EVALUATION OF ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF COLEOPTERAN PESTS AND CHARACTERISATION OF PESTICIDE TOLERANT STRAINS

by

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(2007-21-102)

THESIS

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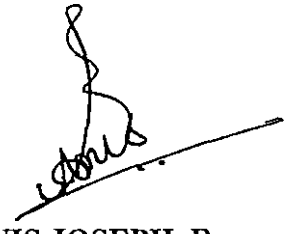
DECLARATION

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



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CERTIFICATE

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Dedicated to my dear loving Husband,

Mr.Shine Kumar, and

Our sweet loving daughter

Careine Anna Shine

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"I can do all things through Christ who strengthens me."
Philippians 4:13

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Anis Joseph. R

(2007- 21- 102)

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LIST OF ABBREVIATIONS

a.i	Active ingredient
AFLP	Amplified fragment length polymorphic DNA
bp	Base pair
B : C	Benefit Cost ratio
CD	Critical difference
cfu	Colony forming unit
cm	Centimetre
Contd.	*Continued*
CRD	Completely randomized design
CTAB	Cetyl trimethyl ammonium bromide
DAI	Days after inoculation
DAT	Days after treatment
dATP	deoxy Adenosine Tri Phosphate
dCTP	deoxy Cytosine Tri Phosphate
dGTP	deoxy Guanosine Tri Phosphate
DNA	Deoxy ribonucleic acid
dNTPs	deoxy Nucleotide Tri Phosphates
dTTP	deoxy Thiamidine Tri Phosphate
EC	Emulsifiable concentrate
EDTA	Ethylene Diamino Tetra Acetic acid disodium salt
EPF	Entomopathogenic fungi
et al.	And others/ co –workers/co-authors
Fig.	Figure
g	Gram
$g\ l^{-1}$	Gram per litre
G	Granules

HCl	Hydrochloric acid
ha	Hectare
IPM	Integrated pest management
ISSR	Inter Simple Sequence Repeats
kg	Kilogram
kg ha^{-1}	Kilogram per hectare
KAU	Kerala Agricultural University
l	Litre
LC	Lethal concentration
LT	Lethal time
m	Metre
m^2	Square metre
m^3	Cubic metre
M	Molar
MAS	Month after storage
ml	Millilitre
ml l^{-1}	Milli litre per litre
mm	Millimetre
mM	Millimolar
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
Ng	Nanogram
PCR	Polymerase Chain Reaction
pH	Per Hydrogen
$plant^{-1}$	Per plant
PVP	Poly Vinyl Pyrrolidone
RAPD	Random Amplified Polymorphic DNA
RBD	Randomized block design

RFLP	Restriction fragment length polymorphism
RH	Relative humidity
rpm	Rotations per minute
SDS	Sodium Dodecyl Sulphate
SL	Soluble liquid
spp.	Species
spores ml^{-1}	spores per milli litre
SSR	Simple Sequence Repeats
t ha^{-1}	tonnes per hectare
TAE	Tris Acetic acid EDTA
Tris-HCl	Tris (hydroxyl methyl) amino methane Hydrochloride
UV	ultra violet
viz.	Namely
WAS	Weeks after spraying
WP	Wettable powder

LIST OF SYMBOLS

@	At the rate of
°C	Degree Celsius
%	per cent
μ	Micro
~	Tilde
p	Pico
∞	Infinite
&	And
χ	Chi

Introduction

1. INTRODUCTION

The order Coleoptera of the class Insecta has the lion's share in animal kingdom with 40 per cent insect species (Hammond, 1992; Hunt, 2007). The coleopteran beetles and weevils, having chewing mouth parts feed on almost all plant tissues and many of them are injurious crop pests.

The Devavriksha - the coconut palm is highly prone to the attack of coleopteran pests in Kerala. Five per cent palms below the age of ten years are killed annually by red palm weevil, *Rhynchophorus ferrugineus* Oliver (Lever, 1979). In the Indian scenario, 12 per cent damage in 5-10 year old palms has been noted (Sekhar, 2000). Yield loss of 10-25 per cent was reported by Murphy and Briscoe (1999). Moreover, it is a polyphagous pest that even erase boundaries. *R. ferrugineus* as a pest of date palm and arecanut palm has been reported by Dutta *et al.* (2010) and Aldawood and Rasool (2011).

Another beetle pest that widely damage coconut leaf and spathe is the rhinoceros beetle, *Oryctes rhinoceros* L. Generally, ten per cent yield loss that sometimes goes up to 50 per cent has been reported due to the pest in Kerala (Sathiamma *et al.*, 2001).

The King of spices, pepper and Queen of spices, cardamom are cultivated in an area of 172182 and 41600 ha, respectively in Kerala (FIB, 2013). These crops are also ravaged by beetle pests. Infestation of the pepper pollu beetle, *Lanka ramakrishnai* Prathapan & Viraktamath, in berries and stalk is estimated to cause an yield loss of 6 to 40 per cent (Krishnamurthy *et al.*, 2010). The root feeding beetle of cardamom *Basilepta fulvicorne* Jacoby, a major pest in the nurseries as well as in the main field in the cardamom growing tracts of Kerala is known to inflict injury to the tune of 10 to 70 per cent (Varadarasan *et al.*, 1993; Varadarasan, 1995, 2002 and 2013).

Banana, a rich source of nutrients and a favourite fruit of Keralites is cultivated year round in an area of 58671 ha (FIB, 2013) for meeting the demands of the domestic as well as foreign markets. The crop is also not spared by beetle pests. One of them, the rhizome weevil, *Cosmopolites sordidus* (Germar), known from all banana growing areas of the world (Peck and Thomas, 1998) caused even up to 100 per cent crop loss in Uganda (Gold *et al.*, 2004), apparently attacks all banana varieties and the infestation causes substantial yield loss.

Neither tuber crops nor vegetables are free from beetle attack. *Cylas formicarius* Fabricius, infesting the vines, tubers and leaves is the most dreaded pest of sweet potato. The weevil causing 4 to 50 per cent yield loss in Kerala (Pillai *et al.*, 1993) and is a widely dispersed pest in the tropics and subtropical regions of the world. The grubs as well as the adults of the tortoise beetle, *Metriona circumdata* H. also damages the leaves of this tuber crop.

The polyphagous pest epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) infesting *Solanum melongena* L., *Lycopersicon esculentum* L., *Cucurbita moschata* Duchesne ex Poir, *Luffa aegyptiaca* Mill and the Pumpkin beetle, *Aulacophora foveicollis* Lucas infesting *Cucumis melo* L. and *Cucumis sativus* L. are recognized as important pests of vegetables.

These pest problems in crops intensified ever since agriculture intensified. The search for molecules that ensure speedy kill of pests culminated in the flooding of markets with highly toxic synthetic chemical pesticides. Though targeted results could be achieved, these chemicals also triggered concerns of pesticide residues and environmental hazards. Hence, the need of the hour is to check such heightening spin off problems by chemical pesticides.

The consensus is that insects need not be eradicated but only regulated. Bioagents consisting of macrobials as well as microbials are pest regulators. Proactive initiatives in the exploitation of microbials in pest management have brought the potentials of entomopathogenic fungi *viz. Beauveria bassiana* (Balsamo) Vuillimen and *Metarhizum anisopliae* (Metschnikoff) Sorokin to lime light. Today,

in view of their environmental safety, pathogenicity and self perpetuating nature these fungi are much focused world over.

The white muscardine fungus, *B. bassiana* and the green muscardine fungus *M. anisopliae* have very wide host range (Tanada and Kaya, 1993) but now it has been recognized that many isolates of these fungi are specific in their action. The susceptibility of an insect to infection by fungi is dependent on the virulence of the fungus, characters of the insect as well as the prevailing environmental conditions. So the pathogenicity and virulence of these fungi to various crop pests and their effective doses need to be unravelled before their adoption in pest management programmes.

In integrated pest management progammes (IPM), a pivotal role is given for biocontrol agents and sometimes it may require applications of biocontrol agents along with chemical pesticides. In this context, it is important that we have sufficient knowledge on the compatibility of these fungi with pesticides. Moreover, if fungi with pesticide tolerance could be developed, would enhance their scope in IPM programmes.

As these fungi can be cultured in artificial media, their potential for utilisation in biological control programmes is also vast but development of economically viable mass production techniques using cost effective substrates are essential. Despite many advantages, the share of entompathogenic fungi in the pesticide market is still meager, which is mainly attributed to the lack of formulations with sufficient shelf life.

To be successful, the organisms should perform well under field situations. Sometimes the performance shown in the laboratory may not be extended to the field, this reiterates the need for evaluation of the fungi under different agroclimatic conditions.

With respect to research in pest management also, we need to follow an approach akin to horses for courses. What is important in terms of local interests, state's interest needs to be addressed. Having the view that even tiny research steps

in this arena of biological control can contribute to giant leaps in the adoption of mycopesticides for pest management in the State, the present project entitled "Evaluation of entomopathogenic fungi for the management of major coleopteran pests and characterisation of pesticide tolerant strains' was chalked out. The following were the objectives:

- To assess the pathogenicity of the entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillimen and *Metarhizium anisopliae* (Metschnikoff) Sorokin to nine important coleopteran pests infesting crops in Kerala
- To determine the LC_{50}, LC_{90} and LT_{50} of the pathogens against the pests acquiring infection through bioassay
- To identify cost effective substrates for the mass production of *B. bassiana* and *M. anisopliae*
- To develop formulations of the fungi
- To evaluate the efficacy of the fungi against two selected coleopteran pests under field conditions
- To study the compatibility of fungi with pesticides and
- To develop and characterise pesticide tolerant strains of *B. bassiana* and *M. anisopliae.*

Review of Literature

2. REVIEW OF LITERATURE

Entomopathogenic fungi play a key role in natural control as well as in biological control of crop pests. The white muscardine fungus, *Beauveria bassiana* (Balsamo) Vuillemin and the green muscardine fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin are the two globally much focused entomopathogenic fungi. The literature pertaining to the pathogenicity, bioassay, mass multiplication, field efficacy, compatibility with pesticides and molecular characterisation of these two fungi are reviewed.

2.1 PATHOGENICITY

2.1.1 *Entomopathogenic fungi*

Roberts and Humber (1984) regarded entomogenous fungi as the pathogens of choice for coleopteran control and the soil is an ideal reservoir of these fungi for the control of subterranean pests (Tanya and Doberski, 1984; Carruthers and Soper, 1987; Klein 1988; Martins, 1988; Milner, 1989 and 1992). Epizootics of entomopathogenic fungi were reported from several scarab species and other soil inhabiting coleopterans (Butt, 1990; Glare, 1992). The growing research on biological protectants has revealed the positive role played by entomopathogens. Investigators since mid 1990s, Adane *et al.* (1996), Hidalgo *et al.* (1998), Bello *et al.* (2000), Ekesi *et al.* (2001) and Padin *et al.* (2002) suggested that isolates of entomopathogenic fungi are potential microbial control agents against coleopteran pests. Hyphomycetous fungi have great potential as biological control agents against insects and they are being developed worldwide for the control of many pests of agricultural importance (Keller *et al.,* 2003; Thungrabeab and Tongma, 2007).

B. bassiana and *M. anisopliae* appear to be the most useful because of their ease of mass production, storage, virulence, and application. The hydrophobic conidia of fungi are able to attach to all body regions, with a preference for surfaces containing hairs and cause infection (Rice and Choo, 2000; Toledo *et al.*, 2008; 2010).

2.1.2 *B. bassiana*

Beauveria spp. were reported as entomopathogenic fungi as early as 1836 (Bassi, 1836). *B. bassiana* was considered as one of the principal organisms for insect pathology research (Steinhaus, 1963). The earliest report of this fungus in the management of pests was by Klochko (1969). *B. bassiana* was found as the most effective and superior fungi than others (Verma *et al.*, 1988). *B. bassiana* is the most common and ubiquitous fungal entomopathogen and has perhaps the longest history as an experimental mycoinsecticide. Its relevant characteristics are the cosmopolitan distribution, easy recognition and isolation, frequent occurrence in nature and the wide broad host range including more than 700 insect species and widely used as biological control agent for crop pests (Goettel *et al.*, 1990; Ferron *et al.*, 1991; Leathers *et al.*, 1993; Chua *et al.*, 1994; Alves *et al.*, 2005; Rehner, 2005; Nirmala *et al.*, 2006).

B. bassiana has emerged as one of the most promising and extensively researched biocontrol agent that can suppress a variety of economically important insect pests (Coates *et al.*, 2002; Mc Guire *et al.*, 2005; Araujo *et al.*, 2009). Prasad and Syed (2010) reported that *B. bassiana* is one such fungus that is especially valuable due to their non toxic nature towards non target animals and humans. It grows naturally in soil throughout the world and acts as a potent parasite on various insect species causing white muscardine disease and can be successfully utilized for the control of crop pests (Karthikeyan and Jacob, 2010).

2.1.2.1 Pathogenicity of B. bassiana to test insects

A perusal of literature on the pathogenicity of *B. bassiana* to the nine test insects in the present study showed that no work has been undertaken on the assessment of the pathogenicity of *B. bassiana* to the pumpkin beetle *Aulacophora foveicollis* Lucas, pepper pollu beetle, *Lanka ramakrishnai* Prathapan and Viraktamath and sweet potato tortoise beetle, *Metriona circumdata* H.

2.1.2.1.1 B. fulvicorne

The natural occurrence of *B. bassiana* on the grubs of *B. fulvicorne* was observed by Varadarasan and Sivasubramonian (1996). Varadarasan (2002 ; 2013) and Varadarasan *et al.* (2006) reported that the adult beetles of *B. fulvicorne* were pathogenic to *B. bassiana* and the mortality of the grubs of *B. fulvicorne* ranged between 65 and 75 per cent at 20 days after treatment with the fungus.

2.1.2.1.2 C. sordidus

Many researchers have studied the effect of *B. bassiana* on *C. sordidus* (Delattre and Bart, 1978; Godonou *et al.*, 2000; Nankinga and Moore, 2000; Gold *et al.*, 2001 ; 2003).

Diaz *et al.* (1986) reported the 100 per cent mortality of *C. sordidus* by the fungus and *B. bassiana* was identified as a potent biocontrol agent of banana rhizome weevil by Filho *et al.* (1989; 1991 ; 1995), Kaaya *et al.* (1993), Schoeman and Schoeman (1999) and Lopez *et al.* (2010).

Laboratory evaluation of different strains of *B. bassiana* was conducted in Uganda, (Nankinga *et al.*, 1994) and in West Africa (Godonou *et al.*, 2000) and the fungus was found to produce 50 per cent to 100 per cent mortality in banana weevil adults in 14 days (Magara *et al.*, 2004). In laboratory bioassays, *B. bassiana* was found highly pathogenic to *C. sordidus*, causing more than 90 per cent mortality within two weeks (Akello *et al.*, 2008 ; 2009).

2.1.2.1.3 C. formicarius

According to Diaz *et al.*, (1986) *B. bassiana* caused 100 per cent mortality of *C. formicarius*. The pathogenicity of the fungus to the adults and grubs of *C. formicarius* was reported by Burdeos and Villacarlos (1989). Su (1991a; 1991b) observed that *B. bassiana* at a concentration of 1.6×10^4 conidia ml^{-1} caused more than 80 per cent mortality. Jansson (1992) reported the natural infection of the fungus to *C. formicarius*.

2.1.2.1.4 H. vigintioctopunctata

B. bassiana as an efficient pathogen of *Epilachna vigintioctopunctata* F. was reported by Klochko (1969) The fungus caused 98 per cent mortality of larvae within 13 days. The susceptibility of *H. vigintioctopunctata* to *B. bassiana* was recorded by Padmaja and Kaur (1998). Jiji *et al.* (2008) observed a mean mortality of 63.33 per cent on epilachna beetle grubs due to *B. bassiana* infection.

2.1.2.1.5 O. rhinoceros

Pathogenicity of *B. bassiana* to *O. rhinoceros* was reported by Latch (1976).

2.1.2.1.6 R. ferrugineus

Entomopathogenic fungi are among the most relevant biological agents suggested to control *R. ferrugineus* (Faleiro, 2006). *R. ferrugineus* has been found infected by *B. bassiana* under natural conditions (Agullo *et al.*, 2010). Francardi *et al.* (2012) observed that *B. bassiana* strain isolated from soil recorded a lower cumulative mortality on larvae (13 per cent) and adults (13 per cent) of red palm weevil. *B. bassiana* strain isolated from *R. ferrugineus* showed cumulative mortality values higher than 50 per cent against treated larvae.

2.1.2.2 Other coleopterans

The most successful use of fungi against scarabs is exemplified by *Beauveria* sp. against the European cockchafer, *Melolontha melolontha* L. (Keller *et al.*, 1986) and also by the application of blastospores to aggregations of the adult beetles (Keller *et al.*, 1989). *B. bassiana* has been used for approximately 100 years to control white grubs and adults in Europe (Zimmerman, 1992). *B. bassiana* is highly pathogenic to white grubs, *Holotrichia serrata* F. and related species (Nehru and Jayarathnam, 1993; Poprawski and Khachatourians, 1994). *B. bassiana* @ 1×10^8 spores ml^{-1} concentration inflicted initial mortality of white

grubs on sixth day and 100 per cent mortality was observed on twelfth day (Mohi-Ud-Din *et al.*, 2006).

The fungus was effectively used for the suppression of coleopteran stored grain pests and rice weevil (Searle and Doberski, 1984; Tanya and Doberski, 1984; Rice *et al.*, 1993; Adane *et al.*, 1996; Moina *et al.*, 1998). *B. bassiana,* has proven its effectiveness against the major coleopteran stored grain pests *viz. Sitophilus oryzae* L., *Rhyzopertha dominica* Fabricius, *Oryzaephilus surinamensis* L., *Prostephanus truncates* Horn. and *Tribolium castaneum* Herbst. (Smith *et al.*, 1999). Wakefield *et al.* (2005) demonstrated that some *B. bassiana* isolates provided 100 per cent mortality in *O. surinamensis.*

The pathogenicity of *B. bassiana* against colorado potato beetles was observed by Anderson *et al.* (1988). Zhang and Groden (1995) studied the pathogenicity of two strains of the fungus *B. bassiana*, which controlled *Leptinotarsa decemlineata* Say.

B. bassiana was pathogenic to the the boll weevil (Wright, 1993), rice hispa (Puzari and Hazarika, 1994; Hazarika and Puzari, 1995 and 1997), coffee berry borer (Varela and Morales, 1996), sugarcane stem borer (Berretta *et al.* 1998) and the darkling beetle (Castrillo and Brooks, 1998). Ekesi (2000) reported higher per cent mortality and antifeedant effect of *B. bassiana* isolate CPD_3 and CPD_{10} on cowpea leaf beetle, *Ootheca mutabilis* Sahlberg. Mc coy *et al.*, (2000) reported that the conidia of *B. bassiana* have suppressed root weevil larval populations when applied at high inoculumn rates. Adult weevils of *Odoiporus longicollis* Oliv. were found naturally infected with entomopathogenic fungi, *B. bassiana* (Padmanabhan *et al.*, 2001, 2002). Infection of *B. bassiana* on mango stone weevil was reported by Verghese *et al.* (2003).

According to Draganova *et al.* (2006) the coleopteran wood borers like *Ips sexdentatus* Boerner and *Ips acuminatus* Gyll. were also susceptible to the fungus, the isolate 426 of *B. bassiana* caused the highest lethal effect to adults of *I. sexdentatus* (96.67 per cent), followed by the isolates 412 and 422 of

B. bassiana which recorded 90.67 per cent and 89.33 per cent, respectively. *B. bassiana* is pathogenic to *Phyrdenus muriceus* Germar *Diabrotica speciosa* (Germar), *Cycloneda sanguinea* L. and *Diloboderus abderus* Sturm (Toledo *et al.*, 2008).

2.1.3 *M. anisopliae*

M. anisopliae is a potential imperfect entomopathogenic fungus found in soil throughout the world. Metschnikoff (1879) reported the pathogenicity of this fungus for the first time in wheat cockchafer. *Metarhizium* spp. are known to infect more than 200 insect species, many of which are major agricultural pests (St. Leger, 1993). *M. anisopliae* is a mitosporic haploid fungus with a global distribution, has a wider host range of important agricultural pests and therefore, it holds great potential for use as biological control agent (Butt *et al.*, 2001; Nahar *et al.*, 2004).

2.1.3.1 Pathogenicity of M. anisopliae to test insects

Infectivity of *M. anisopliae* to the test insects in the present study *viz. A. foveicollis, L. ramakrishnai* and *M. circumdata* has not been documented earlier. However, the infectivity to the test insects *B. fulvicorne, C. sordidus, C. formicarius, H. vigintioctopunctata, O. rhinoceros and R. ferrugineus* has been recorded.

2.1.3.1.1 B. fulvicorne

The adult beetles of *B. fulvicorne* were found to be naturally infected by *M. anisopliae* and the grubs were also prone to infection by the fungus (Varadarasan *et al.*, 1993; Varadarasan *et al.*, 2002; Varadarasan, 2003; Varadarasan and Sydhic, 2005).

2.1.3.1.2 C. sordidus

Metarhizium spp. virulent to *C. sordidus* adults and larvae were isolated from soil (Kaaya *et al.*, 1993; Krauss *et al.*, 2004; Lopes *et al.*, 2011). Lopes

et al. (2013) reported that *C. sordidus* larvae are very sensitive to *M. anisopliae*, with mortality rates around 98 per cent after nine days.

2.1.3.1.3 C. formicarius

Pathogenicity of *M. anisopliae* was reported against sweet potato weevil *C. formicarius* (Khan *et al.*, 1990). *M. anisopliae* caused death of *C. formicarius* larvae in three to five days after inoculation (Rana and Villacarlos, 1991) and the fungus had adverse effect on total fecundity of the weevils. Korada *et al.* (2010) reported the effectiveness of *M. anisopilae* to regulate *C. formicarius*.

2.1.3.1.4 H. vigintioctopunctata

M. anisopliae aqueous formulation @ 5×10^{12} conidia had good effect against the adult *H. vigintioctopunctata* (Swaminathan *et al.*, 2010).

2.1.3.1.5 O. rhinoceros

The fungus, *M. anisopilae* var. *majus,* infected *O. rhinoceros* through spores, germinated and penetrated through the cuticle into the insect where the fungus proliferated (Ferron *et al.,* 1975; Latch and Falloon, 1976). Because of their protected, moist habitat which favoured the persistence of spores, it was the larval stage which was most frequently infected. Increased attention has been paid to the use of *Metarhizium* as a biological control agent against *O. rhinoceros* (Beichle, 1980; Sundarababu *et al.*, 1983; Pillai, 1987). In Samoa, *M. anisopilae* has long been established as a promising pathogen of the coconut rhinoceros beetle (Waterhouse and Norris, 1987). The fungus infected both adults and grubs of rhinoceros beetle. Among the two *Metarhizium* sp., *M. anisopliae* var. *anisopliae* and *M. anisopliae* var. *major*, the later one is more pathogenic to rhinoceros beetle (CPCRI, 1999). Sujatha and Rao (2004) reported natural occurrence of *M. anisopliae* on the grubs of rhinoceros beetle.

2.1.3.1.6 R. ferrugineus

Gindin *et al.* (2006) found that *M. anisopliae* strains were more virulent and the lethal time was shorter on red palm weevil larvae. *M. anisopliae* obtained from *R. ferrugineus* showed the highest efficacy against the weevil larvae and adults. A cumulative larval mortality of 100 per cent and adult mortality of 90 per cent was noticed (Francardi *et al.*, 2012).

2.1.3.2 Pathogenicity of M. anisopliae to other coleopterans

The efficacy of *M. anisopliae* used as a microbial pathogen of the beetle *Popillia japonica* Newman was summarized by Fleming (1968). Evaluation of this fungus revealed fair potential as pathogen of Japanese beetle larvae (Martins, 1988; Krueger *et al.*, 1991; 1992). Khaderkhan *et al.*, (1993) observed the mortality of *Semontus japonica* L. when infected with *M. anisopliae.*

The fungus has been investigated against a range of cane scarabs (Easwaramoorthy and Santhalakshmi, 1991; Milner, 1992; Easwaramoorthy *et al.*, 2004) and peanut scarabs, *Heteronyx* spp.; (Milner *et al.*, 1993). It is an effective pathogen of the pasture scarab, *Adoryphorus couloni* Burmeister in Tasmania, Australia (Rath, 1992). Pathogenicity of *M. anisopliae* was reported against mulberry brown chaffer, *Holotrichia paralella* (Fabr.) (Chen *et al.*,1995).

Fourth instar larvae of Colorado potato beetle, *L. decemlineata* were killed by *M. anisopliae* (Chabchoul and Taborsky, 1991). Bustillo *et al.* (1999) reported the pathogenicity of the fungus towards the coffee berry borer, *Hypothenemus hampei* Ferrari. Mc coy (1995) and Mc coy *et. al.*, (2000) reported that *M. anisopliae* have infected the root weevil when applied at high inoculum rates.

Anitha *et al.* (1998), Yue *et al.* (2003), Anitha (2004), Beegum (2005) and Beegum and Anitha (2008) observed *M. anisopliae* as a potent pathogen of pseudostem weevil, *Odoiporus longicollis* Oliv.

2.1.4. Signs and symptoms of infection

2.1.4.1 *B. bassiana*

B. bassiana infected sugarcane root borer larvae became hard and brittle and body showed pinkish discolouration in initial stages of infection and was covered with fluffy fungal mat in the advanced stages (Easwaramoorthy and Santhalakshmi, 1987; 1993). Alves *et al.* (2002) observed that *B. bassiana* colonizes insect hosts initially through a yeast phase, by production of yeast like cells which are developed by budding, from germinating conidia after 24-h incubation. Cells were typically 5–10 µm and fungal colonies were initially circular and mucoid, but later were covered with mycelia and conidia. Larvae of lepidopteran pests showed various symptoms when sprayed with spore suspension of *B. bassiana*. During the initial stages, the infected larvae were sluggish in their movement, became cream in colour and died from the next day onwards. The infected larvae failed to moult and early pupation of later instars was observed. In case of early instars the food uptake was reduced considerably. Malformed adults were emerged from the infected pupae. These pupae were black in colour, compared to brown colour of normal pupae and white puffy mycelial growth of *B. bassiana* appeared on pupae (Jiji *et al.*, 2008). *B. bassiana* infected eggs of *H. vigintioctopunctata* showed colour change from the yellow to brown and later white mycelial growth appeared on these eggs. Grubs failed to emerge from infected eggs. Infected grubs showed less appetite, later the colour of the spines changed to brownish black and finally grub became black in colour. On dead grubs white mycelial growth of the fungus appeared. Prasad and Syed (2010) observed that the treated larvae stopped feeding and became sluggish. The cuticle of the treated larvae became black due to excessive melanisation.

2.1.4.2 M. anisopliae

Pathogenicity of *M. anisopliae* was studied by many workers. Loss of appetite and sluggishness are the symptoms of infection. The infected grub of rhinoceros beetle died within 10-15 days. Initially white fungal colonies appeared

at the joints and the integument which later turned greenish following sporulation of the fungus. Finally the cadaver became black. The fungus infected both adults and grubs of rhinoceros beetle (CPCRI, 1999). *M. anisopliae* on grubs and adults of pseudostem weevil, caused sluggish movement and reduced feeding. Immediately after death the body of the infected grub became soft and later turned hard. White mycelial mat appeared all over the body of the grubs, later colour changed to green (Butt *et al.,* 1995; Anitha, 2000; Campbell *et al.,* 2000). According to Beegum and Anitha (2008) the grubs of *O. longicollis* infected with *M. anisopliae* became lethargic and became mummified.

2.3 BIOASSAY

2.2.1 *B. bassiana*

2.2.1.1 B. bassiana on C. sordidus

Brenes and Carballo (1994) determined the LC_{50} and LC_{90} values of A_4 strain of *B. bassiana* against *C. sordidus* as 7.89×10^7 and 2.67×10^9 spores ml^{-1}, respectively. They obtained the LT_{50} value between 6.3 and 10 days. Khan and Gangaprasad (2001) reported LC_{50} value of 4.5×10^7 spores ml^{-1} of *B. bassiana* against banana bore weevil, *C. sordidus*.

2.2.1.2 B. bassiana on other coleopterans

The fungus, *B. bassiana* was more effective in the concentration ranged from 10^7 to 10^9 spores ml^{-1} against rice hispa, *Dicladispa armigera* Oliv. (Agarwal, 1990). Ninety five percent mortality of larvae of *Ostrinia fumacata* Hubner was obtained when *B. bassiana* at 50×10^9 spores ml^{-1} was sprayed on them (Zhang *et al.,* 1992). A sharp increase in mortality of the beetle was observed three and four days after treatment with either 10 mg of *B. bassiana* 100 $adults^{-1}$. The LT_{50} values at 10 mg 100 $beetles^{-1}$ for *B. bassiana* was 3.1 days (Lacey *et al.,* 1994).

Wegensteiner (1996) conducted laboratory bioassays using *B. bassiana* against adults of bark borer, *Ips typographus* L. and concluded that a

concentration of 3×10^6 conidia cm^{-2} bark seemed to be a sufficient infection dose to kill more than 90 per cent of beetles. Adults of shot hole borer of tea, when sprayed with *B. bassiana* spore suspensions under *in vitro* conditions, caused 100 per cent mortality within five to six days at 10^7 and 10^8 spores ml^{-1} (Selvasundaram and Muraleedharan, 2000).

Sheeba *et al.,* (2001) conducted bioassays by introducing 25 adult insects of *S. oryzae* on 50 g of *B. bassiana* mixed rice in glass jars maintained at 28±2°C, and 70 per cent RH. At 7.6 log conidia ml^{-1}, *B. bassiana* produced a mortality up to 75.80 per cent.

The fungus at 1.3×10^9 spores ml^{-1} caused cent per cent mortality of mango stone weevil in two to seven days (Verghese *et al.*, 2003). Further reports on bioassay include report of Fabio *et al.*, (2003) and Beron *et al.*, (2005) against *Cyclocephala signaticollis* and sugarcane scarabs (Srikanth *et al.,* 2006 and 2010). *B. bassiana* had a cumulative adult mortality of 56.67 to 80.00 per cent at 10^5 to 10^9 spores ml^{-1} on *L. pygmaea* and the LC_{50} value was 2.26×10^4 spores ml^{-1} under laboratory conditions (Karthikeyan and Jacob, 2010).

