field trial also, almost a similar trend in the effectiveness of the fungal pathogens in reducing the larval population of the *C. medinalis* was seen, Though initially, a slight variation in the performance of the pathogens was noted, on the twenty first day the maximum population reduction was seen in *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (55.09 per cent), followed by talc based *M. anisopliae* (Ma 4 @ 20g $l^{-1}$ (55.09), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (52.17), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (50.74), talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ (49.26) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (31.87), respectively (Fig. 3B).

With respect to the insecticides also, a similar trend as that in first field trial was observed, with the highest percentage reduction in larval population in the chlorantraniliprole @ 30 g a.i $ha^{-1}$ (83.89), followed by thiamethoxam @ 25 g a.i $ha^{-1}$ (80.65), acephate @ 750 g a.i $ha^{-1}$ (80.65) and malathion @ 575 g a.i $ha^{-1}$ (67.73). On the twenty first day also, chlorantraniliprole @ 30 g a.i $ha^{-1}$ maintained its supremacy with 65.22 per cent reduction over untreated followed by acephate @ 750 g a.i $ha^{-1}$ (62.30), thiamethoxam @ 25 g a.i $ha^{-1}$ (60.87) and malathion @ 575 g a.i $ha^{-1}$ (50.74) (Fig. 3B). The suitability of different formulations of chlorantraniliprole in managing the rice leaf folder was reported by Murali *et al.* (2013) and Sarao and Kaur (2013).

Maximum reduction in adult population of *C. medinalis,* both in the first and second field trial was brought about by *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ and talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$, each with 41.48 per cent reduction over untreated in the first field trial. In the second trial, 50.03 and 40.01 per cent reduction was observed in talc based *B. bassiana* (Bb 5) @ 20 g $l^{-1}$ and *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$, respectively (Fig. 4 A and B). However, it was noted that there was no statistical difference among the fungal treatments evaluated at 21 DAT.




(A) Field trial – I




(B) Field trial – II

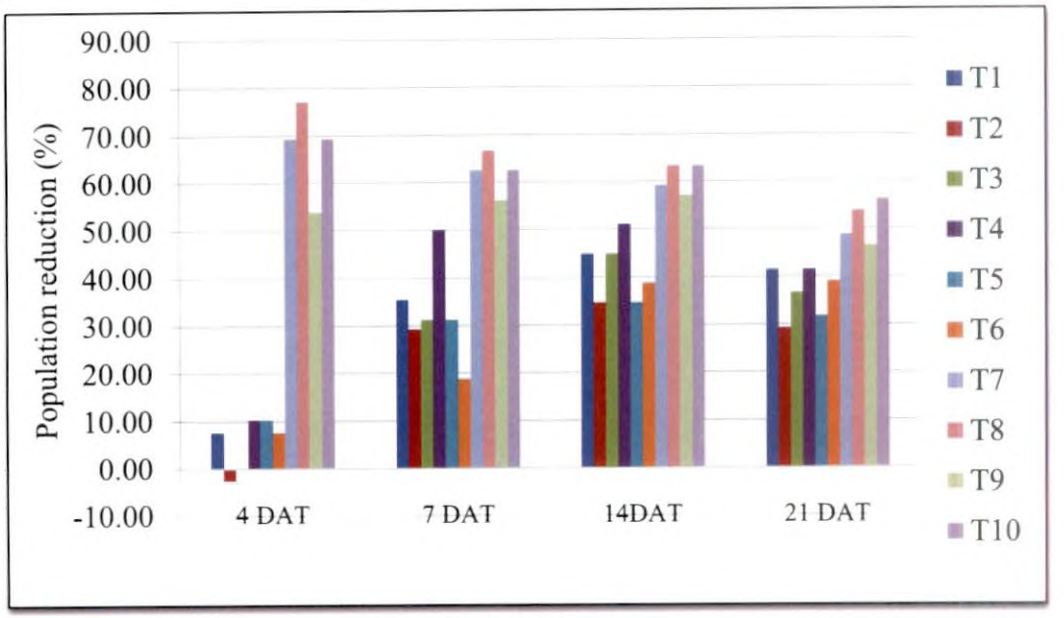
Fig. 3 Reduction in the population of *C.medinalis* larvae in the field experiments

T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$
T3 - Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$
T4 - *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$
T5 - *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$
T6 - *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$
T7 - Acephate @ 750 g a.i $ha^{-1}$
T8 - Chlorantraniliprole @ 30 g a.i $ha^{-1}$
T9 - Malathion @ 575 g a.i $ha^{-1}$
T10 - Thiamethoxam @ 25 g a.i $ha^{-1}$
DAT – Days after treatment

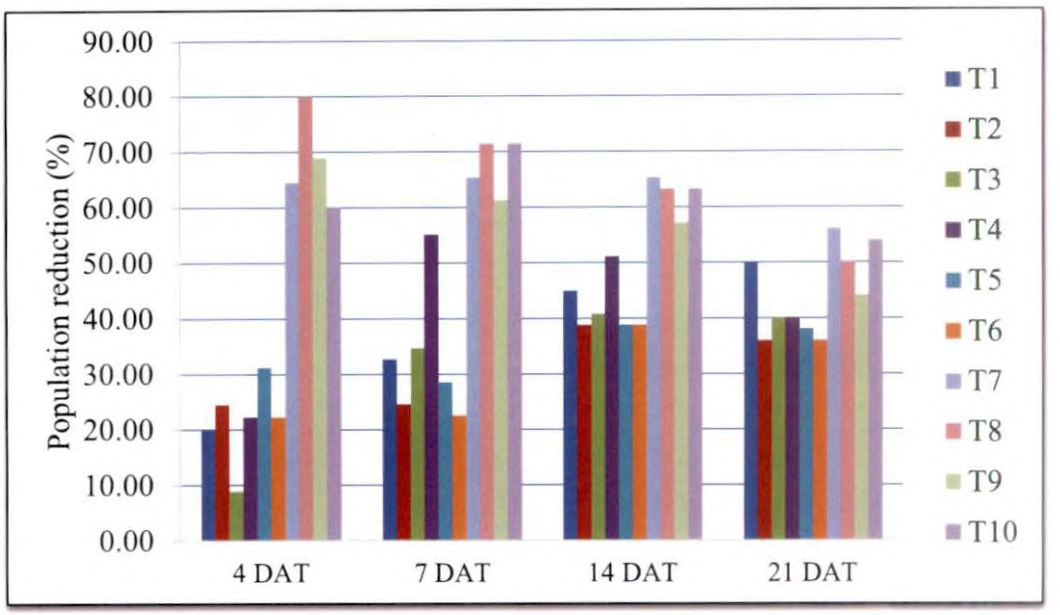
Significant and quicker effect of the chemical pesticides in reducing the adult population of *C. medinalis*, compared to the fungal pathogens was evident in the observations on the fourth day. All the chemicals, except malathion @ 575 g a.i $ha^{-1}$ were on par in their effectiveness, the percentage reduction in population ranged from 76.92 in chlorantraniliprole @ 30 g a.i $ha^{-1}$ to 53.85 in malathion @ 575 g a.i $ha^{-1}$ in the first trial and 80.00 in chlorantraniliprole @ 30 g a.i $ha^{-1}$ to 60.00 in thiamethoxam @ 25 g a.i $ha^{-1}$ in the second trial (Fig.4A and B). However, in the subsequent observations the effect of these chemicals was seen to decline. At 21 DAT, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ and talc based *B. bassiana* (Bb 5) @ 20 $g\ l^{-1}$ were on par with all insecticides evaluated.

Considering the damage caused by *C. medinalis* to rice leaves, it was seen that the maximum reduction in damage was provided by *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ which harboured the lowest larval as well as adult population in both the experiments. At 21 DAT, percentage reduction in damage noted in this treatment was to the tune of 75.81 and 66.44 per cent, respectively, whereas in other treatments with fungal pathogens, the reduction in damage was from 51.75 to 63.78 percentage in the first field trial and 47.31 to 58.76 in the second field trial (Fig.5A and B).

Immediate and significant effect of the chemical pesticides was evident in the damage caused by *C. medinalis* too. Acephate @ 750 g a.i $ha^{-1}$ and thiamethoxam @ 25 g a.i $ha^{-1}$ recorded the highest percentage reduction of 76.33 each, over untreated during the first trial at 4 DAT. The effect of *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ came on par with acephate @ 750 g a.i $ha^{-1}$ at 21 DAT in the first field trial. During the second field trial, chlorantraniliprole @ 30 g a.i $ha^{-1}$ recorded the highest percentage reduction of 81.04 per cent over untreated at 4 DAT. *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ maintained parity with all the chemicals evaluated, except malathion @ 575 g a.i $ha^{-1}$ which was inferior to the other chemicals evaluated on 21 DAT in the second trial(Fig.5A and B).



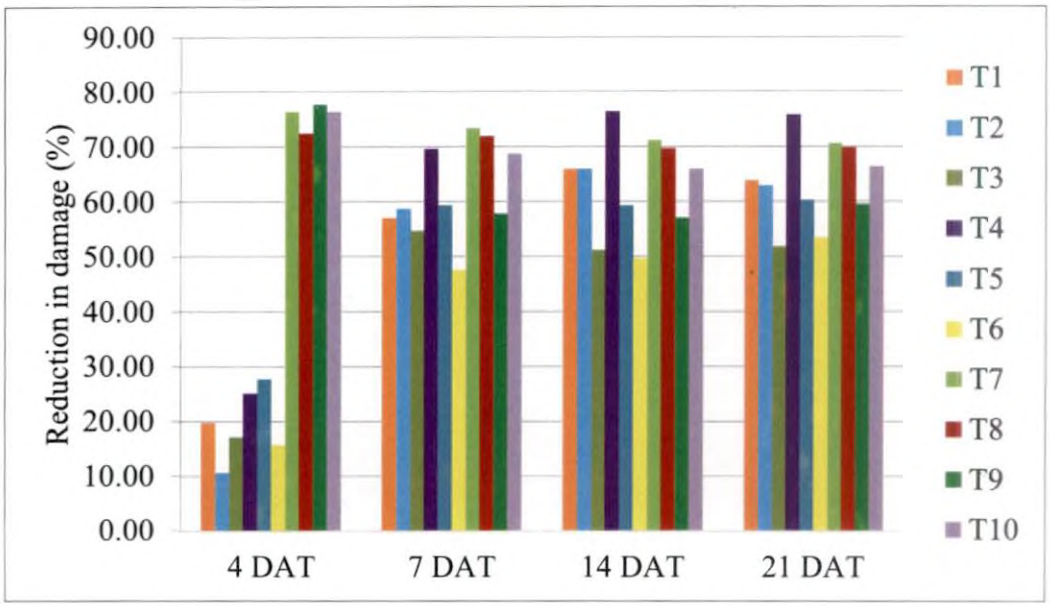

(A) Field trial I



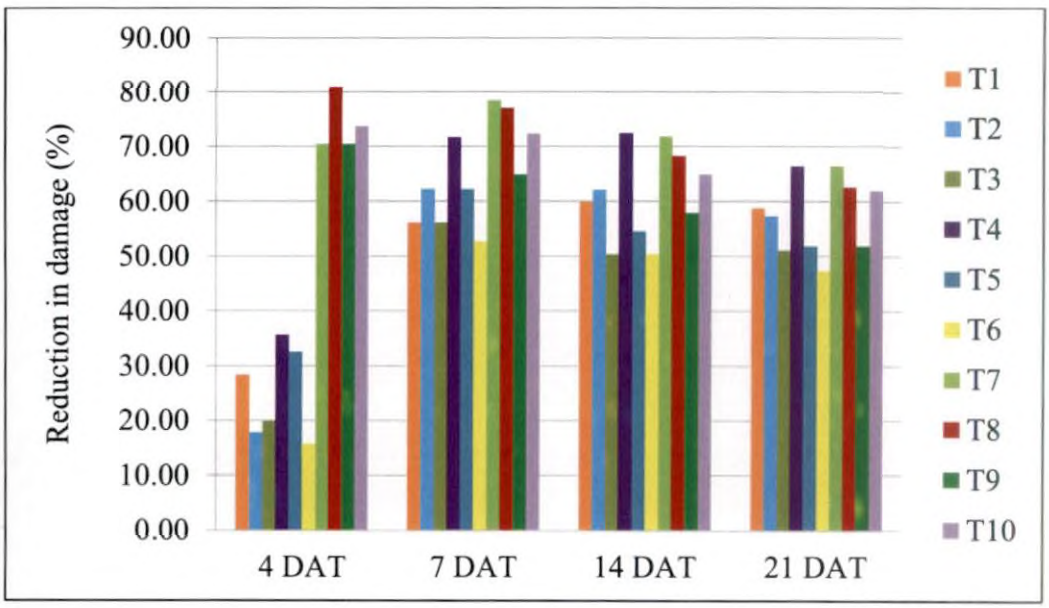

(B) Field trial II

Fig. 4 Reduction in the population of *C. medinalis* adult in the field experiments

T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$
T3 - Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$
T4 - *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$
T5 - *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$
T6 - *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$
T7 - Acephate @ 750 g a.i $ha^{-1}$
T8 - Chlorantraniliprole @ 30 g a.i $ha^{-1}$
T9 - Malathion @ 575 g a.i $ha^{-1}$
T10 - Thiamethoxam @ 25 g a.i $ha^{-1}$
DAT – Days after treatement




(A) Field trial- I




(B) Field trial- II

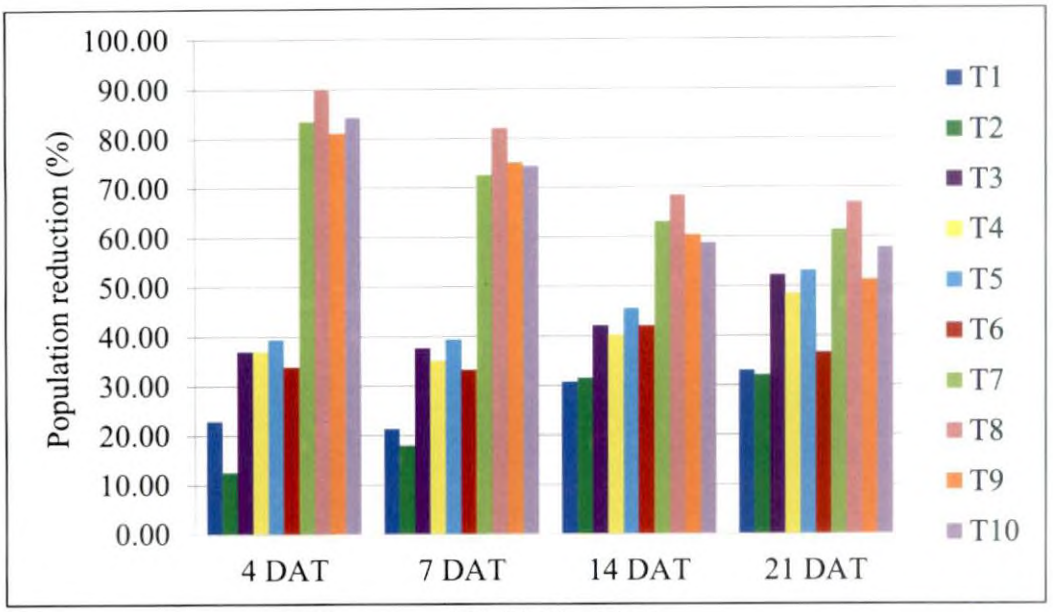
Fig. 5 Reduction in the extent of damage by *C. medinalis* in the field experiments

T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$
T3 - Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$
T4 - *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$
T5 - *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$
T6 - *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$
T7 - Acephate @ 750 g a.i $ha^{-1}$
T8 - Chlorantraniliprole @ 30 g a.i $ha^{-1}$
T9 - Malathion @ 575 g a.i $ha^{-1}$
T10-Thiamethoxam @ 25 g a.i $ha^{-1}$
DAT – Days after treatement

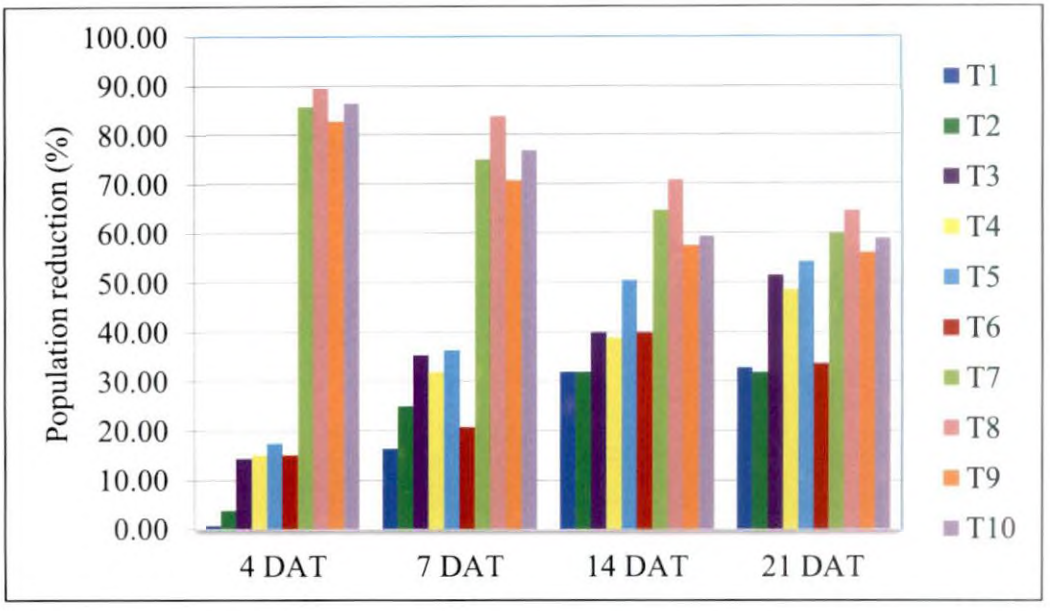
Further, the effect of the fungal pathogens as well as that of the insecticides on the population of *L. acuta* and on the damage by this pest, followed almost the same trend. A slight difference noted was that, the performance of *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$and talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ was slightly better than *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$, which reiterated the performance displayed by *M. anisopliae* (Ma 4) @ $10^{9}$ spores $ml^{-1}$ in the laboratory. The percentage reduction in the population of *L. acuta* in these treatments was 53.21, 52.30 and 48.61, respectively at 21 DAT in the first field trial. The corresponding values being 54.22, 51.42 and 48.61 per cent, respectively in the second field trial (Fig. 6A and B). However, there was no significant difference between these treatments, in both the trials. Loc and Chi (2005) observed *M. anisopliae* as an effective fungal pathogen of rice bug and they recommended the use of three isolates, Ma-OM3-BD, Ma-HG-B and Ma-HG-BD @ 6 x $10^{12}$ conidia $ha^{-1}$ for the management of *L. acuta*.

Among the chemicals, the performance of chlorantraniliprole @ 30 g a.i $ha^{-1}$ was on par with that of acephate @ 750 g a.i $ha^{-1}$ in both the trials. The percentage reduction in population of *L. acuta* observed in chlorantraniliprole @ 30 g a.i $ha^{-1}$ was 89.77 at 4 DAT in the first field trial, later it got reduced to 66.97 on 21 DAT. The values being 83.46 and 61.46, respectively for acephate @ 750 g a.i $ha^{-1}$ (Fig. 6A and B). .

A similar trend was observed in the second trial also. However, the performance of the chlorantraniliprole @ 30 g a.i $ha^{-1}$ was significantly superior to *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ in both the trials. On the twenty first day, the performance of *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ which recorded the least population, among the fungal pathogens came on par with that of thiamethoxam @ 25 g a.i $ha^{-1}$ and malathion @ 575 g a.i $ha^{-1}$ in both trials. The population reduction in thiamethoxam @ 25 g a.i $ha^{-1}$ and malathion @ 575 g a.i $ha^{-1}$ in the first field trial were 57.80 and 51.36 per cent, respectively and that in the second field trial were




(A) Field trial- I




(B) Field trial- II

Fig. 6 Reduction in the population of *L. acuta* in the field experiments

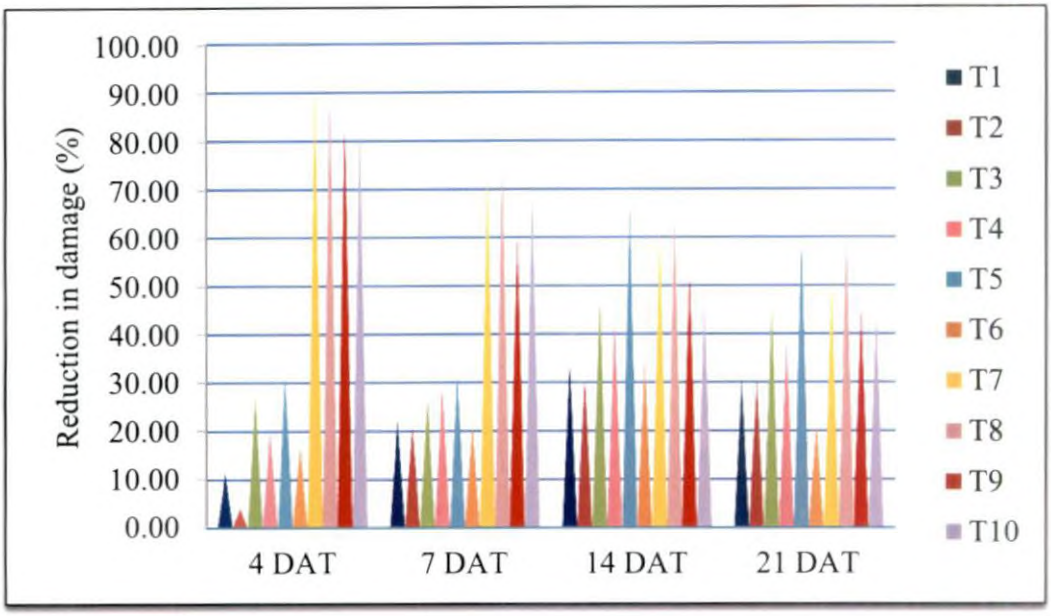
T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$
T3 - Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$
T4 - *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$
T5 - *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$
T6 - *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$
T7 - Acephate @ 750 g a.i $ha^{-1}$
T8 - Chlorantraniliprole @ 30 g a.i $ha^{-1}$
T9 - Malathion @ 575 g a.i $ha^{-1}$
T10 - Thiamethoxam @ 25 g a.i $ha^{-1}$
DAT – Days after treatement

58.87 and 56.07, per cent respectively. Kiran and Veeranna (2012) also made similar observation of equal effectiveness of *M. anisopliae* and thiamethoxam against the hemipteran bug *Nilaparvatha lugens* Stal. infesting rice.

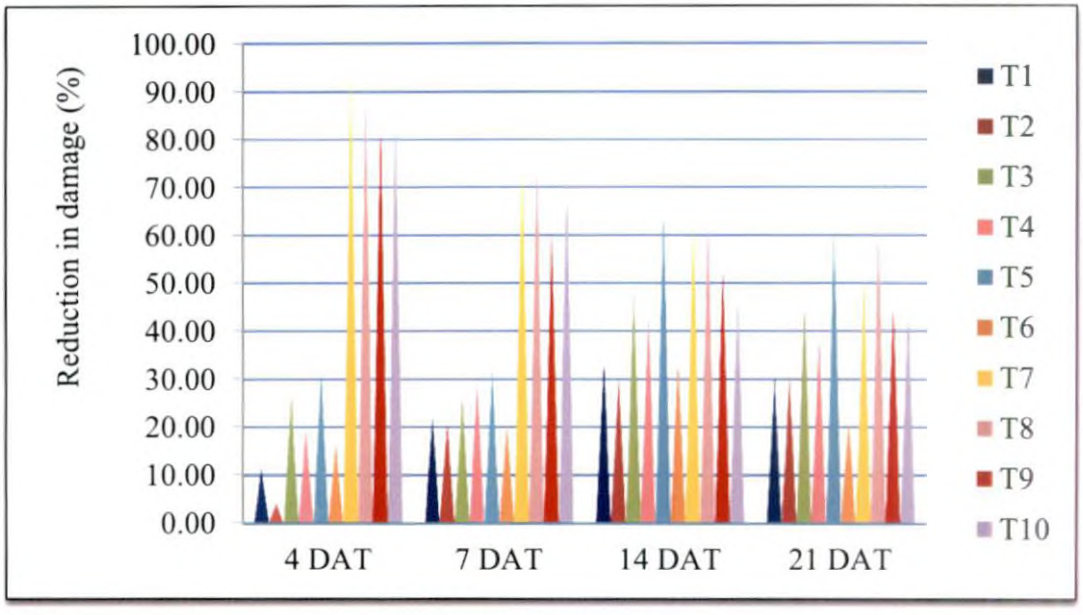
The effect of the treatments *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$, chlorantraniliprole @ 30 g a.i $ha^{-1}$ and acephate @ 750 g a.i $ha^{-1}$ on the extent of damage to the grains by *L. acuta* was on par. The percentage reduction of damaged grains in these treatments over untreated noted at 21 DAT in the first trial were 60.96, 66.31, 55.61, respectively. The corresponding values in the second trial were 60.96, 62.57and 51.87 per cent, respectively (Fig.7A and B). Kalita *et al.* (2009) reported that *M. anisopliae* WP @ 1.15 per cent ($10^6$ spores $ml^{-1}$) and *B. bassiana* WP @ 1.15 per cent ($10^6$ spores $ml^{-1}$) reduced the extent of damage by 4.89 and 5.15 per cent only respectively. The difference in the reduction of damage can be attributed to the variation of isolates used in the study.

Efficacy of entomopathogenic fungi against other rice pests under field conditions was reported by many researchers. The suitability of *M. anisopliae* and *B. bassiana* @ $10^{12}$ spores $ml^{-1}$ in managing *N. lugens* was observed by Aguda and Rombach (1987); Rao (1989) and Reddy *et al.* (2013). *B. bassiana* @$10^6$ spores $ml^{-1}$ was recommended for the management of *Dicladispa armigera* (Oliver) (Hazarika and Puzari, 1990; Puzari and Hazarika, 1991 and Puzari *et al.*, 1994). Karthikeyan and Jacob (2010) recorded the efficiency of *B. bassiana* @ $10^7$ spores $ml^{-1}$ in managing *Leptispa pygmaea* Baly under Kerala conditions. Fazeli- Dinan *et al.* (2012) recommended the use of *B. bassiana* @ $10^7$ spores $ml^{-1}$ as a cost effective method for the management of the green semi looper, *Naranga aenescens* Moore.

The effect of the different fungal treatments was reflected correspondingly in the yield obtained in two field experiments also. The treatments *viz.,B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ which recorded the lowest population and damage of *C. medinalis* and *L. acuta*, respectively produced




(A) Field trial- I




(B) Field trial- II

Fig.7 Reduction in the extent of damage by *L. acuta* in the field experiments

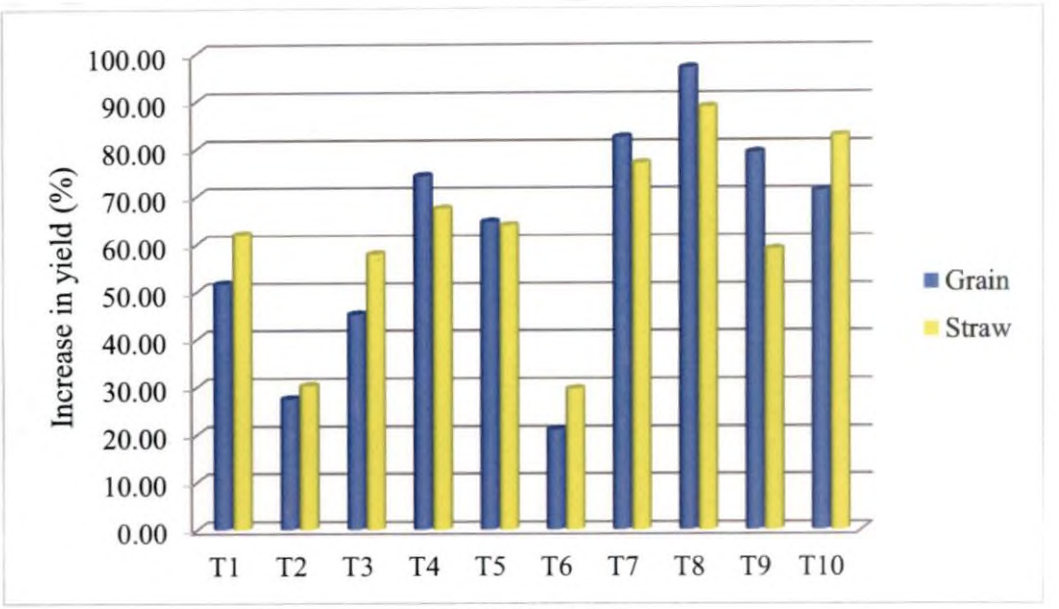
T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$
T3 - Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$
T4 - *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$
T5 - *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$
T6 - *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$
T7 - Acephate @ 750 g a.i $ha^{-1}$
T8 - Chlorantraniliprole @ 30 g a.i $ha^{-1}$
T9 - Malathion @ 575 g a.i $ha^{-1}$
T10 - Thiamethoxam @ 25 g a.i $ha^{-1}$
DAT – Days after treatement

the maximum yield among the fungal treatments. During the first field trial *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ treatments caused 74.19 per cent and 64.52 per cent increase in grain yield over untreated whereas 64.84 and 71.88 per cent increase in grain yield was noted during the second trial (Fig.8A and B).

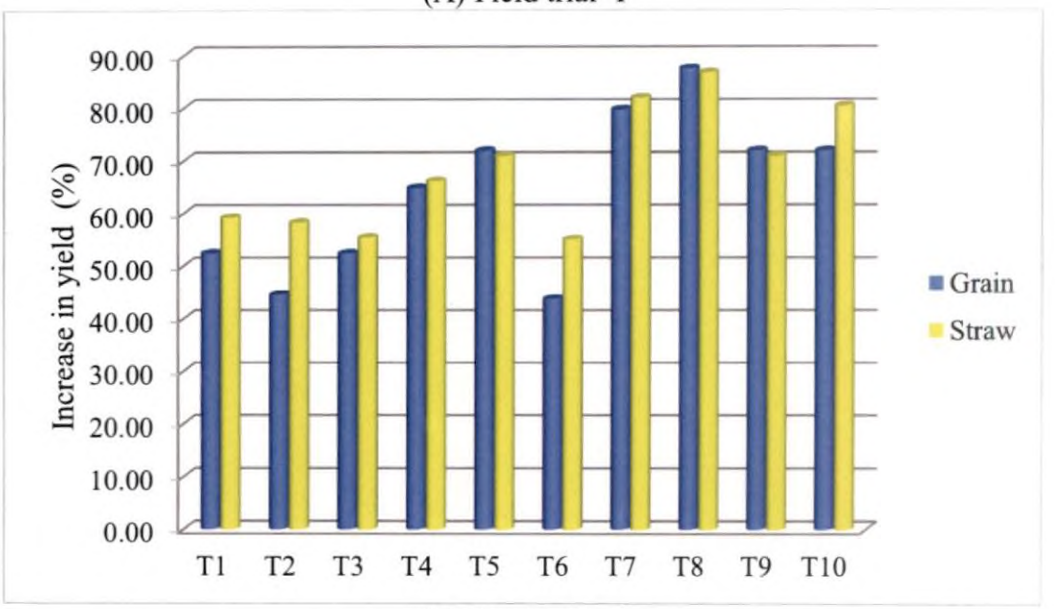
The yield obtained from the different chemical treatments in the first experiment varied significantly. Consistent superiority of the chemical insecticides could not be observed in both the experiments, as thiamethoxam @ 25 g a.i $ha^{-1}$ that yielded significantly lower yield (3.53 kg $plot^{-1}$) compared to chlorantraniliprole @ 30 g a.i $ha^{-1}$ (4.07 kg $plot^{-1}$) in the first experiment came on par with all the other three chemical treatments *viz.*, chlorantraniliprole@ 30 g a.i $ha^{-1}$ (4.00 kg $plot^{-1}$), acephate @ 750 g a.i $ha^{-1}$ (3.83 kg $plot^{-1}$) and malathion@ 575 g a.i $ha^{-1}$ (3.67 kg $plot^{-1}$) with an average yield of 3.67 kg $plot^{-1}$ in the second experiment.