2.2.2 *M. anisopliae*

2.2.2.1 M. anisopliae on test insects

M. anisopliae killed 100 per cent of the *O. rhinoceros* larvae 7 – 16 days after treatment when applied in the form of spore suspension (Latch, 1976). The LD_{50} of third instar larvae of *O. rhinoceros* has been estimated at 416 fungal spores, and infected larvae survived an average of about three weeks (Sundarababu *et al.,* 1983). Ho (1996) observed that the effective dose of fungus for 50 per cent (ED_{50}) mortality of the grubs was 3.79 to 5.80×10^3 spores ml^{-1}. Moslim *et al.* (1999) conducted bioassays with the third instar grubs and the lethal time for fifty per cent mortality came to about 8.9 – 9.1 days. Francardi *et al.* (2012) reported that the cumulative larval mortality of *O. rhinoceros* reached 100 per cent and adult mortality 90 per cent and LT_{50} was 13.10 days when treated with *M. anisopliae.*

Red palm weevil adults treated with *M. anisopliae* strain M.08/I05 showed higher cumulative mortality of 53 per cent and LT_{50} of adults reached in 26.80 days (Francardi *et al.*, 2012).

M. anisopliae caused about 98 per cent mortality of *C. sordidus* by nine days post exposure with a LT_{50} of 4.20 days (Lopes *et al.*, 2013).

2.2.2.2 M. anisopliae on other coleopterans

Chen *et al.* (1995) observed 100 percent mortality of *H. paralella* when an isolate of *M. anisopliae* was applied to soil at a concentration of 2.5×10^6 spores g^{-1} under in vitro conditions. Spore concentration of *M. anisopliae* varying form 1.5×10^5 spores ml^{-1} to 1.5×10^{10} spores ml^{-1}, were reported against various coleopteran soil pests (Chen *et al.*, 1995 Vannien *et al.*, 1999; Wickramatileke *et al.*, 2000; Rachappa *et al.*, 2001; Rajendran, 2002; Pandey, 2003).

The LC_{50} values of *P. japonica* 7 days after exposure to *M. anisopliae* was 0.7 mg conidia 100 $adults^{-1}$, respectively at 22-24^{0}C. A sharp increase in mortality was observed 3 days after treatment with 10 mg of *M. anisopliae* 100 $adults^{-1}$, respectively. The LT_{50} values at 10 mg 100 $beetles^{-1}$ for *M. anisopliae* was 4.2 days (Lacey *et al.*, 1994).

The probit analysis of dose mortality responses of the grubs of *O. longicollis* on the tenth day after inoculation with *M. anisopliae* obtained the LC_{50} value as 3.9×10^6 spores ml^{-1} (Beegum and Anitha, 2008).

2.3 SUBSTRATES AND FORMULATIONS

2.3.1 Mass multiplication on substrates

2.3.1.1 B. bassiana

Batista *et al.* (1989) observed better conidial production of *B. bassiana* in bran broth compared to rice and potato broth. Ibrahim and Low (1993) identified coconut water as the best liquid medium for production of *B. bassiana*. Chaudhuri *et al.* (2001) tested various substrates including grains and agricultural by products for mass multiplication of *B. bassiana* and based on biomass

production, conidial count, radial growth and viability of conidia, sorghum grains was found to be the best substrates to support growth and sporulation of the fungus. According to Haraprasad *et al.* (2001) *B. bassiana* grew well on rice bran (coarse) and wheat bran yielding a maximum sporulation. The suitability of locally available and cheaper substrates, especially the agricultural by products were evaluated for the mass multiplication of *B. bassiana,*. Rice bran, coconut water and rice bran extract were found to be the best substrates for growth and sporulation (Jiji *et al.*, 2008). Francisco and Florez (2008) could produce better conidial yield of *B. bassiana* when mass multiplied on rice.

2.3.1.2 M. anisopliae

Daoust and Roberts (1983) observed rice medium as a better and cheaper substrate than the more complex mycological media for the production of conidia of *M. anisopliae*. Danger *et al.*, (1991) used coconut water from copra making industry for mass production of *M. anisopliae* against rhinoceros beetle *O. rhinoceros*. Rice has been commonly used to mass produce *M. anisopliae* (Mendonca, 1992). Alvarez *et al.*(1997), Narvaez *et al.* (1997) and Jenkins *et al.* (1998) reported sterile humid rice as the best substrate for growing *M. anisopliae*. Moslim *et al.* (2005) observed that the spores of the fungus have been successfully produced using solid state fermentation on broken maize.

2.3.2 Formulations

2.3.2.1 B. bassiana

Walstad *et al.* (1970) noticed that the survival of spores of the muscardine fungi over extended periods of time was affected by the storage temperature. They observed that the spores of *B. bassiana* remained viable for twelve months at 8 ^{0}C, but lost the virulence after 0.5 and 2.5 months at 21 ^{0}C. The spores of *B. bassiana* formulated as wettable powder retained more than 85 per cent spore germination even after eight months when stored under refrigerated condition. The pathogenicity of the fungus was maintained even after one year at room temperature (Zhang *et al.*,1992). Sandhu *et al.*, (1993) found that *B. bassiana*

remained viable for 24 months when formulated. The spores of *B. bassiana* formulated as wettable powder gave a spore germination rate of more than 85 per cent after eight months of storage under refrigeration. The pathogenicity was maintained even after storage for one year at room temperature. Jiji *et al.*, (2008) reported that wettable powder formulation of *B. bassiana* at 10 per cent was found effective under in vitro condition as well as for the field trial.

2.3.2.2 M. anisopliae

The spores of *M. anisopliae* remained viable for twelve months at 8 ^{0}C, but lost the virulence after 0.5 and 2.5 months at 21 ^{0}C (Walstad *et al* ., 1970). Andersch (1992) patented the process of formulating *M. anisopliae* as pellets. Stathers *et al.* (1993) found that the spores of *M. anisopliae* formulated in powder stored better at 5 °C and 15 °C than if formulated in oil. Moore *et al.* (1995) observed 42 percent germination of *M. flavoviridae* after four to five months of storage as powder at 25 - 37 ^{0}C while the germination percentage increased to 97 at 10 - 12 ^{0}C. A long shelf for *M. anisopliae* has been identified by Alves *et al.* (1998) who evaluated 64 formulations of *M. anisopliae*. He found that fungus could be stored upto 660 days with 33 per cent viability. Hamid *et al.* (2005) observed that the stability of the powder formulation was very high and the viability of the spores stored at 5 °C and 15 °C after 15 MAS remained at about 60 per cent, however that which stored at 28 °C showed a reduction up to 52.20 per cent.

2.4 FIELD EFFICACY

2.4.1 *B.bassiana*

2.4.1.1 B. bassiana on test insects

In the management of *C. formicarius, B. bassiana* was applied as a foliar spray or in combination with pheromone trap, for its successful infection and dispersal in the field. Spraying of *B. bassiana* solution (isolated from *C. formicarius*) at a concentration of 1.6×10^4 conidia ml^{-1} at planting and rootstock formation, and broadcasting soybeans containing *B. bassiana* into rows

at planting controlled *C. formicarius* effectively (Su, 1991 a). The success of infection of *B. bassiana* on *C. formicarius* is dependent on the type of soil in the experimental field, which largely affects the survival of the fungus (Su, 1991b). *B. bassiana* densities of 0, 100 and 1000 conidia per gram of soil resulted in 0, 30 and 100 per cent *C. formicarius* mortality, respectively in Taiwan (Jansson, 1992). Yasuda *et al.* (2000) reported the superior infectivity of corn oil formulation of *B. bassiana* against *C. formicarius* in field. Application of *B. bassiana* (Bio-powder 1.5 WP®) @ 6.75 kg ha^{-1} resulted in production of highest marketable yield of tubers in sweet potato (15.4-20.24 t ha^{-1}) in India (Anonymous 2009).

Filho *et al.* (1991) observed a population reduction of 61 per cent for adult *C. sordidus* by applying *B. bassiana* (@ 50 ml / pseudostem trap) at 1 × 10^9 conidia ml^{-1} and obtained reductions in infestation of the pest ranging from 29.00 to 41.30 per cent and 18.30 to 35.60 per cent. Nankinga (1999) applied corn bran containing *B. bassiana* conidia to the topsoil around banana mats and after application, however, five months after treatment, only 20 per cent of banana weevils collected were infected. Godonou *et al.* (2000) applied an oil palm kernel cake-based formulation of conidial powder to planting holes and suckers; and they recorded 41 per cent mortality among banana weevils two months after application.

Under field conditions the wettable powder formulation of *B. bassiana* recorded a mean mortality of 60 per cent against *H. vigintioctopunctata* and 76 per cent against epilachna beetle on brinjal plants (Jiji *et al.*, 2008).

2.4.1.2 B. bassiana on other coleopterans

The field experiment conducted by Lavalee *et al.* (2005) proved the potential of *B. bassiana* application for the control of the pine shoot beetle (*Tomicus piniperda*, Scolytidae). The persistent toxicity of *B. bassiana* in field against rice blue beetle was on par with other eco-friendly

insecticides *viz.*, azadirachtin 1 per cent (Econeem) and neem oil (Karthikeyan and Jacob, 2010).

2.4.2 *M. anisopliae*

2.4:2.1 M. anisopliae on C. formicarius

Villacarlos *et al.* (1995) reported that *M. anisopliae* can be successfully utilised for the control of sweet potato weevil and the adults were found to be more prone to the fungus cultured in cheaper soil amendments.

2.4.2.2 *M. anisopliae on other coleopterans*

The field efficacy of *M. anisopliae* against *O. longicollis* was evaluated by Anitha (2000) and Begum and Anitha (2008).

2.5 COMPATIBILITY WITH PESTICIDES

One of the most promising aspects of microbial control agent of insects pest is its integration with other pest control measures, particularly the chemical method (Urs *et al.*, 1967). Several pesticides have been reported to have varying effects on entomopathogenic fungi from inhibitory to stimulatory.

2.5.1 *B. bassiana*

Anderson and Roberts (1983) found that generally the wettable powder and flowable formulations cause no inhibition, and often increase the colony counts whereas emulsifiable concentrate formulations frequently inhibit *B. bassiana* germination. Carbaryl at 0.1,1,10,100 and 1000 ppm inhibited the germination of conidia of *B. bassiana* (Aguda *et al.*, 1988). Anderson *et al.* (1989) reported that abamectin, triflumuron, thuringiensin and carbaryl demonstrated no significant inhibition of colony growth of the fungus, *B. bassiana*. *B. bassiana* showed better growth on the medium containing 25 per cent diflubenzuron at the two lower doses while, at ten times the field dose, growth was inhibited (Sapieaha and Mietkiewski , 1992). The fungus was found

to be compatible with commonly used insecticides (chlorpyriphos, imidaclorpid, lambda cyhalothrin, amrutneem, carbaryl, malathion, dimethoate and acetamiprid) and fungicides (mancozeb and carbendazim) (Devi *et al.,* 2004; Jini and Varadarasan, 2005; Alizadeh *et al.,* 2007 a ; 2007 b ; Jiji *et al.,* 2008; Shah *et al.,* 2009).

2.5.2 *M. anisopliae*

Carbaryl at 0.1,1,10,100 and 1000 ppm inhibited the germination of conidia of the entomogeneous fungus, *M. anisopliae* (Aguda *et al.,* 1988).

2.6 MOLECULAR CHARACTERISATION

Molecular techniques improve our understanding of the diversity and distribution of entomopathogenic fungi in the environment (Rehner, 2005). The recent studies on fungal populations showed that both the genera *Beauveria* and *Metarhizium* contain cryptic species with features that are often ambiguous and specific molecular markers must be used to identify them (Rehner and Buckley, 2005).

2.6.1 *B. bassiana*

Despite the agricultural importance of some strains, the genetics of these two imperfect fungi, *B. bassiana* and *M. anisopliae* has rarely been studied. Molecular diagnostics (typing) of *B. bassiana* isolates have been attempted by studying isozyme (Bridge *et al.*1990; St.Legar *et al.*1992; Castrillo and Brooks 1998) and esterase profiles (Varela and Morales 1996), telomeric fingerprinting (Viaud *et al.*1996 and 1998; Couteadier and Viaud 1997), polymorphisms in internal transcribed spacer regions of rDNA (Glare and Inwood, 1998) and RAPD analysis (Maurer *et al.*1997; Berrette *et al.*1998; Castrillo and Brooks 1998; Glare and Inwood 1998; Luz *et al.,* 1998).

Like many fungi, *B. bassiana* lacks a conventional sexual cycle but exhibits parasexual recombination (Meirelles and Azevedo, 1991). *B. bassiana* isolates have been reported to differ in host range and virulence to a given insect species. Identification of a molecular marker linked to a virulent phenotype to a

target pest would be useful in screening for isolates effective against it. RAPDs, the PCR-based DNA fingerprints (produced by amplifying DNA using short arbitrarily chosen oligonucleotide primers) provide a rapid and reliable means of identifying neutral genetic markers. It has been possible to correlate particular fungal genotypes defined by RAPD markers with particular pathogenicity groups (Goodwin and Annis 1991; Mills *et al.*1992). The RADP analysis showed a high (~70%) level of similarity among the *B. bassiana* isolates though they had been isolated from different climatic zones spreading over both hemispheres and from varied hosts. Large genetic distances have been observed among *B. bassiana* isolates in the RADP analysis of Berretta *et al.* (1998) and Castrillo and Brooks (1998).

In the random amplified polymorphic DNA (RAPD) analysis conducted by Devi *et al.*, (2001) 30 per cent variability was observed among the isolates of *B. bassiana*; which clustered into three major groups. The groups based on virulence rating did not match with the RAPD clusters. One of the highly aggressive isolates clustered with less aggressive isolates in one cluster and the other grouped along with the medium aggressive isolates in a different cluster. The RAPD characterization would be useful for proprietary reasons when fungal isolates are introduced into new ecosystems for pest control, it ensures a means of detecting the introduced pathogen versus the native one. It will be useful for patenting purposes when the isolates are used in commercial formulations (Devi *et al.*, 2001). Literature pertaining to molecular characterisation of pesticide tolerant strains is meagre.

2.6.2 *M. anisopliae*

Repeated subculturing of EPF such as *M. anisopliae* on nutrient agar media is known to lead to sector formation, phenotypic degeneration, changes in the ability to produce secondary metabolites and enzymes and pleomorphic deterioration (Lesile, 1993; Masel *et al.*, 1996; Kamp and Bidochka, 2002; Ryan *et al.* 2002). The diversity of soil and insect isolates of *M. anisopliae* was studied by using molecular tools like PCR and RAPD by Fungaro *et al.* (1996). Isolates

of *M. anisopliae* suffer pleomorphic deterioration (Kamp and Biodochka, 2002), with an associated decline in virulence due to rapid changes in the surface properties of conidia (Shah *et al.*, 2007).

Wang (2002) observed that the successive subculturing of *M. anisopliae* strain V-275, led to the discovery of mutants with distinctive morphological, biochemical and genetic characteristics in contrast with the parent strain and the presence of such new genotypes indicated that mitotic recombination events occurred and resulted in loss of pigmentation and different enzyme profiles compared with the parents. Genetic similarities for *M. anisopliae* was recorded to be 63-90 per cent (Muro *et al.*, 2003) in ISSR and AFLP studies of the different isolates collected from different parts, respectively. On the other hand, repeated in vivo transfers (10-30 times) of *M. anisopliae* and different EPF did not always lead to loss of virulence against their hosts (Nahar *et al.*, 2008). Inter-simple sequence repeats (ISSR) fingerprinting and phylogenetic analyses were used to assess the genetic variability of monosporic isolates of *B. bassiana sensu lato (s.l.)* and *M. anisopliae (s.l.),* respectively, which are species complexes comprising genetically distinct but morphologically similar taxa (Lopes *et al.*, 2013).

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Evaluation of entomopathogenic fungi for the management of major coleopteran pests and characterisation of pesticide tolerant strains" was conducted in the Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram between 2007 and 2014. The details of the materials and methods adopted for the investigation are described below.

3.1 PATHOGENICITY OF ENTOMOPATHOGENIC FUNGI

Laboratory experiments were carried out to test the pathogenicity of two fungi, *viz. Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin on the following coleopteran pests of crops.

1. Banana rhizome weevil (*Cosmopolites sordidus* Germ.)
2. Cardamom root grub (*Basilepta fulvicorne* Jacoby)
3. Coconut rhinoceros beetle (*Oryctes rhinoceros* Linn.)
4. Epilachna beetle (*Henosepilachna vigintioctopunctata* F.)
5. Pepper pollu beetle (*Lanka ramakrishnai* Prathapan & Viraktamath)
6. Red palm weevil (*Rhynchophorus ferrugineus* F.)
7. Red pumpkin beetle (*Aulacophora foveicollis* Lucas)
8. Sweet potato tortoise beetles (*Metriona circumdata* H.)
9. Sweet potato weevil (*Cylas formicarius* F.)

3.1.1 Maintenance of fungal cultures

B. bassiana isolate, PDBC Bb 5 and *M. anisopliae* isolate, PDBC Ma 4 used for the studies were obtained from Project Directorate of Biological control (PDBC), Bangalore, later renamed as National Bureau of Agriculturally Important Insect Pests

(NBAII). The fungal isolates were subcultured and maintained on Potato Dextrose Agar (PDA) media at 27 ± 5 ° C. Mass production of the fungi for laboratory experiments was done in Potato Dextrose Broth (PDB).

3.1.2 Maintenance of insect stock culture

The adults as well as the immature stages of all the test insects were captured initially from the field. The insects thus collected were kept in rearing jars along with their respective host plants for fifteen days in order to screen diseased insects. The healthy disease free insects were further reared in the laboratory to obtain the stock culture for the experiments.

3.1.2.1 A. foveicollis

The grubs of *A.foveicollis* were reared on roots and stems of pumpkin/cucurbits kept in soil in plastic trays of size 30 cm diameter and 20 cm height. The decayed plant parts were removed periodically to keep the trays clean. The grubs preferred fresh and juicy stems and they moved from decayed stems to fresh stems in the rearing trays. On maturity, the grubs pupated in soil and these were collected and kept in jars for adult emergence. Further, the adults were transferred to jars containing fresh stems for egg laying. Leaves were also provided as food for the adults. The stems with oviposition punctures were then transferred to trays for larval rearing.

3.1.2.2 B. fulvicorne

The field collected healthy adult beetles were reared on polypet jars of size 30 cm height and 15 cm diameter, the mouth of which were covered with muslin cloth. Cardamom leaves and jack fruit leaves were provided as food. Soil and stubbles of Cardamom plants were also placed inside these jars for enabling egg laying. The plant parts bearing egg masses were removed periodically to rear the emerging grubs in trays. The grubs of *B. fulvicorne* were reared on cardamom rhizomes bearing fresh

roots that were kept in soil in plastic trays of size 30 cm diameter and 20 cm deep. On pupation in soil, they were collected and kept in jars for adult emergence.

3.1.2.3 C. sordidus

C. sordidus was reared in plastic trays of 30 cm diameter and 20 cm deep having vertically cut healthy pieces of banana rhizomes (approximately 150 g). Healthy adults and grubs collected from field were released into these trays and covered with muslin cloth. The adults oviposited on the surface of the rhizome bits and the emerging grubs developed within the rhizome pieces and pupated at the end of the tunnel/ on rhizomes. The insects thus reared were used for the experiments. New pieces of rhizome were provided regularly as food.

3.1.2.4 C. formicarius

The healthy field collected grubs and adults were released on small sweet potato tubers with fresh cuts as well as on stem cuttings with roots, that were kept in soil in trays and the whole unit was covered with muslin cloth. Every week the food was changed due to browning of the tubers, as these discoloured tubers and vines were not preferred for feeding. Pupae collected from the vines, tubers and soil were kept for adult emergence in bottles.

3.1.2.5 H. vigintioctopunctata

Field collected adults and grubs were reared on fresh brinjal leaves kept in polypet jars and covered with muslin cloth. Fresh brinjal leaves were given as food for both the adults and grubs. To avoid overcrowding, only 15 adults or 15 grubs were confined per jar. The egg masses obtained were transferred to fresh jars alongwith food for the emerging grubs which later pupated on the leaves.

3.1.2.6 L. ramakrishnai

Adults and grubs were reared on young flushes of pepper and twigs bearing spikes, that were kept in polypet jars and covered with muslin cloth. The cut ends of the twigs were rolled with moistened cotton balls so as to keep them fresh and green. A thin layer of wet soil was kept at the bottom of the rearing jar for pupation of the grubs. The emerged adults were kept in jars provided with tender berries for egg laying.

3.1.2.7 O. rhinoceros

The field collected healthy grubs were transferred to plastic rearing trays of size 30 cm diameter and 20 cm height containing powdered cow dung moistened with 50 per cent water. The cow dung was changed once in every five days. About 15 grubs were kept in each tray to prevent overcrowding and cannibalism. The pupae were collected and kept in polypet jars for adult emergence. The healthy adults were reared on young coconut fronds and very tender leaves placed in 15 L plastic buckets with lid. Adults were also fed with a juice prepared by mixing 100 g jaggery and 10 g salt in 250 ml water. In addition, mashed ripe palayamkodan banana fruits were also provided for the adults. The food was changed once in every five days due to browning of the tender plant parts. Moistened cow dung was provided in the buckets to enable egg laying. The eggs were collected and reared on separate trays.

3.1.2.8 R. ferrugineus

Adults and grubs collected from field were reared separately on coconut trunk waste obtained from saw mills and damaged plantations, that were kept in 15 L plastic buckets with lid. Regular cleaning and transferring of the insects to clean buckets were done to prevent diseases. Overcrowding was also avoided to prevent cannibalism by keeping only 20 grubs / adults in each bucket. The grubs that pupated

inside the buckets were transferred to jars for adult emergence and the mated adults were allowed to oviposit in the trunk wastes.

3.1.2.9 M. circumdata

The grubs and adults of *M. circumdata* were reared on fresh sweet potato twigs with young leaves which were kept in polypet jars. The cut ends of the sweet potato vines were rolled with moist cotton balls for keeping them fresh and green. The eggs were laid singly on the ventral sides of the leaves and the grubs that emerged from these eggs were further transferred to fresh trays along with food. Those pupated on the plant parts were collected and kept separately for adult emergence.

3.1.3 Pathogenicity tests

The pathogenicity was evaluated against the third instar grubs and adults of all the nine test insects, and also against the eggs of *H. vigintioctopunctata*, *M. circumdata* and *O. rhinoceros* and pupae of *B. fulvicorne* , *C. sordidus*, *C. formicarius*, *H. vigintioctopunctata*, *M. circumdata*, *O. rhinoceros* and *R. ferrugineus*. Preliminary evaluation on the pathogenicity of the *B. bassiana* and *M. anisopliae* was done using spore suspensions of the respective fungi. Potato dextrose broth was used for the culturing of the fungi. 14 day old culture of the respective fungus was blended in a mixer / grinder for two minutes, strained and then the spore suspension was directly applied on the test insects using an atomizer. Observations on the symptoms and mortality of the insects were recorded from 24 hours to 14 days after treatment. The dead insects were removed and examined for symptom development. The dead insects were placed over a wet filter paper inside a Petri dish for the development of symptoms. The death of the insects due to the fungus was confirmed by microscopic observation of the spores with lactophenol cotton blue.

3.1.4 Assessment of the virulence of *B. bassiana* and *M. anisopliae* to the test insects

3.1.4.1 Virulence of B. bassiana

3.1.4.1.1 To Adults

B. bassiana isolate Bb 5 at a concentration of 10^8 spores ml^{-1} was prepared from 14 day old culture of the fungus grown in potato dextrose broth. Estimation of the spore count was done using an improved Neubauer's Haemocytometer. Fifteen numbers of adults of each of the nine test insects were separately confined in clean jars and the spore suspension was sprayed onto the insects uniformly and kept as such for ten minutes and then transferred to another rearing jar with fresh food. This was replicated thrice. Test insects sprayed with water alone served as control.

Observations on the symptoms and mortality of the insects were recorded up to 14 DAT. From the data, the percentage of mortality was calculated, afterwards corrected using Abbott's formula (Abbott, 1925) and subjected to statistical analysis.

3.1.4.1.2 To Grubs

Virulence of Bb 5 was assessed against the third instar grubs of all the nine test insects at the concentration of 10^8 spores ml^{-1} . The procedure followed for assessing the virulence of the pathogen was as described in 3.1.4.1.1. The experiment was done as CRD with three replications with fifteen grubs each per replication.

3.1.4.1.3 To Eggs

The pathogenicity of Bb 5 was evaluated against the eggs of *H. vigintioctopunctata* and *M. circumdata* at 10^8 spores ml^{-1} concentration. The methodology followed was as given in 3.1.4.1.1. The experiment was conducted as CRD with eight replications, and each replication had fifteen eggs of the test insects.

3.1.4.1.4 To Pupa

The effect of Bb 5 on pupa of *C. formicarius*, *H. vigintioctopunctata* and *M. circumdata* was tested at 10^8 spores ml^{-1} concentration. The procedure explained in 3.1.4.1.1 was followed to assess the pathogenicity. The experiment was carried out as CRD with eight replications, and each replication had fifteen pupae of the test insects.

3.1.4.2 Virulence of M.anisopliae

Virulence of *M. anisopliae* isolate Ma 4 was also assessed at the concentration 10^8 spores ml^{-1} to the adults, third instar grubs, eggs and pupa of the coleopterans following the procedure mentioned in 3.1.4.1.

3.2 BIOASSAY

Bioassay of the two fungi *viz., B. bassiana* and *M. anisopliae* was carried out against the adults and grubs of all the nine coleopteran pests listed under 3.1 to determine the LC_{50}, LC_{90} and LT_{50} values. The doses for bioassay against adults and grubs of each pest were fixed based on preliminary studies. Five serial dilutions of the corresponding fungal spore suspensions were prepared from 14 day old stock culture of the fungus grown in potato dextrose broth (PDB) (Table 1). Third instar grubs and newly emerged adults from the culture stock of the insects (3.1.2) were used for the study. The fungal spore suspension was uniformly sprayed on to the adults and grubs using an atomizer and were then released into rearing jars with fresh food. Three replications were maintained for each adults and grubs. The number of test stages used differed for each pest and it ranged from 8 to 30 per treatment. The insects treated with distilled water alone served as control for the experiment. The mortality of the adults / grubs was recorded every day. The log dose probit mortality data was statistically analysed after necessary correction using Abbott's formula (Abbott, 1925) and the LC_{50}, LC_{90} and LT_{50} values and fiducial limits and other

Table 1. Details of test insects, fungi and doses selected for bioassay

Sl. No	Test insect	Number treatment	Stage of Pest	Fungus (spores ml^{-1})	
				B. bassiana	*M. anisopliae*
1	*A. foveicollis*	18	A	2.9×10^{8}	5.2×10^{10}
				2.9×10^{7}	5.2×10^{9}
				2.9×10^{6}	5.2×10^{8}
				2.9×10^{5}	5.2×10^{7}
				2.9×10^{4}	5.2×10^{6}
		15	G	2×10^{7}	2.3×10^{7}
				2×10^{6}	2.3×10^{6}
				2×10^{5}	2.3×10^{5}
				2×10^{4}	2.3×10^{4}
				2×10^{3}	2.3×10^{3}
2	*B. fulvicorne*	15	A	2.2×10^{8}	2.7×10^{8}
				2.2×10^{7}	2.7×10^{7}
				2.2×10^{6}	2.7×10^{6}
				2.2×10^{5}	2.7×10^{5}
				2.2×10^{4}	2.7×10^{4}
		15	G	1×10^{6}	1.5×10^{8}
				1×10^{5}	1.5×10^{7}
				1×10^{4}	1.5×10^{6}
				1×10^{3}	1.5×10^{5}
				1×10^{2}	1.5×10^{4}
3	*C. sordidus*	15	A	2.3×10^{9}	2.7×10^{10}
				2.3×10^{8}	2.7×10^{9}
				2.3×10^{7}	2.7×10^{8}
				2.3×10^{6}	2.7×10^{7}
				2.3×10^{5}	2.7×10^{6}
		12	G	1.9×10^{8}	3.1×10^{8}
				1.9×10^{7}	3.1×10^{7}
				1.9×10^{6}	3.1×10^{6}
				1.9×10^{5}	3.1×10^{5}
				1.9×10^{4}	3.1×10^{4}
4	*C. formicarius*	20	A	2.1×10^{7}	3.5×10^{9}
				2.1×10^{6}	3.5×10^{8}
				2.1×10^{5}	3.5×10^{7}
				2.1×10^{4}	3.5×10^{6}
				2.1×10^{3}	3.5×10^{5}
		18	G	1×10^{7}	2.7×10^{8}
				1×10^{6}	2.7×10^{7}
				1×10^{5}	2.7×10^{6}
				1×10^{4}	2.7×10^{5}
				1×10^{3}	2.7×10^{4}
5	*H. vigintioctopunctata*	18	A	2.1×10^{8}	3.4×10^{8}
				2.1×10^{7}	3.4×10^{7}
				2.1×10^{6}	3.4×10^{6}
				2.1×10^{5}	3.4×10^{5}
				2.1×10^{4}	3.4×10^{4}

Table 1. Contd.

		30	G	1×10^{7} 1×10^{6} 1×10^{5} 1×10^{4} 1×10^{3}	1.8×10^{7} 1.8×10^{6} 1.8×10^{5} 1.8×10^{4} 1.8×10^{3}
6	*L. ramakrishnai*	12	A	3.4×10^{8} 3.4×10^{7} 3.4×10^{6} 3.4×10^{5} 3.4×10^{4}	2.9×10^{11} 2.9×10^{10} 2.9×10^{9} 2.9×10^{8} 2.9×10^{7}
		15	G	3×10^{6} 3×10^{5} 3×10^{4} 3×10^{3} 3×10^{2}	1.63×10^{8} 1.63×10^{7} 1.63×10^{6} 1.63×10^{5} 1.63×10^{4}
7	*M. circumdata*	15	A	2.52×10^{9} 2.52×10^{8} 2.52×10^{7} 2.52×10^{6} 2.52×10^{5}	3.97×10^{10} 3.97×10^{9} 3.97×10^{8} 3.97×10^{7} 3.97×10^{6}
		18	G	1.3×10^{8} 1.3×10^{7} 1.3×10^{6} 1.3×10^{5} 1.3×10^{4}	2×10^{9} 2×10^{8} 2×10^{7} 2×10^{6} 2×10^{5}
8	*O. rhinoceros*	8	A	1×10^{15} 1×10^{14} 1×10^{13} 1×10^{12} 1×10^{11}	7×10^{13} 7×10^{12} 7×10^{11} 7×10^{10} 7×10^{9}
		15	G	4.5×10^{11} 4.5×10^{10} 4.5×10^{9} 4.5×10^{8} 4.5×10^{7}	3.7×10^{8} 3.7×10^{7} 3.7×10^{6} 3.7×10^{5} 3.7×10^{4}
9	*R. ferrugineus*	8	A	5.7×10^{13} 5.7×10^{12} 5.7×10^{11} 5.7×10^{10} 5.7×10^{9}	2.86×10^{15} 2.86×10^{14} 2.86×10^{13} 2.86×10^{12} 2.86×10^{11}
		8	G	3.1×10^{11} 3.1×10^{10} 3.1×10^{9} 3.1×10^{8} 3.1×10^{7}	3.9×10^{13} 3.9×10^{12} 3.9×10^{11} 3.9×10^{10} 3.9×10^{9}

A-Adult G-Grub

regression parameters were worked out using SPSS Statistics Version 21 (IBM CORP., 2012) as explained by Fang *et al.* (2005).