The straw yield recorded from the different treatments varied significantly in both the field trials. During the first field trial, among the fungal treatments, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ caused 67.30 per cent and 63.81 per cent increase in straw yield over untreated whereas 66.14 and 70.89 per cent increase in straw yield was noted during second trial.

Kay *et al.* (1993) recorded cent per cent mortality of *L. acuta* with the application of acephate @ 750 g a.i $ha^{-1}$. Zhong *et al.* (2002) observed 88.90 per cent reduction in the larval population of *C. medinalis* at three DAT while Smitha (2004) reported the efficiency of acephate @750 g a.i $ha^{-1}$ in reducing the population and damage of both *C. medinalis* and *L. acuta*. Ashokappa (2011) recommended thiamethoxam @ 25 g a.i $ha^{-1}$ for the management of *L. acuta* while Murali *et al.* (2013) used a combination product of chlorantraniliprole five per cent GR and thiamethoxam 10 per cent GR @ 6 kg $ha^{-1}$ and observed significant reduction in the




(A) Field trial- I




(B) Field trial- II

Fig. 8 Increase in yield in different treatments over untreated.

T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$
T3 - Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$
T4 - *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$
T5 - *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$
T6 - *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$
T7 - Acephate @ 750 g a.i $ha^{-1}$
T8 - Chlorantraniliprole @ 30 g a.i $ha^{-1}$
T9 - Malathion @ 575 g a.i $ha^{-1}$
T10 - Thiamethoxam @ 25 g a.i $ha^{-1}$

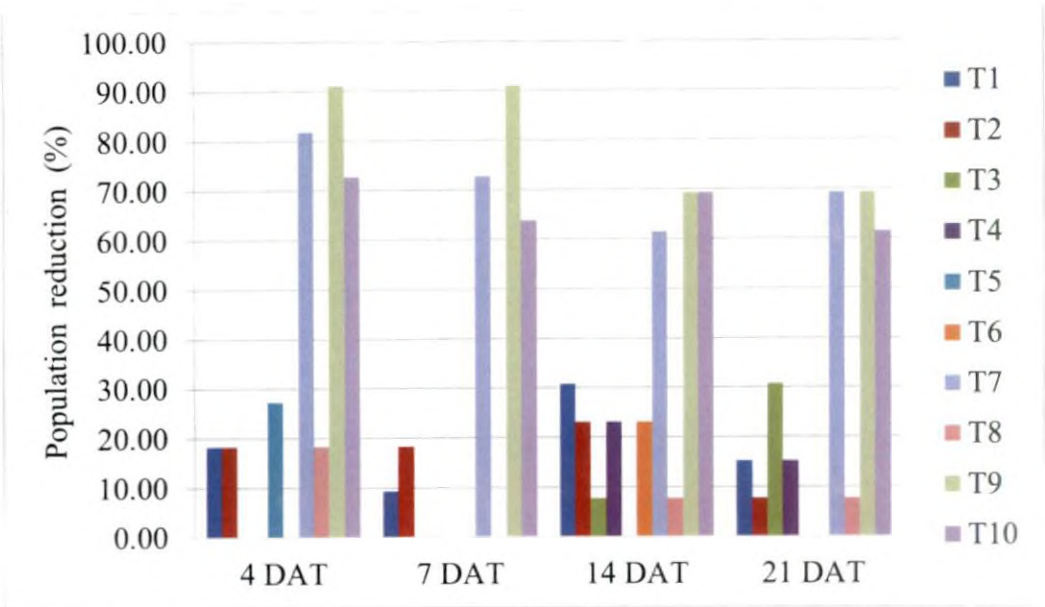
population of *C. medinalis*. Sarao and Kaur (2013) reported the suitability of chlorantraniliprole @ 30 g a.i $ha^{-1}$ in reducing the population of rice leaf roller.

A perusal of the impact of the fungal pathogens and chemical insecticides on the population of the hymenopteran parasitoids indicated significantly lower number of hymenopteran parasitoids in plots treated with chemical pesticides except chlorantraniliprole @ 30 g a.i $ha^{-1}$, over untreated on the fourth day after the application of treatments and in the subsequent observations until 21 DAT in both the trials. At 21 DAT, the population reduction over untreated ranged from zero to 15.24 and from zero to 9.26 for the fungal treatments in the first and second trials, respectively, whereas the corresponding population reduction ranged from 7.62 to 69.28 and from 9.26 to 81.74, respectively in chemical insecticide treated plots (Fig. 9A and B).

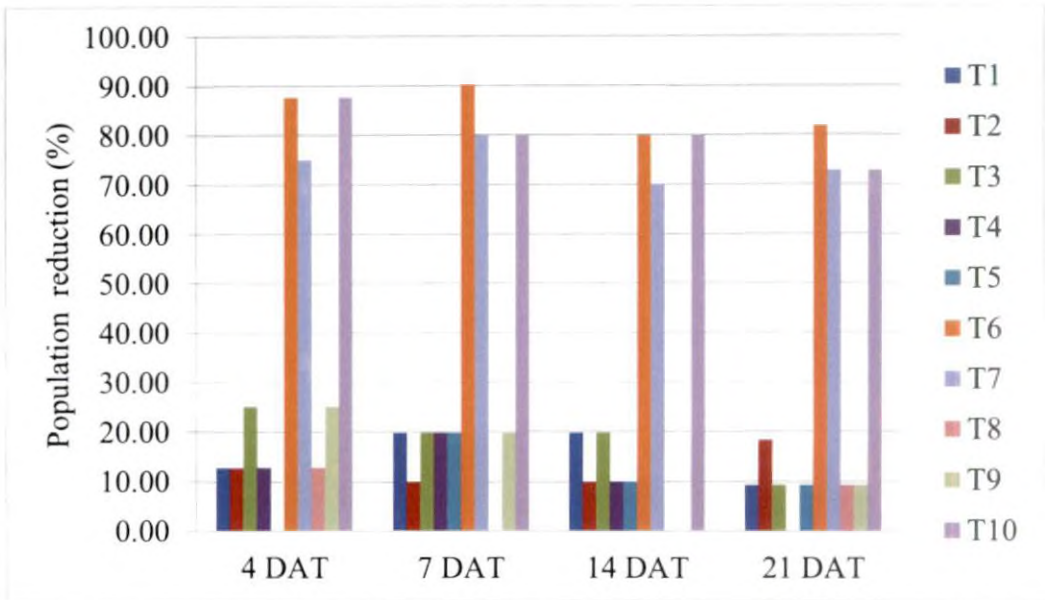
A similar trend was observed on the effect of fungal and chemical treatments on the population of insect and spider predators also. At 21 DAT, the population reduction of insect predators over untreated ranged from zero to 6.82 per cent and 9.13 to 56.85 per cent in the fungal and chemical treatments, respectively, in the first trial. The corresponding values in second field trial ranged from 2.15 to 10.83 per cent and 13.05 to 58.71 per cent, respectively (Fig. 10A and B).

The safety of the fungal pathogens to spider predators was evident from the population reduction that varied from zero to 16.64 per cent compared to the reduction in the chemical treatments that ranged from 9.50 to 38.07 per cent in first field trial at 21 DAT. The corresponding values in the second field trial ranged from 6.38 to 19.14 and 10.66 to 42.57 per cent in fungal and chemical treatments, respectively (Fig.11A and B).

Chi *et al.* (2005) revealed the safety of entomopathogenic fungi against predatory insects and spiders. Further, Reddy *et al.* (2013) recorded significantly higher population of insect and spider predators in *B. bassiana* (0.5 per cent) and



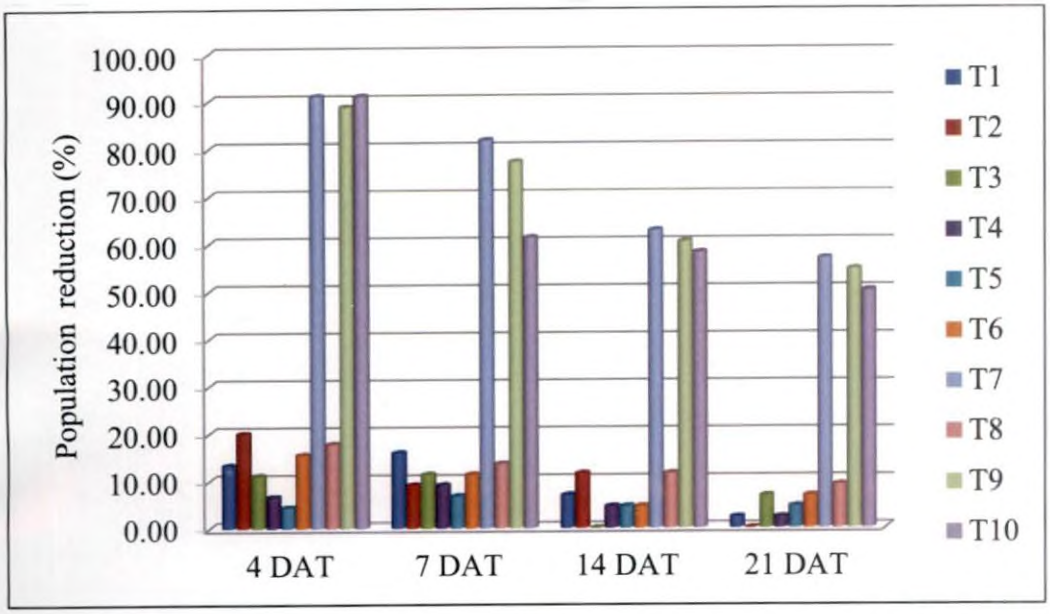

(A) Field trial- I



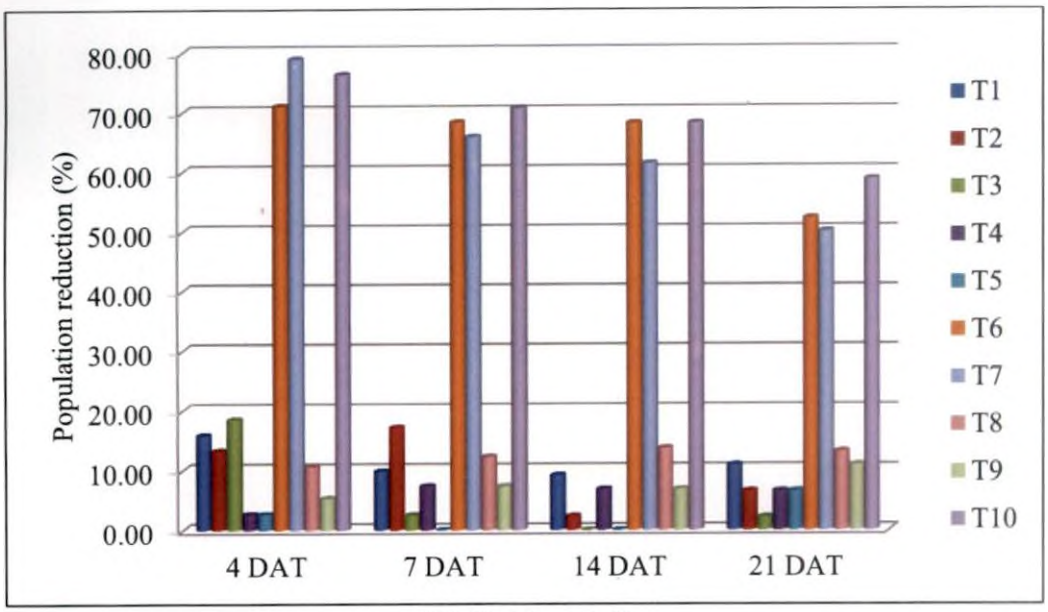

(B) Field trial-II

Fig. 9 Reduction in the population of hymenopteran parasitoids in the field experiments

T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$
T3 - Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$
T4 - *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$
T5 - *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$
T6 - *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$
T7 - Acephate @ 750 g a.i $ha^{-1}$
T8 - Chlorantraniliprole @ 30 g a.i $ha^{-1}$
T9 - Malathion @ 575 g a.i $ha^{-1}$
T10- Thiamethoxam @ 25 g a.i $ha^{-1}$
DAT – Days after treatement



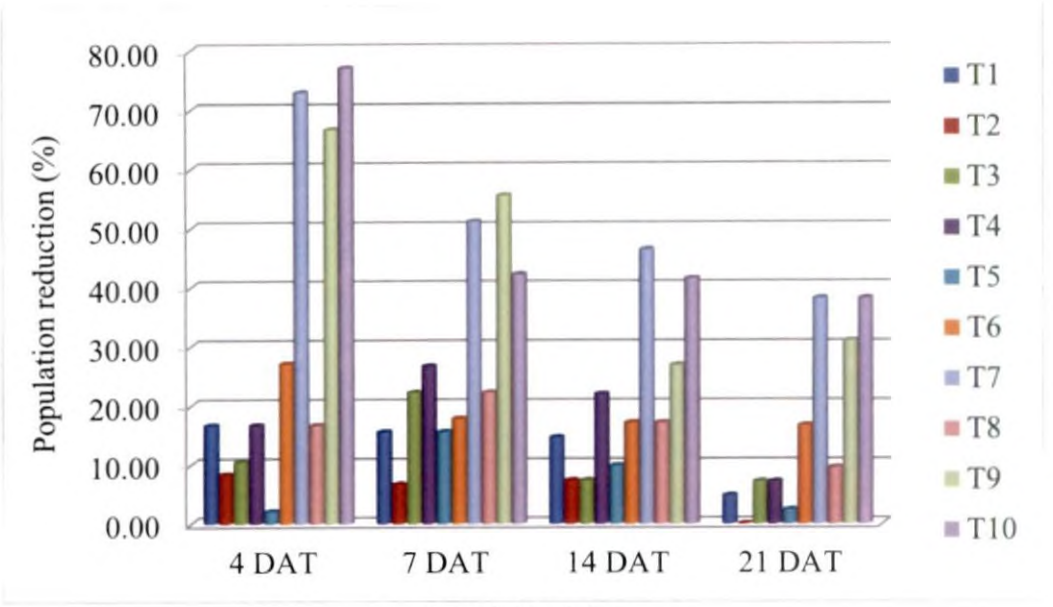

(A) Field trial- I



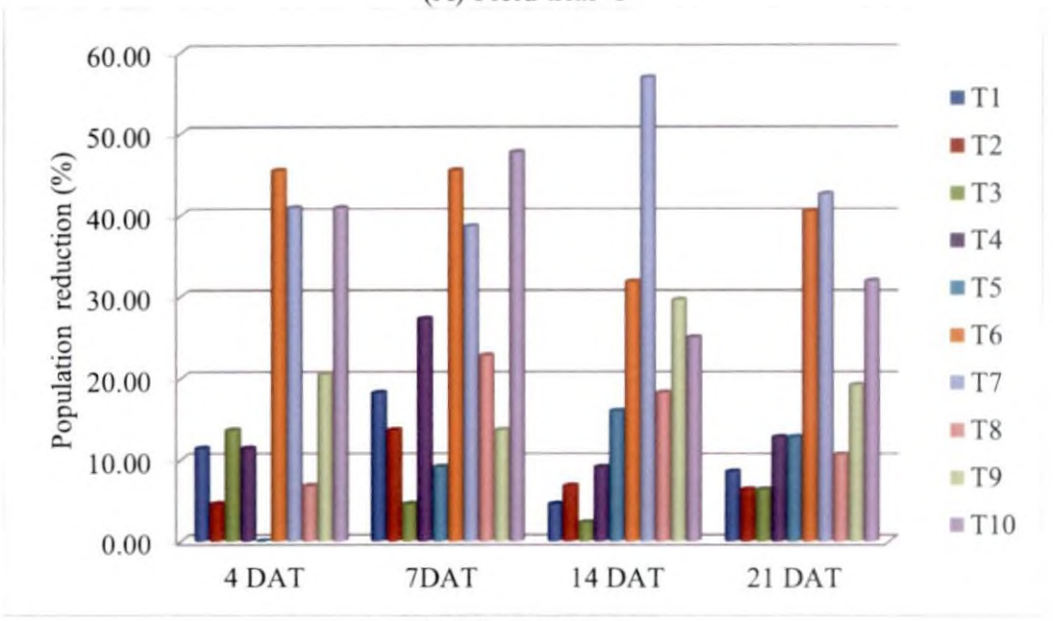

(B) Field trial- II

Fig. 10 Reduction in the population of insect predators in the field experiments

T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$
T3 - Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$
T4 - *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$
T5 - *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$
T6 - *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$
T7 - Acephate @ 750 g a.i $ha^{-1}$
T8 - Chlorantraniliprole @ 30 g a.i $ha^{-1}$
T9 - Malathion @ 575 g a.i $ha^{-1}$
T10 - Thiamethoxam @ 25 g a.i $ha^{-1}$
DAT – Days after treatement




(A) Field trial- I




(B) Field trial- II

Fig.11 Reduction in the population of spider predators in the field experiments

T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g $l^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g $l^{-1}$
T3 - Talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$
T4 - *B. bassiana* (Bb 5) @$10^{10}$ spores $ml^{-1}$
T5 - *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$
T6 - *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$
T7 - Acephate @ 750 g a.i $ha^{-1}$
T8 - Chlorantraniliprole @ 30 g a.i $ha^{-1}$
T9 - Malathion @ 575 g a.i $ha^{-1}$
T10 - Thiamethoxam @ 25 g a.i $ha^{-1}$
DAT- Days after treatement

*M. anisopliae* (0.5 per cent) treated plots, compared to that in acephate 75 SP @ 1.5 g $l^{-1}$ treated plots.

Earlier, Smitha (2004) reported the toxicity of acephate 0.05 per cent to predators and parasitoids in rice ecosystem while broad toxicity of organophosphates to natural enemies was observed by Beers (2008). Preetha *et al.* (2010) also recorded high toxicity of organophosphorous insecticides to insect predators in rice ecosystem.

The relative safety of chlorantraniliprole, an anthranilic diamide to natural enemies observed in the present study can be attributed to its mode of action in sensitive species. According to Cordova *et al.* (2006) when fed by insects, chlorantraniliprole activates ryanodine receptors (RyRs) and stimulates the release and depletion of intracellular calcium stores from the sarcoplasmic reticulum of muscle cells, causing impaired muscle regulation, paralysis and ultimately death of sensitive species. The selectivity of chlorantraniliprole to beneficial insects was earlier reported by Bassi *et al.* (2008) and Dinter *et al.* (2008). Safety of the insecticide, chlorantraniliprole to spiders in rice ecosystem was reported by Jaafar *et al.* (2013) and Karthick *et al.* (2014).

Harmful effect of neonicotinoids and thiamaethoxam to parasitoids and predators was documented by Cloyd and Bethke (2011) and Prabhaker *et al.* (2011).

Only economically viable technologies will be conceived by farmers, hence, computation of benefit cost ratio (BCR) for the different treatments is also inevitable. The perspective of the Keralites to chemical free agriculture produce favours its market in spite of the higher price. 25 per cent hike in price for chemical free unhusked paddy grains are offered currently in the market. Considering this, the BCR worked out in the first trial, ranked in the following order *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (1.65), chlorantraniliprole @ 30 g a.i $ha^{-1}$ (1.58), *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (1.55), acephate @ 750 g a.i $ha^{-1}$ (1.48), malathion @ 575 g a.i $ha^{-1}$ (1.46), talc based *B. bassiana* (Bb 5) @ 20g $l^{-1}$ (1.44), thiamethoxam @ 25 g a.i

$ha^{-1}$ (1.39), talc based *M. anisopliae* (Ma 4) @ 20g $l^{-1}$ (1.38), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (1.20) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$ (1.12).

In the second trial BCR followed the order *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ (1.63), *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ (1.56), chlorantraniliprole @ 30 g a.i $ha^{-1}$ (1.50), acephate @ 750 g a.i $ha^{-1}$ (1.46), talc based *B. bassiana* (Bb 5) @ 20g $l^{-1}$ (1.45), *M. anisopliae* (Ma 4) in talc @ 20g $l^{-1}$ (1.45), thiamethoxam @ 25 g a.i $ha^{-1}$ (1.39), talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ (1.38), malathion @ 575 g a.i $ha^{-1}$ (1.39) and *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$(1.35).

Reddy *et al.* (2013) recorded BCR of 1.9 and 1.7 for the treatments with *B. bassiana* 0.5 per cent and *M. anisopliae* 0.5 per cent, respectively in rice plots.

An attempt was also made to grade the different treatments, considering their overall effect on the population and damage caused by *C. medianlis* and *L. acuta*, population of natural enemies and benefit-cost ratio in the two trials. The grading of the different treatments on a one to 10 scale indicated the following order chlorantraniliprole @ 30 g a.i $ha^{-1}$ > *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ > acephate @ 750 g a.i $ha^{-1}$ > *M. anisopliae* @ $10^{10}$ spores $ml^{-1}$ (Ma 4) > thiamethoxam @ 25 g a.i $ha^{-1}$ > talc based *B. bassiana* (Bb 5) = talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ > malathion @ 575 g a.i $ha^{-1}$ > talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ > *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$, which secured the grade points of 156, 143, 130, 129, 120, 110, 110, 100, 93 and 82, respectively (Fig.12). From the above, it was seen that *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ ranked the best among the fungal treatments and chlorantraniliprole @ 30 g a.i $ha^{-1}$ ranked the best among the insecticide treatments.

Though the social and environmental costs of chemical pesticides are high, they need to be retained as one of the tools in pest management for containing serious pest outbreaks. Therefore, the compatibility of insecticides with other components of IPM, especially with that of the bioagents needs elucidation. An assessment of the

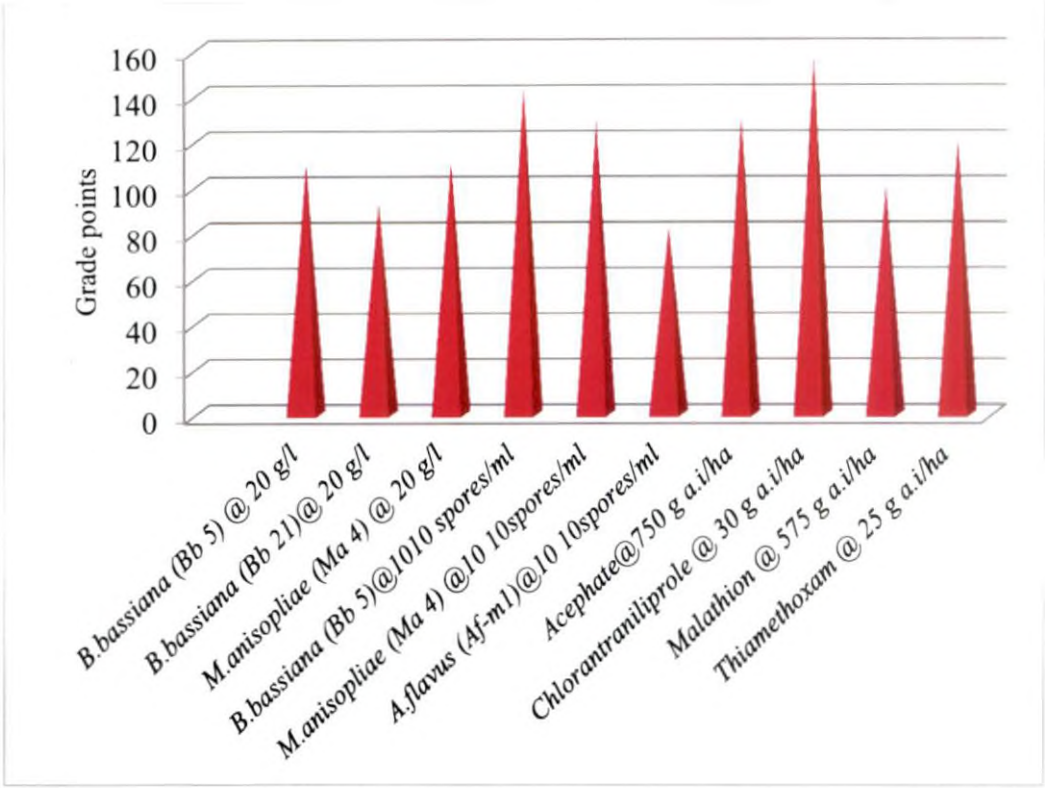



Fig.12 Grading of treatments in the field trials.

compatibility of fungal pathogens, *B. bassiana* (Bb 5 and Bb 21), *M. anisopliae* (Ma 4), *A. flavus* (Af-m1) with three inseciticides *viz*., acephate, chloratraniliprole and thiamethoxam at three different concentrations (3.5) carried out in the laboratory revealed that all the insecticides were compatible with the pathogens evaluated. The grading by Hassan (1989) suggested that if the inhibition by insecticides on growth of fungi was less than 50 per cent, that chemical could be considered as harmless. In the present study all the insecticides were harmless as they showed only less than 50 per cent inhibition in growth with the exception of the organophosphorus insecticide, acephate 0.150 and 0.225 per cent, which recorded slightly higher inhibition of 54.51 and 56.35 per cent respectively in *M. anisopliae* (Ma 4). It was also seen that the inhibition exerted was concentration dependent. Findings of Asi *et al.,* 2010 and Rashid *et al.,* 2010 also support these results. Babu *et al.* (2014) reported high toxicity of organophosphorus insecticide, quinalphos 0.30 per cent to *M. anisopliae*. Among the different insecticides evaluated, chlorantraniliprole was the least inhibitory which caused only 8.47 per cent inhibition in *M. anisopliae* (Ma 4) to 45.60 per cent inhibition in *A. flavus* (Afm-1) even at the highest concentration of 0.008 per cent in the medium. This was followed by the neonicotinoid insecticide, thiamethoxam 0.008 per cent, which showed only 18.97 per cent inhibition in *M. anisopliae* (Ma 4) to 43.21 per cent inhibition in *A. flavus* (Af-m1). Acephate belonging to organophoshorus group was the most inhibitory to the fungi at all the concentrations evaluated (Fig.13A). Similar observation made by Rachappa *et al.* (2007) was that the insecticides belonging to neonicotinoid caused only 11.10 per cent inhibition of *M. anisopliae* compared to 46.66 per cent inhibition by organophosphorus insecticides. Analogous findings by Anis (2014) were that imidacloprid, a neonicotinoid insecticide was less inhibitory to *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) compared to organophosphorus insecticide, malathion. In the studies conducted by Lokesh (2014) another newer molecule, emamectin benzoate 0.002 per cent was found less inhibitory to *B. bassiana* and *M. anisopliae*.

Whereas the growth of fungi was found to decrease with increase in concentration of insecticide in the media, the spore count was found to increase with increase in insecticides concentration. However, the percentage reduction in spore count in poisoned media over unpoisoned is presented in Fig.13B. It is known that factors such as nutrients, chemicals etc. enhances sporulation of fungi (Palma-Guerrero *et al.*, 2010) this may be one of the reasons for the higher spore count noted invariably for all the fungi when grown in higher doses of pesticides in the compatibility studies. Good sporulation of *B. bassiana* and *M. anisopliae* in poisoned media was observed by Dhar and Kaur (2009), who attributed this behavior to the physiological resistance offered by the fungi to the pesticides. Zimmermann (1975) opined that inhibition of mycelial growth by insecticides was not indicative of reduction in sporulation and conidial germination. Another observation of Tamai *et al.* (2002) was that, there was no positive relationship between vegetative growth and conidial yield.

Sporulation was inhibited to varying extent by insecticides. Chlorantraniliprole at its highest concentration of 0.008 per cent was the least inhibitory, the percentage reduction varied from only 2.75 to 10.42 in *A. flavus* (Af-m1) and *B. bassiana* (Bb 5). Thiamethoxam 0.008 per cent also exhibited low inhibition that varied from 4.60 per cent in *B. bassiana* (Bb 21) to 8.59 per cent in *B. bassiana* (Bb 5), respectively. Acephate 0.075 per cent produced the maximum inhibition of 28.37 per cent in *A. flavus* (Af-m1). Variations in the compatibility of *B. bassiana* to various insecticides were earlier reported by Anderson and Roberts (1983) and Rajanikanth *et al.* (2010).