3.3 SELECTION OF SUBSTRATES FOR THE MASS MULTIPLICATION OF THE FUNGI

B. bassiana and *M. anisopliae* were multiplied in the substrates mentioned below and stored for a period of three months. The mycelial growth pattern was observed visually. The spore count, colony forming units (cfu) and bioefficacy of the fungi grown in these materials were estimated at monthly intervals. Visual scoring of the mycelial growth in the different substrates was also done.

3.3.1 Substrates

1. Rice bran	2. Rice husk	3. Wheat bran
4. Coconut oil cake	5. Groundnut oil cake	6. Neem cake
7. Coir pith compost	8. Cow dung	9. Saw dust

3.3.1.1 Preparation of the medium

Thirty grams each of the substrates were weighed separately and water was added just enough to wet the material. The moistened substrates were then taken in 250 ml conical flasks, plugged with cotton and sterilized at 1.1 kg cm^{-2} pressure at 121 ° C for 40 minutes. After cooling, the sterilized materials were inoculated with five mm fungal discs of the respective fungus, cut from the outer edges of ten day old actively growing fungal culture in potato dextrose agar using a five mm cork screw borer in a laminar air flow chamber. The conical flasks were then incubated at room temperature. Nine such flasks were maintained for each substrate for drawing samples at monthly intervals. The moistened substrates without the inoculum in conical flasks served as control for the experiment. The experiment was replicated

thrice. The growth of fungi in the different substrates was visually observed at different intervals and was scored under three categories as follows:

1.Profuse growth - >75 per cent growth in the conical flask within10 days (+++)
2.Moderate growth - > 50 per cent growth in the conical flask within 10 days (++)
3.Slight growth - < 25 per cent growth in the conical flask within 10 days (+)

3.3.1.2 Estimation of spore count

For estimating the spore load, 100 ml of sterile distilled water was added to each of the conical flasks and rotated vigorously for two minutes. After shaking, the filtrate was separated by filtering through a double layered muslin cloth into another conical flask using a sterile glass funnel. The spore count of the filtrate was estimated using a Haemocytometer after making necessary dilutions. At monthly intervals filtrate was collected separately from the culture maintained in three flasks for each fungus and the spore counts were determined.

3.3.1.3 Estimation of cfu

The filtrate obtained from one gram of the substrate was used for evaluating the viability of the spores of the fungi. To enumerate the viable spores in the substrates, plate count technique described by Aneja (1996 and 2003) was followed. The filtrate was serially diluted to get concentrations of 10^{-2}, 10^{-4} and 10^{-6}. From the serially diluted filtrate one ml was transferred to sterile Petri dishes and 15 ml molten cooled potato dextrose rose bengal agar was poured over the fungal suspension, the plates were then rotated gently to mix the contents and distribute the spores throughout the medium. This was incubated at room temperature in an inverted position, once the agar was solidified. The experiment was replicated thrice. The plates were observed daily for the development of fungal colonies. Four days after inoculation, the number of colonies developed were counted by placing each Petri

dish in a Quebec colony counter and the observations on cfu were recorded. Petri dishes with potato dextrose rose bengal agar alone served as the control. The calculations were made as follows:

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the diluents}}$$

$$\text{Number of cfu g}^{-1} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution}}$$

3.3.1.4 Bioefficacy

The bioefficacy of the fungi in the respective substrates at monthly intervals was assessed against the adults and grubs of *C. formicarius*. The test stages were obtained from the insect stock culture maintained as described in 3.1.2. The fungal filtrate was collected as given in 3.3.4 and the test stages were uniformly sprayed with the fungal spore suspension using an atomizer and kept as such for ten minutes for drying and then transferred to a new polypet jar with their respective feed kept inside and covered with muslin cloth. Insects sprayed with sterile water served as control. Three replications for each treatment were maintained and each replication had a batch of 18 test insects. Observations were taken daily for the development of symptoms and mortality. The cumulative mortality at 14 days after treatment was recorded and percentage mortality was calculated.

3.3.2 Talc based formulation of fungi

B. bassiana and *M. anisopliae* were cultured in potato dextrose broth at 27 ± 5 ° C as mentioned under 3.1.1. Fourteen day old fungal culture of the respective fungus was blended thoroughly in a mixer for two minutes at 18000 rpm and 30 ml of the blended fungal culture of the respective fungus was added to 100 g

of previously sterilized talc and mixed thoroughly in trays. This was shade dried for two days. The formulated material was then packed in sterile polythene bags and stored under room temperature.

3.3.2.1 Estimation of Spore count

One gram of the talc based formulation of the respective fungus was weighed out and mixed in 10 ml sterile water and filtered through a double layered muslin cloth and one drop from the filtrate was used to assess the spore count of the fungus in a Haemocytometer. Three replications were taken separately for both the fungus. The spores present in one ml of the filtrate was estimated using the formula,

$$\text{Number of spores ml}^{-1} = \frac{D\,x}{n\,k}$$

where, D – dilution factor

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

n – number of small squares counted

k – volume of one small square in cm^3

3.3.2.2 Estimation of cfu

One gram of the talc based formulation was mixed with 100 ml sterile water to get dilution of 10^{-2} and this was serially diluted to get concentrations of 10^{-4} and 10^{-6}. Colony forming units of the fungi were estimated as described in 3.3.2.1. The cfu was computed following the method of Aneja (1996 and 2003).

3.3.2.3 Bioefficacy

20 g of the talc formulation was mixed in one litre of water to obtain the spore suspension and followed the method given in 3.3.1.4 to evaluate the bioefficacy.

3.4 FIELD EVALUATION OF ENTOMOPATHOGENIC FUNGI

3.4.1 Banana

The spore suspensions, fungal culture in different substrates and talc formulations of *B. bassiana* and *M. anisopliae* and synthetic chemicals were evaluated for their efficacy against *C. sordidus* in the field. The field trial was conducted in the Instructional Farm, College of Agriculture, Vellayani. The experiment was done during 2008 - '10. The banana variety selected for the trial was 'Nendran'. The crop was raised and maintained as per the package of practices recommendations of the KAU (2007). The experiment was carried out during two seasons, the second experiment was conducted in the succeeding crop.

3.4.1.1 Design and Lay out

Design	: RBD
Treatments	: 15
Replications	: 3
Spacing	: 2 m × 2 m
Plot size	: 6 m × 2 m
Pit size	: 0.50 m^3

3.4.1.2 Treatments

T1 : *B. bassiana* @ 5×10^9 spores ml^{-1}

T2 : *B. bassiana* @ 5×10^{11} spores ml^{-1}

T3 : Talc based formulation of *B. bassiana* @ 20 g l^{-1}

T4 : Talc based formulation of *B. bassiana* @ 30 g l^{-1}

T5 : *M. anisopliae* @ 5×10^{10} spores ml^{-1}

T6 : *M. anisopliae* @ 5×10^{12} spores ml^{-1}

T7 : Talc based formulation of *M. anisopliae* @ 20 g l^{-1}

T8 : Talc based formulation of *M. anisopliae* @ 30 g l^{-1}

T9 : *B. bassiana* in cow dung @ 50 g

T10 : *M. anisopliae* in cow dung @ 50 g

T11 : *B. bassiana* in neem cake @ 50 g

T12 : *M. anisopliae* in neem cake @ 50 g

T13 : Carbofuran 3G @ 20 g $plant^{-1}$

T14 : Chlorpyriphos 0.03 per cent

T15 : Control (Untreated)

B. bassiana and *M. anisopliae* were cultured in PDA broth and spore suspensions were prepared from 14 day old culture and the spore count of the respective fungi was adjusted to the required spore concentrations as mentioned in the treatments (T1, T2, T5 and T6) in 3.4.1.2 using an improved Neubauer's Haemocytometer. The talc formulation of both the fungi as given in the treatments (T3, T4, T7 and T8) was prepared as mentioned under 3.3.2 and the required quantity of the talc formulation was mixed in one litre of water to prepare the spray solution. The fungi was cultured in cow dung and neem cake as described under 3.3.1.1 and the respective fungi cultured in cow dung and neem cake as mentioned in the treatments under 3.4.1.2 and stored for two months was used for the experiment. Furadan 3G was used for the treatment T13 @ 20 g $plant^{-1}$. Tagban 20 per cent EC @ 1.5 ml l^{-1} was used as the treatment T14.

3.4.1.3 Application

Only one soil drenching was given. Application of spore suspensions of fungi, fungal culture along with substrates, talc formulations and insecticides was done around individual plants in a circular manner in a shallow furrow taken 15 cm away from the base of the plant. Drenching of the spray solution was done in the early morning hours during the early vegetative stage of the crop at three months after planting. Around the control plants also shallow furrows were taken.

3.4.1.4 Population of pests

3.4.1.4.1 Population of C. sordidus in soil

The base of the banana plant was cleared and the surface soil to a depth of 5 cm was removed just before harvesting of the crop. 250 g soil each from the four quadrants around the plant was taken and mixed together to make to get one kilogram soil and the adult weevil population in the soil was counted. For each treatment, three replicated samples were taken.

3.4.1.5.2 Population of C.sordidus in rhizomes and extent of damage

After harvesting the bunches plants were uprooted and the rhizomes were separated, the roots were removed, cleaned. The rhizomes were then cut into several vertical pieces of 2 cm thickness. Observations on the number of galleries and the number of grubs present inside each piece were made. Three replicated samplings were made for each treatment.

3.4.1.5.3 Yield

The harvested bunches were weighed separately and the data was recorded.

3.4.1.5.4 Benefit : Cost ratio

The yield per hectare was worked out from the mean yield of banana per treatment. The Benefit : Cost ratio of the treatments was also calculated after a value addition of 25 per cent (in rupees terms) for the banana receiving microbial treatment compared to the chemical pesticide treatment (Sangeetha, 2003).

3.4.1.5 Maintenance of succeeding crop

Two healthy needle suckers were maintained in each pit after removal of the harvested plants. The weeds, stubbles, crop wastes and unhealthy suckers of the previous crop were removed and the healthy suckers retained were raised as per the

package of practices recommendations of the KAU (2007) for the second experiment. The treatments and replications were as in the main crop (3.4.1.2). The treatments were given 300 days after first treatment application and when the succeeding crop was in the early vegetative stage.

3.4.2 Sweet potato

The two fungi, *B.bassiana* and *M.anisopliae* in the form of spore suspension, talc formulation and the fungal culture in different substrates and synthetic chemicals *viz.* imidacloprid and lambda cyhalothrin were tested against *C. formicarius* in the field. Two methods of application *viz.* soil drenching and foliar application was also evaluated. The field experiments were carried out in the Instructional Farm, College of Agriculture, Vellayani. The variety selected for the trial was 'Sree Bhadra'. The crop was raised and maintained as per the package of practices recommendations of the KAU (2007). The two experiments on soil drenching were carried out during February, 2008 to April, 2008 and June, 2008 to August, 2008. The experiment to evaluate the effect of foliar application was conducted simultaneously during February, 2008 to April, 2008.

3.4.2.1 Experiment to assess the effect of soil drenching

3.4.2.1.1 Design and Lay out

Design	: RBD
Treatments	: 15
Replications	: 3
Spacing	: 75 cm × 75 cm
Plot size	: 4.50 m × 2 m
Mounds per plot	: 3
Mound size	: 0.50 m^3

3.4.2.1.2 Treatments involved

T1 : *B. bassiana* @ 10^9 spores ml^{-1}

T2 : *B. bassiana* @ 10^{11} spores ml^{-1}

T3 : Talc based *B. bassiana* @ 20 g l^{-1}

T4 : Talc based *B. bassiana* @ 30 g l^{-1}

T5 : *M. anisopliae* @ 10^{10} spores ml^{-1}

T6 : *M. anisopliae* @ 10^{12} spores ml^{-1}

T7 : Talc based *M. anisopliae* @ 20 g l^{-1}

T8 : Talc based *M. anisopliae* @ 30 g l^{-1}

T9 : *B. bassiana* in cow dung @ 50 g

T10 : *M. anisopliae* in cow dung @ 50 g

T11 : *B. bassiana* in neem cake @ 50 g

T12 : *M. anisopliae* in neem cake @ 50 g

T13 : Imidacloprid 0.006 per cent

T14 : Lambda cyhalothrin 0.025 per cent

T15 : Control (Untreated)

3.4.2.1.3 Preparation of spray solutions

The spore suspensions of *B. bassiana* and *M. anisopliae* as mentioned in the treatments in 3.4.2.2 (T1, T2, T5 and T6) were prepared as given in 3.4.1.3 and estimated using an improved Neubauer's haemocytometer. The talc formulation of both the fungi as given in the treatments (T3, T4, T7 and T8) were also prepared as mentioned under 3.3.2 and the required quantity of the talc formulation was mixed in one litre of water to prepare the spray solution. The fungi was cultured in cow dung and neem cake as described under 3.3.1.1 and the respective fungi cultured in cow dung and neem cake were stored for two months before field application was used for the experiment. Hilmida 17.8 per cent SL was used for the treatment T13 @ 0.337

ml l^{-1} per mound. Karate 5 per cent EC @ 2.5 ml l^{-1} was used as the treatment T14 and the control treatment was also maintained.

3.4.2.1.4 Application of treatments

The field experiment was laid out as per the statistical design given in 3.4.2.2. The treatments were given one month after planting (MAP). Drenching of spore suspensions of fungi / insecticides was done in the individual mounds after making shallow furrow in a circular manner 10 cm away from the base of the plant in the soil without disturbing the roots and plants. The spray volume to drench individual mounds was standardized as one litre. The fungi grown in cow dung and neem cake were incorporated into soil in the furrows made in the mounds. Application of treatments was done in the early morning hours. The control plot was maintained with water spraying.

3.4.2.2 Experiment to assess the effect of foliar application

3.4.2.2.1 Design and Lay out

Design	: RBD
Treatments	: 10
Replications	: 3
Spacing	: 75 cm × 75 cm
Plot size	: 4.50 m × 2 m
Mounds per plot	: 3
Mound size	: 0.50 m^3

3.4.2.2.2 Treatments involved

T1 : *B. bassiana* @ 10^9 spores ml^{-1}

T2 : *B. bassiana* @ 10^{11} spores ml^{-1}

T3 : Talc based *B. bassiana* @ 20 g l^{-1}

T4 : Talc based *B. bassiana* @ 30 g l^{-1}

T5 : *M. anisopliae* @ 10^{10} spores ml^{-1}

T6 : *M. anisopliae* @ 10^{12} spores ml^{-1}

T7 : Talc based *M. anisopliae* @ 20 g l^{-1}

T8 : Talc based *M. anisopliae* @ 30 g l^{-1}

T9 : Imidacloprid 0.006 per cent

T10 : Control (Untreated)

3.4.2.2.3 Preparation of spray solutions

The spray solutions were prepared as described in 3.4.2.1.3.

3.4.2.2.4 Application of treatments

The treatments were given one month after planting (MAP). The foliar spraying was done using a hand sprayer. One litre of spray fluid was used for treating plants in each mound. While spraying drift was avoided using gunny sheets.

3.4.2.3. Estimation of population of adult C. formicarius

The population of *C. formicarius* present in the foliage as well as in soil was determined. The pre treatment and post treatment counts of the weevil present in two square feet area of the foliage was taken. Post treatment counts were taken at weekly intervals. For estimation of population in soil, the mounds were cleared and the surface soil at a depth of 5 cm was removed. Approximately 250 g soil from each of the four quadrants around the mound was taken and mixed together to make into one kg soil and the adult weevil population in soil was counted, just before the harvesting of the crop. For each treatment, three replicated samples were taken.

3.4.2.4 Population of natural enemies

Pre treatment and post treatment counts on the number of natural enemies present in the foliage of each plants were taken at weekly intervals.

3.4.2.5 Sampling of the tubers

Three tubers per mound were sampled for estimating the damage and the population of grubs. The tubers were cleaned and cut into one cm thick circular discs. The number of galleries and the population of grubs present inside the cut pieces were counted. Three replicated samplings were made for each treatment.

3.4.2.6 Yield

The yield in terms of weight and number of total tubers and marketable tubers was recorded from all the treatments and the benefit cost ratio was worked out.

3.4.2.7 Benefit : Cost ratio

The yield per hectare was worked out from the mean yield of sweet potato per treatment. The Benefit : Cost ratio of the treatments was also calculated as given under 3.4.1.5.4.

3.5 COMPATIBILITY OF FUNGI WITH PESTICIDES

The compatibility of two fungicides and six insecticides with *B. bassiana* and *M. anisopliae* was determined in the lab adopting poison food technique (Zentmeyer, 1955). The growth, sporulation and bioefficacy of the fungi grown on the poison food media was assessed. The experiment was conducted in CRD with three replications. The pesticides used are listed below:

1. Carbendazim 0.1 per cent (Arrest 50 per cent WP)
2. Carbofuran 3G 0.30 ppm (Technical material from Sigma Aldrich)
3. Carbosulfan 6G 0.10 ppm (Technical material from Sigma Aldrich)
4. Chlorpyriphos 0.03 per cent (Tagban 20 per cent EC)
5. Imidacloprid 0.006 per cent (Hilmida 17.8 per cent SL)
6. Lambda cyhalothrin 0.025 per cent (Karate 5 per cent EC)
7. Malathion 0.15 per cent (Milthion 50 per cent EC)
8. Mancozeb 0.2 per cent (Indofil-M-45 75 per cent WP)

The required quantity of the chemicals were measured and added separately to 100 ml of sterilized PDA in conical flasks in its molten stage when the temperature was below 45 ° C and mixed thoroughly. The media thus prepared was poured into sterile 9 cm Petri plates inside the laminar air flow chamber and allowed to solidify. Circular fungal discs of 5 mm size, cut from the growing outer edges of 14 day old culture of the respective fungi, using a sterile cork borer was placed in the centre of these Petri plates with solidified poisoned PDA. Fungus inoculated into media without pesticide served as the control. The inoculated plates were incubated at room temperature.

3.5.1 Estimation of growth of the fungi

Observations on the radial growth of the fungi was measured daily, until the fungi fully covered the control plates.

3.5.2 Sporulation of the fungi

To assess the sporulation of the fungi, at 15 days after inoculation, 10 ml sterile water was poured into each Petri plate with sporulating fungi and the spore suspension was collected and from this one ml of the spore suspension was used to estimate the spore count using an improved Neubauer's haemocytometer after making necessary dilutions (Rombach *et al.*, 1986). The spore count in the spore suspension obtained from Petri plates maintained as control was also estimated.

3.5.3 Bioefficacy of the fungus

The bioefficacy of the fungus cultured in poisoned media was also tested against the newly emerged adults and grubs of *C. formicarius*. The insects were obtained from the insect stock culture maintained (3.1.2). The spore suspension prepared as mentioned in 3.5.2 was applied on the test insects. The experiment was done in CRD with three replications and in each replication there were 15 test stages.

Observations on mortality were taken daily and the cumulative percentage mortality was assessed. Insects sprayed with the culture without pesticide served as control.

3.6 DEVELOPMENT OF PESTICIDE TOLERANT STRAIN AND MOLECULAR CHARACTERISATION

3.6.1 Culturing of fungi for pesticide tolerance

Inorder to develop pesticide tolerant strains of *B. bassiana* and *M. anisopliae*, the chemicals listed in Table 2 were used. Both the fungi were grown separately in poison food media continuously for ten passages with increase in concentrations of pesticides, considering the growth of the fungus in the respective poisoned media in each passage as given in Table 2. The culturing of the respective fungi was carried out as described in 3.5.

3.6.1.1 Estimation of radial growth of the fungi

The radial growth of both the fungi was measured regularly until the control plate was covered with the pure culture of the respective fungi as given in 3.5.1. The growth of both the fungi during the ten passages were recorded accordingly.

3.6.1.2 Estimation of spore count

The spore count of the fungi cultured in poisoned media was computed using a haemocytometer as explained in 3.5.2. The spore count of the respective fungi during all the ten passages was worked out.

3.6.1.3 Evaluation of bioefficacy

The bioefficacy of the respective fungi grown on poison food media was tested against newly emerged adults of *C. formicarius* as mentioned in 3.5.3. The virulence of both the fungi during the ten passages through chemicals listed was evaluated separately in the same manner.

Table 2. Dosage of pesticides used in media for culturing of the fungi for the development of pesticide tolerant strains

Treatments	Unit	Passages									
		I	II	III	IV	V	VI	VII	VIII	IX	X
Carbendazim 50 % WP	g l^{-1}	2.00	4.00	4.00	8.00	8.00	8.00	16.00	16.00	16.00	32.00
Chlorpyriphos 20 % EC	ml l^{-1}	1.50	3.00	3.00	3.00	6.00	6.00	6.00	12.00	12.00	12.00
Carbofuran (Technical material 3G)	ppm	0.30	0.30	0.60	0.60	0.60	1.20	1.20	1.20	2.40	2.40
Carbosulfan (Technical material 6G)	ppm	0.10	0.20	0.20	0.40	0.40	0.80	0.80	0.80	1.60	1.60
Imidacloprid 17.8 % SL	ml l^{-1}	0.337	0.674	1.348	2.696	2.696	5.392	5.392	10.784	10.784	10.784
Lambda cyhalothrin 5 % EC	ml l^{-1}	2.50	5.00	5.00	5.00	10.00	10.00	10.00	20.00	20.00	20.00
Mancozeb 75%WP	g l^{-1}	2.67	2.67	5.34	5.34	10.68	10.68	10.68	21.36	21.36	21.36
Malathion 50 % EC	ml l^{-1}	3.00	3.00	6.00	6.00	6.00	6.00	12.00	12.00	24.00	24.00

3.6.2 Molecular characterisation

3.6.2.1 Sample details

The mycelium of *B. bassiana* and *M. anisopliae* harvested from 15 day old potato dextrose agar culture poisoned with the chemicals listed in Table 2 was used for DNA isolation. The sample details are listed in Table 3. The experiment was laid out in CRD with three replications.

3.6.2.2 DNA isolation

DNA was isolated using GenElute Plant Genomic DNA Miniprep Kit (Sigma). 20 mg of the mycelium was transferred to a microcentrifuge tube and ground in 350 µl of lysis solution A and 50 µl of lysis solution B using a micro pestle. The mixture was incubated at 65°C for 10 minutes with occasional inversion. 130 µl of precipitation solution was added to the mixture, mixed completely by inversion and the sample was placed on ice for five minutes. The sample was centrifuged at 14,000 rpm (Eppendorf Centrifuge 5804R) for five minutes to pellet the cellular debris, proteins and polysaccharides. The supernatant was transferred to the GenElute filtration column tube and centrifuged at 14,000 rpm for one minute. This removed any cellular debris not removed in the previous step. The filtration column was discarded and 700µl of binding solution was added directly to the flow through liquid and mixed thoroughly by inversion. 700 µl of this mixture was added into GenElute nucleic acid binding column and centrifuged at 14,000 rpm for one minute.

The flow through liquid was discarded and the collection tube was retained. The column was returned to the collection tube and the remaining sample was applied to the column. Centrifugation was repeated as above and the flow through liquid and the collection tube were discarded. The binding column was placed into a fresh 2 ml collection tube. 500 µl ethanol-added wash solution was added to the binding column and centrifuged at 14,000 rpm for one minute. The flow through liquid was discarded

Table 3. Sample details used for the isolation of DNA for molecular analysis

Sample Code	Poison in the media	Fungus
1	T_1 Carbendazim	*M. anisopliae*
2	T_2 Mancozeb	*M. anisopliae*
3	T_3 Carbofuran	*M. anisopliae*
4	T_4 Carbosulfan	*M. anisopliae*
5	T_5 Imidacloprid	*M. anisopliae*
6	T_6 Chlorpyriphos	*M. anisopliae*
7	T_7 Lambda cyhalothrin	*M. anisopliae*
8	T_8 Malathion	*M. anisopliae*
9	T_9 Control (Pure culture)	*M. anisopliae*
10	T_1 Carbendazim	*B. bassiana*
11	T_2 Mancozeb	*B. bassiana*
12	T_3 Carbofuran	*B. bassiana*
13	T_4 Carbosulfan	*B. bassiana*
14	T_5 Imidacloprid	*B. bassiana*
15	T_6 Chlorpyriphos	*B. bassiana*
16	T_7 Lambdacyhalothrin	*B. bassiana*
17	T_8 Malathion	*B. bassiana*
18	T_9 Control (Pure culture)	*B. bassiana*

and the collection tube was retained. The wash was repeated once more. The binding column was transferred to a new collection tube. 50 µl of elution solution (pre-warmed to 65 °C) was added to the binding column and centrifuged at 14,000 rpm for one minute. The stock DNA was properly labelled and stored at 4 °C.

3.6.2.3 Agarose Gel Electrophoresis

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1 µl of 6 X gel-loading buffer (0.25 per cent bromophenol blue, 30 per cent sucrose in TE buffer pH - 8.0) was added to 5 µl of DNA. The samples were loaded to 0.8 per cent agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg ml^{-1} ethidium bromide. Electrophoresis was performed with 0.5 X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.6.2.4 DNA Fingerprinting

3.6.2.4.1 PCR amplification

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X PCR buffer (contains 1.5 mM $MgCL_2$), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), ~10ng DNA, 0.4 µl of Taq DNA polymerase enzyme (Genei), 0.1 mg ml^{-1} BSA, and 5 pM of primer. The reaction was carried out in triplicate for getting concurrent results. Ten primers were used for the reactions. The details of the primers are given in Table 4. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

3.6.2.4.2 PCR amplification profile

95 °C - 5.00 min

Table 4. Primers involved in the reaction and their sequences

Sl.No	Primer Name	Sequence of the primers
1	RFu 1	CCTGGGCCAG
2	RFu 2	CCTGGGCGAG
3	RFu 3	CCTGGGCTGG
4	RFu 4	CCTGGGCTAT
5	RFu 5	CCTGGGCTTG
6	RFu 6	CCTGGGCTAC
7	RFu 7	CCTGGGCTTA
8	RFu 8	CCTGGGTCGA
9	RFu 9	CCTGGGTGCA
10	RFu 10	CCTGGGTGAC

94 °C	-	0.45 min	10 cycles
35 °C	-	1.00 min	
72 °C	-	1.30 min	
94 °C	-	0.45 min	30 cycles
37 °C	-	0.45 min	
72 °C	-	1.00 min	
72 °C	-	10.00 min	
4 °C	-	∞	

3.6.2.4.3 Agarose Gel Electrophoresis of PCR products

The PCR products were checked in 1.5 per cent agarose gels prepared in 0.5 X TBE buffer containing 0.5 µg ml^{-1} ethidium bromide. 1 µl of 6 X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5 X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder (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).

3.6.2.5 Data analysis

The PCR product was scored for the presence (+) or absence (-) of bands. The number of monomorphic bands, number of polymorphic bands were recorded. Thus, banding pattern of all the ten primers for the eighteen samples as given in Table 3 were scored as 1 and 0 in the excel sheet and then subjected for further statistical analysis.

3.6.2.6 Tree matrix and similarity coefficient

A genetic similarity matrix was constructed using Jaccard's similarity coefficient values and this matrix was subjected to an un-weighted pair-group method for arithmetic average analysis (UPGMA) to generate a dendrogram using average linkage procedure. All these computations were carried out using NTSYS-pc version 2.02 (Rohlf, 1998) software and the dendrogram constructed was used to assess the association and distance between the fungi cultured in different chemicals under study.

3.7 ISOLATION OF NATURAL ISOLATES FROM INSECTS AND IDENTIFICATION BY ITS SEQUENCING

3.7.1 Isolation of natural isolates

The naturally infected test insects obtained from field were isolated from the healthy insects, surface cleaned and incubated in moist humid chamber until mycelial growth appears. Once the fungal growth was expressed, the cadaver was transferred to PDA slants and later the active growing mycelia were transferred to fresh PDA slants and further subcultured and replicated into more slants and kept under refrigeration for further studies and identification.

3.7.2 DNA Barcoding using universal primers of ITS

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

About 100 mg of the mycelium is homogenized using liquid nitrogen and the powdered tissue is transferred to a microcentrifuge tube. Four hundred microlitres of buffer PL1 is added and vortexed for one minute. Ten microlitres of RNase A solution is added and inverted to mix. The homogenate is incubated at 65 °C for 10

minutes. The lysate is transferred to a Nucleospin filter and centrifuged at 11000 x g for two minutes. The flow through liquid is collected and the filter is discarded. Four hundred and fifty microlitres of buffer PC is added and mixed well. The solution is transferred to a Nucleospin Plant II column, centrifuged for one minute and the flow through liquid is discarded. Four hundred microlitre buffer PW1 is added to the column, centrifuged at 11000 x g for one minute and flow though liquid is discarded. Then, 700 µl PW2 is added, centrifuged at 11000 x g and flow through liquid is discarded. Finally, 200 µl of PW2 is added and centrifuged at 11000 x g for two minutes to dry the silica membrane. The column is transferred to a new 1.7 ml tube and 50 µl of buffer PE is added and incubated at 65°C for five minutes. The column is then centrifuged at 11000 x g for one minute to elute the DNA. The eluted DNA was stored at 4 °C.

3.7.2.2 Agarose Gel Electrophoresis for DNA Quality check

The quality of the DNA isolated was checked using agarose gel electrophoresis. One µl of 6 X gel-loading buffer (0.25 per cent bromophenol blue, 30 per cent sucrose in TE buffer pH-8.0) was added to 5 µl of DNA. The samples were loaded to 0.8 per cent agarose gel prepared in 0.5 X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg ml^{-1} ethidium bromide. Electrophoresis was performed with 0.5 X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (*Genei*) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.7.2.3 PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X PCR buffer (contains 1.5 mM $MgCL_2$), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 10 ng DNA, 0.4 µl of Phire HotStart II DNA polymerase enzyme (Thermo scientific), 0.1 mg ml^{-1} BSA, 5 pM of forward and

reverse primers (Table 5). The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

3.7.2.4 PCR amplification profile

ITS

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

3.7.2.5 Agarose Gel electrophoresis of PCR products

The PCR products were checked in 1.2 per cent agarose gels prepared in 0.5 X TBE buffer containing 0.5 μg ml^{-1} ethidium bromide. One μl of 6 X loading dye was mixed with 5 μl of PCR products and was loaded and electrophoresis was performed at 75 V power supply with 0.5 X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.7.2.6 ExoSAP-IT Treatment

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

Table 5. Primers used for ITS sequencing of the new isolates

Target	Primer Name	Direction	Sequence (5' → 3')	Reference/Remarks
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTTGCGG	White *et al.*, 1990
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC	

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

3.7.2.7 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 v3.1 Cycle sequencing Kit (Applied Biosystems , USA) following manufactures protocol.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)	-	10-20 ng
Primer	-	3.2 pM (either Forward or Reverse)
Sequencing Mix	-	0.28 µl
5x Reaction buffer	-	1.86 µl
Sterile distilled water	-	make up to 10 µl

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

3.7.2.8 Post Sequencing PCR Clean up

1. Make master mix I of 10 µl milli Q and 2 µl 125 mM EDTA per reaction
2. Add 12 µl of master mix I to each reaction containing 10 µl of reaction contents and are properly mixed.
3. Make master mix II of 2 µl of 3 M sodium acetate pH 4.6 and 50 µl of

ethanol per reaction.