Further evidence on compatibility of these fungi with pesticides was noted from the conidial viability of these fungi grown in poisoned media (Fig.13C). The reduction in conidial viability was less than 20 per cent in all the fungi grown in the different poisoned media. *B. bassiana* (Bb 21) and *M. anisopliae* (Ma 4) when grown in the higher doses of chlorantraniliprole (0.008 per cent) and thiamethoxam (0.008

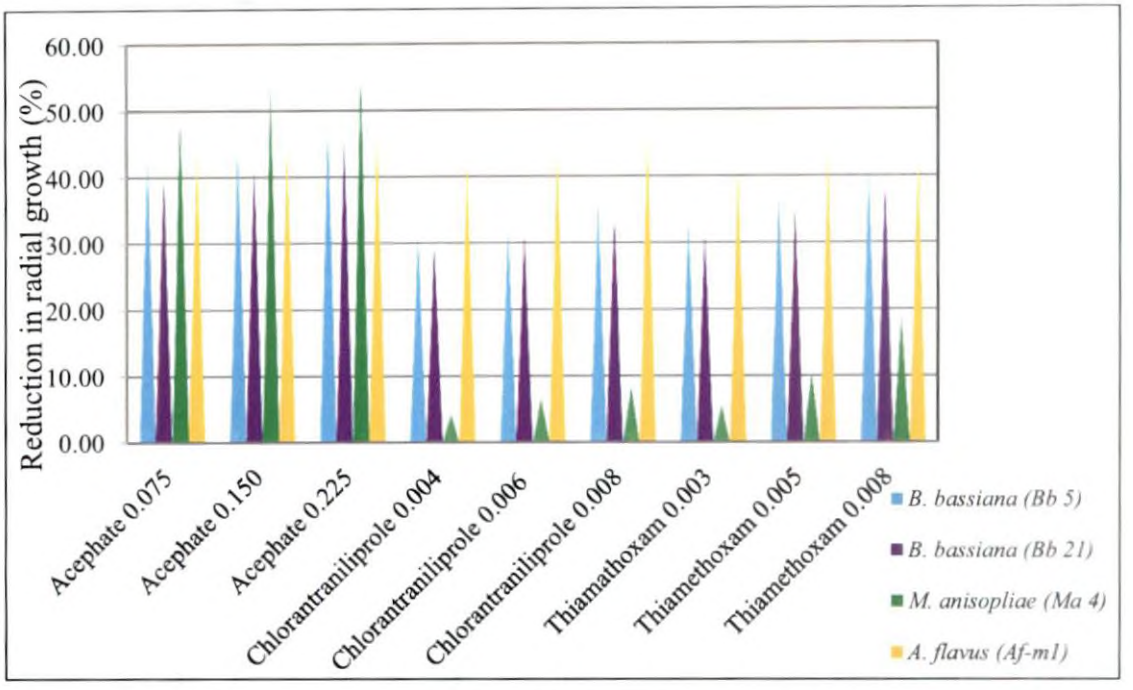



Fig. 13 (A) Reduction in growth of fungi in the poisoned media

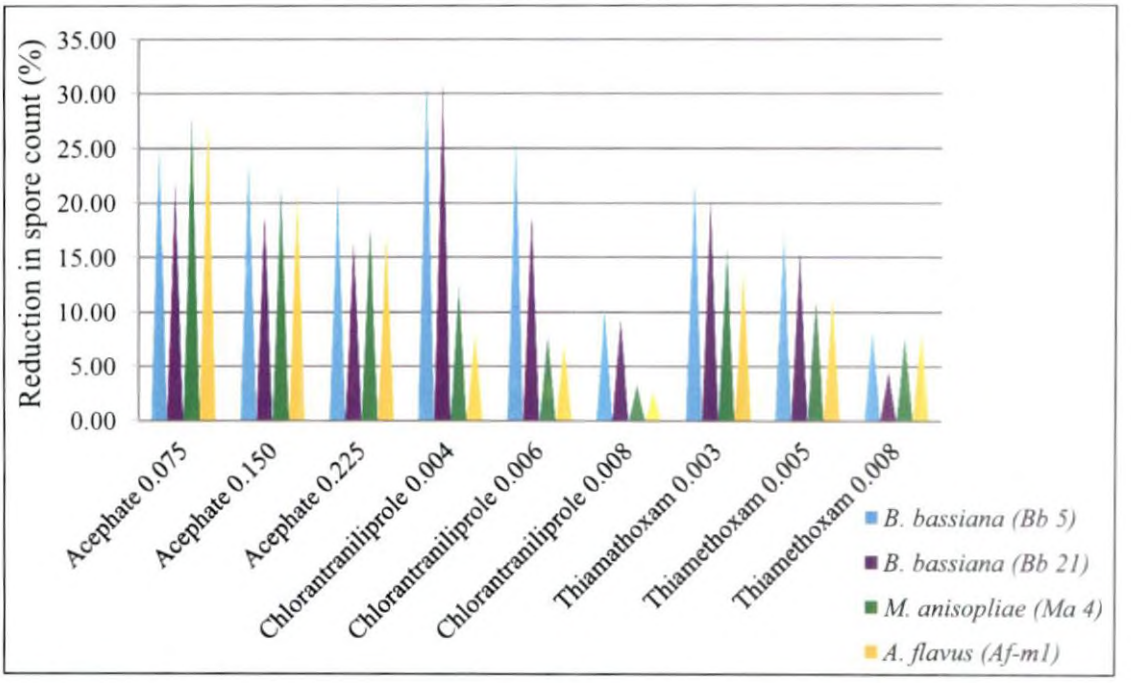



Fig. 13 (B) Reduction in spore count of fungi in the poisoned media

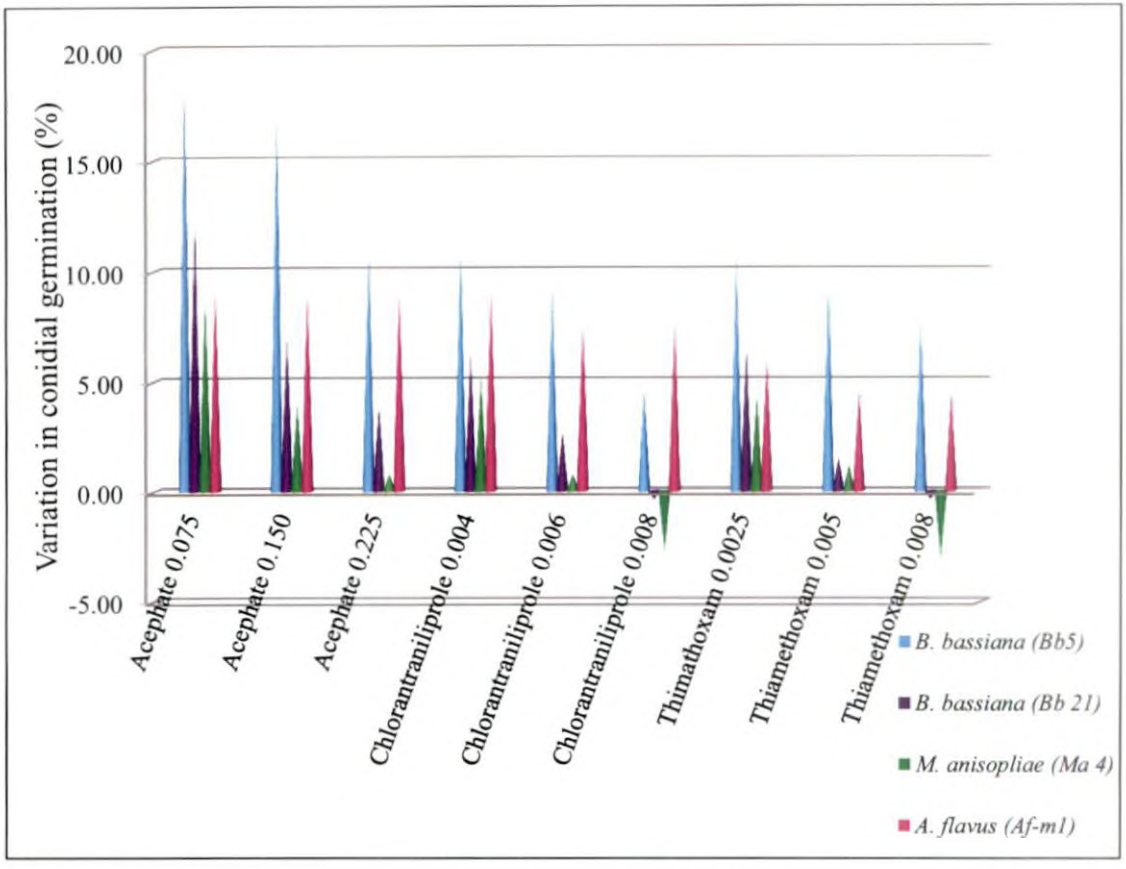



Fig.13 (C) Effect of insecticides on the conidial germination of fungi

per cent) the conidial viability was even higher than that in the unpoisoned. Silva *et al.* (2013) also reported that thiamethoxam did not affect the conidial germination and categorized it as compatible with *M. anisopliae* which supports the present findings.

It was seen from the present study that the compatibility varied with fungi and insecticides. This is supported by the statements of Alves and Leucona (1998) and Tanzini *et al.* (2002) that the toxicity of pesticides to fungal entomopathogens varied with the fungus species, chemical nature of the active ingredient, mode of action, product formulation and recommended label rate.

To examine the bioefficacy of the fungi grown in poisoned media, the spore suspension from 14 day old culture at $10^9$ spores $ml^{-1}$ was sprayed on the adults of *L. acuta* collected from the stock culture maintained in the laboratory. There was no significant difference in the mean mortality of *L. acuta* treated with different fungi grown in poisoned media as well as that grown in untreated. Overtly, it is evident from the growth, spore count, conidial viability and bioefficacy that, *B.bassiana* (Bb 5 and Bb 21) and *M. anisopliae* (Ma 4) are suitable for integration in IPM programmes.

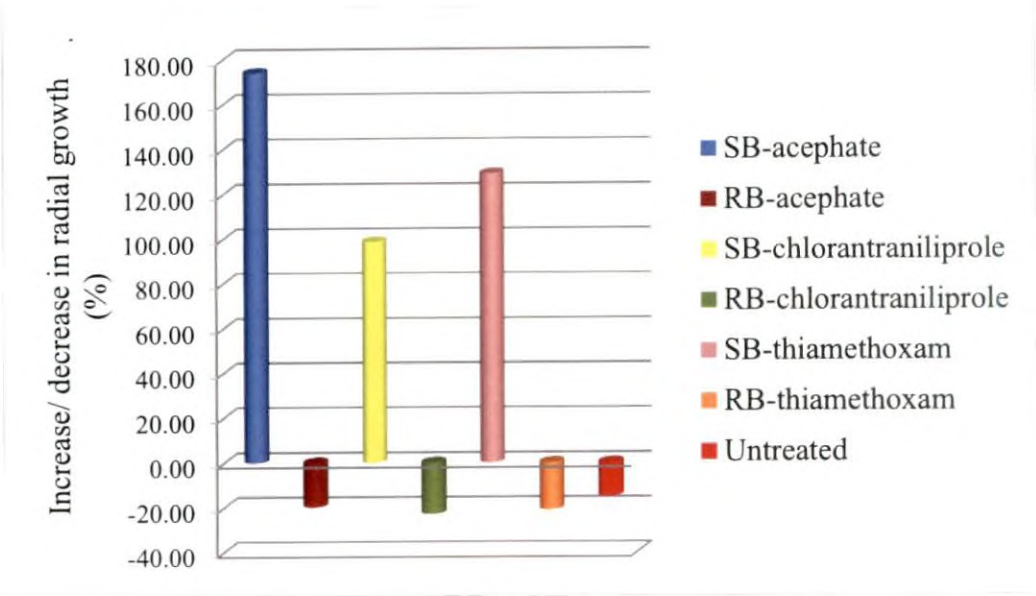
Improvement of parasitoids and predators for pesticide tolerance and other desirable traits, through artificial selection and other molecular techniques have been attempted by researchers (Hoy, 1986 and Charles *et al.*, 2011). Shapiro *et al.* (2002; 2011) have developed fungicide tolerant strains of *B. bassiana* and *M. brunneum*. Nonetheless, artificial selection of entomopathogenic fungi for insecticide tolerance is a less explored domain. The studies undertaken by Sudharma (2006) and Anis (2014) shed some light in this arena; their studies indicated that, genetic variations were induced in fungi *viz., Fusarium pallidoroseum* Cook (Sacc.), *B. bassiana* and *M. anisopliae*, by chemical pesticides. With this backdrop, attempts were made in the present investigation, as it is highly essential in gaining in depth

knowledge on pesticide tolerance of fungi, but a slightly different methodology was followed (3.6.1.2).

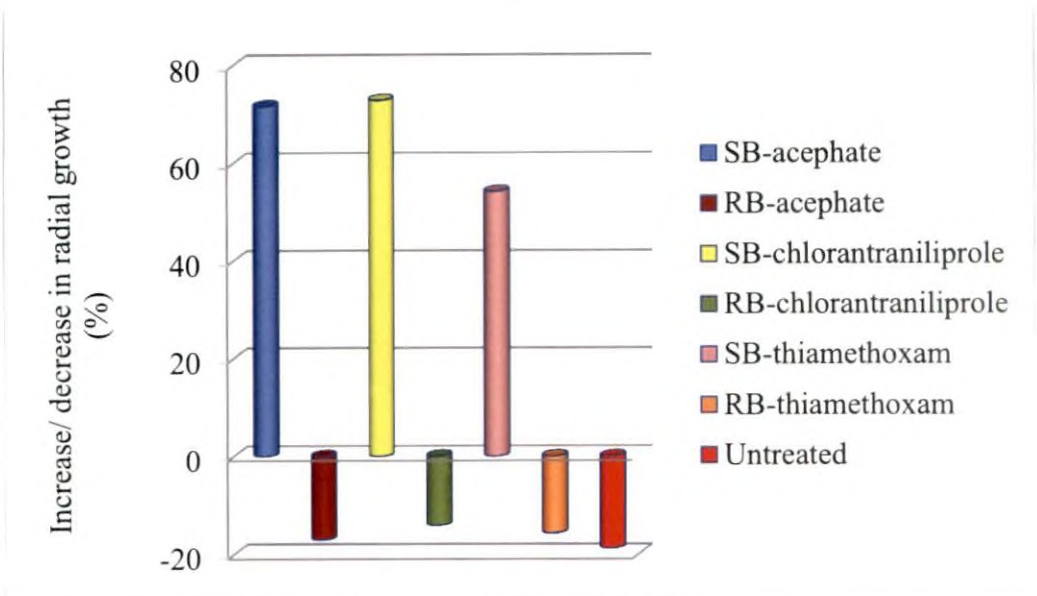
Growth, spore count and the bioefficacy of selected cultures (fungi grown initially in media containing highest tolerable dose of insecticide and further grown in poisoned media for 10 passages), relaxed cultures (fungi gown initially in media containing highest tolerable dose of insecticide and further grown in unpoisoned media for 10 passages) and untreated cultures (fungi grown only in unpoisoned media) of *B. bassiana* (Bb 5) / *M. anisopliae* (Ma 4) differed significantly.

It was seen that the growth of selected cultures of *B. bassiana* (Bb 5) *i.e.,* SB-acephate, SB-thiamethoxam and SB-chlorantraniliprole increased after 10 passages through respective poisoned media which was to the tune of 174.15, 129.11 and 98.59 per cent over the growth of the fungus noted in the first passage. However, relaxed *B. bassiana* (Bb 5) *i.e.,* RB-acephate, RB-thiamethoxam and RB-chlorantraniliprole had a reduction in growth to the extent of 19.88, 21.10 and 23.06 per cent, respectively and untreated *B. bassiana* (Bb 5) (UB) showed 15.10 per cent reduction in growth (Fig.14 A). In the process of artificial selection of *B. bassiana* for fungicide resistance using fenbuconazole Shapiro *et al.*, 2002 observed increased mycelial growth which is in tune with the present results.

The growth of selected cultures of *M. anisopliae* (Ma 4) (SM-chlorantraniliprole, SM-acephate and SM-thiamethoxam) increased and it was 72.87, 71.67 and 54.15 per cent, respectively. However, the relaxed and untreated cultures showed reduction in growth after 10 passages through respective media. Relaxed cultures of *M. anisopliae* (Ma 4) (RM-chlorantraniliprole, RM-thiamethoxam and RM-acephate) showed reduction to the extent of 14.04, 15.64 and 17.03 per cent, respectively and untreated *M. anisopliae* (Ma 4) showed 18.76 per cent reduction (Fig.14 B).




(A) *B. bassiana*

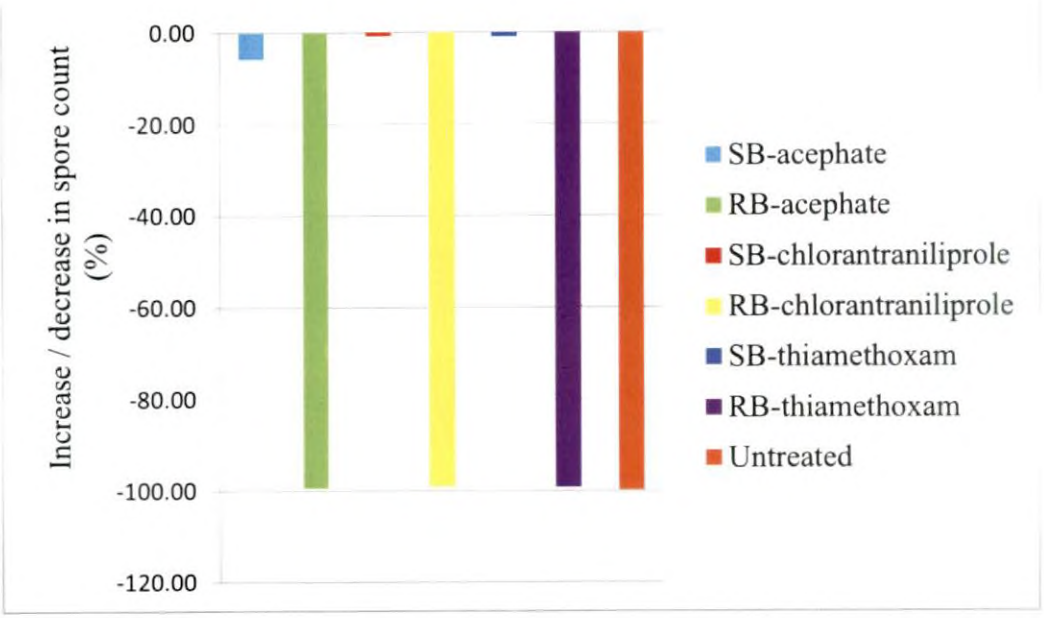



(B) *M. anisopliae*

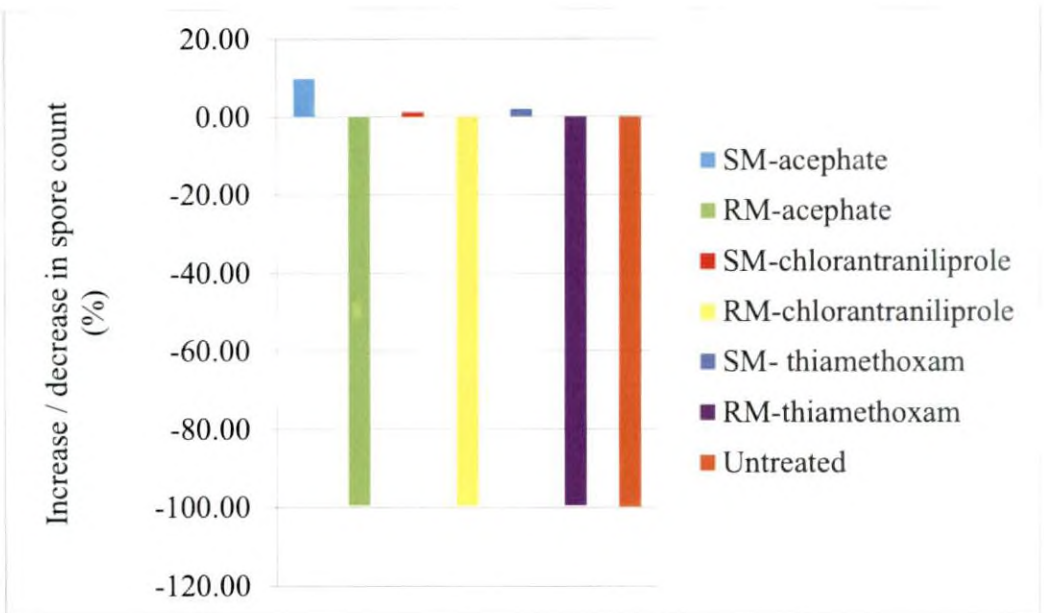
Fig. 14 Growth of selected, relaxed and untreated fungi after ten passages.

With respect to spore count, reduction was seen invariably, in selected, relaxed and untreated *B. bassiana* (Bb 5) after 10 passages through poisoned / unpoisoned media, however the percentage reduction was less in the selected, 0.91, 0.94 and 5.92 only in SB-chlorantraniliprole, SB-thiamethoxam and SB-acephate, respectively while that of the relaxed was 99.19, 99.24 and 99.48 in RB-chlorantraniliprole, RB-thiamethoxam and RB-acephate, respectively. Untreated *B. bassiana* (Bb 5) (UB) showed 99.81 percentage reduction in spore count (Fig.15 A).

Contrastingly, selected *M. anisopliae* (Ma 4) *i.e.,* SM-acephate SM-thiamethoxam and SM-chlorantraniliprole showed an increase in spore count which was to the extent of 9.77, 2.00 and 1.17 per cent, respectively. As observed in the case of relaxed cultures of *B. bassiana* (Bb 5), the spore count of the relaxed cultures of *M. anisopliae* (Ma 4) *i.e.,* RM-thiamethoxam RM-chlorantraniliprole and RM-acephate also reduced to the tune of 99.52, 99.53 and 99.54 per cent, respectively and the untreated *M. anisopliae* (Ma 4) showed 99.80 per cent reduction (Fig.15 B). With respect to bioefficacy also, reduction was seen in selected, relaxed and untreated *B. bassiana* (Bb 5) after 10 passages through poisoned / unpoisoned media, but the percentage reduction was less in the selected. It was seen that the percentage reduction in the bioefficacy of selected cultures of *B. bassiana* (Bb 5) was very less compared to that of the relaxed and untreated cultures, the percentage reduction being 8.70, 16.00 and 19.23 per cent only in SB-acephate, SB-chlorantraniliprole and SB-thiamethoxam, respectively. In the case of relaxed cultures of *B. bassiana* (Bb 5), RB-acephate, RB-chlorantraniliprole and RB-thiamethoxam, the percentage reduction was 43.49, 36.36 and 36.36 per cent, respectively. The bioefficacy of untreated *B. bassiana* (Bb 5) (UB) declined to the extent of 62.50 per cent (Fig.16A). The present study is in agreement with that of Shapiro et al. (2002) who observed no virulence changes in *B. bassiana* after 10 passages in poisoned media.




(A) *B. bassiana* (Bb 5)

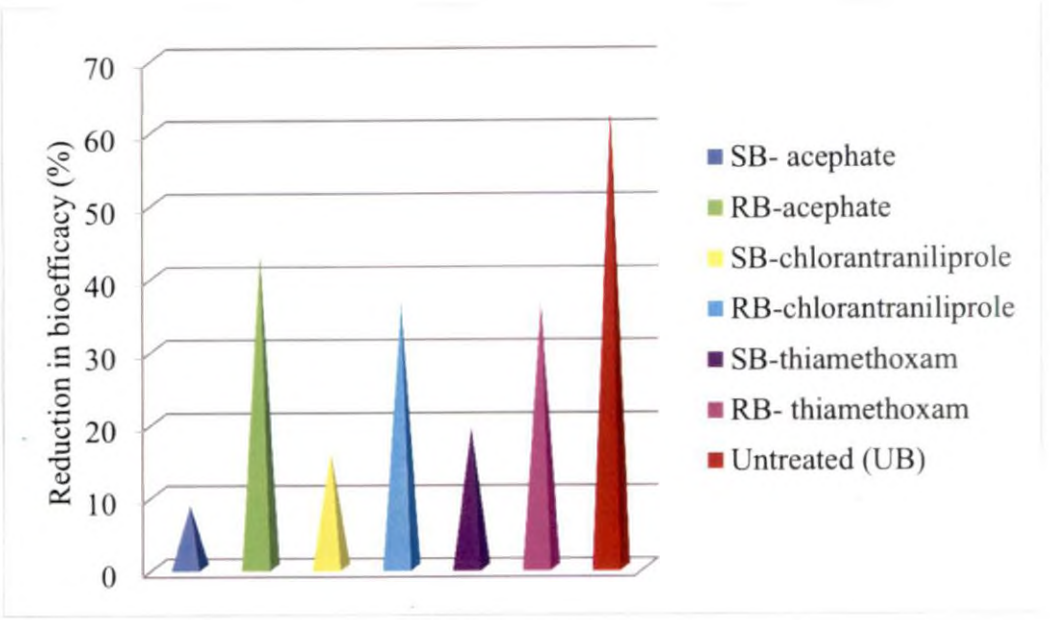



(B) *M. anisopliae* (Ma 4)

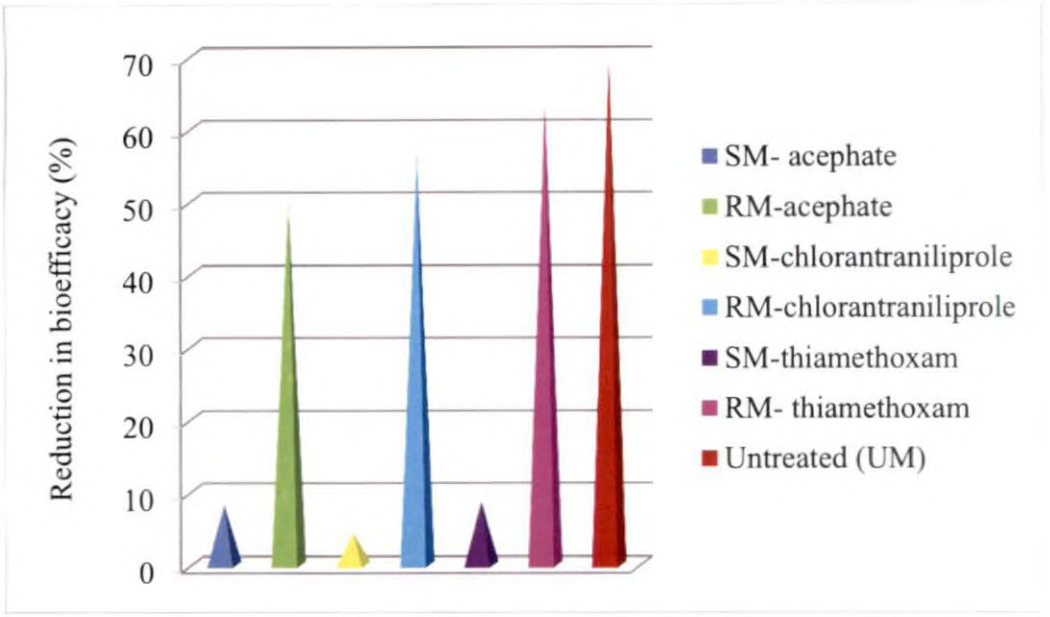
Fig. 15 Spore count of selected, relaxed and untreated fungi after ten passages

The bioefficacy of the selected, relaxed and untreated cultures of *M. anisopliae* (Ma 4) followed the same trend as that of *B. bassiana* (Bb 5) while the reduction in SM-chlorantraniliprole, SM-acephate and SM-thiamethoxam was only 4.36, 8.34 and 8.70 per cent and it was 49.99, 56.53 and 63.63 per cent for RM-acephate, RM-chlorantraniliprole and RM-thiamethoxam. The bioefficacy of untreated *M. anisopliae* (Ma 4) declined to the extent of 69.23 per cent, respectively on 10 passages (Fig. 16B). Mohammadbeigi (2013) who studied the effect of continuous passage of *B. bassiana* and *M. anisopliae* through Potato Dextrose Agar (PDA) was of the opinion that the virulence of both the fungi reduced after four subcultures. Similar observations were also made by Anis (2014) who observed reduction in growth, spore count and bioefficacy of the fungi, *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) on 10 passages through PDA and also through media poisoned with carbofuran, carbosulfan, imidacloprid, chlorpyriphos, lambda cyhalothrin, malathion, carbendazim and mancozeb , and that the effect varied with fungi.

After 10 passages through the poisoned / unpoisoned media, the selected, relaxed and untreated *B. bassiana* (Bb 5) / *M. anisopliae* (Ma 4) were further grown in media containing doses of insecticides higher than the maximum tolerable dose *i.e.,* 4.5 x for acephate and 8.5 x for both chlorantraniliprole and thiamethoxam to check their tolerance to higher concentrations. It was seen that the selected cultures of *B. bassiana* (Bb 5) (SB-thiamethoxam, SB-chlorantraniliprole, and SB-acephate) only could grow in these higher concentrations. The mean growth (1.50, 1.47 and 1.43 cm) observed was statistically similar. Similarly, all the selected cultures of *M. anisopliae* (Ma 4) (SM-thiamethoxam, SM-chlorantraniliprole, and SM- acephate) also grew in these higher concentrations and the growth noted was 1.83, 1.73 and 1.67 cm, respectively but they were significantly different. It was seen that tolerance of *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) to acephate increased by 12.50 per cent and that to chlorantraniliprole and thiamethoxam increased by 6.25 per cent. No growth for all the relaxed and untreated cultures of both *B. bassiana* (Bb 5) /




(A) *B. bassiana* (Bb 5)




(B) *M. anisopliae* (Ma 4)

Fig.16 Bioefficacy of selected, relaxed and untreated fungi after ten passages

*M. anisopliae* (Ma 4) was seen in media containing higher concentrations of these insecticides.

It is inferred that the tolerance of fungi to insecticides enhanced on passage through insecticide poisoned media continuously, as the relaxed fungi failed to tolerate the higher doses of insecticides and at the same time the selected culture tolerated doses higher to their maximum tolerable level after 10 passages. It seems that the tolerance was dependent on the stressors in the media, and that the stressors might have induced some physiological changes in the fungi, which might have contributed to the difference in the tolerance noted between the different selected fungal cultures in the present study. Shapiro *et al.* (2011) studied the impact of artificial selection for fungicide resistance on *B. bassiana* and *M. brunneum* and they have stated that fungicide sensitivity and selection potential differed based on the medium and the fungal species. Strikingly, it is seen that *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) tolerated higher doses of insecticides and hence are ideal tools for IPM even in agroecosystems where high load of insecticides are used.

After 10 passages through poisoned / unpoisoned media, molecular analysis of all the selected, relaxed and untreated cultures of both *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) was done, to identify the changes induced in the DNA of the fungi by the insecticides, if any, adopting RAPD. The number of bands developed per amplification was primer dependent and it varied from six to 18 and five to 18 for *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4), respectively (Plate 5 and 6). Among the 10 primers, RFu 1 to 10 evaluated, only RFu- 4 showed two specific bands, at 800 and 500 bp and that too only in selected culture of *B. bassiana* (SB-chlorantraniliprole) and the polymorphism exhibited was only 12.5 per cent. Overall polymorphism exhibited by all the primers in *B. bassiana* (Bb 5) was only 1.61 per cent. When *M. anisopliae* (Ma 4) was subjected to RAPD, no polymorphic bands developed. As the polymorphic bands developed were negligible and none in *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4), respectively it is inferred that the

insecticides *viz.*, acephate, chlorantraniliprole and thiamethoxam, do not induce any change in the genetic makeup of these fungi.

In the earlier studies, it was seen that the insecticides differed in their ability to induce variations in the genetic profile of the fungi (Sudharma, 2006). While the synthetic pyrethroid insecticides, fenvalerate and lambdacyhalothrin, induced significant variations in the genetic makeup of the fungus, *F. pallidoroseum*, no variations were induced by the insecticide chlorpyriphos, belonging to the organophosphorous group. Anis (2014) observed that the fungicide, carbendazim induced significant variations in the genetic makeup of *B. bassiana* and *M. anisopliae*. Further, she observed that the variations exhibited were higher for *B. bassiana* (82.30 per cent) compared to *M. anisopliae* (26.58 per cent). This indicates that, the induction of genetic variation is dependent on the fungus also. The results of the present study reveals that the organophosphorus insecticide, acephate, the diamide, chlorantraniliprole, and the neonicotinoid, thiamethoxam are suitable, for integration with *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) for pest management in rice. Such characters of the insecticides necessitate selective alignments of the fungal pathogens and insecticides during concurrent exploitation of these tools in IPM programmes.

It has been proved from the present studies that *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ are effective fungal pathogens for the management of the two major pests, *C. medinalis* and *L. acuta* in rice besides being safe to the natural enemies and favour good economic returns. As both fungi are compatible with insecticides *viz.*, acephate, chlorantraniliprole and thiamethoxam even at higher doses, and as they do not undergo genetic variations on continuous exposure to these insecticides, they are suitable for integration with these insecticides. Integration of *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ with acephate @ 750 g a.i $ha^{-1}$, chlorantraniliprole @ 30 g a.i $ha^{-1}$ and thiamethoxam @ 25

g a.i $ha^{-1}$ is possible. The insecticide suggested as the best for integration for rice pest management is chlorantraniliprole @ 30 g a.i $ha^{-1}$ as it is safe to natural enemies.