4. Add 52 µl of master mix II to each reaction.
5. Contents are mixed by inverting.
6. Incubate at room temperature for 30 minutes
7. Spin at 14,000 rpm for 30 minutes
8. Decant the supernatant and add 100 µl of 70 per cent ethanol
9. Spin at 14,000 rpm for 20 minutes.
10. Decant the supernatant and repeat 70 per cent ethanol wash
11. Decant the supernatant and air dry the pellet.

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

3.7.2.9 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 sequence obtained was identified using BLAST (Basic Local Alignment Search Tool) as demonstrated by Morgulis *et al.* (2008) and Zhang *et al.* (2000).

3.8 STATISTICAL ANALYSIS

The data generated from each of the experiments were tabulated and analysed using ANOVA (Analysis of Variance) / ANOCOVA (Analysis of Co-variance) (Panse and Sukhatme, 1967).

Results

4. RESULTS

4.1 PATHOGENICITY

The pathogenicity of *B. bassiana* and *M. anisopliae* was tested against nine coleopteran pests of crops using spore suspensions prepared from fourteen day old culture of the respective fungi in potato dextrose broth. Both the fungi produced mycoses on all the nine coleopteran pests (Table 6) and the infection was confirmed through Koch's postulate also. The pupal stage of *R. ferrugineus* was the only stage that survived the infection among the different stages tested and the survival of the pupa of *R. ferrugineus* was seen only when treated with *M. anisopliae*. Pupa treated with *B. bassiana* succumbed to infection. The results on pathogenicity of the fungi is presented in Tables 6 to 14.

4.1.1 Signs and symptoms

4.1.1.1 B. bassiana

On acquiring infection, the symptoms exhibited by all the nine coleopterans were the same except for minor variations, but the period for acquisition of the symptoms slightly varied with insects. Both grubs and adults lost their vigour and remained sluggish, food uptake was reduced and feeding gradually stopped. The adults lost their brightness. As and when the pathogenesis progressed dull white mycelia developed on the surface of the cadaver.

The size of the grubs were reduced and they appeared dull with shrunken integument. Once they were dead,irregular dull-beige spots developed on the intersegmental regions of the body, later these spots coalesced to form enlarged patches and the grubs turned dark. The cadavers were stiff, hard and mummified. Initially white mycelia developed over the body. During advanced stages of mycosis thick fluffy cottony white powdery mass covered the entire body, giving a white bloomy appearance for the cadaver (Plates 1 to 7).

Table 6. Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to different stages of coleopteran pests

Pest	Stage of Pest	*B. bassiana*	*M. anisopliae*
A. foveicollis	A	+	+
	G	+	+
B. fulvicorne	A	+	+
	G	+	+
	P	+	+
C. sordidus	A	+	+
	G	+	+
	P	+	+
C. formicarius	A	+	+
	G	+	+
	P	+	+
H. vigintioctopunctata	A	+	+
	E	+	+
	G	+	+
	P	+	+
L. ramakrishnai	A	+	+
	G	+	+
M. circumdata	A	+	+
	E	+	+
	G	+	+
	P	+	+
O. rhinoceros	A	+	+
	E	+	+
	G	+	+
	P	+	+
R. ferrugineus	A	+	+
	G	+	+
	P	+	-

A-Adult, E-Egg, G-Grub, P-Pupa

\+ Pathogenic

\- Non-Pathogenic

4.1.1.1.1 A. foveicollis

The treated adults of *A. foveicollis* were actively flying and moving around during the first three days. On the fourth day, movements were reduced and the beetles were found to hide under the leaves provided as food and they showed reduced feeding. Mating and egg laying were hindered. Mortality of the adults were seen from the sixth day onwards and the body became dark brownish and soft, later it became hard. Upon incubation white spores emerged over the cadavers within three days.

The grubs sprayed with spore suspension of *B. bassiana* did not show any visible change during the first day of observation. On the second day, the grubs remained in the same bore hole in the stems with reduced feeding and activity. The feeding stopped on the fourth day and some of the grubs came outside of the bore holes and were found dead whereas some were found dead within the bore holes. The pearl white colour of the grubs changed to dull off white with yellowish spots, which later coalesced and darkened. Sporulation was observed two days after incubation of the cadaver.

4.1.1.1.2 B. fulvicorne

The adults of *B. fulvicorne* were actively seen on cardamom seedlings provided as food during the first two to three days after treatment with *B. bassiana.* Active flight and food uptake was reduced on the fourth day and the beetles were seen resting in an upside down position most of the time (Plate 14). Later, the insect became moribund and were dead on the sixth day. The dead insects lost their metallic sheen of their elytra and became dull. Thread like white mycelia appeared along the neck and in the integument two days after incubation in a moist chamber.

The grubs did not show any visible symptoms of pathogenesis for two days after treatment. Feeding was reduced on the third day the grubs became sluggish and most of them were found on the surface of the soil. The grubs were seen dead on the fourth day and the cadaver was initially soft and pinkish, which

later became dark and hard. Two days after incubation, cottony white mycelium emerged and engulfed the entire body of the cadaver.

The treated pupa remained as such for two days. Prominent discolouration of the pupa was observed four days after treatment and the pupa became dark and brittle, later white mycelial growth appeared over the body.

4.1.1.1.3 C. formicarius

Adult *C. formicarius* treated with *B. bassiana* showed no visual symptoms for two days, but on the third day they became sluggish, lost vigour and feeding was low. The infected beetles remained outside the tubers and were seen dead on the fourth day.

The treated grubs did not exhibit any visible change during the first day and they were actively boring and feeding on the fresh sweet potato tubers provided to them. On the second day, they succumbed to infection, with less feeding and movements. The grubs were dead on the third day and the creamy white colour of the grubs changed to yellowish white with light brown spots over the body, which later coalesced to form mustard coloured patches over the cadaver. The symptom development in the pupa was similar to that described for *B. fulvicorne.*

4.1.1.1.4 C. sordidus

The sequence of development of symptoms in adults, grubs and pupae of *C. sordidus* was similar to that observed in *B. fulvicorne.* The beetles were dead from the seventh day onwards and the grubs were found dead from the fifth day onwards.

4.1.1.1.5 H. vigintioctopunctata

H. vigintioctopunctata adults treated with the fungus were active for the first three days but on the fourth day the beetles clustered beneath the leaves. Egg

laying was reduced and the infected females laid eggs singly, in a scattered manner. On the sixth day the insects were seen dead.

B. bassiana treated eggs of the beetle became dull coloured one day after treatment. Three days after treatment mycelia developed over the egg mass. The treated grubs of *H. vigintioctopunctata,* after moving actively for two days developed symptoms and were dead on the fifth / sixth day after treatment.

Symptom development in pupa was similar to that in *B. fulvicorne.* Those pupae which survived the infection moulted but some of them developed into malformed adults but were dead immediately after moulting and thread like mycelium developed on sixth day.

4.1.1.1.6 L. ramakrishnai

On the sixth day *B. bassiana* infected beetles were seen dead and later white mycelium developed in the cadaver. The treated grubs became moribund and remained outside the berries most of the time until death that initiated on the fourth day.

4.1.1.1.7 M. circumdata

Up to four days, the treated adults of *M. circumdata* did not show any visible pathogenic symptoms but later it developed and were dead on the sixth day. The cadaver lost its metallic sheen and subsequently developed mycelial growth over the body and always seen above the foliage (Plate 14).

The infected eggs turned dark within two days. The hay coloured eggs on acquiring infection, became brownish and thread like fine mycelium developed over them on the fourth day.

The grubs remained healthy for three days without exhibiting any visible pathogenesis when sprayed with the fungus. The food uptake was reduced on the fourth day and they were dead on the sixth day. The symptoms of infection in the treated pupa was similar to that observed for the pupa of *H. vigintioctopunctata.*

4.1.1.1.8 O. rhinoceros

Up to twelve days the adult *O. rhinoceros* treated with *B. bassiana* did not exhibit any visual symptoms. Mortality was observed from the fifteenth day onwards.

The infected eggs turned dark on fourth day and were shrunk with wrinkles over the smooth bead like surface, later, on the eighth day thread like mycelium started sprawling around the infected egg.

The treated grubs did not show any visual changes for a week. On the eighth day, they were weak, and became sluggish and moribund. The infected grubs remained most of the time on the soil surface and failed to moult to next instars. Dark brown spots and patches appeared on the grubs. Mortality of the grubs initiated from eleventh day onwards. Subsequently the cadavers were covered with mycelium.

The infected pupa became shrunk and wrinkles appeared over the surface and mycelium developed on the tenth day.

4.1.1.1.9 R. ferrugineus

Symptoms of infection of *B. bassiana* in adults appeared only after fifteen days. Slowly the food uptake was reduced and they became sluggish and lost vigour. The mortality of the beetles started from the eighteenth day onwards. The ferric brown colour of the adults changed to dark brown upon infection.

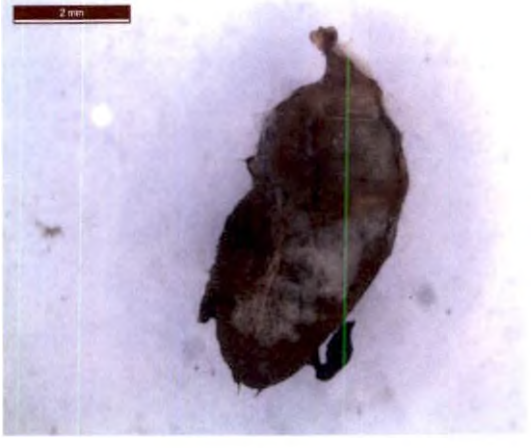
The grubs remained healthy without any disease symptoms for three days. On the fourth day feeding was poor. As pathogenesis advanced, the grubs totally stopped feeding and remained outside the bore holes. The grubs were dead on the sixth day.

The treated pupa did not show any visual changes for five days. On the sixth day, the pupa upon touching did not rattle and on the eighth day, white mycelium emerged over the fibrous cocoon.

Adult

Grub

B. bassiana infected *A. foveicollis*



Pupa

Adult

Grub

B. bassiana infected *B. fulvicorne*

Plate 1. *B. bassiana* infection on coleopteran insects

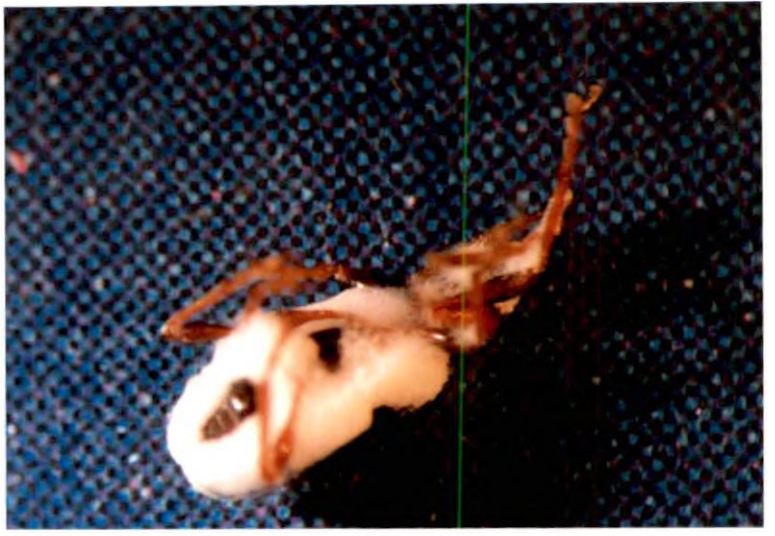
Adult

Egg

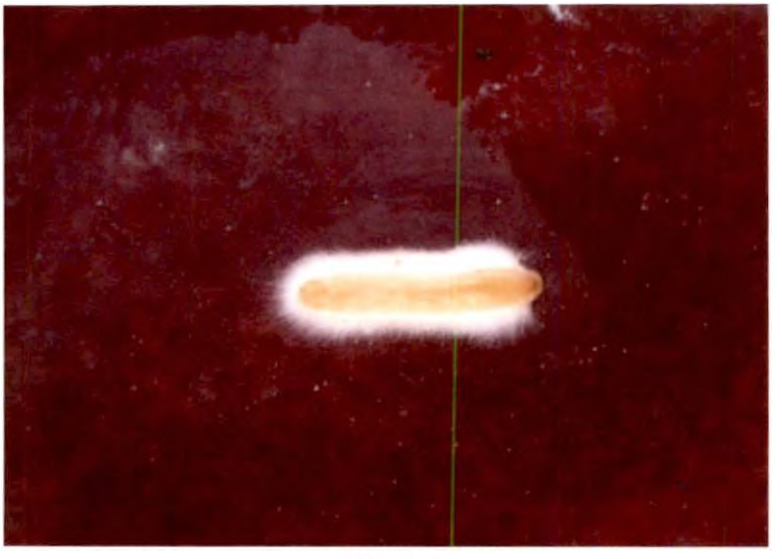
Grub

Pupa

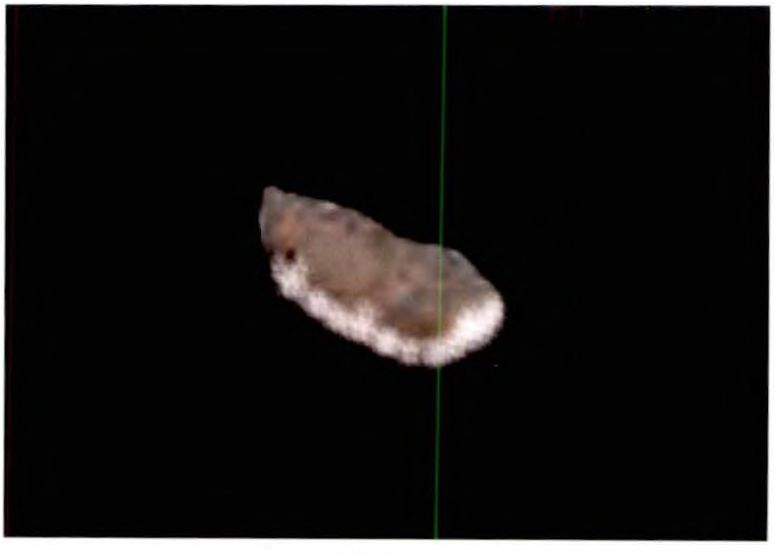
Plate 2. *B. bassiana* infection on *C. sordidus*



Adult



Grub



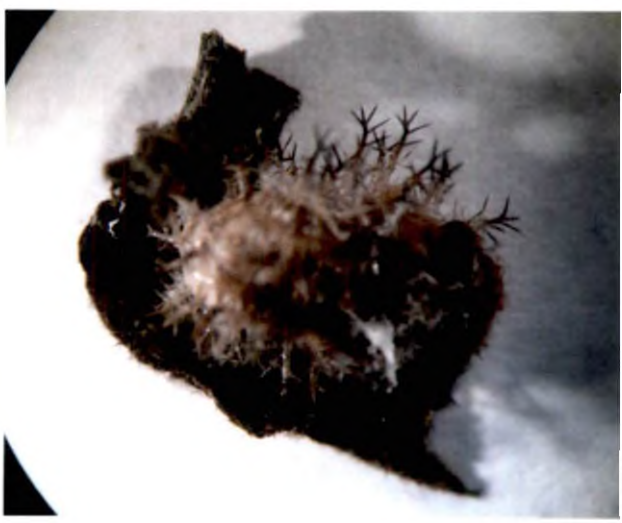
Pupa

Plate 3. ***B. bassiana*** **infection on** ***C. formicarius***

Adult

Grub

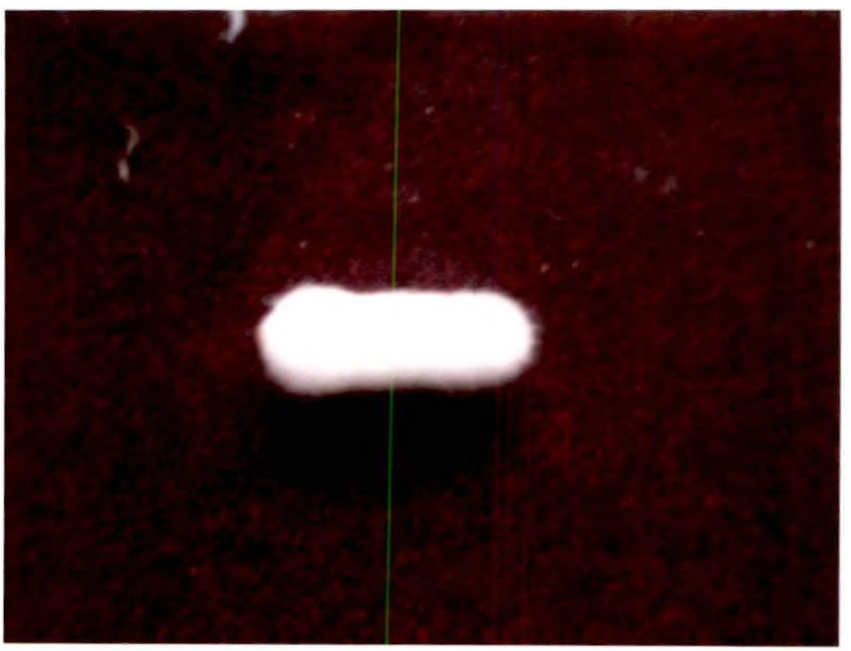
Egg



Pupa

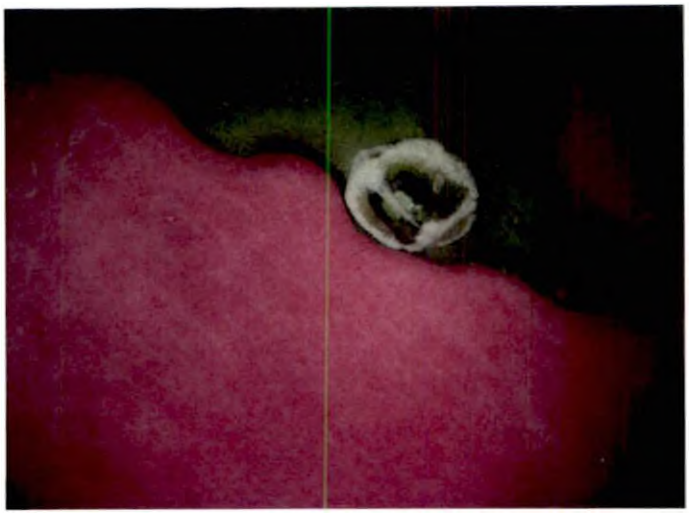
Plate 4. *B. bassiana* infection on *H. vigintioctopunctata*

Adult



Grub

Plate 5. ***B. bassiana*** **infection on** ***L. ramakrishnai***



Adult

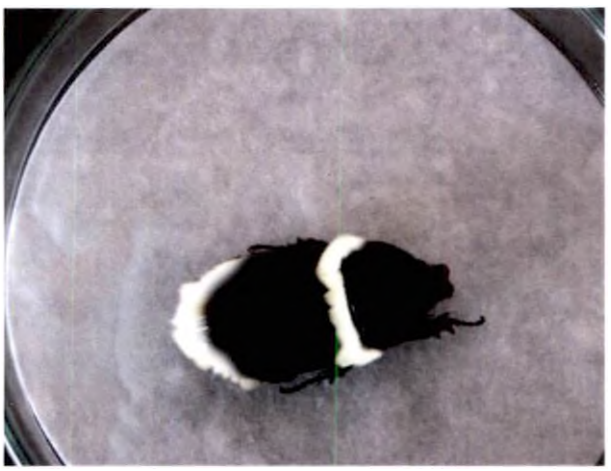
Grub

Egg

Plate 6. ***B. bassiana*** **infection on** ***M. circumdata***

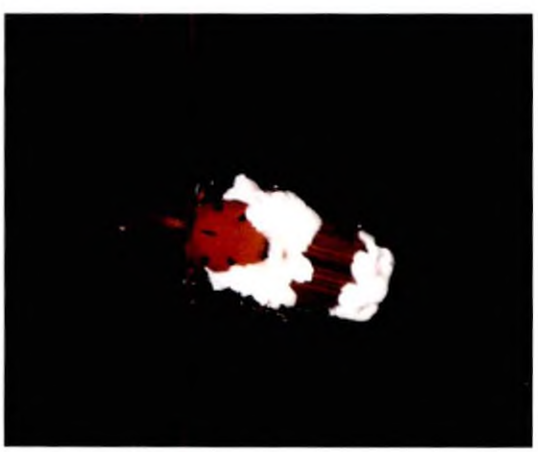
Pupa

Grub

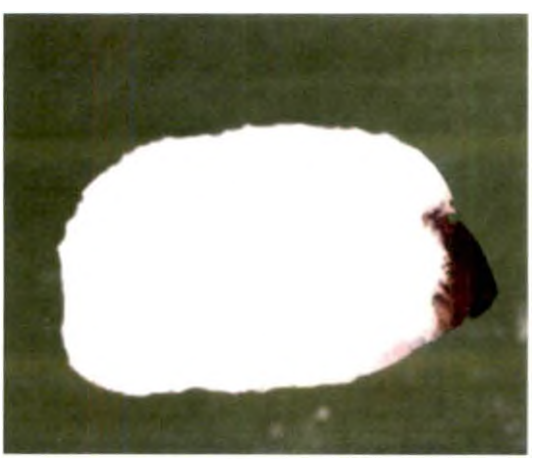


Adult

B. bassiana infection on *O. rhinoceros*



Adult



Grub

B. bassiana infection on *R. ferrugineus*

Plate 7. *B. bassiana* infection on coleopteran insects

4.1.1.2 M. anisopliae

. The pathogenesis observed in *M. anisopliae* treated coleopterans was similar to that observed in *B. bassiana* infection with the exception of the colour of the mycelium produced; reduced feeding, sluggishness. Size reduction and loss of shiny appearance was also noted. When dead, the body was soft initially, later the cadaver became dark, hard and mummified. Mycelium that was initially white changed to profuse olive green mass that further changed to dark green (Plates 8 to 13).

4.1.1.2.1 A. foveicollis

The adults of *A. foveicollis* treated with the fungi did not show any remarkable change for five days and were actively feeding and mating. Later, on the sixth day, the symptoms were noted and the mortality was observed from seventh day onwards. The infected grubs became humped and weak on the fifth day and was found dead on the sixth day. The typical colour of the fungus was also seen over the dead adults and grubs.

4.1.1.2.2 B. fulvicorne

The adult *B. fulvicorne* became less active on the fourth day. The insect was dead on the eighth day. The grubs that acquired infection exhibited symptoms three days after inoculation with the fungus and were dead on the sixth day. On the infected pupa, after five days olive green mycelia appeared.

4.1.1.2.3 C. formicarius

The adult weevils of *C. formicarius* showed symptoms of infection five days after inoculation. The mortality started from the seventh day onwards. The grubs became infected three days after inoculation and were dead on the sixth day. The infected pupae turned hay coloured with wrinkles and later mycelial growth appeared over them.

4.1.1.2.4 C. sordidus

The development of symptoms in the adults and grubs of *C. sordidus* was similar to that of *C. formicarius*.

4.1.1.2.5 H. vigintioctopunctata

The course of development of symptoms in the case of *H. vigintioctopunctata* was also similar to that of *C. formicarius*.

4.1.1.2.6 L. ramakrishnai

The adults of *L. ramakrishnai* developed similar pathogenesis as in the case of *B. fulvicorne*. The grubs turned pinkish upon infection and were dead from the fifth day onwards.

4.1.1.2.7 M. circumdata

The infected adults of *M. circumdata* became sluggish and remained on the top of leaves. Mortality started from the seventh day onwards. The eggs turned dark on third day. The treated grubs turned dark and were found dead from the fifth day onwards. The infected pupa wrinkled, became brownish and mycelial growth developed over the body.

4.1.1.2.8 O. rhinoceros

Adults of *O. rhinoceros* on acquiring infection became less active and stopped feeding. Mortality was observed from the eighth day onwards. The treated eggs turned dark on the third day. The grubs became moribund on the fifth day. Ultimately, all these stages were covered with mycelia. The infected pupa became shrunk and disfigured and mycelium developed over the surface on the fifth day.

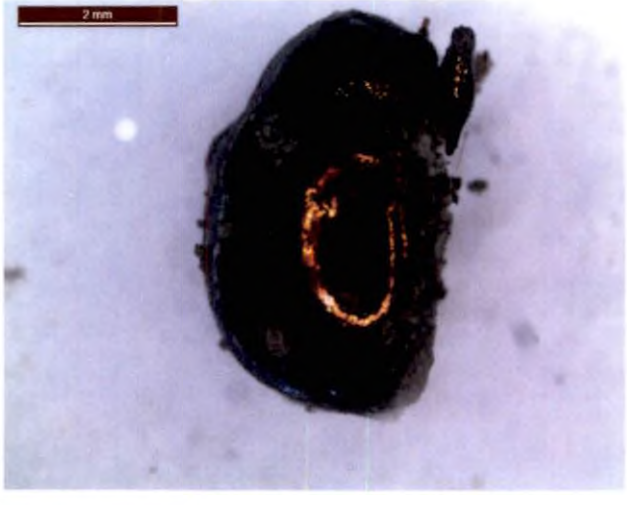
4.1.1.2.9 R. ferrugineus

The symptoms of *M. anisopliae* infection on adults and grubs of *R. ferrugineus* was similar to that of *O. rhinoceros*. In adults, the mortality

Adult

Grub

M. anisopliae infection on *A. foveicollis*



Adult

Grub

M. anisopliae infection on *B. fulvicorne*

Plate 8. *M. anisopliae* infection on coleopteran insects

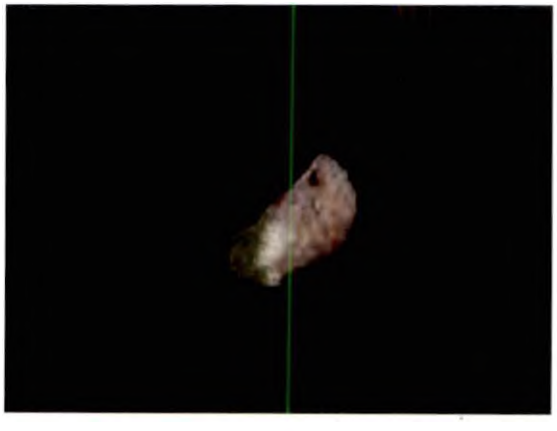
Adult

Grub

M. anisopliae infection on *C. sordidus*

Adult

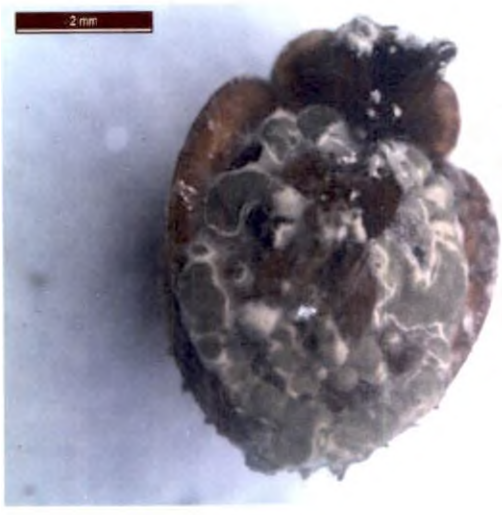
Grub



Pupa

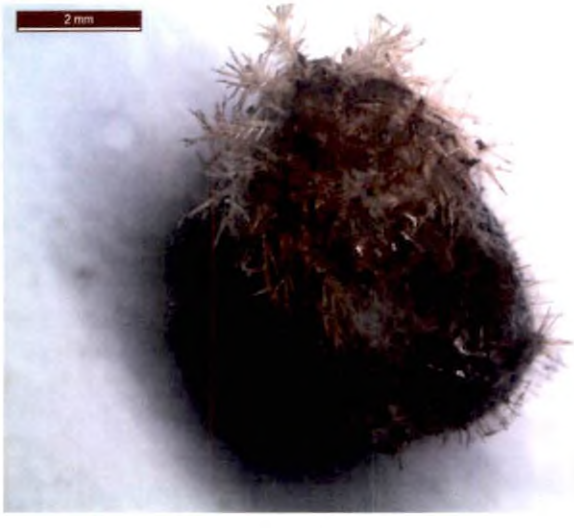
M. anisopliae infection on *C. formicarius*

Plate 9. *M. anisopliae* infection on coleopteran insects



Adult

Egg



Pupa

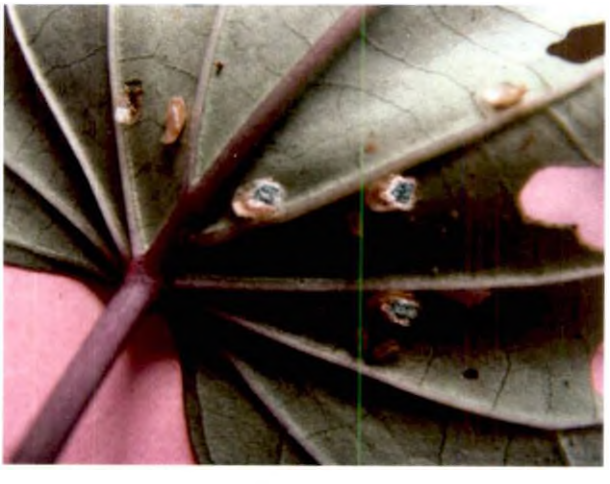
Grub

Plate 10. ***M. anisopliae*** **infection on** ***H. vigintioctopunctata***

M. anisopliae infection on adults of *L. ramakrishnai*

Adult

Grub



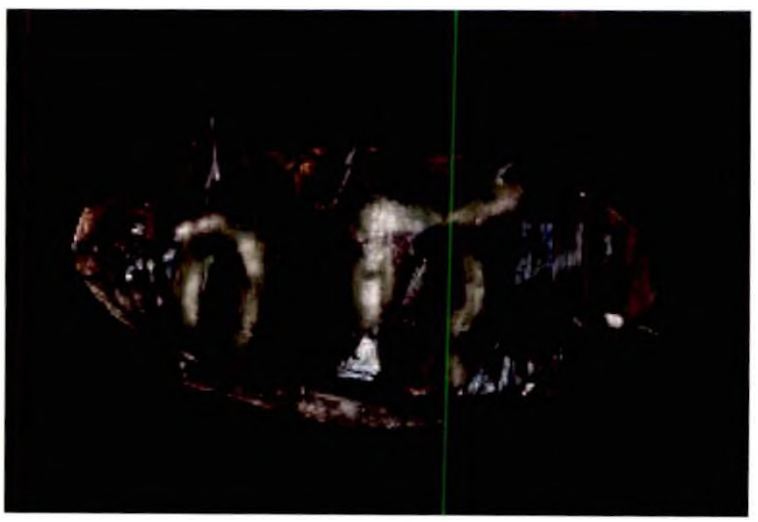
Eggs

M. anisopliae infection on *M. circumdata*

Plate 11. *M. anisopliae* infection on coleopteran insects

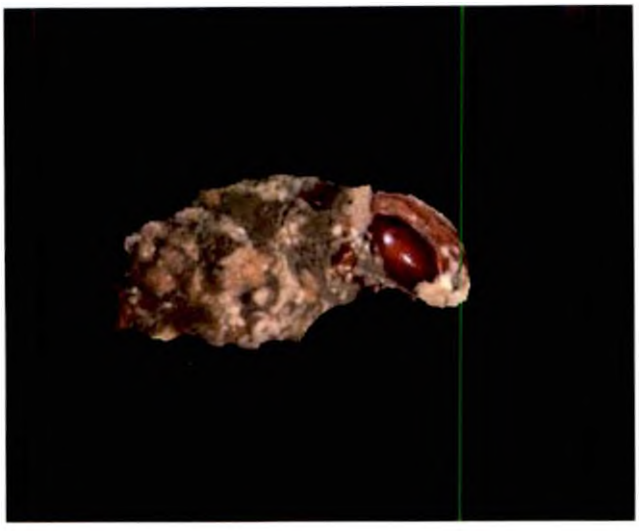
Grub

Pupa



Adult

Plate 12. ***M. anisopliae*** **infection on** ***O. rhinoceros***



Grub

Adult

Plate 13. ***M. anisopliae*** **infection on** ***R. ferrugineus***

B. bassiana infected *M. circumdata* on foliage

B. bassiana infected *B. fulvicorne* on foliage

Plate 14. Summit symptom in the coleopterans

initiated only after 20 days but in grubs mortality was seen from the tenth day onwards.

4.1.2 Assessment of the virulence of *B. bassiana* and *M. anisopliae*

4.1.2.1 B. bassiana @ 10^8 spores ml^{-1}

4.1.2.1.1 To Adults

Upon exposure to *B. bassiana* at a concentration of 10^8 spores ml^{-1}, among the nine adult coleopteran pests tested, at 7 DAT the highest mean per cent mortality of 35.52 was observed for the adults of *H. vigintioctopunctata,* (Table 7) which was on par with the mortality percentage observed for the adults of *C. formicarius* (33.22). The adult *L. ramakrishnai* were susceptible to the fungus and the infection caused a mean mortality of 28.84 per cent, which was statistically on par with the mortality in the adults of *A. foveicollis* and *M. circumdata* (24.37). The mean per cent mortality recorded for the adult *B. fulvicorne* (17.66) was significantly lower and found to be on par with the mean per cent mortality of *C. sordidus* adults (13.33). *B. bassiana* did not produce pathogenesis in the adults of *R. ferrugineus* and *O. rhinoceros* at the dose tested.

At 14 DAT, the highest mean mortality rate was observed for the adult *L. ramakrishnai* (78.15), which was found to be statistically on par with that observed for the adults of *C. formicarius* and *H. vigintioctopunctata,* which recorded a mean per cent mortality of 77.85 and 71.16, respectively. The mean mortality observed for adult *M. circumdata* was 64.48 per cent which was on par with that for the adults of *A. foveicollis* (62.43). The adult weevils of *C. sordidus* showed a mean per cent mortality of 42.21, and it was on par with that of the adult *B. fulvicorne* (33.22). Even at 14 DAT, the adult beetles of *O. rhinoceros* and the adult weevils of *R. ferrugineus* were found uninfected by the fungus at the concentration of 10^8 spores ml^{-1}.

Table 7. Mean percentage mortality of adults of different coleopteran pests when treated with *B. bassiana* @ 10^8 spores ml^{-1}

Pest	7 DAT	14 DAT
A. foveicollis	24.37 (29.57)	62.43 (52.17)
B. fulvicorne	17.66 (24.84)	33.22 (35.18)
C. sordidus	13.33 (21.41)	42.21 (40.50)
C. formicarius	33.22 (35.18)	77.85 (61.90)
H. vigintioctopunctata	35.52 (36.57)	71.16 (57.49)
L. ramakrishnai	28.84 (32.47)	78.15 (62.11)
M. circumdata	24.37 (29.57)	64.48 (53.39)
O. rhinoceros	0 (0)	0 (0)
R. ferrugineus	0 (0)	0 (0)
C.D (0.05)	(4.047)	(5.944)

Figures in parentheses are angular transformed values

DAT: Days after treatment

4.1.2.1.2 To Grubs

The results on virulence of *B. bassiana,* Bb 5 to the grubs of the coleopteran pests are presented in Table 8. When *B. bassiana* at a concentration of 10^8 spores ml^{-1} was sprayed on the grubs, at 7 DAT, the highest mean mortality of 84.57 per cent was recorded for the grubs of *B. fulvicorne.* This was statistically on par with the mean per cent mortality recorded for the grubs of *L. ramakrishnai* (80.29). The pathogen could produce a mean per cent mortality of 66.78 in the grubs of *A. foevicollis*, which was on par with that for the grubs of *H. vigintioctopunctata* and grubs of *C. formicarius,* which showed a mean mortality of 64.48 and 57.79 per cent, respectively. The mean mortality observed for the grubs of *C. sordidus* was 42.21 per cent and was significantly lower than that noticed for other coleopterans. *B. bassiana* produced infection in the grubs of *M. circumdata* but it was significantly lower, the mortality percentage was 21.68. The mortality recorded for the grubs of *R. ferrugineus* and *O. rhinoceros* was the same and this was significantly lower than that recorded for the grubs of other insects (0.76 per cent).

At 14 DAT, there was a considerable increase in the mortality rates of the grubs of the coleopterans. The grubs of *B. fulvicorne, C. formicarius* and *L. ramakrishnai* could not survive the fungal infection and the mortality achieved was 99.99 per cent. The mean per cent mortality recorded for the grubs of *A. foveicollis* was 95.60, and was on par with that of the grubs of *H. vigintioctopunctata* with a mean mortality rate of 89.11 per cent.

The mortality observed for the grubs of *M. circumdata* was 66.78. On 14 DAT also, the infectivity of *B. bassiana* to *O. rhinoceros* and *R. ferrugineus* grubs were on par and significantly lower than that recorded for all the other coleopterans, the mean mortality were 8.66 and 6.67 per cent, respectively.

4.1.2.1.3 To pupa

The mortality of the pupa of *H. vigintioctopunctata* was significantly high when treated with a spore concentration of 10^8 spores ml^{-1} at 7 DAT and recorded

Table 8. Mean percentage mortality of grubs of coleopteran pests when treated with *B. bassiana* @ 10^8 spores ml^{-1}

Pest	7 DAT	14 DAT
A. foveicollis	66.78 (54.78)	95.60 (77.85)
B. fulvicorne	84.57 (66.84)	99.99 (90.00)
C. sordidus	42.21 (40.50)	85.37 (67.48)
C. formicarius	57.79 (49.46)	99.99 (90.00)
H. vigintioctopunctata	64.48 (53.39)	89.11 (70.71)
L. ramakrishnai	80.29 (63.61)	99.99 (90.00)
M. circumdata	21.68 (27.74)	66.78 (54.78)
O. rhinoceros	0.76 (4.99)	8.66 (17.11)
R. ferrugineus	0.76 (4.99)	6.67 (14.96)
C.D (0.05)	(8.034)	(8.525)

Figures in parentheses are angular transformed values

DAT: Days after treatment

a mean mortality of 91.34 per cent. The pathogenicity to the pupa of *M. circumdata* and *C. formicarius* were 42.12 and 37.75 per cent, respectively and were on par (Table 9).

The pupa of *H. vigintioctopunctata* recorded cent per cent mortality at 14 DAT, followed by the pupa of *M. circumdata* and *C. formicarius* which had a mean mortality of 95.60 and 77.85 per cent and differed significantly.

4.1.2.1.4 To eggs

At 7 DAT, the mean mortality of the eggs of *H. vigintioctopunctata* was 91.34 per cent and *M. circumdata* eggs had 42.12 per cent mortality (Table 10).

The mean percentage mortality of *H. vigintioctopunctata* and *M. circumdata* were 99.99 and 95.60 at 14 DAT and were statistically on par.

4.1.2.2 M. anisopliae @ 10^8 spores ml^{-1}

4.1.2.2.1 To Adults

When the adults of the coleopteran pests were treated with spore suspension of *M. anisopliae* at a concentration of 10^8 spores ml^{-1}, the highest mean per cent mortality was recorded for the adults of *B. fulvicorne* (22.15) at 7 DAT, which was on par with that of *H. vigintioctopunctata* which recorded a mean per cent mortality of 17.66 and differed significantly (Table 11) from that recorded for the other coleopterans. The adults *M. circumdata* recorded a mean per cent mortality of 8.66 at 7 DAT which was on par with the mortality rates of *A. foveicollis* and *C. formicarius, C. sordidus, L. ramakrishnai,*, all of which recorded the same mean per cent mortality of 6.67. The lowest mean per cent mortality of 0.76 was noticed for the adults of *O. rhinoceros* which was on par with that for *R. ferrugineus* which had survived the pathogenesis caused by *M. anisopliae* at 7 DAT.

The mortality rates were found to increase over the time, at 14 DAT the highest mean per cent mortality was observed for the adult beetles of

Table 9. Mean percentage mortality of pupa of coleopteran pests when treated with *B. bassiana* @ 10^8 spores ml^{-1}

Pest	7 DAT	14 DAT
C. formicarius	37.75 (37.89)	77.85 (61.90)
H.vigintioctopunctata	91.34 (72.85)	99.99 (90.00)
M. circumdata	42.12 (40.45)	95.60 (77.85)
C.D (0.05)	(11.831)	(9.274)

Figures in parentheses are angular transformed values

DAT: Days after treatment

Table 10. Mean percentage mortality of eggs of coleopteran pests when treated with *B. bassiana* @ 10^8 spores ml^{-1}

Pest	7 DAT	14 DAT
H.vigintioctopunctata	91.34 (72.85)	99.99 (90.00)
M. circumdata	42.12 (40.45)	95.60 (77.85)
C.D (0.05)	(22.507)	(34.586)

Figures in parentheses are angular transformed values

DAT: Days after treatment

Table 11. Mean percentage mortality of the adults of different coleopteran pests when treated with *M. anisopliae* @ 10^8 spores ml^{-1}

Pest	7 DAT	14 DAT
A. foveicollis	6.67 (14.96)	39.94 (39.18)
B. fulvicorne	22.15 (28.06)	46.65 (43.06)
C. sordidus	6.67 (14.96)	31.06 (33.86)
C. formicarius	6.67 (14.96)	44.43 (41.79)
H.vigintioctopunctata	17.66 (24.84)	60.06 (50.78)
L. ramakrishnai	6.67 (14.96)	44.43 (41.79)
M. circumdata	8.66 (17.11)	39.94 (39.18)
O. rhinoceros	0.76 (4.99)	10.89 (19.26)
R. ferrugineus	0 (0)	0 (0)
C.D (0.05)	(5.835)	(5.431)

Figures in parentheses are angular transformed values

DAT: Days after treatment

H. vigintioctopunctata (60.06) and it differed significantly from that for the other beetles. A mean per cent mortality of 46.65 was recorded for the adult *B. fulvicorne*, and it was followed by *C. formicarius* and *L. ramakrishnai* both of which recorded a mean per cent mortality of 44.43 and were on par with the mortality rates of *A. foveicollis* and *M. circumdata* (39.94). The mean per cent mortality noticed for the adults of *C. sordidus* and *O. rhinoceros* were 31.06 and 10.89, respectively and the infectivity of *M. anisopliae* to these beetles differed significantly. *R. ferrugineus* adults did not develop mycoses at the spore concentration of 10^8 spores ml^{-1} of *M. anisopliae,* isolate Ma 4.

4.1.2.2.2 To Grubs

When the grubs of the coleopteran pests were inoculated with *M. anisopliae* at a concentration of 10^8 spores ml^{-1} (Table 12) at 7 DAT, the highest mean per cent mortality was recorded for the grubs of *O. rhinoceros* (66.78). The percentage of mortality recorded for the grubs of *H. vigintioctopunctata* was 42.21 which was on par with that for the grubs of *B. fulvicorne* (35.52). The mean per cent mortality of the grubs of *L. ramakrishnai* was 28.84, and it was on par with that for the grubs of *A. foevicollis,* grubs of *C. sordidus and* grubs of *C. formicarius,* for which mortality was 24.37, 22.14 and 22.14 per cent, respectively. The mortality observed for the grubs of *M. circumdata* was significantly lower (10.89 per cent). The fungus did not infect the grubs of *R. ferrugineus.*

At 14 DAT, the mean mortality observed for the grubs of *O. rhinoceros* and grubs of *H. vigintioctopunctata* were 87.18 per cent and 84.57 per cent respectively and were statistically on par. The grubs of *B. fulvicorne* and grubs of *A. foveicollis* showed a mean mortality rate of 71.16 per cent and this was on par with the per cent mortality noted for the grubs of *C. formicarius* (64.48). The mean per cent mortality noticed for the grubs of *L. ramakrishnai* was 62.25, which was statistically on par with that of *C. sordidus* grubs (60.06) and grubs of *M. circumdata* (60.06 per cent). The grubs of *R. ferrugineus* were not dead at the treated concentration of 10^8 spores ml^{-1}, of Ma 4 isolate of *M. anisopliae.*

Table 12. Mean percentage mortality of grubs of different coleopteran pests when treated with *M. anisopliae* @ 10^8 spores ml^{-1}

Pest	7 DAT	14 DAT
A. foveicollis	24.37 (29.58)	71.16 (57.49)
B. fulvicorne	35.52 (36.57)	71.16 (57.49)
C. sordidus	22.14 (28.06)	60.06 (50.78)
C. formicarius	22.14 (28.06)	64.48 (53.39)
H.vigintioctopunctata	42.21 (40.50)	84.57 (66.84)
L.ramakrishnai	28.84 (32.47)	62.25 (52.07)
M. circumdata	10.89 (19.26)	60.06 (50.78)
O. rhinoceros	66.78 (54.78)	87.18 (68.99)
R. ferrugineus	0 (0)	0 (0)
C.D (0.05)	(6.701)	(5.059)

Figures in parentheses are angular transformed values

DAT: Days after treatment

4.1.2.2.3 To pupa

When the pupa of *H. vigintioctopunctata, C. formicarius* and *M. circumdata* were treated with *M.anisopliae* at a spore concentration of 10^8 spores ml^{-1} a mean percentage mortality of 15.43, 8.66 and 3.00 respectively at 7 DAT and differed significantly (Table 13).

At 14 DAT, the highest pupal mortality of 55.57 per cent was noticed for *H. vigintioctopunctata* and it differed significantly from others. The mortality of the pupa of *C. formicarius* and *M. circumdata* were 31.06 and 24.37 per cent respectively and were on par.

4.1.2.2.4 To eggs

The eggs of *H. vigintioctopunctata* recorded a mean mortality of 64.65 per cent at 7 days after treatment, whereas the eggs of *M. circumdata* produced 8.66 per cent mortality when treated with *M. anisopliae* and differed significantly (Table 14).

Cent per cent mortality of the eggs of *H. vigintioctopunctata* was noted at 14 DAT and *M. circumdata* eggs produced a mean mortality of 24.37 per cent.

4.2 BIOASSAY

Bioassay of the fungi *B. bassiana* and *M. anisopliae* against the adults and grubs of all the nine test insects was carried out and LC_{50}, LC_{90} and LT_{50} values were calculated and the results are given in Tables 15 to 50.

4.2.1 *B. bassiana*

4.2.1.1 A. foveicollis

4.2.1.1.1 Adult

Mortality of the adult *A. foveicollis* inoculated with different spore concentrations of *B. bassiana* was noticed from the fifth day after inoculation and the mortality rate increased with increase in the spore concentration (Table 15).

Table 13. Mean percentage mortality of pupa of coleopteran pests when treated with *M. anisopliae* @ 10^8 spores ml^{-1}

Pest	7 DAT	14 DAT
C. formicarius	3.00 (9.97)	31.06 (33.86)
H.vigintioctopunctata	15.43 (23.12)	55.57 (48.18)
M. circumdata	8.66 (17.11)	24.37 (29.57)
C.D (0.05)	(6.359)	(9.240)

Figures in parentheses are angular transformed values

DAT: Days after treatment

Table 14. Mean percentage mortality of eggs of coleopteran pests when treated with *M. anisopliae* @ 10^8 spores ml^{-1}

Pest	7 DAT	14 DAT
H.vigintioctopunctata	64.65 (53.50)	99.99 (90.00)
M. circumdata	8.66 (17.11)	24.37 (29.57)
C.D (0.05)	(13.682)	(21.475)

Figures in parentheses are angular transformed values

DAT: Days after treatment

Table 15. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *A. foveicollis* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	10	15	18	
2.9×10^8	25.93	50.00	74.07	100.00	9.494
2.9×10^7	18.52	40.74	66.67	87.04	11.374
2.9×10^6	14.82	25.93	53.71	64.82	14.659
2.9×10^5	3.71	18.52	29.63	40.74	19.891
2.9×10^4	0	12.96	18.52	25.93	23.520
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores $ml^{-1} \times 10^8$)	Fiducial limit for LC_{50} (spores $ml^{-1} \times 10^8$)	LC_{90} (spores $ml^{-1} \times 10^8$)	Fiducial limit for LC_{90} (spores $ml^{-1} \times 10^8$)	χ^2	Regression equation
5	5.27	3.78 - 6.75	10.22	8.52 - 10.97	13.808	$Y = 3.325 + 1.363 x$
10	2.75	2.27 - 4.21	7.61	5.94 - 9.39	10.491	$Y = 3.826 + 1.724 x$
15	0.73	0.34 - 1.89	4.62	3.28 - 6.04	28.334	$Y = 4.553 + 2.241 x$
18	0.05	0.02 - 0.09	0.31	0.22 - 0.36	12.410	$Y = 5.689 + 2.224 x$

The mortality percentage ranged from 12.96 to 50, 18.52 to 74.07 and 25.93 to 100 per cent at 10, 15 and 18 DAT, respectively in the spore concentration ranging from 2.9×10^4 to 2.9×10^8 spores ml^{-1}.

The minimum time (9.494 days) required for 50 per cent kill of the test insect was obtained at a spore concentration of 2.9×10^8 spores ml^{-1}. At the lowest dose of 2.9×10^4 spores ml^{-1}, 23.52 days was required to achieve 50 per cent kill of the beetle.

The LC_{50} values obtained by the probit analysis of dose-mortality responses of the insect on the fifth, tenth, fifteenth and eighteenth DAT were 5.27 $\times 10^8$, 2.75 $\times 10^8$, 0.73 $\times 10^8$ and 0.05 $\times 10^8$ spores ml^{-1}, respectively. The corresponding LC_{90} values were 10.22 $\times 10^8$, 7.61 $\times 10^8$, 4.62 $\times 10^8$ and 0.31 $\times 10^8$ spores ml^{-1} at 5, 10, 15 and 18 DAT respectively.

4.2.1.1.2 Grub

The grubs of *A. foveicollis* treated with *B. bassiana* was also found dead on the fifth day and the mortality ranged from 11.11 per cent to 46.67 per cent at spore concentration of 2×10^3 to 2×10^7 spores ml^{-1}. As the spore concentration increased from 2×10^3 to 2×10^7 spores ml^{-1}, the mortality increased from 24.45 per cent to 64.45 per cent and 44.45 per cent to 100 per cent at 7 and 14 DAT, respectively (Table 16).

At the highest concentration of 2×10^7 spores ml^{-1}, the time taken for 50 per cent kill was 5.382 days and the time increased as the concentration of spores decreased and at a concentration of 2×10^3 spores ml^{-1} 14.238 days were taken to bring about 50 per cent kill.

The LC_{50} values corresponding to the fifth, seventh and fourteenth DAT were 2.15×10^7, 1.09×10^7 and 0.009×10^7 spores ml^{-1}, respectively. Based on the cumulative percentage mortality, the LC_{90} values obtained were 5.46×10^7, 4.02×10^7 and 0.25×10^7 spores ml^{-1} at 5, 7 and 14 DAT, respectively.

Table 16. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *A. foveicollis* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	5	7	14	
2×10^7	46.67	64.45	100.00	5.382
2×10^6	31.11	44.45	84.45	7.927
2×10^5	24.45	35.55	66.67	10.432
2×10^4	17.78	26.67	53.33	12.859
2×10^3	11.11	24.45	44.45	14.238
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores $ml^{-1} \times 10^7$)	Fiducial limit for LC_{50} (spores $ml^{-1} \times 10^7$)	LC_{90} (spores $ml^{-1} \times 10^7$)	Fiducial limit for LC_{90} (spores $ml^{-1} \times 10^7$)	χ^2	Regression equation
5	2.15	1.46 - 4.32	5.46	3.66 - 11.74	4.871	$Y = 3.511 + 1.109 x$
7	1.09	0.69 - 1.91	4.02	2.79 - 7.54	3.726	$Y = 3.969 + 2.100 x$
14	0.009	0.0063 - 0.015	0.25	0.17 - 0.48	3.127	$Y = 4.725 + 2.077 x$

4.2.1.2 B. fulvicorne

4.2.1.2.1 Adult

Death of the adult *B. fulvicorne* inoculated with *B. bassiana* was noticed from the seventh day onwards and the rate of mortality ranged from 6.67 to 17.78 per cent, the mortality increased to 8.89 to 40.00 per cent and 66.67 to 100 per cent on the fourteenth and eighteenth day after treatment, respectively (Table 17).

The shortest time span required for the mortality of half the population of the test insect was 12.845 days at the highest concentration of 2.2×10^8 spores ml^{-1} and the longest duration of 17.275 days was required for the lowest spore concentration of 2.2×10^4 spores ml^{-1}.

A spore concentration of 6.53×10^8 spores ml^{-1} was recorded as the LC_{50} value at 7 DAT. Whereas the LC_{50} values obtained for the fourteenth and eighteenth DAT were 2.82×10^8 and 0.02×10^8 spores ml^{-1}, respectively. The LC_{90} values on 7, 14 and 18 DAT were 12.65×10^8, 6.35×10^8 and 0.19×10^8 spores ml^{-1}, respectively.

4.2.1.2.2 Grub

The mortality of the third instar grubs of *B. fulvicorne* was observed from the third day after inoculation with *B. bassiana* but there was no mortality at the lower concentration of 10^2 spores ml^{-1}. The per cent mortality was 6.67 and 20 at 10^4 and 10^6 spores ml^{-1}. As time elapsed mortality also increased and on the fourteenth day cent per cent mortality was observed in the grubs treated with 10^6 spores ml^{-1}. At the lowest dose of 10^2 spores ml^{-1} mortality to the tune of 53.33 per cent was observed (Table 18).

The minimum period of 6.598 days was recorded for obtaining 50 per cent kill of the grubs at the highest spore concentration of 10^6 spores ml^{-1} whereas a period of 13.848 days was required to kill half the population of the test insects at the lowest dosage of 10^2 spores ml^{-1}.

Table 17. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *B. fulvicorne* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	7	14	18	
2.2×10^8	17.78	40.00	100.00	12.845
2.2×10^7	13.33	26.67	91.11	14.298
2.2×10^6	8.89	17.78	82.22	15.538
2.2×10^5	6.67	11.11	77.78	16.257
2.2×10^4	6.67	8.89	66.67	17.275
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} spores (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
7	6.53	5.93 - 6.81	12.65	11.48 - 13.23	1.288	$Y = 2.368 + 1.759 x$
14	2.82	1.93 - 5.66	6.35	4.27 - 13.53	5.033	$Y = 3.024 + 2.534 x$
18	0.02	0.01 - 0.04	0.19	0.13 - 0.22	2.455	$Y = 5.402 + 2.389 x$

Table 18. Cumulative per cent mortality, LT_{50} and probit analysis of dose- mortality responses of grubs of *B. fulvicorne* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	3	5	10	14	
10^6	20.00	44.45	64.44	100.00	6.598
10^5	11.11	26.67	42.22	95.55	8.827
10^4	6.67	22.22	26.67	75.55	11.258
10^3	0.00	6.67	17.78	64.45	12.860
10^2	0.00	4.45	15.55	53.33	13.848
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^6$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^6$)	LC_{90} (spores ml^{-1} $\times 10^6$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^6$)	χ^2	Regression equation
3	1.87	1.73 - 2.01	3.23	2.97 - 3.67	8.312	Y = 1.751 + 3.372 x
5	1.11	0.98 - 1.24	2.43	2.18 - 2.59	11.367	Y = 1.079 + 4.293 x
10	0.63	0.59 - 0.68	1.78	1.56 - 1.84	7.686	Y = 6.699 + 4.985 x
14	0.002	0.001 - 0.004	0.06	0.04 - 0.12	2.924	Y = 3.977 + 2.698 x

The LC_{50} values derived from the probit analysis of log dose-mortality of the insects on the third, fifth, tenth and fourteenth DAT were 1.87×10^6, 1.11×10^6, 0.63×10^6 and 0.002×10^6 spores ml^{-1}, respectively and the LC_{90} values recorded for the corresponding days were 3.23×10^6, 2.43×10^6, 1.78×10^6 and 0.06×10^6 spores ml^{-1}, respectively.

4.2.1.3 C. sordidus

4.2.1.3.1 Adult

The mortality of adult *C. sordidus* initiated from the seventh day after treatment and a mortality range of 6.67 to 20.00 per cent was recorded in the different treatments. A mortality rate ranging from 15.56 to 51.11 per cent was observed on the fourteenth day and still higher mortality of 46.67 to 95.55 per cent on the twentieth day in the spray concentrations of 2.3×10^5 to 2.3×10^9 spores ml^{-1} (Table 19).

The least time required for the death of 50 per cent of test insects was 12.544 days which was recorded in the adults sprayed with the highest fungal concentration of 2.3×10^9 spores ml^{-1}. Maximum time of 19.829 days was required for the lowest concentration of 2.3×10^5 spores ml^{-1}.

The log-dose mortality probit analysis indicated that the LC_{50} values were 4.24×10^9, 2.58×10^9 and 0.08×10^9 spores ml^{-1} at 7, 14 and 20 DAT and the spore strength required for killing 90 per cent of the test insects for the corresponding days were about 7.76×10^9, 9.23×10^9 and 1.65×10^9 spores ml^{-1}, respectively.

4.2.1.3.2 Grub

The morality of the third instar grubs of *C. sordidus* was noticed from the third day and the rate of mortality ranged between 8.33 to 19.45 per cent. The per cent mortality ranged from 5.55 to 38.89 per cent, 16.67 to 50.00 per cent and 58.33 to 97.22 per cent at 7, 10 and 14 DAT, respectively (Table 20).

Table 19. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *C. sordidus* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	7	14	20	
2.3×10^9	20.00	51.11	95.55	12.544
2.3×10^8	17.78	57.78	91.11	14.123
2.3×10^7	13.33	44.44	80.00	16.250
2.3×10^6	11.11	33.33	60.00	17.043
2.3×10^5	6.67	15.56	46.67	19.829
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^9$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^9$)	LC_{90} (spores ml^{-1} $\times 10^9$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^9$)	χ^2	Regression equation
7	4.24	2.65 - 14.28	7.76	4.74 - 27.98	3.644	$Y = 2.636 + 1.540\ x$
14	2.58	1.39 - 12.65	9.23	7.15 - 22.52	3.678	$Y = 2.674 + 0.498\ x$
20	0.08	0.02 - 0.10	1.65	1.01 - 1.95	10.397	$Y = 2.822 + 1.245\ x$

Table 20. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *C. sordidus* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	3	7	10	14	
1.9×10^8	19.45	38.89	50.00	97.22	8.181
1.9×10^7	13.89	25.00	38.89	86.11	9.967
1.9×10^6	8.33	13.89	27.78	80.55	11.286
1.9×10^5	0	5.55	22.22	69.45	12.376
1.9×10^4	0	5.55	16.67	58.33	13.284
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
3	3.83	2.92 - 5.65	6.82	5.32 - 14.21	8.650	Y = 1.640 + 2.680 x
7	2.39	1.98 - 4.33	4.97	3.11 - 12.56	6.772	Y = 1.195 + 3.667 x
10	1.82	1.12 - 4.98	5.35	3.31 - 16.32	4.013	Y = 0.660 + 2.834 x
14	0.76	0.54 - 1.07	0.91	0.70 - 6.32	6.528	Y = 1.586 + 3.003 x

The minimum time required for 50 per cent kill of the grubs of *C. sordidus* was 8.181 days at the higher spore concentration of 1.9×10^8 spores $ml^{-1}$and the maximum time of 13.284 days was taken for the lowest dose of 1.9×10^4 spores ml^{-1}.

The spore concentration required to kill 50 per cent population of the test insects on the third day was 3.83 $\times10^8$ spores ml^{-1}. Application of lower concentrations of 2.38 $\times10^8$, 1.82 $\times10^8$ and 0.76 $\times10^8$ spores ml^{-1} resulted in 50 per cent mortality on 7, 10 and 14 DAT, respectively. The LC_{90} values obtained were 6.82 $\times$ 10^8, 4.97 $\times10^8$, 5.35 $\times10^8$ and 0.91 $\times10^8$ spores ml^{-1} at 3, 7, 10 and 14 days, respectively.

4.2.1.4 C. formicarius

4.2.1.4.1 Adult

The mortality of the adults of *C. formicarius* ranged between 5.00 to 21.67 per cent on the fifth day after inoculation with the fungus. The rate of mortality increased from 13.33 to 45.00 per cent and 26.67 to 73.33 per cent on the tenth and fourteenth day after inoculation, respectively. The peak mortality of 45.00 to 100 per cent was observed on the eighteenth DAT (Table 21).

At a higher spore concentration of 2.1 $\times$ 10^7 spores ml^{-1}, the time required to kill 50 per cent population of the test insect was the lowest (9.869 days), whereas at lower spore concentration of 2.1 $\times$ 10^3 spores ml^{-1}, a period of 19.162 days was required for obtaining 50 per cent mortality of the test insects.