# *Summary*

## 6. SUMMARY

Entomopathogenic fungi are now recognised as important biocontrol agents of insect pests of crops. For a quantum leap in their adoption for pest management, virulent isolates against target pests are requisites. In this context, isolation of fungi in continuum gains importance. Moreover their identification at molecular level is of prime importance, since morphological identification alone is unambiguous. It is also necessary to garner information on their pathogenicity, effective field dose, field performance and impact on natural enemies of pests. Better exploitation of pathogens in integrated pest management could be made possible with pesticide tolerant strains. Though the entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin are being exploited for pest management in vegetable ecosystems, little attempts have been made to tap the potential of these fungi for rice pest management in Kerala. Considering these issues, a project entitled "Entomopathogenic fungi for the management of insect pests in rice ecosystem" was undertaken during 2011 - 2014 at College of Agriculture, Vellayani, and the objectives were to isolate and identify indigenous entomopathogenic fungi, assessment of their pathogenicity to selected rice pests, determination of their virulence, $LC_{50}$ and $LT_{50}$ values and their evaluation in the field. Studies on their compatibility with chemical insecticides and development of pesticide tolerant strains were the other two objectives in order to generate information regarding their suitability in integrated pest management programmes.

- Six entomopathogenic fungi were collected from insect cadaver / soil, collected from rice fields in Thiruvananthapuram district.
- The fungus isolated from the cadaver of rice bug, *Leptocorisa acuta* (Thunb) on ITS sequencing was identifed as, *Aspergillus flavus* Link. The accession number assigned by National Centre for Biotechnology Information (NCBI) was KP 739825.
- Using Galleria bait method two isolates of *B. bassiana* (Bb-m2 and Bb-m5) and one isolate of *M anisopliae* (Ma-m1) were collected from soil samples and accession

number for these isolates, obtained from NCBI were KP 739828, KP 739831, KP 739826, respectively.

- Using modified Veens semiselective media two isolates of *B. bassiana* (Bb-m3 and Bb-m4) were obtained from soil samples and accession number for these isolates obtained from NCBI were KP 739829 and KP 739830.

- Pathogenicity studies showed that the NBAIR isolates *B. bassiana* (Bb 5), *M. anisopliae* (Ma 4), and the isolate *B. bassiana* (Bb 21) from Department of Microbiology, College of Agriculture, Vellayani and the six new indigenous isolates, *A. flavus* (Af-m1), *B. bassiana* (Bb-m2, Bb-m3, Bb-m4 and Bb-m5) and *M. anisopliae* (Ma-m1) are pathogenic to larvae of *Cnaphalocrocis medinalis* Guen. and nymphs and adults of *L. acuta*. Only *B. bassiana* (Bb 5) was pathogenic to eggs.

- The symptoms of infection by all the fungi were more or less similar. Reduced feeding, movement and mating were seen in infected insects. Colour of the mycelia on the cadaver varied.

- Single dose assay of all the nine fungal isolates at $10^8$ spores $ml^{-1}$ against third instar larvae of *C. medinalis*, showed that *B. bassiana* (Bb 5) which recorded cent per cent mortality at eight DAT was significantly superior to other treatments, and it was followed by *B. bassiana* (Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1) with 90.00, 73.33 and 66.67 per cent mortality, respectively.

- Single dose assay of all the nine fungal isolates at $10^8$ spores $ml^{-1}$ against *L. acuta* nymphs showed that *M. anisopliae* (Ma 4) which caused cent per cent mortality and *B. bassiana* (Bb 5) that caused 90.00 per cent mortality at 10 DAT were statistically on par. Among the different fungi evaluated against adult *L. acuta*, at $10^9$ spores $ml^{-1}$, *M. anisopliae* (Ma 4) showed superior performance with cent per cent mortality at 10 DAT.

- The new indigenous fungal isolates collected from soil were inferior, the morality percentage ranged from zero to 33.33 per cent against *C. medinalis* and *L. acuta* nymphs and from zero to 20.00 per cent against adult *L. acuta*.

- The lethal concentration of *B. bassiana* (Bb 5), *B. bassiana* (Bb 21), *M. anisopliae* (Ma 4) required to bring fifty per cent mortality at four DAT were 1.70 x $10^8$, 1.54 x $10^8$ and 1.02 x $10^8$ spores $ml^{-1}$, respectively and that of *A. flavus* (Af-m1) at six DAT was 1.66 x $10^8$ spores $ml^{-1}$ against *C. medinalis*. The corresponding $LC_{90}$ values were 3.50 x $10^8$, 3.40 x $10^8$, 2.25 x $10^8$ and 3.37 x $10^8$ spores $ml^{-1}$, respectively.

- Against nymphs of *L. acuta*, lethal concentration to get fifty per cent mortality at five DAT were 1.63 x $10^8$, 3.55 x $10^8$, 0.95 x $10^8$ and 1.74 x $10^8$ spores $ml^{-1}$ for *B. bassiana* (Bb 5), *B. bassiana* (Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1), respectively. The corresponding $LC_{90}$ values of the fungi were 3.92 x $10^8$, 7.55 x $10^8$, 2.96 x $10^8$ and 3.46 x $10^8$ spores $ml^{-1}$.

- The $LC_{50}$ and $LC_{90}$ values against adult *L. acuta*, were 2.08 x $10^{10}$, 1.08 x $10^{10}$, 1.53 x $10^{10}$, 4.84 x $10^{10}$ spores $ml^{-1}$ and 4.23 x $10^{10}$, 4.11 x $10^{10}$, 2.85 x $10^{10}$ and 8.72 x $10^{10}$ spores $ml^{-1}$ for *B. bassiana* (Bb 5), *B. bassiana* (Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1), respectively.

- The $LT_{50}$ recorded were 5.38, 5.08, 4.69 and 7.39 days for *B. bassiana* (Bb 5), *B. bassiana* (Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1), respectively against *C. medinalis* at a concentration of $10^8$ spores $ml^{-1}$.

- At a concentration of $10^8$ spores $ml^{-1}$, $LT_{50}$ of *B. bassiana* (Bb 5), *B. bassiana* (Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1) against nymphs of *L. acuta* were 6.02 , 7.08 , 5.26 and 8.22 days and against adult *L. acuta*, the values were 6.65, 6.92, 7.13 and 7.96 days, respectively at $10^9$ spores $ml^{-1}$.

- The results of the field experiments showed that, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ which recorded a population reduction of *C. medinalis* larvae to the extent of 58.61 in the first trial and 55.09 per cent in the second trial over the untreated and which also showed 41.48 per cent and 40.01 per cent reduction of adult *C. medinalis* in the first and second trials, respectively was the best among the fungal treatments.

- The insecticide, chlorantraniliprole @ 30 g a.i $ha^{-1}$ recorded the highest population reduction of *C. medinalis* larvae and adult, the values being 91.04 and 76.92 per cent in the first and 83.89 and 80.00 per cent in the second trial, respectively.

- *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ that showed a reduction in damage by *C. medinalis* to the extent of 75.81 and 66.44 per cent at 21 DAT, in the first and second field trials, respectively was the best among the fungi evaluated,.

- Among the insecticides evaluated, chlorantraniliprole @ 30 g a.i $ha^{-1}$ thiamethoxam @ 25 g a.i.$ha^{-1}$ and acephate @ 750 g a.i $ha^{-1}$ were on par in reducing the damage caused by *C. medinalis* in both the trials. Acephate @ 750 g a.i $ha^{-1}$ and thiamethoxam @ 25 g a.i $ha^{-1}$ recorded the highest percentage reduction of 76.33 each, over untreated during the first trial and chlorantraniliprole @ 30 g a.i $ha^{-1}$ showed the highest percentage reduction of 81.04 per cent over untreated during the second trial.

- Among the fungal treatments evaluated maximum reduction in the population of *L. acuta* was obtained with *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ which recorded 53.21 and 54.22 per cent reduction at 21 DAT in the first and second field trials, respectively.

- In insecticide treated plots, initial reduction in the population of *L. acuta* was high compared to fungal treatments. Chlorantraniliprole @ 30 g a.i $ha^{-1}$ showed

89.77 reduction in the population of *L. acuta* in the first field trial at four DAT, but the reduction was less at 21 DAT (66.97).

- The effect of *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$, chlorantraniliprole @ 30 g a.i $ha^{-1}$ and acephate @ 750 g a.i $ha^{-1}$ were on par in the extent of damage by *L. acuta*. The percentage reduction of damaged grains in these treatments over untreated, noted at 21 DAT in the first trial were 60.96, 66.31 and 55.61 per cent, respectively. The corresponding values in the second trial were 60.96, 62.57 and 51.87 per cent, respectively.

- The treatments, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ showed the highest yield of grain and straw, the percentage increase over untreated for grains being 74.19 and 64.52 per cent and 67.30 and 63.81 per cent for straw in the first trial, respectively. The corresponding values in the second trial being 64.84 and 71.88 per cent for grain and 66.14 and 70.89 per cent for straw, respectively.

- Chlorantraniliprole @ 30 g a.i $ha^{-1}$ recorded the highest grain and straw yield among all the treatments evaluated and it was 96.77 and 88.57 per cent higher than that in the untreated during the first trial, the corresponding values in the second field trial being 87.50 and 86.71 per cent, respectively.

- An assessment of the effect of fungal and chemical treatments on the population of hymenopteran parasitoids, insect and spider predators revealed that entomopathogenic fungi and the insecticide, chlorantraniliprole @ 30 g a.i $ha^{-1}$ favoured significantly higher population of natural enemies in both the field trials compared to the insecticide treatments, acephate @ 750 g a.i $ha^{-1}$, malathion @ 575 g a.i $ha^{-1}$ and thiamethoxam @ 25 g a.i $ha^{-1}$.

- The treatments, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ , chlorantraniliprole @ 30 g a.i $ha^{-1}$ and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-}$ , ranked top with benefit-

cost ratio (BCR) of 1.65, 1.58 and 1.55, respectively in the first trial whereas in the second trial, *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$, *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ and chlorantraniliprole @ 30 g a.i $ha^{-1}$ raked the best with BCR of 1.63, 1.56 and 1.50, respectively.

- Overall grading of all the treatments on a one to ten scale on the basis of the population and damage of *C. medinalis* and *L. acuta*, population of natural enemies and BCR in both the field trials, showed that the grade was the highest for the insecticide treatment chlorantraniliprole @ 30 g a.i $ha^{-1}$ and it was followed by the treatments *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ > acephate @ 750 g a.i $ha^{-1}$ > *M. anisopliae* @ $10^{10}$ spores $ml^{-1}$ (Ma 4) > thiamethoxam @ 25 g a.i $ha^{-1}$ > talc based *B. bassiana* (Bb 5) = talc based *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$ > malathion @ 575 g a.i $ha^{-1}$ > talc based *B. bassiana* (Bb 21) @ 20 g $l^{-1}$ > *A. flavus* (Af-m1) @ $10^{10}$ spores $ml^{-1}$.

- Compatibility studies of the fungal pathogens, *B. bassiana* (Bb 5 and Bb 21), *M. anisopliae* (Ma 4), *A. flavus* (Af-m1) with the three inseciticides *viz.*, acephate, chloratraniliprole and thiamethoxam and at three different concentrations revealed that all the insecticides were compatible with the fungi evaluated. All the insecticides were harmless as they showed only less than 50 per cent inhibition in growth with the exception of the organophosphorus insecticide, acephate 0.150 and 0.225 per cent, which recorded slightly higher inhibition of 54.51 and 56.35 per cent, respectively only in *M. anisopliae* (Ma 4).

- The percentage reduction in the spore count was less even in media poisoned with the highest concentration of 0.008 per cent of chlorantraniliprole and thiamethoxam and it was only 2.75 to 10.42 per cent and 4.60 to 8.59 per cent, respectively over the untreated. The maximum inhibition of 28.37 per cent in sporulation was produced by acephate 0.075 per cent.

- *B. bassiana* (Bb 21) and *M. anisopliae* (Ma 4) when grown in the higher doses of chlorantraniliprole (0.008 per cent) and thiamethoxam (0.008 per cent) the conidial viability was even higher than that in the unpoisoned. No significant difference in the mean mortality of *L. acuta* treated with different fungi grown in different poisoned media as well as that grown in untreated, also revealed the compatibility of these fungi and insecticides.

- During the studies conducted to develop pesticide tolerant strains of fungi it was observed that the growth, spore count and the bioefficacy of selected cultures (fungi gown initially in media containing highest tolerable dose of insecticide and further grown in poisoned media for ten passages), relaxed cultures (fungi gown initially in media containing highest tolerable dose of insecticide and further grown in unpoisoned media for ten passages) and untreated cultures (fungi grown only in unpoisoned media) of *B. bassiana* (Bb 5) / *M. anisopliae* (Ma 4) differed significantly.

- The growth of selected cultures of *B. bassiana* (Bb 5) *i.e.,* SB-acephate, SB-thiamethoxam and SB-chlorantraniliprole increased after ten passages through respective poisoned media and it was to the tune of 174.15, 129.11 and 98.59 per cent over the initial growth, whereas a reduction was noticed in the relaxed and untreated (UB) cultures and the extent of reduction was 19.88, 21.10 and 23.06 per cent in RB-acephate, RB-thiamethoxam and RB-chlorantraniliprole, and 15.10 per cent in untreated respectively. Similarly, the growth of selected cultures of *M. anisopliae* (Ma 4) (SM-chlorantraniliprole, SM-acephate and SM-thiamethoxam) increased to the extent of 72.87, 71.67 and 54.15 per cent, respectively while that of the relaxed (RM-chlorantraniliprole, RM-thiamethoxam and RM-acephate) and untreated cultures showed 14.04, 15.64 and 17.03 and 18.76 per cent reduction in growth, respectively.

- With respect to spore count, reduction was seen invariably, in selected, relaxed and untreated *B. bassiana* (Bb 5) after ten passages through poisoned / unpoisoned media, however the percentage reduction was less in the selected, 0.91, 0.94 and 5.92 per cent only, in SB-chlorantraniliprole, SB-thiamethoxam and SB-acephate, respectively while that of the relaxed was 99.19, 99.24 and 99.48 per cent in RB-chlorantraniliprole, RB-thiamethoxam and RB-acephate, respectively. Untreated *B. bassiana* (Bb 5) (UB) showed 99.81 percentage reduction in spore count. Contrastingly, selected cultures of *M. anisopliae* (Ma 4) *i.e.,* SM-acephate SM-thiamethoxam and SM-chlorantraniliprole showed an increase in spore count to the extent of 9.77, 2.00 and 1.17 per cent, respectively while the relaxed cultures, RM-thiamethoxam RM-chlorantraniliprole RM-acephate and untreated (UM) showed reduction to the tune of 99.52, 99.53 and 99.54 and 99.80 per cent, respectively.

- After ten passages through poisoned / unpoisoned media, reduction in bioefficacy of both *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) was seen for all the cultures, *i.e.,* selected, relaxed and untreated but the reduction was very less in selected cultures compared to that of the relaxed and untreated. In the selected cultures of *B. bassiana* (Bb 5) the percentage reduction was only 8.70, 16.00 and 19.23 per cent, in SB-acephate, SB-chlorantraniliprole and SB-thiamethoxam, respectively whereas the reduction was 43.49, 36.36 and 36.36 in relaxed and 62.50 per cent in untreated. A similar effect was noted in *M. anisopliae* (Ma 4) also, the reduction in bioefficacy of SM-chlorantraniliprole, SM-acephate and SM-thiamethoxam was only 4.36, 8.34 and 8.70 per cent and it was 49.99, 56.53 and 63.63 per cent in RM-acephate, RM-chlorantraniliprole and RM-thiamethoxam. The bioefficacy of untreated *M. anisopliae* (Ma 4) declined to the extent of 69.23 per cent.