The LC_{50} values obtained from the probit analysis of dose-mortality responses of the insect on the fifth, tenth, fourteenth and eighteenth DAT were 5.62 $\times$ 10^7, 2.44 $\times$ 10^7, 0.56 $\times$ 10^7 and 0.0011 $\times$ 10^7 spores ml^{-1}, respectively, and their corresponding LC_{90} values recorded were 11.54 $\times$ 10^7, 6.89 $\times$ 10^7, 3.47 $\times$ 10^7 and 0.22 $\times$ 10^7 spores ml^{-1}, respectively.

Table 21. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *C. formicarius* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	10	14	18	
2.1×10^7	21.67	45.00	73.33	100.00	9.869
2.1×10^6	18.33	36.67	61.67	88.33	11.507
2.1×10^5	13.33	28.33	48.33	78.33	13.482
2.1×10^4	10.00	21.67	30.00	61.67	16.642
2.1×10^3	5.00	13.33	26.67	45.00	19.162
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^7$)	LC_{90} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^7$)	χ^2	Regression equation
5	5.62	3.12 – 7.10	11.54	6.23 – 15.39	4.858	Y = 2.111 + 1.214 x
10	2.44	1.99 - 3.75	6.89	5.36 - 10.51	8.078	Y = 3.191 + 2.701 x
14	0.56	0.13 - 0.85	3.47	2.47 - 5.22	16.559	Y = 4.677 + 2.954 x
18	0.0011	0.0009 - 0.02	0.22	0.12 - 0.43	11.241	Y = 4.151 + 2.510 x

4.2.1.4.2 Grubs

The mortality of the third instar grubs treated with *B. bassiana* initiated on the second day after inoculation and it ranged from 3.71 to 20.37 per cent. The rate of mortality was between 14.82 to 42.59, 27.78 to 59.26 and 51.85 to 100 per cent at 5, 7 and 12 DAT, respectively (Table 22).

The minimum time required for the 50 per cent kill of the test insects was 5.485 days at the highest spore concentration of 10^7 spores ml^{-1} and the lowest concentration of 10^3 spores ml^{-1} a maximum time of 11.338 days was needed.

The probit analysis results showed that the LC_{50} was 2.94×10^7 spores ml^{-1} at two days after treatment, and the LC_{50} values were 1.27×10^7, 0.59×10^7 and 0.011×10^7 spores ml^{-1} respectively at 5, 7 and 12 DAT. The LC_{90} values recorded were 6.02×10^7, 3.41×10^7, 2.55×10^7 and 0.089×10^7 spores ml^{-1} respectively at 2, 5, 7 and 12 DAT.

4.2.1.5 H. vigintioctopunctata

4.2.1.5.1 Adult

The adult *H. vigintioctopunctata* was noticed dead from fifth day after inoculation and the mortality ranged from 1.85 to 25.93 per cent. The mortality ranges of the test insects on the tenth, fifteenth and eighteenth DAT were 18.52 to 51.85 per cent, 35.18 to 81.48 per cent and 55.56 to 100 per cent, respectively in the different spore concentrations of the fungi varying from 2.1×10^4 to 2.1×10^8 spores ml^{-1} (Table 23).

The LT_{50} value for the highest dose came to about 9.114 days and the lowest dose recorded 17.089 days to bring about death of 50 per cent population of the test insect.

The LC_{50} values obtained after the log dose-mortality probit analysis of the data were 3.99×10^8, 1.86×10^8, 0.23×10^8 and 0.013×10^8 spores ml^{-1}at 5, 10, 15 and 18 days after inoculation, respectively. The concentration required to

Table 22. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *C. formicarius* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	2	5	7	12	
10^7	20.37	42.59	59.26	100.00	5.485
10^6	18.52	33.33	48.15	92.59	6.571
10^5	12.96	25.93	35.18	81.48	8.115
10^4	11.11	18.52	31.48	68.52	9.515
10^3	3.71	14.82	27.78	51.85	11.338
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^7$)	LC_{90} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^7$)	χ^2	Regression equation
2	2.94	2.06 - 4.19	6.02	4.96 - 7.47	5.403	Y = 2.812 + 1.218 x
5	1.27	0.81 - 3.28	3.41	2.14 - 9.51	5.070	Y = 2.977 + 1.759 x
7	0.59	0.35 - 1.25	2.55	1.67 - 5.94	4.219	Y = 3.313 + 2.394 x
12	0.011	0.009 - 0.057	0.089	0.07 - 0.14	8.361	Y = 3.874 + 1.738 x

Table 23. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *H. vigintioctopunctata* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	10	15	18	
2.1×10^8	25.93	51.85	81.48	100.00	9.114
2.1×10^7	18.52	40.74	68.52	92.59	11.026
2.1×10^6	12.96	35.18	59.26	79.63	12.794
2.1×10^5	7.41	25.93	46.29	68.52	14.995
2.1×10^4	1.85	18.52	35.18	55.56	17.089
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
5	3.99	3.61 - 4.09	7.91	7.43 - 8.09	8.013	$Y = 3.070 + 1.304 x$
10	1.86	1.61 - 1.99	6.20	5.95 - 6.40	6.357	$Y = 3.129 + 1.547 x$
15	0.23	0.12 - 0.52	2.86	2.30 - 2.98	11.485	$Y = 4.145 + 2.023 x$
18	0.013	0.001 - 0.57	0.17	0.11 - 0.32	5.249	$Y = 3.919 + 2.417 x$

cause 90 per cent kill of the test insect came to 7.91×10^8, 6.20×10^8, 2.86×10^8 and 0.17×10^8 spores ml^{-1}at 5, 10, 15 and 18 DAT, respectively.

4.2.1.5.2 Grub

Three days after inoculation with the fungi the mortality of the grubs of *H. vigintioctopunctata* was initiated and it ranged between 3.33 to 14.44 per cent and later increased to 13.33 to 37.78 per cent, 20.00 to 64.44 per cent and 56.67 to 98.89 per cent at 7, 10 and 14 DAT, respectively in the different concentrations ranging from 10^7 to 10^3 spores ml^{-1}(Table 24).

The minimum time required for 50 per cent kill of the test insect was 7.84 days at the highest concentration of 10^7 spores ml^{-1}, whereas at the lowest concentration of 10^3 spores ml^{-1} the maximum number of days was recorded for causing 50 per cent mortality of the insects (13.287 days).

The LC_{50} value computed three days after inoculation was 2.79×10^7 spores ml^{-1}, whereas it was 1.54×10^7, 0.48×10^7 and 0.03×10^7 spores ml^{-1}on the seventh, tenth and fourteenth day respectively after inoculation. The corresponding LC_{90} values recorded were 5.04×10^7, 3.95×10^7, 2.10×10^7 and 0.32×10^7 spores ml^{-1}, at 3, 7, 10 and 14 days after inoculation respectively.

4.2.1.6 L. ramakrishnai

4.2.1.6.1 Adult

The adults of *L. ramakrishnai* was found dead from the fifth day after inoculation with the fungus. There was no mortality at the lower concentration of 3.4×10^4 spores ml^{-1}. In others, the rate of mortality was between 2.78 to 22.22 per cent. The range increased to 8.33 to 44.45 per cent, 30.55 to 80.55 per cent and 47.22 to 100 per cent at 10, 15 and 20 DAT, respectively when applied at different spore concentration of the fungus (Table 25).

Table 24. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *H. vigintioctopunctata* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	3	7	10	14	
10^7	14.44	37.78	64.44	98.89	7.840
10^6	12.22	31.11	52.22	91.11	8.976
10^5	7.78	25.56	42.22	84.45	10.074
10^4	3.33	15.56	31.11	72.22	11.675
10^3	0.00	13.33	20.00	56.67	13.287
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^7$)	LC_{90} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^7$)	χ^2	Regression equatio
3	2.79	2.57 - 2.91	5.04	4.79 - 5.14	12.712	Y = 2.864 + 1.601
7	1.54	1.30 - 1.77	3.95	3.59 - 4.06	9.760	Y = 3.373 + 1.817
10	0.48	0.41 - 0.52	2.10	1.79 - 2.17	19.186	Y = 5.079 + 1.381
14	0.03	0.01 - 0.04	0.32	0.19 - 0.35	26.913	Y = 3.909 + 1.651

Table 25. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *L. ramakrishnai* treated with different spore concentrations of *B. bassiana*.

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	10	15	20	
3.4×10^8	22.22	44.45	80.55	100.00	9.910
3.4×10^7	16.67	30.55	72.22	91.67	11.845
3.4×10^6	11.11	25.00	52.78	69.45	15.242
3.4×10^5	2.78	19.45	36.11	61.11	17.627
3.4×10^4	0	8.33	30.55	47.22	19.714
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
5	6.76	6.18 - 7.33	12.65	11.52 - 12.91	8.118	$Y = 2.573 + 1.470 x$
10	3.94	2.51 - 10.24	9.94	6.25 - 28.09	5.095	$Y = 2.940 + 1.842 x$
15	0.31	0.10 - 0.73	4.54	3.73 - 5.18	12.596	$Y = 3.804 + 2.095 x$
20	0.01	0.007 - 0.05	0.30	0.20 - 0.59	2.516	$Y = 3.665 + 1.439 x$

The minimum time required for the kill of 50 per cent population of *L. ramakrishnai* was 9.91 days and the lowest concentration of 3.4×10^4 spores ml^{-1} recorded the lengthy duration for 50 per cent kill (19.714 days).

From the probit analysis of the dose-mortality data, the LC_{50} values were found as 6.76×10^8, 3.94×10^8, 0.31×10^8 and 0.01×10^8 spores ml^{-1} at 5, 10, 15 and 20 days after treatment, respectively. The spore concentrations required for the mortality of 90 per cent of the test insects were 12.65×10^8, 9.94×10^8, 4.54×10^8 and 0.30×10^8 spores ml^{-1}, at 5, 10, 15 and 20 DAT, respectively.

4.2.1.6.2 Grub

The grubs of *L. ramakrishnai* were found dead from the fifth day onwards and the mortality ranged from 2.22 to 24.45 per cent when sprayed with spore suspensions having concentrations ranging from 3×10^3 to 3×10^6 spores ml^{-1}, whereas there was no mortality when sprayed with spore suspension having 3×10^2 spores ml^{-1}. Later on the mortality increased from 11.11 to 51.11 per cent, 17.78 to 64.45 per cent and 46.67 to 100 per cent at 8, 10 and 14 days, respectively after inoculation with the fungi (Table 26).

The least time required for causing 50 per cent mortality of the test insects was 7.859 days when sprayed with 3×10^6 spores ml^{-1} and the longest duration of 14.181 days was recorded when sprayed with 3×10^2 spores ml^{-1}.

The LC_{50} computed were 5.72×10^6, 2.72×10^6, 1.33×10^6 and 0.007×10^6 spores ml^{-1} at 5, 8, 10 and 14 days after treatment, respectively. The concentration of the fungus for causing 90 per cent kill of the test insects at 5, 8, 10 and 14 DAT were 11.02×10^6, 8.18×10^6, 6.51×10^6 and 0.31×10^6 spores ml^{-1}, respectively.

Table 26. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *L. ramakrishnai* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	8	10	14	
3×10^6	24.45	51.11	64.45	100.00	7.859
3×10^5	17.78	42.22	55.55	88.89	9.023
3×10^4	15.55	35.55	44.45	71.11	10.655
3×10^3	2.22	24.45	35.55	64.45	12.044
3×10^2	0	11.11	17.78	46.67	14.181
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^6$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^6$)	LC_{90} (spores ml^{-1} $\times 10^6$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^6$)	χ^2	Regression equation
5	5.72	4.67 - 6.24	11.02	9.20 - 11.53	12.896	Y = 3.904 + 1.385 x
8	2.72	2.18 - 3.25	8.18	7.14 - 8.43	12.308	Y = 3.234 + 2.103 x
10	1.33	0.82 - 1.84	6.51	4.73 - 7.29	12.832	Y = 3.378 + 2.329 x
14	0.007	0.0011 - 0.009	0.31	0.21 - 0.61	4.632	Y = 3.659 + 2.003 x

4.2.1.7 M. circumdata

4.2.1.7.1 Adult

On the fifth day after inoculation with *B. bassiana*, the mortality of the adult *M. circumdata* was noticed and it ranged from 2.22 to 17.78 per cent. The mortality percentage ranged from 11.11 to 40.00 per cent, 20.00 to 84.45 per cent and 35.55 to 100 per cent on 10, 15 and 20 DAT in the different doses ranging from 2.52×10^5 to 2.52×10^9 spores ml^{-1}, respectively (Table 27).

Death of 50 per cent adults of *M. circumdata* was seen at 10.23 days at the higher spore concentration of 2.52×10^9 spores ml^{-1} whereas 23.533 days was required to bring 50 per cent kill at the lowest concentration of 2.52×10^5 spores ml^{-1}.

The probit analysis of log dose- mortality responses between the fungus and the insect indicated the LC_{50} values as 7.26×10^9, 3.73×10^9, 0.07×10^9 and 0.0056×10^9 spores ml^{-1} at fifth, tenth, fifteenth and twentieth DAT, respectively. A spore concentration of 14.04×10^9, 10.76×10^9, 2.91×10^9 and 0.16×10^9 spores ml^{-1} was necessary to cause 90 per cent kill of the test insects at the fifth, tenth, fifteenth and twentieth days after inoculation respectively.

4.2.1.7.2 Grub

The percentage of mortality ranging from 7.41 to 20.37 was noticed on the fifth day after inoculation for the grubs of *M. circumdata* and the rate increased as days progressed. The mortality in the ranges of 14.82 to 40.74 per cent, 27.78 to 68.52 per cent and 51.85 to 98.15 per cent was recorded at 10, 15 and 20 DAT respectively (Table 28).

The LT_{50} was computed for the various doses and the shortest duration was 10.892 days and the highest was 20.052 days, corresponding to the highest and lowest doses of 1.3×10^8 spores ml^{-1} and 1.3×10^4 spores ml^{-1}, respectively.

Table 27. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *M. circumdata* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	10	15	20	
2.52×10^9	17.78	40.00	84.45	100.00	10.230
2.52×10^8	15.55	37.78	75.55	95.55	11.157
2.52×10^7	11.11	31.11	68.89	84.45	12.775
2.52×10^6	6.67	22.22	37.78	64.45	17.073
2.52×10^5	2.22	11.11	20.00	35.55	23.533
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^9$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^9$)	LC_{90} (spores ml^{-1} $\times 10^9$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^9$)	χ^2	Regression equation
5	7.26	5.22 - 10.83	14.04	11.71 - 17.21	4.983	$Y = 2.818 + 1.373 x$
10	3.73	2.34 - 7.43	10.76	6.61 - 12.76	8.694	$Y = 3.090 + 1.680 x$
15	0.07	0.01 - 0.35	2.91	1.57 - 5.23	33.196	$Y = 4.448 + 2.735 x$
20	0.006	0.009 - 0.009	0.16	0.09 - 0.32	18.241	$Y = 4.298 + 1.889 x$

Table 28. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *M. circumdata* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	10	15	20	
1.3×10^8	20.37	40.74	68.52	98.15	10.892
1.3×10^7	18.52	31.48	55.56	87.04	12.893
1.3×10^6	16.67	25.93	44.44	72.22	14.515
1.3×10^5	12.96	24.07	31.48	66.67	16.175
1.3×10^4	7.41	14.82	27.78	51.85	20.052
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
5	4.96	3.23 - 8.77	10.76	9.11 - 13.47	3.015	$Y = 2.271 + 1.099 x$
10	1.85	1.12 - 7.19	5.13	3.01 - 21.76	3.621	$Y = 2.528 + 1.096 x$
15	0.26	0.09 - 1.05	2.88	1.31 - 4.47	10.218	$Y = 3.137 + 1.456 x$
20	0.005	0.001 - 0.05	0.58	0.16 - 1.11	11.65	$Y = 3.807 + 1.451 x$

The spore concentration required to cause 50 per cent mortality of the test insects was 4.96×10^8, 1.85×10^8, 0.26×10^8 and 0.005×10^8 spores ml^{-1} at 5, 10, 15 and 20 DAT, respectively, and the corresponding LC_{90} values were 10.76×10^8, 5.13×10^8, 2.88×10^8 and 0.58×10^8 spores ml^{-1}, respectively.

4.2.1.8 O. rhinoceros

4.2.1.8.1 Adult

With respect to *O. rhinoceros* the mortality of the adults initiated from only on the thirtieth day and it ranged between 4.17 and 25.00 per cent at 10^{13} to 10^{15} spores ml^{-1}. There was no mortality at the lower doses of 10^{11} and 10^{12} spores ml^{-1}. On the fortieth and fiftieth day after inoculation the rate of mortality recorded were 8.33 to 62.50 per cent and 37.50 to 95.83 per cent, respectively (Table 29).

At the higher spore concentration of 10^{15} spores ml^{-1} the minimum time required to cause 50 per cent kill of the test population was 34.401 days, whereas the maximum time of 52.553 days for killing half the population of beetles was recorded at the spore concentration of 10^{11} spores ml^{-1}.

The spore concentrations of 1.56×10^{15}, 0.67×10^{15} and 0.02×10^{15} spores ml^{-1} brought about 50 per cent mortality of adults on the thirtieth, fortieth and fiftieth DAT, respectively and the corresponding LC_{90} values on these days were 2.67×10^{15}, 1.85×10^{15} and 0.63×10^{15} spores ml^{-1}, respectively.

4.2.1.8.2 Grub

B. bassiana was observed to cause mortality of the grubs on the fourteenth day after inoculation and the rate of mortality ranging between 2.22 and 28.89 per cent was observed. The rate of mortality was between 11.11 to 57.78 per cent and 37.78 to 97.78 per cent at twenty first and thirty second days after treatment, respectively (Table 30).

Table 29. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *O. rhinoceros* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	30	40	50	
10^{15}	25.00	62.50	95.83	34.401
10^{14}	12.50	41.67	83.33	41.371
10^{13}	4.17	29.17	70.83	45.047
10^{12}	0	20.83	50.00	49.317
10^{11}	0	8.33	37.50	52.553
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^{15}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^{15}$)	LC_{90} (spores ml^{-1} $\times 10^{15}$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^{15}$)	χ^2	Regression equation
30	1.56	1.06 - 3.59	2.67	1.77 - 6.63	5.180	$Y = 3.074 + 1.796 x$
40	0.67	0.54 - 0.78	1.85	1.63 - 1.91	6.192	$Y = 3.545 + 1.719 x$
50	0.02	0.009 - 0.04	0.63	0.52 - 0.67	10.132	$Y = 3.216 + 1.540 x$

Table 30. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *O. rhinoceros* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	14	21	32	
4.5×10^{11}	28.89	57.78	97.78	18.933
4.5×10^{10}	11.11	51.11	91.11	21.863
4.5×10^{9}	2.22	40.00	73.33	25.742
4.5×10^{8}	0	22.22	64.44	28.596
4.5×10^{7}	0	11.11	37.78	34.450
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times10^{11}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times10^{11}$)	LC_{90} (spores ml^{-1} $\times10^{11}$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times10^{11}$)	χ^2	Regression equation
14	6.22	5.93 - 6.62	10.39	9.95 - 10.59	9.206	$Y = 4.929 + 1.910\ x$
21	3.07	3.01 - 3.11	10.49	10.21 - 10.63	17.999	$Y = 3.562 + 1.530\ x$
32	0.04	0.01 - 0.08	2.08	2.01 - 2.12	24.504	$Y = 3.690 + 1.366\ x$

The time taken for 50 per cent kill of *O. rhinoceros* grubs was the least (18.933 days) at the highest dose of 10^5 spores ml^{-1} and the lowest dose of 10^7 spores ml^{-1} recorded the maximum time of 34.45 days.

The spore concentration required to cause mortality of 50 per cent population of the grubs was 6.22×10^{11} spores ml^{-1} at fourteenth DAT, whereas at spore concentrations of 3.07×10^{11} and 0.04×10^{11} spores ml^{-1} at 21 and 32 days respectively were required to bring about 50 per cent mortality. The LC_{90} values corresponding to 14, 21 and 32 DAT were 10.39×10^{11}, 10.49×10^{11} and 2.08×10^{11} spores ml^{-1}, respectively.

4.2.1.9 R. ferrugineus

4.2.1.9.1 Adults

The mortality of the adult *R. ferrugineus* was noticed from the thirty fifth day onwards and the mortality ranged from 8.33 to 66.67 per cent, but this was seen only in higher spore concentrations ranging from 5.7×10^{10} to 5.7×10^{13} spores ml^{-1}. The mortality rate increased from 25 to 100 per cent on the forty eighth day in the highest concentration tested, i.e., 5.7×10^{13} spores ml^{-1} (Table 31).

The time taken for 50 per cent kill of the test insects was 30.728 days at the highest dose of 5.7×10^{13} spores ml^{-1}, whereas at the lowest dose of 5.7×10^9 spores ml^{-1}, the time taken was 51.029 days.

The probit analysis of log dose-mortality responses of the fungus and the insect indicated 3.76×10^{13} spores/ml as the LC_{50} value 35 DAT and at 48^{th} day, the spore concentration needed for killing half the population of the test insects was 0.15×10^{13} spores/ml. The concentration at which 90 per cent of the test insect killed was 8.79×10^{13} and 0.89×10^{13} spores/ml at 35 and 48 DAT respectively.

Table 31. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *R. ferrugineus* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)		LT_{50} (Days)
	35	48	
5.7×10^{13}	66.67	100.00	30.728
5.7×10^{12}	41.67	75.00	38.874
5.7×10^{11}	25.00	58.33	44.754
5.7×10^{10}	8.33	41.67	49.795
5.7×10^{9}	0	25.00	51.029
Control	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^{13}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^{13}$)	LC_{90} (spores ml^{-1} $\times 10^{13}$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^{13}$)	χ^2	Regression equation
35	3.76	1.69 – 5.82	8.79	6.41 – 10.23	6.746	$Y = 4.362 + 3.255 x$
48	0.15	0.07 – 2.52	0.89	0.51 – 10.94	2.184	$Y = 2.108 + 1.184 x$

4.2.1.9.2 Grub

The grubs treated with the fungus were noticed to be dead on the twentieth day and the range of mortality observed was 8.33 to 29.17 per cent, but there was no mortality in the lower doses of 3.1×10^{7} and 3.1×10^{8} spores ml^{-1}. At 34 DAT, mortality to the tune of 29.17 per cent was observed in the lowest concentration of 3.1×10^{7} spores ml^{-1}, also in the highest dose 91.67 per cent mortality was seen (Table 32).

The minimum time required for the kill of 50 per cent population of the grubs of *R. ferrugineus* was 36.143 days at the highest spore concentration and a longest duration of 50.89 days was recorded for the lowest spore concentration.

The spore concentration required to cause 50 per cent kill of the test insects was 4.64×10^{13} and 0.05×10^{13} spores ml^{-1} at 20 and 34 DAT, respectively. The spore concentrations of 8.56×10^{13} and 2.63×10^{13} spores ml^{-1}were necessary to cause 90 per cent mortality of *R. ferrugineus* at 20 and 34 DAT, respectively.

4.2.2 *M. anisopliae*

4.2.2.1 A. foveicollis

4.2.2.1.1 Adult

A. foveicollis treated with *M. anisopliae* were seen dead on the seventh day, mortality ranging from 3.71 to 18.52 per cent was also recorded. The rate of mortality at 10, 15 and 22 DAT ranged between 7.41 to 40.74, 29.63 to 75.93 and 44.44 to 98.15 per cent, respectively in the spore concentrations ranging from 5.2×10^{6} to 5.2×10^{10} spores ml^{-1} (Table 33).

The time required for the mortality of 50 per cent population of the *A. foveicollis* adults was recorded as 11.374 days at the highest spore concentration of 5.2×10^{10} spores ml^{-1}, whereas the lowest spore concentration of 5.2×10^{6} spores ml^{-1} recorded the maximum time of 21.973 days.

Table 32. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *R. ferrugineus* treated with different spore concentrations of *B. bassiana*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)		LT_{50} (Days)
	20	34	
3.1×10^{11}	29.17	91.67	36.143
3.1×10^{10}	20.83	79.17	40.992
3.1×10^{9}	8.33	58.33	44.128
3.1×10^{8}	0	45.83	48.852
3.1×10^{7}	0	29.17	50.890
Control	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times10^{11}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times10^{11}$)	LC_{90} (spores ml^{-1} $\times10^{11}$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times10^{11}$)	χ^2	Regression equation
20	4.64	4.25 - 5.02	8.56	7.81 - 8.75	9.028	$Y = 2.947 + 1.514 x$
34	0.05	0.01 - 0.09	2.63	2.11 - 2.75	10.258	$Y = 3.571 + 0.860 x$

Table 33. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *A. foveicollis* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	7	10	15	22	
5.2×10^{10}	18.52	40.74	75.93	98.15	11.374
5.2×10^{9}	12.96	31.48	62.96	92.59	13.179
5.2×10^{8}	7.41	20.37	50.00	72.22	16.511
5.2×10^{7}	3.71	18.52	35.18	59.26	19.263
5.2×10^{6}	0	7.41	29.63	44.44	21.973
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^{10}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^{10}$)	LC_{90} (spores ml^{-1} $\times 10^{10}$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^{10}$)	χ^2	Regression equation
7	11.42	9.61 - 13.24	20.62	17.46 - 22.01	7.692	$Y = 2.933 + 1.591 x$
10	6.73	4.82 - 8.64	16.39	13.08 - 18.45	8.583	$Y = 3.388 + 2.893 x$
15	0.99	0.28 - 1.69	8.14	6.77 - 8.82	12.092	$Y = 4.396 + 2.001 x$
22	0.02	0.009 - 0.07	2.22	1.62 - 2.52	23.994	$Y = 3.817 + 1.092 x$

Analysis of log dose-mortality responses between the fungi and the test insect showed that the spore concentration required to bring 50 per cent kill of the beetles was 11.42×10^{10}, 6.73×10^{10}, 0.99×10^{10} and 0.02×10^{10} spores ml^{-1} on 7, 10, 15 and 22 days after inoculation, respectively. The LC_{90} values for the corresponding days were 20.62×10^{10}, 16.39×10^{10}, 8.14×10^{10} and 2.22×10^{10} spores ml^{-1}, respectively.

4.2.2.1.2 Grub

The *A. foveicollis* infected with *M. anisopliae* were found dead on the seventh day after inoculation and the mortality ranged from 2.22 to 17.78 per cent in the different test doses of the fungi. Mortality ranging from 13.33 to 35.55, 22.22 to 51.11 and 51.11 to 97.78 per cent were observed at 9, 12 and 16 DAT respectively (Table 34).

To obtain 50 per cent kill of the grubs the minimum time required was 10.708 days at the highest spore concentration of 2.3×10^7 spores ml^{-1} whereas in the lowest concentration of 2.3×10^3 spores ml^{-1} the maximum time span of 15.688 days was required for achieving mortality of half the population of the test grubs.

The spore concentration for causing mortality of 50 per cent of the grubs was obtained as 4.91×10^7, 3.79×10^7, 2.08×10^7 and 0.13×10^7 spores ml^{-1} at 7, 9, 12 and 16 DAT, respectively. The corresponding LC_{90} values recorded were 8.66×10^7, 9.19×10^7, 7.59×10^7 and 1.07×10^7 spores ml^{-1} respectively.

4.2.2.2 B. fulvicorne

4.2.2.2.1 Adult

From the seventh day onwards, mortality was noticed in *M. anisopliae* treated adult *B. fulvicorne* and it ranged between 2.22 to 31.11 per cent in the different test doses from 2.7×10^4 to 2.7×10^8 spores ml^{-1}. This increased and ranged between 8.89 to 53.33 and 53.33 to 95.55 per cent at 14 and 22 DAT respectively (Table 35).

Table 34. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *A. foveicollis* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	7	9	12	16	
2.3×10^7	17.78	35.55	51.11	97.78	10.708
2.3×10^6	11.11	24.45	42.22	88.89	11.957
2.3×10^5	6.67	22.22	35.55	71.11	13.411
2.3×10^4	2.22	15.55	28.89	62.22	14.480
2.3×10^3	0	13.33	22.22	51.11	15.688
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^7$)	LC_{90} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^7$)	χ^2	Regression equation
7	4.91	3.79 - 6.04	8.66	6.88 - 9.71	6.026	$Y = 2.835 + 1.685 x$
9	3.79	2.26 - 17.41	9.19	5.31 - 45.54	2.015	$Y = 2.423 + 2.111 x$
12	2.08	1.19 - 8.12	7.59	4.43 - 34.39	3.838	$Y = 2.476 + 1.483 x$
16	0.13	0.06 - 0.53	1.07	0.79 - 1.23	11.671	$Y = 4.151 + 2.418 x$

Table 35. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *B. fulvicorne* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	7	14	22	
2.7×10^8	31.11	53.33	95.55	23.465
2.7×10^7	17.78	31.11	82.22	29.111
2.7×10^6	8.89	15.55	73.33	40.056
2.7×10^5	4.47	8.89	68.89	43.163
2.7×10^4	2.22	8.89	53.33	52.314
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
7	3.99	3.64 - 4.35	7.58	6.89 - 7.74	6.682	$Y = 1.429 + 3.947 x$
14	2.42	2.13 - 2.71	5.39	5.11 - 5.54	8.109	$Y = 1.040 + 5.161 x$
22	1.18	1.02 - 1.25	1.67	1.17 - 1.79	7.134	$Y = 4.624 + 3.647 x$

The time taken for attaining 50 per cent kill of the test insects at the highest spore concentration of 2.7×10^8 spores ml^{-1} was 23.465 days, whereas in the lowest concentration of 2.7×10^4 spores ml^{-1} took 52.314 days.

The LC_{50} values of *M. anisopliae* for *B. fulvicorne* was obtained as 3.99×10^8, 2.42×10^8 and 1.18×10^8 spores ml^{-1} on 7, 14 and 22 days respectively after treatment. The LC_{90} values for the corresponding days were 7.58×10^8, 5.39×10^8 and 1.67×10^8 spores ml^{-1}.

4.2.2.2.2 Grub

The grubs of *B. fulvicorne* were found to be dead on the fifth day after treating with *M. anisopliae,* and the mortality ranged between 4.45 to 6.67 per cent in the different spore concentrations ranging from 1.5×10^6 to 1.5×10^8 spores ml^{-1} whereas there was no mortality in the lower doses of 1.5×10^4 and 1.5×10^5 spores ml^{-1}. The mortality rate gradually increased and ranged from 4.45 to 33.33 per cent, 8.89 to 37.78 per cent and 66.67 to 97.78 per cent at 10, 14 and 20 days after inoculation with the fungus with spore concentrations ranging from 1.5×10^4 to 1.5×10^8 spores ml^{-1} (Table 36).

Inorder to kill half the population of the grubs of *B. fulvicorne* 11.17 days was taken at the highest spore concentration of 1.5×10^8 spores ml^{-1}, whereas in the lowest concentration of 1.5×10^4 spores ml^{-1} the maximum time span of 38.305 days was taken.

The LC_{50} values computed based on the log dose-mortality data of the fungus and the test insect showed that a spore concentration of 6.09×10^8, 2.10×10^8, 2.11×10^8 and 0.08×10^8 spores ml^{-1} were essential at 5, 10, 14 and 20 DAT, respectively, to attain 50 per cent kill of the grubs of *B. fulvicorne* and that a higher concentration of 10.11×10^8, 3.99×10^8, 4.99×10^8 and 0.56×10^8 spores ml^{-1} could bring 90 per cent mortality of the test insects at 5, 10, 14 and 20 days after inoculation, respectively.

Table 36. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *B. fulvicorne* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	10	14	20	
1.5×10^8	6.67	33.33	37.78	97.78	11.170
1.5×10^7	6.67	15.55	33.33	91.11	17.667
1.5×10^6	4.45	8.89	17.78	84.45	21.387
1.5×10^5	0	4.45	13.33	73.33	28.972
1.5×10^4	0	4.45	8.89	66.67	38.305
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
5	6.09	4.85 - 7.99	10.11	8.32 - 12.16	5.295	$Y = 1.946 + 1.351 x$
10	2.10	1.53 - 3.56	3.99	2.86 - 7.08	3.536	$Y = 1.426 + 4.185 x$
14	2.11	1.83 - 2.39	4.99	4.45 - 5.13	8.649	$Y = 1.937 + 2.958 x$
20	0.08	0.05 - 0.13	0.56	0.29 - 0.62	7.530	$Y = 2.939 + 6.998 x$

4.2.2.3 C. sordidus

4.2.2.3.1 Adult

The adult *C. sordidus* acquired infection of *M. anisopliae* and was found dead on the tenth day after inoculation with the fungus and the rate of mortality was between 6.67 to 20.00 per cent when applied with spore concentration ranging from 2.7×10^6 to 2.7×10^{10} spores ml^{-1} and it gradually increased from 15.56 to 57.78 per cent and 46.67 to 95.55 per cent at 14 and 24 DAT respectively (Table 37).

The highest spore concentration of 2.7×10^{10} spores ml^{-1} took a shorter period of 14.224 days for attaining 50 per cent kill of the adults, whereas the lowest concentration of 2.7×10^6 spores ml^{-1} recorded a time span of 24.31 days for killing half the population of the adults.

The lethal concentrations of *M. anisopliae* to bring about 50 per cent kill in the adult population of *C. sordidus* were 8.86×10^{10}, 1.68×10^{10} and 1.25×10^{10} spores ml^{-1} at 10, 14 and 24 days respectively after treatment. The concentration of spores required to kill 90 per cent population of the adults were computed as 18.48×10^{10}, 7.29×10^{10} and 1.57×10^{10} spores ml^{-1} at 10, 14 and 24 DAT, respectively.

4.2.2.3.2 Grub

M. anisopliae treated grubs of *C. sordidus* were dead on the seventh day and the mortality percentage ranged from 5.55 to 36.11. Further, 27.78 to 80.55 and 44.45 to 97.22 per cent mortality ranges were observed at 10 and 16 days after inoculation with the fungus, respectively in the concentrations ranging from 3.1×10^4 to 3.1×10^8 spores ml^{-1} (Table 38).

The time required for killing 50 per cent of *C. sordidus* grubs was recorded as 8.309 days at the highest spore concentration of 3.1×10^8 spores ml^{-1} and the lowest dose of 3.1×10^4 spores ml^{-1} recorded the maximum duration of 16.019 days.

Table 37. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *C. sordidus* treated with different spore concentrations of *M. anisopliae*.

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	10	14	24	
2.7×10^{10}	20.00	57.78	95.55	14.224
2.7×10^{9}	17.78	51.11	91.11	15.322
2.7×10^{8}	13.33	44.44	80.00	17.227
2.7×10^{7}	11.11	33.33	60.00	20.670
2.7×10^{6}	6.67	15.56	46.67	24.310
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^{10}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^{10}$)	LC_{90} (spores ml^{-1} $\times 10^{10}$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^{10}$)	χ^2	Regression equation
10	8.86	5.32 - 11.13	18.48	16.22 - 19.85	2.376	$Y = 1.181 + 1.436 x$
14	1.68	1.12 - 1.93	7.29	6.21 - 7.54	12.896	$Y = 3.882 + 2.847 x$
24	1.25	0.98 - 1.41	1.57	1.31 – 1.68	21.273	$Y = 0.459 + 3.742 x$

Table 38. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *C. sordidus* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	7	10	16	
3.1×10^8	36.11	80.55	97.22	8.309
3.1×10^7	33.33	66.67	86.11	9.727
3.1×10^6	22.22	50.00	77.78	11.415
3.1×10^5	16.67	30.55	50.00	15.163
3.1×10^4	5.55	27.78	44.45	16.019
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores $ml^{-1} \times 10^8$)	Fiducial limit for LC_{50} (spores $ml^{-1} \times 10^8$)	LC_{90} (spores $ml^{-1} \times 10^8$)	Fiducial limit for LC_{90} (spores $ml^{-1} \times 10^8$)	χ^2	Regression equation
7	4.81	3.13 - 5.17	11.73	9.24 - 14.96	8.308	$Y = 1.891 + 2.284 x$
10	0.55	0.41 - 0.65	4.06	3.89 - 4.22	11.773	$Y = 1.871 + 4.181 x$
16	0.36	0.29 - 0.42	1.64	1.34 – 1.73	15.857	$Y = 2.835 + 3.557 x$

Analysis of the dose-mortality responses between the fungus and insect brought out that a spore concentration of 4.81×10^8, 5.54×10^8 and 3.55×10^8 spores ml^{-1} were required to cause 50 per cent kill of the test insects at 7, 10 and 14 days after inoculation. The LC_{90} values for the corresponding days were 11.73×10^8, 4.06×10^8 and 1.64×10^8 spores ml^{-1}, respectively.

4.2.2.4 C. formicarius

4.2.2.4.1 Adult

C. formicarius adults were found dead on the tenth day after inoculation with *M. anisopliae* and the rate of morality ranging between 6.67 and 31.67 per cent was observed at spore concentrations ranging from 3.5×10^5 to 3.5×10^9 spores ml^{-1}. Higher ranges of mortality from 15.00 to 65.00 and 25.00 to 98.33 per cent were recorded at 15 and 23 DAT, respectively (Table 39).

Fifty per cent kill of the adult weevils was brought about in 12.012 days at the highest spore concentration of 3.5×10^9 spores ml^{-1} whereas 30.371 days was required at the lowest spore concentration of 3.5×10^5 spores ml^{-1}.

Spore concentrations of 6.27×10^9, 1.84×10^9 and 0.09×10^9 spores ml^{-1} were required to achieve 50 per cent kill of the adult *C. formicarius* at 10, 15 and 20 DAT respectively. The LC_{90} values computed for the corresponding days were 14.21×10^9, 6.76×10^9 and 1.61×10^9 spores ml^{-1}, respectively.

4.2.2.4.2 Grub

The grubs of *C. formicarius* were noticed dead on the fifth day after treatment with *M. anisopliae* and the rate of mortality ranged between 9.26 and 18.52 per cent, at 2.7×10^6 to 2.7×10^8 spores ml^{-1}. The mortality ranging from 3.71 to 31.48 per cent, 12.96 to 68.52 per cent and 25.93 to 98.15 per cent was observed at 7, 14 and 20 DAT respectively in the various test doses ranging from 2.7×10^4 to 2.7×10^8 spores ml^{-1} (Table 40).

Table 39. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *C. formicarius* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	10	15	23	
3.5×10^9	31.67	65.00	98.33	12.012
3.5×10^8	26.67	51.67	86.67	14.485
3.5×10^7	18.33	36.67	65.00	18.902
3.5×10^6	13.33	28.33	46.67	23.092
3.5×10^5	6.67	15.00	25.00	30.371
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^9$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^9$)	LC_{90} (spores ml^{-1} $\times 10^9$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^9$)	χ^2	Regression equation
10	6.27	5.49 - 7.05	14.21	11.48 - 15.90	8.202	$Y = 2.830 + 1.012 x$
15	1.84	1.31 - 3.32	6.76	5.82 - 7.80	16.088	$Y = 4.763 + 2.087 x$
23	0.09	0.01 - 0.43	1.61	1.03 - 1.87	40.000	$Y = 4.293 + 2.060 x$

Table 40. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *C. formicarius* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	7	14	20	
2.7×10^8	18.52	31.48	68.52	98.15	10.195
2.7×10^7	14.82	25.93	44.44	83.33	13.376
2.7×10^6	9.26	18.52	40.74	66.67	16.045
2.7×10^5	0	9.26	25.93	50.00	19.636
2.7×10^4	0	3.71	12.96	25.93	25.740
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
5	5.83	4.91 - 7.24	10.51	8.90 - 13.33	14.126	$Y = 2.991 + 1.597 x$
7	4.56	2.96 - 6.17	9.88	8.05 - 12.37	11.405	$Y = 3.061 + 1.097 x$
14	1.37	0.72 - 3.11	4.67	3.53 - 5.87	13.545	$Y = 5.129 + 2.092 x$
20	0.04	0.008 - 0.19	1.34	0.85 - 1.64	31.026	$Y = 4.350 + 1.949 x$

Fifty per cent of the grubs of *C. formicarius* were killed after a lapse of 10.195 days after application of the fungus at a concentration of 2.7×10^8 spores ml^{-1}. The lowest dose tested, @ 2.7×10^4 spores ml^{-1} took 25.74 days to achieve 50 per cent kill of the grubs.

At 5, 7, 14 and 20 DAT spore concentrations having 5.83×10^8, 4.56×10^8, 1.37×10^8 and 0.04×10^8 spores ml^{-1}, respectively, were required for killing half the population of the grubs. The dosage of *M. anisopliae* required for the mortality of 90 per cent of the grubs corresponding to these days were computed as 10.51×10^8, 9.88×10^8, 4.67×10^8 and 1.34×10^8 spores ml^{-1}, respectively.

4.2.2.5 H. vigintioctopunctata

4.2.2.5.1 Adult

Adults of *H. vigintioctopunctata* that acquired infection when treated with *M. anisopliae* were seen dead on the fifth day with 18.52 per cent mortality at the highest dose of 3.4×10^8 spores ml^{-1}. But there was no mortality at the lower concentrations of 3.4×10^5 and 3.4×10^4 spores ml^{-1}. As time elapsed mortality also increased and 100 per cent morality was achieved at the higher dose and 46.29 per cent mortality in the lowest dose (Table 41).

The time required for the 50 per cent kill of the test insects was 12.06 days, which was recorded at the highest spore concentration. At the same time more than double the time, 24.671 days was required at the lowest dose to bring 50 per cent kill.

The probit analysis data indicated that spore concentrations of 6.96×10^8, 4.39×10^8, 1.98×10^8 and 1.12×10^8 spores ml^{-1} was essential to bring 50 per cent kill at 5, 10, 20 and 25 DAT respectively. The corresponding LC_{90} values computed were 12.27×10^8, 10.92×10^8, 5.99×10^8 and 1.88×10^8 spores ml^{-1}, respectively.

Table 41. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *H. vigintioctopunctata* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	10	20	25	
3.4×10^8	18.52	40.74	77.78	100.00	12.060
3.4×10^7	12.96	35.18	68.52	92.59	14.242
3.4×10^6	7.41	25.93	68.67	85.18	16.192
3.4×10^5	0	12.96	57.41	72.22	19.562
3.4×10^4	0	7.41	37.04	46.29	24.671
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
5	6.96	6.44 - 7.13	12.27	12.01 - 12.52	12.006	$Y = 3.258 + 1.683 x$
10	4.39	3.92 - 4.78	10.92	9.67 - 11.21	14.037	$Y = 3.288 + 8.586 x$
20	1.98	1.78 - 2.14	5.99	5.23 - 6.47	12.564	$Y = 2.965 + 1.842 x$
25	1.12	1.01 - 1.29	1.88	1.57 - 2.21	16.244	$Y = 3.868 + 3.861 x$

4.2.2.5.2 Grub

The grubs of *H. vigintioctopunctata* treated with *M. anisopliae* acquired infection and were dead on the fifth day. There was no mortality in the dose 1.8×10^3 spores ml^{-1} but in higher concentrations the mortality ranged from 3.33 to 12.22 per cent. The rate of mortality ranged between 4.44 to 30, 12.22 to 50 and 57.78 to 97.78 per cent at 10, 14 and 18 DAT, respectively in the different doses tested which ranged from 1.8×10^3 to 1.8×10^7 spores ml^{-1} (Table 42).

The minimum time required for killing 50 per cent population of the grubs was recorded for the highest dose of 1.8×10^7 spores ml^{-1}, which was 12.389 days and the maximum time period of 17.537 days was recorded on the lowest dosage of 1.8×10^3 spores ml^{-1}.

The LC_{50} computed were 4.95×10^7, 3.33×10^7, 1.69×10^7 and 0.89×10^7 spores ml^{-1}at 5, 10, 14 and 18 days after inoculation, respectively. The spore concentration required for the corresponding days to kill 90 per cent population of the grubs of *H. vigintioctopunctata* was computed as 8.48×10^7, 7.30×10^7, 5.43×10^7 and 1.67×10^7 spores ml^{-1}, respectively.

4.2.2.6 L. ramakrishnai

4.2.2.6.1 Adult

The mortality of adult *L. ramakrishnai* was observed only on the tenth day after inoculation with *M. anisopliae* and the mortality was seen at spore concentrations of 2.9×10^8 spores ml^{-1} and above only, in which the percentage ranged from 11.11 to 38.89. But mortality was noticed at the lowest concentrations at 20 DAT. The rate of mortality ranged from 19.45 to 69.45 per cent and 36.11 to 97.22 per cent at 20 and 32 days after treatment, respectively (Table 43).

At the highest spore concentration of 2.9×10^{11} spores ml^{-1}, the time taken for 50 per cent kill of the test insects was 13.683 days, whereas at the lowest concentration the time taken was 34.93 days.

Table 42. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *H. vigintioctopunctata* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	10	14	18	
1.8×10^7	12.22	30.00	50.00	97.78	12.389
1.8×10^6	7.79	24.44	44.44	92.22	13.411
1.8×10^5	4.44	16.67	35.56	85.56	14.526
1.8×10^4	3.33	13.33	24.45	76.67	15.615
1.8×10^3	0	4.44	12.22	57.78	17.537
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^7$)	LC_{90} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^7$)	χ^2	Regression equation
5	4.95	2.94 - 6.17	8.48	7.41 - 9.87	6.486	$Y = 3.036 + 1.796 x$
10	3.33	3.05 - 3.52	7.30	6.99 - 7.68	13.053	$Y = 3.519 + 1.076 x$
14	1.69	1.32 - 175	5.43	5.11 - 5.61	22.844	$Y = 4.016 + 8.005 x$
18	0.89	0.73 - 0.96	1.67	1.51 - 1.82	29.468	$Y = 4.256 + 9.567 x$

Table 43. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *L. ramakrishnai* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	10	20	32	
2.9×10^{11}	38.89	69.45	97.22	13.683
2.9×10^{10}	27.78	61.11	86.11	17.810
2.9×10^{9}	13.89	55.55	77.78	21.767
2.9×10^{8}	11.11	27.78	44.45	32.502
2.9×10^{7}	0	19.45	36.11	34.930
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^{11}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^{11}$)	LC_{90} (spores ml^{-1} $\times 10^{11}$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^{11}$)	χ^2	Regression equation
10	3.66	3.26 - 4.05	7.69	6.92 - 7.87	10.612	$Y = 3.582 + 1.163 x$
20	0.93	0.71 - 1.37	5.41	4.56 - 5.62	16.889	$Y = 3.307 + 1.265 x$
32	0.05	0.01 - 0.07	1.56	1.20 - 1.65	22.492	$Y = 3.728 + 1.969 x$

The log dose-mortality data analysis showed that the lethal concentrations to obtain 50 per cent mortality was 3.66×10^{11}, 0.93×10^{11} and 0.05×10^{11} spores ml^{-1} at 10, 20 and 32 DAT, respectively. For obtaining 90 per cent mortality of the adults of *L. ramakrishnai* 7.69×10^{11}, 5.41×10^{11} and 1.56×10^{11} spores ml^{-1} respectively are required on the corresponding days.

4.2.2.6.2 Grub

Mortality of the grubs of *L. ramakrishnai* also initiated on the tenth day after treatment with *M. anisopliae*. On the eighteenth day after treatment with 1.63×10^8 spores ml^{-1} 97.78 per cent mortality was achieved. At the lowest dose evaluated, *i.e.*, @ 1.63×10^4 spores ml^{-1} 42.22 per cent mortality was seen (Table 44).

The time required for killing half the number of test insects treated were 11.664 and 19.075 days, at the highest and lowest spore concentration, respectively.

Fifty per cent mortality was seen in ten days when infected with the fungus at 2.42×10^8 spores ml^{-1} and on 15 and 18 days after treatment, the spore concentration required for killing 50 per cent population of the grubs was 1.29×10^8 and 0.06×10^8 spores ml^{-1}. The spore concentrations required for achieving LC_{90} on 10, 15 and 18 days were 4.55×10^8, 3.23×10^8 and 0.83×10^8 spores ml^{-1}, respectively.

4.2.2.7 M. circumdata

4.2.2.7.1 Adult

M. circumdata treated with *M. anisopliae* was found dead from the tenth day after inoculation and the rate of mortality was between 8.89 to 35.55 per cent. The mortality further increased to 28.89 to 71.11 per cent and 48.89 to 97.78 per cent at 15 and 20 DAT, respectively in the spore concentrations ranging from 3.97×10^6 to 3.97×10^{10} spores ml^{-1} (Table 45).

Table 44. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *L. ramakrishnai* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	10	15	18	
1.63×10^8	31.11	57.78	97.78	11.664
1.63×10^7	13.33	31.11	82.22	15.316
1.63×10^6	8.89	24.45	71.11	16.504
1.63×10^5	6.67	15.55	62.22	17.501
1.63×10^4	2.22	11.11	42.22	19.075
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
10	2.42	1.74 - 4.26	4.55	3.21 - 8.39	3.095	$Y = 3.996 + 1.459\ x$
15	1.29	0.95 - 1.98	3.23	2.39 - 5.14	4.968	$Y = 4.877 + 2.862\ x$
18	0.06	0.03 - 0.09	0.83	0.62 - 0.88	12.793	$Y = 3.875 + 3.138\ x$

Table 45. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *M. circumdata* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	10	15	20	
3.97×10^{10}	35.55	71.11	97.78	11.361
3.97×10^{9}	24.44	68.89	88.89	12.968
3.97×10^{8}	17.78	55.55	77.78	14.970
3.97×10^{7}	15.55	48.89	66.67	16.390
3.97×10^{6}	8.89	28.89	48.89	19.608
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^{10}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^{10}$)	LC_{90} (spores ml^{-1} $\times 10^{10}$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^{10}$)	χ^2	Regression equation
10	6.13	3.88 - 17.64	14.03	8.67 - 43.22	3.241	$Y = 2.831 + 1.994 x$
15	0.0913	0.30 - 0.25	8.29	4.41 - 13.23	13.489	$Y = 3.757 + 1.096 x$
20	0.008	0.001 - 0.013	1.79	0.81 - 2.86	14.980	$Y = 4.760 + 2.485 x$

The fungus killed 50 per cent of *M. circumdata* in 11.361 days at the highest spore concentration of 3.97×10^{10} spores ml^{-1}, where as a period of 19.608 days was needed at the lowest concentration.

The spore concentrations of 6.13×10^{10}, 0.0913×10^{10} and 0.008×10^{10} spores ml^{-1} was necessary to kill 50 per cent population of the test insects at 10, 15 and 20 DAT, respectively. For the corresponding days, the dosage required for killing 90 per cent of the *M. circumdata* was computed as 14.03×10^{10}, 8.29×10^{10} and 1.79×10^{10} spores ml^{-1} respectively.

4.2.2.7.2 Grub

The percentage mortality in the grubs of *M. circumdata* treated with *M. anisopliae* initiated on the fifth day after inoculation. After a lapse of 20 days, the mortality percentage reached 98.15 per cent at the highest dose of 2×10^9 spores ml^{-1} whereas at the lower doses of 2×10^5 spores ml^{-1} the mortality percentage was only 44.44 (Table 46).

At the highest spore concentration of 2×10^9 spores ml^{-1} the time taken for killing 50 per cent population of test insects was 10.885 days and the lowest concentration recorded 20.347 days.

The lethal concentration to kill 50 per cent *M. circumdata* were 4.64×10^9, 2.39×10^9, 0.74×10^9 and 0.001×10^9 spores ml^{-1} at 5, 10, 15 and 20 days after treatment, respectively. The corresponding LC_{90} were 8.92×10^9, 5.88×10^9, 4.12×10^9 and 0.89×10^9 spores ml^{-1} respectively.

4.2.2.8 O. rhinoceros

4.2.2.8.1 Adult

The adults of *O. rhinoceros* were noticed to be dead 30 days after inoculation with *M. anisopliae*. High spore concentration ranging from 7×10^9 to 7×10^{13} spores ml^{-1} was required to bring 16.67 to 83.33 per cent mortality, but

Table 46. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *M. circumdata* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)				LT_{50} (Days)
	5	10	15	20	
2×10^9	20.37	42.59	66.67	98.15	10.885
2×10^8	18.52	35.18	59.26	87.04	12.462
2×10^7	12.96	25.93	42.59	79.63	14.881
2×10^6	3.71	14.82	35.19	62.97	17.645
2×10^5	0	3.71	24.07	44.44	20.347
Control	0	0	0	0	0

Probt analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^9$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^9$)	LC_{90} (spores ml^{-1} $\times 10^9$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^9$)	χ^2	Regression equation
5	4.64	2.89 - 7.58	8.92	8.11 - 10.92	13.493	$Y = 2.553 + 1.388 x$
10	2.39	2.05 - 4.69	5.88	5.21 - 7.98	17.007	$Y = 3.625 + 1.881 x$
15	0.74	0.41 - 2.52	4.12	2.96 - 5.71	12.370	$Y = 3.750 + 2.137 x$
20	0.001	0.0009 - 0.026	0.89	0.26 - 1.95	21.625	$Y = 4.578 + 2.422 x$

cent per cent mortality was achieved on the forty fifth day after treatment at the highest dose tested (Table 47).

Fifty per cent mortality of the test insects was recorded at 23.465 days in the highest spore concentration and a period of 52.314 days was recorded in the lowest dose.

A spore concentration of 2.58×10^{13} and 0.03×10^{13} spores ml^{-1} were required at 30 and 45 DAI, respectively for obtaining 50 per cent mortality of adults of *O. rhinoceros*. The concentration required to kill 90 per cent of the insect population was 7.92×10^{13} and 0.62×10^{13} spores ml^{-1} at 30 and 45 days after inoculation respectively.

4.2.2.8.2 Grub

The treated grubs of *O. rhinoceros* were found dead in a shorter period of ten days after inoculation with *M. anisopliae* at concentrations ranging from 3.7×10^6 to 3.7×10^8 spores ml^{-1} and the rate of mortality ranged from 22.22 to 46.67 per cent. Subsequently the mortality increased and reached 100 per cent 30 days after treatment in the highest dose of 3.7×10^8 spores ml^{-1} (Table 48).

At the highest spore concentration, the time required for 50 per cent kill of the grubs was 11.17 days whereas in the lowest concentration slightly longer period of 38.305 days was taken.

At 10, 20 and 30 DAT, spore concentrations of 3.79×10^8, 1.08×10^8 and 0.04×10^8 spores ml^{-1} was required to bring 50 per cent mortality of the gubs, whereas the concentrations required for LC_{90} were 8.04×10^8, 4.67×10^8 and 0.49×10^8 spores ml^{-1}, respectively.

4.2.2.9 R. ferrugineus

4.2.2.9.1 Adult

R. ferrugineus treated with different spore concentrations of *M. anisopliae* were noticed to be dead only after 50 days. The rate of mortality was between

Table 47. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *O. rhinoceros* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)		LT_{50} (Days)
	30	45	
7×10^{13}	83.33	100.00	23.465
7×10^{12}	58.33	91.67	29.111
7×10^{11}	20.83	66.67	40.056
7×10^{10}	20.83	54.17	43.163
7×10^{9}	16.67	45.83	52.314
Control	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^{13}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^{13}$)	LC_{90} (spores ml^{-1} $\times 10^{13}$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^{13}$)	χ^2	Regression equation
30	2.58	2.05 - 2.91	7.92	7.13 - 8.16	9.541	$Y = 4.810 + 2.619\ x$
45	0.03	0.01 - 0.15	0.62	0.42 - 1.24	5.308	$Y = 3.635 + 1.068\ x$

Table 48. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *O. rhinoceros* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)			LT_{50} (Days)
	10	20	30	
3.7×10^8	46.67	80.00	100.00	11.170
3.7×10^7	33.33	66.67	80.00	17.667
3.7×10^6	22.22	51.11	73.33	21.387
3.7×10^5	0	26.67	48.89	28.972
3.7×10^4	0	4.45	20.00	38.305
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
10	3.79	3.37 - 4.21	8.04	7.23 - 8.24	28.345	$Y = 4.895 + 1.145 x$
20	1.08	0.72 - 1.42	4.67	3.98 - 4.84	38.157	$Y = 3.883 + 1.099 x$
30	0.04	0.01 - 0.09	0.49	0.41 - 0.52	22.277	$Y = 4.179 + 1.105 x$

16.67 and 50.00 per cent at spore concentrations of 2.86×10^{13} to 2.86×10^{15} spores ml^{-1}. Though mortality increased, 91.67 per cent mortality was achieved only 64 days after inoculation with a spore concentration of 2.86×10^{15} spores ml^{-1} (Table 49).

The minimum time required for killing 50 per cent population of the adults was recorded as 48.622 days at the highest spore concentration of 2.86×10^{15} spores ml^{-1} and at the lowest concentration recorded a time period of 69.658 days was required.

From the probit analysis it is seen that for obtaining 50 per cent mortality of the adults, a spore concentration of 2.73×10^{15} and 0.14×10^{15} spores ml^{-1} was needed at 50 and 64 DAT respectively. The corresponding concentration for LC_{90} were 5.59×10^{15} and 0.53×10^{15} spores ml^{-1} at 50 and 64 days after inoculation, respectively.

4.2.2.9.2 Grub

The mortality of the grubs of *R. ferrugineus* were noticed on 30 days after inoculation of *M. anisopliae* and the rate of mortality ranging from 4.17 to 12.50 per cent was observed. 91.67 per cent mortality was seen at 45 DAT at the highest concentration of 3.9×10^{13} spores ml^{-1} (Table 50).

At the highest spore concentration the time taken for 50 per cent mortality was 36.143 days and a period of 50.89 days was required at the lowest spore concentration of 3.9×10^{9} spores ml^{-1}.

The LC_{50} and LC_{90} computed were 10.29×10^{13} and 17.69×10^{13} spores ml^{-1}, respectively at 30 DAT and 0.75×10^{13} and 3.40×10^{13} spores ml^{-1} at 45 days after inoculation, respectively.

Table 49. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of *R. ferrugineus* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)		LT_{50} (Days)
	50	64	
2.86×10^{15}	50.00	91.67	48.622
2.86×10^{14}	33.33	66.67	57.414
2.86×10^{13}	16.67	50.00	63.286
2.86×10^{12}	0	33.33	66.350
2.86×10^{11}	0	16.67	69.658
Control	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^{15}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^{15}$)	LC_{90} (spores ml^{-1} $\times 10^{15}$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^{15}$)	χ^2	Regression equation
50	2.73	1.22 – 3.91	5.59	4.24 – 7.94	7.253	$Y = 4.998 + 2.865 x$
64	0.14	0.02 – 1.02	0.53	0.29 – 6.03	2.446	$Y = 2.132 + 1.482 x$

Table 50. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of *R. ferrugineus* treated with different spore concentrations of *M. anisopliae*

Concentration (spores ml^{-1})	Cumulative per cent mortality at different intervals after treatment (Days)		LT_{50} (Days)
	30	45	
3.9×10^{13}	12.50	91.67	36.143
3.9×10^{12}	12.50	66.67	40.992
3.9×10^{11}	4.17	54.17	44.128
3.9×10^{10}	0	20.83	48.852
3.9×10^{9}	0	12.50	50.890
Control	0	0	

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^{13}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^{13}$)	LC_{90} (spores ml^{-1} $\times 10^{13}$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^{13}$)	χ^2	Regression equation
30	10.29	9.56 - 11.01	17.69	16.83 - 17.95	5.738	$Y = 2.021 + 1.779 x$
45	0.75	0.49 - 0.83	3.40	3.12 - 3.53	16.868	$Y = 4.492 + 2.689 x$

4.3 SELECTION OF SUBSTRATES FOR MASS MULTIPLICATION OF FUNGI

With a view to select suitable substrates for the mass multiplication of *B. bassiana* and *M. anisopliae,* these fungi were multiplied in rice husk, rice bran, wheat bran, coconut oil cake, groundnut oil cake, neem cake, coir pith compost, cow dung and saw dust and the growth pattern, sporulation and colony forming units in these substrates were assessed. The results are presented in Tables 51 to 59.

B. bassiana grew profusely in cow dung, neem cake, rice bran and wheat bran (Table 51). Moderate growth was observed in the case of coconut oil cake and groundnut oil cake, where as in coir pith compost, saw dust and rice husk mycelial growth was only slight.

The growth of *M. anisopliae* was high in cow dung, wheat bran and ground nut oil cake, moderate in neem cake and rice bran. Mycelium development was only slight in the case of coconut oil cake, coir pith compost, saw dust and rice husk.

4.3.1 Spore count of the fungi in different substrates

4.3.1.1 B. bassiana

One month after storage under room temperature, *B. bassiana* cultured in cow dung recorded significantly higher concentration of spores and the spore count obtained was 127.69×10^5 spores ml^{-1} (Table 52). The fungus grown on wheat bran had a spore load of 11.29×10^5 spores ml^{-1}, which was statistically on par with the spore counts on neem cake, rice bran, coconut oil cake, groundnut oil cake, coir pith compost, rice husk and saw dust, which recorded a concentration of 0.302×10^5, 0.199×10^5, 0.018×10^5, 0.013×10^5, 0.010×10^5, 0.005×10^5 and 0.004×10^5 spores ml^{-1}, respectively.