- The selected, relaxed and untreated *B. bassiana* (Bb 5) / *M. anisopliae* (Ma 4) when further grown in media containing doses of insecticides higher than the maximum tolerable dose *i.e.,* 4.5 x for acephate and 8.5 x for both chlorantraniliprole and thiamethoxam, only selected cultures of *B. bassiana* (Bb 5) and *M. anisopliae*

(Ma 4) could grow in these higher concentrations. No growth for all the relaxed and untreated cultures of both *B. bassiana* (Bb 5) / *M. anisopliae* (Ma 4) was seen in media containing higher concentrations of these insecticides.

- On molecular analysis of the selected, relaxed and untreated cultures of both *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) after ten passages through poisoned / unpoisoned media it was seen that there was no polymorphism in *M. anisopliae* (Ma 4) and that the polymorphism exhibited in *B. bassiana* (Bb 5) was only 1.61 per cent.

It is concluded that entomopathogenic fungi *viz., B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ are pathogens not to be put on the backburner as they are found ideal for rice pest management. Overuse and abuse of any single tactic for pest management can lead to serious aftermaths, in the long run. Henceforth, these fungal pathogens are not to be dependent upon as a sole tactic for pest management. As the insecticide, chlorantraniliprole @ 30 g a.i $ha^{-1}$ was effective against rice pests without affecting natural enemies in the rice ecosystem and as it favours economic returns, integration of *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) with this insecticide for rice pest management is suggested.

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# *Appendices*

Appendix-I

Estimation of benefit-cost ratio (Field trial-I)

| Treatment | Plant protection cost | | | | | | Benefit | | | | | | Benefit-cost ratio (BCR) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Application cost | | Material cost | | | Total cost | Grain* (kg ha$^{-1}$) | Amount*for grain yield (Rs) | Straw kg ha$^{-1}$) | Amount for straw yield @Rs.5 kg$^{-1}$ | Total amount (Rs) | Total benefit = total amount-total cost (Rs) | |
| | Labour (8 no;s @Rs. 600 labour$^{-1}$) | Sprayer hiring Charges (Rs) | Quantity | Rate (Rs.) | Amount (Rs.) | | | | | | | | |
| T1 | 4800.00 | 200.00 | 10 kg | 100 kg$^{-1}$ | 1000.00 | 6000.00 | 3784.22 | 94605.48 | 6319.65 | 31598.23 | 126203.70 | 120203.70 | 1.44 |
| T2 | 4800.00 | 200.00 | 10 kg | 100 kg$^{-1}$ | 1000.00 | 6000.00 | 3180.35 | 79508.86 | 5311.19 | 26555.96 | 106064.81 | 100064.81 | 1.20 |
| T3 | 4800.00 | 200.00 | 10 kg | 100 kg$^{-1}$ | 1000.00 | 6000.00 | 3623.19 | 90579.71 | 6050.72 | 30253.62 | 120833.33 | 114833.33 | 1.38 |
| T4 | 4800.00 | 200.00 | 10 l | 280 l$^{-1}$ | 2800.00 | 7800.00 | 4347.83 | 108695.65 | 7260.87 | 36304.35 | 145000.00 | 137200.00 | 1.65 |
| T5 | 4800.00 | 200.00 | 10 l | 280 l$^{-1}$ | 2800.00 | 7800.00 | 4106.28 | 102657.00 | 6857.49 | 34287.44 | 136944.44 | 129144.44 | 1.55 |
| T6 | 4800.00 | 200.00 | 10 l | 280 l$^{-1}$ | 2800.00 | 7800.00 | 3019.32 | 75483.09 | 5042.27 | 25211.35 | 100694.44 | 92894.44 | 1.12 |
| T7 | 4800.00 | 200.00 | 1 kg | 800 kg$^{-1}$ | 800.00 | 5800.00 | 4549.11 | 90982.29 | 7597.02 | 37985.10 | 128967.39 | 123167.39 | 1.48 |
| T8 | 4800.00 | 200.00 | 0.15 l | 20000 l$^{-1}$ | 3000.00 | 8000.00 | 4911.43 | 98228.66 | 8202.09 | 41010.47 | 139239.13 | 131239.13 | 1.58 |
| T9 | 4800.00 | 200.00 | 1.15 l | 470 l$^{-1}$ | 540.50 | 5540.50 | 4468.60 | 89371.98 | 7462.56 | 37312.80 | 126684.78 | 121144.28 | 1.46 |
| T10 | 4800.00 | 200.00 | 0.10 | 5600 l$^{-1}$ | 560.00 | 5560.00 | 4267.31 | 85346.22 | 7126.41 | 35632.05 | 120978.26 | 115418.26 | 1.39 |
| T11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2495.97 | 62399.36 | 4168.28 | 20841.38 | 83240.74 | 83240.74 | - |

*- Rs.25 kg$^{-1}$ (for the grain yield from fungal treated and untreated plots)
- Rs.20 kg$^{-1}$(for the grain yield from chemical treated plots)

T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g l$^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g l$^{-1}$
T3 - Talc based formulation of *M. anisopliae*(Ma 4) @ 20 g l$^{-1}$
T4 - *B. bassiana* (Bb 5) @$10^{10}$ spores ml$^{-1}$
T5: *M. anisopliae* (Ma 4) @ $10^{10}$ spores ml$^{-1}$
T6: *A. flavus* (Af-m1) @ $10^{10}$ spores ml$^{-1}$
T7:Acephate @@ 750 g a.i ha$^{-1}$
T8: Chlorantraniliprole@ 30 g a.i ha$^{-1}$
T9: Malathion@ 575 g a.i ha$^{-1}$
T10:Thiamethoxam@ 25 g a.i ha$^{-1}$
T11: Untreated

Appendix-II

Estimation of benefit-cost ratio (Field trial-II)

| Treatment | Plant protection cost | | | | | | Benefit | | | | | | Benefit-cost ratio (BCR) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Application cost | | Material cost | | | Total cost | Grain* (kg ha$^{-1}$) | Amount*for grain yield (Rs) | Straw kg ha$^{-1}$) | Amount for straw yield @Rs.5 kg$^{-1}$ | Total amount (Rs) | Total benefit = total amount-total cost (Rs) | |
| | Labour (8 no;s @Rs. 600 labour$^{-1}$) | Sprayer hiring Charges (Rs) | Quantity | Rate (Rs.) | Amount (Rs.) | | | | | | | | |
| T1 | 4800.00 | 200.00 | 10 kg | 100 kg$^{-1}$ | 1000.00 | 6000.00 | 3925.12 | 98128.02 | 6554.95 | 32774.76 | 130902.78 | 124902.78 | 1.45 |
| T2 | 4800.00 | 200.00 | 10 kg | 100 kg$^{-1}$ | 1000.00 | 6000.00 | 3723.83 | 93.95.81 | 6218.80 | 31094.00 | 124189.81 | 118189.81 | 1.38 |
| T3 | 4800.00 | 200.00 | 10 kg | 100 kg$^{-1}$ | 1000.00 | 6000.00 | 3925.12 | 98128.02 | 6536.84 | 32684.18 | 130812.00 | 124812.00 | 1.45 |
| T4 | 4800.00 | 200.00 | 10 l | 280 l$^{-1}$ | 2800.00 | 7800.00 | 4247.18 | 106179.55 | 7092.79 | 35463.97 | 141643.52 | 133843.52 | 1.56 |
| T5 | 4800.00 | 200.00 | 10 l | 280 l$^{-1}$ | 2800.00 | 7800.00 | 4428.34 | 110708.53 | 7395.33 | 36976.65 | 147685.19 | 139885.19 | 1.63 |
| T6 | 4800.00 | 200.00 | 10 l | 280 l$^{-1}$ | 2800.00 | 7800.00 | 3703.70 | 92592.59 | 6185.19 | 3092.93 | 123518.52 | 115718.52 | 1.35 |
| T7 | 4800.00 | 200.00 | 1 kg | 800 kg$^{-1}$ | 800.00 | 5800.00 | 4629.63 | 92592.59 | 7731.48 | 38657.41 | 131250.00 | 125450.00 | 1.46 |
| T8 | 4800.00 | 200.00 | 0.15 l | 20000 l$^{-1}$ | 3000.00 | 8000.00 | 4830.92 | 96618.36 | 8067.63 | 40338.16 | 136956.52 | 128956.52 | 1.50 |
| T9 | 4800.00 | 200.00 | 1.15 l | 470 l$^{-1}$ | 540.50 | 5540.50 | 4428.34 | 88566.83 | 7264.49 | 36322.46 | 124889.29 | 119348.79 | 1.39 |
| T10 | 4800.00 | 200.00 | 0.10 | 5600 l$^{-1}$ | 560.00 | 5560.00 | 4428.34 | 88566.83 | 7338.97 | 36694.85 | 125261.67 | 119701.67 | 1.39 |
| T11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2576.49 | 64412.24 | 4302.74 | 21513.69 | 85925.93 | 85925.93 | - |

*- Rs.25 kg$^{-1}$ (for the grain yield from fungal treated and untreated plots)
- Rs.20 kg$^{-1}$(for the grain yield from chemical treated plots)

T1 - Talc based formulation of *B. bassiana* (Bb 5) @ 20 g l$^{-1}$
T2 - Talc based formulation of *B. bassiana* (Bb 21) @ 20 g l$^{-1}$
T3 - Talc based formulation of *M. anisopliae*(Ma 4) @ 20 g l$^{-1}$
T4 - *B. bassiana* (Bb 5) @10$^{10}$ spores ml$^{-1}$
T5: *M. anisopliae* (Ma 4) @ 10$^{10}$ spores ml$^{-1}$
T6: *A. flavus* (Af-m1) @ 10$^{10}$ spores ml$^{-1}$
T7:Acephate @@ 750 g a.i ha$^{-1}$
T8: Chlorantraniliprole@ 30 g a.i ha$^{-1}$
T9: Malathion@ 575 g a.i ha$^{-1}$
T10:Thiamethoxam@ 25 g a.i ha$^{-1}$
T11: Untreated

# ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF INSECT PESTS IN RICE ECOSYSTEM

*by*

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**Abstract of the thesis**

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## ABSTRACT

The study entitled "Entomopathogenic fungi for the management of insect pests in rice ecosystem" was carried out during 2011 - 2014 at College of Agriculture, Vellayani. The objectives were to isolate and identify indigenous strains of entomopathogenic fungi, to evaluate the fungal pathogens *viz., Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* Metschnikoff (Sorokin) and indigenous fungi against insect pests of rice, to fix their effective dose, to assess the compatibility of the fungal pathogens with new generation pesticides and to develop pesticide tolerant strains of the fungi.

Six new isolates of entomopathogenic fungi *viz., Aspergillus flavus* Link. (Af-m1), *B. bassiana* (Bb-m2, Bb-m3, Bb-m4 and Bb-m5) and one isolate of *M. anisopliae* (Ma-m1) were collected and identified on the basis of morphological and molecular characters. The accession numbers obtained for the isolates from National Center for Biotechnology Information (NCBI) were KP 739825, KP 739828, KP 739829, KP 739830, KP 739831 and KP 739826, respectively.

The pathogenicity of fungal isolates *viz., B. bassiana* (Bb 5), *M. anisopliae* (Ma 4) from NBAIR, *B. bassiana* (Bb 21) from Department of Microbiology, College of Agriculture, Vellayani and the six new isolates *A. flavus* (Af-m1), *B. bassiana* (Bb-m2, Bb-m3, Bb-m4 and Bb-m5) and *M. anisopliae* (Ma-m1) were evaluated against *Cnaphalocrocis medinalis* Guen. and *Leptocorisa acuta* (Thunb). All isolates were pathogenic to larvae of *C. medinalis*, nymphs and adults of *L. acuta*. The isolates *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) were found superior among the fungi evaluated.

The bioassay of the potent fungal pathogens *viz., B. bassiana* (Bb 5, Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1) was conducted against *C. medinalis* larvae and nymphs and adults of *L. acuta* and field doses were fixed on the basis of $LC_{90}$ values. Spore concentration dependent mortality of the insects was seen. The $LC_{90}$ values were $3.50 \times 10^8$, $3.40 \times 10^8$, $2.25 \times 10^8$ and $3.37 \times 10^8$ spores $ml^{-1}$

for *B. bassiana* (Bb 5, Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1) against *C. medinalis* larvae, 3.92 x $10^8$, 7.55 x $10^8$, 2.96 x $10^8$ and 3.46 x $10^8$ spores $ml^{-1}$ against *L. acuta* nymphs and 4.23 x $10^{10}$, 4.11 x $10^{10}$, 2.85 x $10^{10}$ and 8.72 x $10^{10}$ spores $ml^{-1}$ against *L. acuta* adults.

Two field trials carried out at Cropping System Research Centre, Karamana during November 2012 to March 2013 (Puncha) and June 2013 to October 2013 (Virippu) to assess the efficacy of entomopathogens revealed that *B. bassiana* (Bb 5) @ $10^{10}$ spores $ml^{-1}$ was the best treatment, on the basis of pest population and reduction in damage of pests, population of natural enemies and benefit-cost ratio. This was followed by *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$, talc based formulation of Bb 5 @ 20 g $l^{-1}$, talc based formulation of *M. anisopliae* (Ma 4) @ 20 g $l^{-1}$, talc based formulation of Bb 21 @ 20 g $l^{-1}$ and *A. flavus* @ $10^{10}$ spores $ml^{-1}$. Among the insecticides evaluated, chlorantraniliprole @ 30 g a.i $ha^{-1}$ was the best.

Assessment of the compatibility of fungal pathogens with insecticides on the basis of the effect of insecticides on the growth, spore count, germination and bioefficacy revealed that, *B. bassiana* (Bb5 and Bb 21), *M. anisopliae* (Ma 4) and *A. flavus* (Af-m1) were compatible with acephate (0.075, 0.150, 0.225 per cent), chlorantraniliprole (0.004, 0.006 and 0.008 per cent) and thiamethoxam (0.003, 0.005 and 0.008 per cent).

Investigations on pesticide tolerance of entomopathogens showed that, *B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) tolerated 4.0, 8.0 and 8.0 times higher the field dose of acephate, chlorantraniliprole and thiamethoxam, respectively. The growth, spore count and the bioefficacy of selected cultures (fungi gown initially in media containing highest tolerable dose of insecticide and further grown in poisoned media for 10 passages), relaxed cultures (fungi gown initially in media containing highest tolerable dose of insecticide and further grown in unpoisoned media for 10 passages) and untreated cultures (fungi grown only in unpoisoned media) of *B. bassiana* (Bb 5) / *M. anisopliae* (Ma 4) differed

significantly. On culturing of the selected, relaxed and untreated cultures of the fungi after 10 passages, in still higher dose of the insecticides *i.e.,* 4.5x, 8.5x and 8.5x times higher the field dose of acephate, chlorantraniliprole and thiamethoxam, respectively, only the selected cultures tolerated the higher doses.

Molecular characterisation of the selected, relaxed and untreated cultures of *B. bassiana* (Bb 5) / *M. anisopliae* (Ma 4), which were continuously grown for 10 passages in poisoned / unpoisoned media, respectively showed no molecular variations, except a minor polymorphism of 1.61 per cent exhibited in *B. bassiana* (Bb 5).

To conclude, the six new indigenous fungi isolated are pathogenic to *C. medinalis* and *L. acuta. B. bassiana* (Bb 5) and *M. anisopliae* (Ma 4) @ $10^{10}$ spores $ml^{-1}$ are effective and economical for the management of *C. medinalis* and *L. acuta* besides being safe to natural enemies. As these fungi are compatible with acephate @ 750 g a.i $ha^{-1}$, chlorantraniliprole @ 30 g a.i $ha^{-1}$ and thiamethoxam @ 25 g a.i $ha^{-1}$, and as they tolerated higher doses of insecticides, without undergoing any genetic variation they are suitable for integration with these insecticides, the best chemical suggested for integration is chlorantraniliprole @ 30 g a.i $ha^{-1}$ as it is safe to natural enemies.