Table 51. Mycelial growth of *B. bassiana* and *M. anisopliae* on different substrates based on visual scoring

Substrate	*B.bassiana*	*M.anisopliae*
Rice husk	+	+
Rice bran	+++	++
Wheat bran	+++	+++
Coconut oil cake	++	+
Groundnut oil cake	++	+++
Neem cake	+++	++
Coir pith compost	+	+
Cow dung	+++	+++
Saw dust	+	+

+++ Profuse growth

++ Moderate growth

+ Slight growth

Table 52. Spore count of *B. bassiana* in various substrates at different intervals after storage under room temperature

Substrates	Spore count (spores $ml^{-1} \times 10^5$)		
	1MAS	2MAS	3MAS
Rice husk	0.005 (0.073)	9.49 (3.08)	93.53 (9.67)
Rice bran	0.199 (0.445)	2263.83 (47.58)	8.38 (2.89)
Wheat bran	11.297 (3.361)	11676.96 (108.06)	72.56 (8.52)
Coconut oil cake	0.018 (0.135)	23.04 (4.79)	205.94 (14.35)
Groundnut oil cake	0.013 (0.113)	116.67 (10.80)	1.31 (1.14)
Neem cake	0.302 (0.549)	745.86 (27.31)	2.02 (1.42)
Coir pith compost	0.010 (0.100)	5.91 (2.43)	0.28 (0.53)
Cow dung	127.693 (11.299)	10650.47 (103.20)	2.33 (1.53)
Saw dust	0.004 (0.061)	1.22 (1.10)	25.51 (5.05)
C.D (0.05)	(4.9206)	(12.310)	(5.790)

Figures in parentheses are square root transformed values

*MAS : Months After Storage

The spore count of the fungus two months after storage in wheat bran was very high (11676.96 × 10^5 spores ml^{-1}) and was on par with that of cow dung (10650.47 × 10^5 spores ml^{-1}), where as in the substrates like rice bran and neem cake, a mean spore count of 2263.83 × 10^5 and 745.86 spores ml^{-1}, respectively, was recorded and it differed significantly from the spore counts in other substrates. Relatively lower sporulation was observed in the substrates like groundnut oil cake, coconut oil cake, rice husk, coir pith compost and saw dust and recorded 116.67 × 10^5, 23.04 × 10^5, 9.49 × 10^5, 5.91 × 10^5 and 1.22 × 10^5 spores ml^{-1}, respectively.

At three months after storage fungus grown in coconut oil cake had a spore load of 205.94 × 10^5 spores ml^{-1} and was on par with that in rice husk (93.53 spores ml^{-1}). The fungus cultured in wheat bran, saw dust and rice bran recorded moderate spore concentrations of 72.56 × 10^5, 25.51 × 10^5 and 8.38 × 10^5 spores ml^{-1} respectively and were on statistically on par. The fungus mass cultured in cow dung, neem cake, groundnut oil cake and coir pith compost recorded lower spore loads of 2.33 × 10^5, 2.02 × 10^5, 1.31 × 10^5 and 0.28 × 10^5 spores ml^{-1} , respectively, and were on par.

4.3.1.2 M. anisopliae

M. anisopliae grown on cow dung showed significantly higher spore concentration of 202.93 × 10^5 spores ml^{-1} at one month after storage under room temperature. The substrates *viz.* wheat bran, rice bran, groundnut oil cake, neem cake, rice husk, coconut oil cake, coir pith compost and saw dust recorded a spore load of 1.52 × 10^5, 0.026 × 10^5, 0.021 × 10^5, 0.018 × 10^5, 0.017 × 10^5, 0.005 × 10^5, 0.004 × 10^5 and 0.004 × 10^5 spores ml^{-1}, respectively, and were on par statistically (Table 53).

The spore load obtained from cow dung and wheat bran were 8573.66 × 10^5 and 8447.06 × 10^5 spores ml^{-1}, respectively, at the second month and were statistically on par. The concentration of spores present in groundnut oil cake was 1422.62 × 10^5 spores ml^{-1}. The substrates like rice bran, neem cake, rice husk,

Table 53. Spore count of *M. anisopliae* in various substrates at different intervals after storage under room temperature

Substrates	Spore count (spores $ml^{-1} \times 10^5$)		
	1MAS	2MAS	3MAS
Rice husk	0.017 (0.132)	10.76 (3.28)	20.79 (4.56)
Rice bran	0.026 (0.162)	179.16 (13.38)	3.29 (1.81)
Wheat bran	1.524 (1.234)	8447.06 (91.91)	7.71 (2.78)
Coconut oil cake	0.005 (0.069)	3.02 (1.74)	86.38 (9.29)
Groundnut oil cake	0.021 (0.145)	1422.62 (37.72)	24.08 (4.91)
Neem cake	0.018 (0.135)	42.42 (6.51)	0.08 (0.28)
Coir pith compost	0.004 (0.059)	0.73 (0.86)	0.09 (0.31)
Cow dung	202.931 (14.253)	8573.66 (92.59)	293.62 (17.14)
Saw dust	0.004 (0.059)	0.10 (0.32)	11.29 (3.36)
C.D (0.05)	(3.5057)	(11.255)	(5.101)

Figures in parentheses are square root transformed values

*MAS : Months After Storage

coconut oil cake, coir pith compost and saw dust recorded a concentration of 179.16 × 10^5, 42.42 × 10^5, 10.76 × 10^5, 3.02 × 10^5, 0.73 × 10^5 and 0.10 × 10^5 spores ml^{-1}, respectively and were statistically on par.

At three months after storage, the spore concentration in cow dung was the highest (293.62 × 10^5 spores ml^{-1}) and it was significantly different from that in other substrates. The spore concentration on the coconut oil cake and ground nut oil cake were 86.38 × 10^5 and 24.08 × 10^5 spores ml^{-1} respectively and were on par. The spore count recorded from the substrates, rice husk (20.79 × 10^5), saw dust (11.29 × 10^5), wheat bran (7.71 × 10^5), rice bran (3.29 × 10^5), coir pith compost (0.09 × 10^5) and neem cake (0.08 × 10^5) were statistically on par.

4.3.2 Colony forming units of fungi in carrier materials

4.3.2.1 B. bassiana

The concentration of viable spores in cow dung at one month after storage was the highest and came to about 1.99 × 10^5 cfu g^{-1} and differed significantly from the other carrier materials (Table 54). The number of colony forming units present in wheat bran was 0.34 × 10^5, followed by neem cake, rice bran, coconut oil cake with a viable spore count of 0.025 × 10^5, 0.011 × 10^5, 0.009 × 10^5 cfu g^{-1} respectively, and these were significantly higher than that in the following substrates like groundnut oil cake, coir pith compost, saw dust and rice husk, which recorded a viable spore load of 0.005 × 10^5, 0.003 × 10^5, 0.001 × 10^5 and 0.001 × 10^5 cfu g^{-1}, respectively and were statistically on par with each other.

The colony forming units of *B. bassiana* present in the cow dung was the highest at two months after storage and was 7717.42 × 10^5 cfu g^{-1}. The viable spores present in wheat bran (785.02 × 10^5) was on par with that present in rice bran and neem cake which had a concentration of 196.09 × 10^5, 177.23 × 10^5 cfu g^{-1} respectively, and these were significantly higher than that in ground nut oil cake, rice husk, coconut oil cake, coir pith compost and saw dust, which had a concentration of 3.68 × 10^5, 0.87 × 10^5, 0.35 × 10^5, 0.22 × 10^5 and 0.07 × 10^5 colony forming units per gram, respectively.

Table 54. Colony forming units of *B. bassiana* per gram of substrates at different intervals after storage under room temperature

Substrates	cfu g^{-1} ($\times 10^5$)		
	1MAS	2MAS	3MAS
Rice husk	0.001 (0.037)	0.87 (0.93)	9.56 (3.09)
Rice bran	0.011 (0.104)	196.09 (14.00)	0.66 (0.82)
Wheat bran	0.34 (0.58)	785.02 (28.02)	10.91 (3.30)
Coconut oil cake	0.009 (0.092)	0.35 (0.59)	6.09 (2.47)
Groundnut oil cake	0.005 (0.074)	3.68 (1.92)	0.04 (0.19)
Neem cake	0.025 (0.159)	177.23 (13.31)	0.09 (0.30)
Coir pith compost	0.003 (0.058)	0.22 (0.47)	0.01 (0.10)
Cow dung	1.99 (1.41)	7717.42 (87.85)	0.03 (0.17)
Saw dust	0.001 (0.037)	0.07 (0.26)	1.75 (1.32)
C.D (0.05)	(0.4998)	(21.105)	(2.408)

Figures in parentheses are square root transformed values

*MAS : Months After Storage

At the end of third month, among the different stored carrier materials inoculated with the fungus, wheat bran recorded a highest concentration of 10.91 $\times 10^5$ cfu g^{-1} and was on par with rice husk, coconut oil cake and saw dust which produced a cfu concentration of 9.56 $\times 10^5$, 6.09 $\times 10^5$, 1.75 $\times 10^5$ cfu g^{-1} respectively. The substrates like rice bran, neem cake, groundnut oil cake, cow dung and coir pith compost showed statistical similarity with a concentration of 0.66 $\times 10^5$, 0.30 $\times 10^5$, 0.04 $\times 10^5$, 0.03 $\times 10^5$ and 0.01 $\times 10^5$ colony forming units per gram, respectively.

4.3.2.2 M. anisopliae

M. anisopliae when cultured on cow dung had the highest concentration of viable spores at one month after storage under room temperature and recorded 23.98 $\times 10^5$ cfu g^{-1} and differed significantly from the other carrier materials (Table 55). The concentration of colony forming units was 0.19 $\times 10^5$, 0.007 $\times 10^5$, 0.005 $\times 10^5$, 0.004 $\times 10^5$, 0.002 $\times 10^5$, 0.008 $\times 10^5$, 0.007 $\times 10^5$ and 0.0001 $\times 10^5$ cfu g^{-1} in wheat bran, neem cake, rice bran, rice husk, groundnut oil cake, coir pith compost, coconut oil cake and saw dust, respectively and were statistically on par.

The concentration of viable spores in wheat bran at two months after storage was the highest among the different carrier materials and recorded 658.24 $\times 10^5$ cfu g^{-1} and was on par with that of cow dung (266.98 $\times 10^5$) and groundnut oil cake (207.12 $\times 10^5$). Relatively lower concentration of colony forming units were observed in case of neem cake (13.14 $\times 10^5$), rice bran (11.57 $\times 10^5$), rice husk (1.12 $\times 10^5$), coconut oil cake (0.06 $\times 10^5$), coir pith compost (0.02 $\times 10^5$) and saw dust (0.008 $\times 10^5$) and exhibited statistical similarity.

Three months after storage of different inoculated substrates, the fungus cultured in cow dung recorded a concentration of 7.29 $\times 10^5$ cfu g^{-1}, which was the highest and differed significantly from other carrier materials. Cfu count in groundnut oil cake, rice husk, coconut oil cake, wheat bran, saw dust and rice bran were 2.08 $\times 10^5$, 1.14 $\times 10^5$, 0.96 $\times 10^5$, 0.99 $\times 10^5$, 0.82 $\times 10^5$ and 0.11 $\times 10^5$ cfu

Table 55. Colony forming units of *M. anisopliae* per gram of substrates at different intervals after storage under room temperature

Substrates	cfu g^{-1} ($\times 10^5$)		
	1MAS	2MAS	3MAS
Rice husk	0.004 (0.059)	1.125 (1.061)	1.14 (1.07)
Rice bran	0.005 (0.073)	11.573 (3.401)	0.11 (0.34)
Wheat bran	0.192 (0.438)	658.249 (25.656)	0.82 (0.90)
Coconut oil cake	0.007 (0.026)	0.069 (0.263)	0.96 (0.98)
Groundnut oil cake	0.002 (0.049)	207.121 (14.391)	2.08 (1.44)
Neem cake	0.007 (0.087)	13.147 (3.626)	0.07 (0.27)
Coir pith compost	0.008 (0.029)	0.027 (0.164)	0.01 (0.10)
Cow dung	23.986 (4.897)	266.982 (16.339)	7.29 (2.69)
Saw dust	0.001 (0.012)	0.008 (0.087)	0.70 (0.84)
C.D (0.05)	(1.3329)	(15.8031)	(1.155)

Figures in parentheses are square root transformed values

*MAS : Months After Storage

g^{-1} respectively and were on par. Neem cake recorded a mean concentration of 0.07×10^5 cfu g^{-1} and coir pith compost (0.13×10^5 cfu g^{-1}) had the lowest and were on par.

4.3.3 Bioefficacy of the fungi multiplied in different substrates

The bioefficacy of the fungi multiplied in different substrates was tested against the third instar grubs and newly emerged adults of *C. formicarius*.

4.3.3.1 B. bassiana

4.3.3.1.1 On adults of C. formicarius

The mortality of the adults of *C. formicarius* at 14 DAT when treated with spore suspension of *B. bassiana* cultured in cow dung and stored for one month was 38.84 per cent and it was statistically on par with the mortality caused by the spore suspension from wheat bran, which caused a mean mortality of 31.45 per cent (Table 56). The fungus cultured on neem cake, groundnut oil cake, rice bran, coconut oil cake and rice husk caused a moderate mortality with a mean percentage mortality of 20.30, 20.30, 18.45, 14.71 and 14.71, respectively and were statistically on par. Relatively lower mortality was recorded for spore suspension obtained from coir pith compost (11.11 per cent). The lowest mortality was recorded for the fungus cultured in saw dust (3.66 per cent).

The spore suspension of *B. bassiana* cultured in different substrates and stored for two months when applied on the adults of *C. formicarius* the maximum mortality was caused by the fungus cultured in cow dung (53.71 per cent) and it was on par with that in wheat bran (48.14 per cent). A mean mortality of 38.84, 33.26 and 31.45 was recorded for *B. bassiana* grown in neem cake, rice bran and groundnut oil cake respectively and were on par. Relatively lower mortality was observed when treated with coconut oil cake (22.05), coir pith compost (20.30) and rice husk (18.45) and showed statistical significance and were on par. Saw dust caused the lowest mortality of 14.71 per cent.

Table 56. Mean percentage mortality of *C. formicarius* when treated with spore suspensions of *B. bassiana* cultured in different substrates and stored for three months

Substrates	Mortality (%) of *C. formicarius* at 14 DAT					
	Adult			Grub		
	1MAS	2MAS	3MAS	1MAS	2MAS	3MAS
Rice husk	14.71 (22.55)	18.45 (25.43)	25.88 (30.57)	20.30 (26.77)	29.59 (32.95)	37.02 (37.46)
Rice bran	18.45 (25.43)	33.26 (35.20)	22.22 (28.11)	31.45 (34.09)	48.14 (43.92)	35.16 (36.35)
Wheat bran	31.45 (34.09)	48.14 (43.92)	31.45 (34.09)	55.58 (48.18)	66.74 (54.76)	40.73 (39.64)
Coconut oil cake	14.71 (22.55)	22.05 (27.99)	25.88 (30.57)	27.78 (31.79)	37.02 (37.46)	40.73 (39.64)
Groundnut oil cake	20.30 (26.77)	31.45 (34.09)	18.45 (25.43)	40.73 (39.64)	59.27 (50.32)	31.45 (34.09)
Neem cake	20.30 (26.77)	38.84 (38.53)	29.59 (32.95)	36.96 (37.43)	55.58 (48.18)	40.73 (39.64)
Coir pith compost	11.11 (19.46)	20.30 (26.77)	12.86 (21.00)	16.41 (23.89)	31.45 (34.09)	24.03 (29.34)
Cow dung	38.84 (38.53)	53.71 (47.11)	35.16 (36.35)	72.34 (58.25)	81.55 (64.53)	50.00 (44.98)
Saw dust	3.66 (11.03)	14.71 (22.55)	16.67 (24.09)	12.86 (21.00)	24.03 (29.34)	25.88 (30.57)
C.D (0.05)	(7.071)	(4.597)	(3.420)	(4.696)	(3.814)	(3.446)

Figures in parentheses are angular transformed values

*MAS : Months After Storage

When applied with spore suspension of different substrates stored for three months, the fungus grown on cow dung, wheat bran and neem cake caused 35.16 per cent, 31.45 per cent and 29.59 per cent mortality respectively and were on par. The mean mortality caused by the fungus multiplied in coconut oil cake, rice husk and rice bran were 25.88 per cent, 25.88 per cent and 22.22 per cent respectively and these were on par. Relatively lower mortality was found when treated with groundnut oil cake (18.45) and saw dust (16.67) which were on par. The least mortality of 12.86 per cent was recorded in fungus cultured in coir pith compost.

4.3.3.1.2 On grubs of C. formicarius

B. bassiana when applied against the grubs of *C. formicarius* after storage for one month in different substrates, maximum mortality was recorded for cow dung and wheat bran, which recorded mortality to the tune of 72.34 and 55.58 respectively, and these differed significantly from the effect of *B. bassiana* grown in other substrates (Table 56). A mean mortality of 40.73 per cent and 36.96 per cent was recorded for the groundnut oil cake and neem cake, respectively and were on par. When treated with rice bran and coconut oil cake, moderate per cent mortality of 31.45 and 27.78, respectively was caused and were statistically on par. Relatively lower mortality was caused by rice husk (20.30) and coir pith compost (16.41) and were on par. The lowest mortality of 12.86 per cent was recorded for saw dust.

After two months of storage the fungus grown on cow dung and wheat bran recorded a mean mortality of 81.55 per cent and 66.74 per cent respectively against the grubs and these differed significantly from the culture grown in other substrates. The mean mortality of 59.27 per cent and 55.58 per cent was recorded in the case of *B. bassiana* grown in groundnut oil cake and neem cake respectively and were on par. Rice bran based fungi caused a mean per cent mortality of 48.14 and differed significantly from the rest of the treatments. Culture in coconut oil cake and coir pith compost caused a mortality of 37.02 and 31.45 per cent respectively and were on par. The grubs of *C. formicarius* were

least affected by the fungi grown on rice husk and saw dust that recorded a mean mortality of 29.59 and 24.03 per cent, respectively which were on par.

The fungus cultured in cow dung recorded a mean mortality of 50 per cent and differed significantly from that in other substrates at three months after storage. The pathogenicity of fungi cultured in coconut oil cake, neem cake, wheat bran, rice husk and rice bran recorded a mortality of 40.73, 40.73, 40.73, 37.02 and 35.16 per cent, respectively and were on par. The mean mortality percentage of 31.45, 25.88 and 24.03 were noted when sprayed with spore suspension of the fungi grown in groundnut oil cake, saw dust and coir pith compost respectively which were statistically on par in their pathogenicity.

4.3.3.2 M. anisopliae

4.3.3.2.1 On adults of C. formicarius

When the spore suspension from different carrier materials inoculated with *M. anisopliae* and stored for one month was applied on the adults of *C. formicarius*, significant highest mortality was recorded for cow dung (31.45 per cent) (Table 57). The rate of mortality caused for fungi grown in wheat bran, groundnut oil cake and rice bran were 20.30, 14.71 and 12.86 per cent respectively and were statistically on par. The substrates *viz.* neem cake, rice husk and coconut oil cake caused a mean mortality of 9.07, 7.22 and 6.29 per cent respectively and were on par. The coir pith and sawdust inoculated with the fungus could not cause any pathogenicity.

When applied with spore suspension of fungi stored for two months, the cow dung and wheat bran cultured fungus caused a mean mortality of 38.84 and 35.16 per cent, respectively and were on par. Mortality in the range of 29.59, 20.30, 18.45, 18.45 and 16.67 per cent was recorded for groundnut oil cake, rice bran, neem cake, rice husk and coconut oil cake respectively and were statistically on par. The least mortality of 9.07 per cent was recorded for both the coir pith compost and saw dust.

Table 57. Mean percentage mortality of *C. formicarius* when treated with spore suspensions of *M. anisopliae* cultured in different substrates and stored for three months

Substrates	Mortality (%) of *C. formicarius* at 14 DAT					
	Adult			Grub		
	1MAS	2MAS	3MAS	1MAS	2MAS	3MAS
Rice husk	7.22 (15.58)	18.45 (25.43)	7.22 (15.58)	14.71 (22.55)	24.03 (29.34)	12.86 (21.00)
Rice bran	12.86 (21.00)	20.30 (26.77)	2.49 (9.09)	24.03 (29.34)	37.02 (37.46)	12.86 (21.00)
Wheat bran	20.30 (26.77)	35.16 (36.35)	18.45 (25.43)	35.16 (36.35)	55.58 (48.18)	38.84 (38.53)
Coconut oil cake	6.29 (14.52)	16.67 (24.09)	20.30 (26.77)	24.03 (29.34)	35.16 (36.35)	42.58 (40.72)
Groundnut oil cake	14.71 (22.55)	29.59 (32.95)	18.45 (25.43)	31.45 (34.09)	40.73 (39.64)	29.40 (32.82)
Neem cake	9.07 (17.52)	18.45 (25.43)	20.30 (26.77)	24.03 (29.34)	40.73 (39.64)	38.84 (38.53)
Coir pith compost	0 (0)	9.07 (17.52)	0.63 (4.54)	2.49 (9.09)	18.45 (25.43)	9.07 (17.52)
Cow dung	31.45 (34.09)	38.84 (38.53)	27.66 (31.72)	48.14 (43.92)	72.34 (58.25)	53.71 (47.11)
Saw dust	0 (0)	9.07 (17.52)	2.49 (9.09)	0 (0)	20.30 (26.77)	7.22 (15.58)
C.D (0.05)	(8.009)	(4.202)	(8.375)	(5.869)	(4.071)	(5.312)

Figures in parentheses are angular transformed values

*MAS : Months After Storage

The mortality observed was 27.66, 20.30, 20.30, 18.45 and 18.45 per cent for fungus cultured in cow dung, coconut oil cake, neem cake, groundnut oil cake and wheat bran respectively and were on par. The fungus cultured on rice husk, rice bran and saw dust caused a mortality of 7.22, 2.49 and 2.49 per cent respectively and were on par. The lowest mortality was observed when treated with coir pith compost (0.63) at the third month after storage.

4.3.3.2.2 On Grubs of C. formicarius

At one month after storage, *M. anisopliae* grown on cow dung produced significantly higher mortality of 48.14 per cent. The mean mortality caused by fungus in wheat bran and groundnut oil cake were 35.16 and 31.45 per cent respectively and showed statistical parity (Table 57). The fungus grown in coconut oil cake, rice bran and neem cake had same pathogenicity on the grubs of *C. formicarius* and recorded a mortality of 24.03 per cent each and were on par. Relatively lower mortality was observed for the fungus in rice husk (14.71) and the lowest was recorded in the case of *M. anisopliae* in coir pith compost (2.49), whereas the fungus grown in saw dust could not cause any mortality.

The fungus cultured in cow dung caused significantly higher mortality of 72.34 per cent, followed by that in wheat bran (55.58) at two months after storage. The mortality caused by *M. anisopliae* in groundnut oil cake and neem cake were similar and recorded 40.73 per cent mortality and was on par with that in rice bran (37.02) and coconut oil cake (35.16 per cent). Mean mortality percentages of 24.03, 20.30 and 18.45 were caused by the fungus grown on substrates viz. rice husk, saw dust and coir pith compost, respectively and were on par.

At three months after storage, the fungus mass multiplied in cow dung caused a mean mortality of 53.71 per cent and showed statistical similarity with that in coconut oil cake (42.58). Neem cake and wheat bran caused the same effect to the grubs of *C. formicarius* and each recorded a mean mortality of 38.84 per cent each and were on par. The mortality observed for groundnut oil cake was 29.40 per cent. Rice husk and rice bran caused a mean percentage mortality of

12.86 each and were on par with coir pith compost (9.07). The least mortality was caused in the case of saw dust (7.22 per cent).

4.3.4 Formulation of the fungi

4.3.4.1 B. bassiana

The talc based formulation of *B. bassiana* recorded the highest spore count of 21.05 × 10^9 spores ml^{-1}at one month after storage, followed by 9.99 × 10^9 spores ml^{-1} in the second month of storage, which was on par with the spore load recorded during the third month after storage (1.57 × 10^9 spores ml^{-1}) (Table 58).

The concentration of viable spores in the talc formulation during one month after storage under room temperature was significantly higher and recorded 3.43 × 10^9 cfu g^{-1}. At two months after storage, the viable spore load in the talc formulation obtained was 0.87 × 10^9 cfu g^{-1}, which was on par with the cfus during the third month after storage (0.20 × 10^9 cfu g^{-1}).

The mean mortality of adult *C. formicarius* when applied with spore suspensions during the first month after storage of the formulation was 88.83 per cent at 14 days after treatment, which was on par with that treated during the second and third months after storage, where the percentage mortality was 87.02 and 85.16 respectively.

When the grubs of *C. formicarius* were subjected to treatment with the talc based formulation of *B. bassiana*, 100 per cent mortality was observed during the first, second and third months after storage of the formulation and showed statistical parity.

4.3.4.2 M. anisopliae

The spore load present in the *M. anisopliae* formulated talc at one month after storage under room temperature was significantly higher when compared to the other months and recorded a mean spore count of 1.86 × 10^9 spores ml^{-1}. During the second month after storage, a spore concentration of 0.33 × 10^9 spores ml^{-1} was obtained, which was on par with the spore count during the third month after storage (0.29 × 10^9 spores ml^{-1}) (Table 59).

Table 58. Mean spore count and cfu of talc based formulation of *B. bassiana* at different intervals after storage and its bioefficacy

Sl.No.	Months after storage	Spore count (spores ml^{-1}) $\times 10^9$	cfu (cfu g^{-1}) $\times 10^9$	Mean percentage mortality of *C. formicarius* at 14 DAT	
				Adult	Grub
1	1 MAS	21.05 (4.59)	3.43 (1.85)	88.83 (9.42)	100.00 (10.00)
2	2 MAS	9.99 (3.16)	0.87 (0.93)	87.02 (9.33)	100.00 (10.00)
3	3 MAS	1.57 (1.25)	0.20 (0.45)	85.16 (9.23)	100.00 (10.00)
	C.D (0.05)	(3.551)	(0.659)	(0.442)	(0.00)

Figures in parentheses are square root transformed values

*MAS – Months after storage

Table 59. Mean spore count and cfu of talc based formulation of *M. anisopliae* at different intervals after storage and its bioefficacy

Sl.No.	Months after storage	Spore count (spores ml^{-1}) $\times 10^9$	cfu (cfu g^{-1}) $\times 10^9$	Mean percentage mortality of *C. formicarius* at 14 DAT	
				Adult	Grub
1	1 MAS	1.86 (1.36)	0.97 (0.98)	66.59 (8.16)	77.71 (8.82)
2	2 MAS	0.33 (0.57)	0.41 (0.64)	62.94 (7.93)	75.90 (8.71)
3	3 MAS	0.29 (0.54)	0.24 (0.48)	55.46 (7.45)	68.49 (8.28)
	C.D (0.05)	(0.782)	(0.267)	(0.627)	(0.476)

Figures in parentheses are square root transformed values

*MAS – Months after storage

The viability of the talc formulation was significantly higher at one month after storage with a concentration of 0.97×10^9 cfu g^{-1}. The concentration of viable spores at two months after storage was 0.41×10^9 cfu g^{-1} which was on par with the cfu during the third month after storage (0.24×10^9 cfu g^{-1}).

When the efficacy of the formulated *M. anisopliae* on the adults of *C. formicarius* was tested, the mean mortality of the fungus stored for one, two and three months after storage came to 66.59, 62.94 and 55.46 per cent at 14 days after treatment respectively and were on par.

For the grubs of *C. formicarius* the mean percentages of mortality recorded were 77.71, 75.90 and 68.49 when treated with the talc formulation after storage for one, two and three months, respectively, and were on par.

4.4 FIELD EVALUATION

Field efficacy of both the fungi was tested in banana and sweet potato and the results are presented in Tables 60 to 81.

4.4.1 Banana

4.4.1.1 Population of C. sordidus in rhizome and intensity of damage

The control plants without any treatment recorded significantly higher number of galleries in the rhizome when compared to the other treatments and had a mean of 8.33 galleries (Table 60). When *M. anisopliae* grown in cow dung was applied, the mean number of galleries was 6.67 and was on par with *B. bassiana* in neem cake, *B. bassiana* in cow dung, *M. anisopliae* @ 5×10^{10} spores ml^{-1}, *B. bassiana* @ 5×10^9 spores ml^{-1} and *M. anisopliae* @ 5×10^{12} spores ml^{-1} showed mean galleries of 6.67, 6.64, 5.98, 5.32 and 5.27, respectively. The damage intensity was less in the treatments when applied with *M. anisopliae* in neem cake @ 50 g, talc based *M. anisopliae* @ 20 g l^{-1} and talc based *M. anisopliae* @ 30 g l^{-1} in which the mean number of galleries made by *C. sordidus* grubs were 4.32, 4.32 and 4.00, respectively and showed statistical

Table 60. Mean number of galleries and grubs in rhizome in the various treatments during the first field trial in banana

Sl.No.	Treatments	No. of galleries	No. of grubs
T1	*B. bassiana* @ 5×10^9 spores ml^{-1}	5.32 (2.51)	3.65 (2.16)
T2	*B. bassiana* @ 5×10^{11} spores ml^{-1}	1.31 (1.52)	2.32 (1.82)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	2.32 (1.82)	2.96 (1.99)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	0.91 (1.38)	0.91 (1.38)
T5	*M. anisopliae* @ 5×10^{10} spores ml^{-1}	5.98 (2.64)	6.66 (2.77)
T6	*M. anisopliae* @ 5×10^{12} spores ml^{-1}	5.27 (2.50)	5.98 (2.64)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	4.32 (2.31)	6.66 (2.77)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	4.00 (2.24)	3.97 (2.23)
T9	*B. bassiana* in cow dung @ 50 g	6.64 (2.76)	7.98 (2.99)
T10	*M. anisopliae* in cow dung @ 50 g	6.67 (2.76)	9.33 (3.21)
T11	*B. bassiana* in neem cake @ 50 g	6.67 (2.75)	5.32 (2.51)
T12	*M. anisopliae* in neem cake @ 50 g	4.32 (2.31)	4.00 (2.24)
T13	Carbofuran 3G @ 20 g $plant^{-1}$	0.99 (1.41)	1.31 (1.52)
T14	Chlorpyriphos 0.03 %	0.63 (1.28)	0.29 (1.14)
T15	Control (Untreated)	8.33 (3.05)	12.27 (3.64)
	C.D (0.05)	(0.352)	(0.319)

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

similarity. A mean number of 2.32, 1.31, 0.99 and 0.91 galleries were recorded in the treatments with *B. bassiana* in talc @ 20 g l^{-1}, *B. bassiana* @ 5×10^{11} spores ml^{-1}, carbofuran 3G @ 20 g $plant^{-1}$ and *B. bassiana* in talc @ 30 g l^{-1} and were statistically on par. The plants drenched with chlorpyriphos 0.03 per cent recorded significantly lower number of 0.63 galleries per rhizome and differed significantly from the other treatments.

The control treatment recorded the maximum number of grubs in the rhizome and the mean number recorded was 12.27 and showed statistical significance from the other treatments. The population of pests in the treatments with *M. anisopliae* in cow dung @ 50 g and *B. bassiana* in cow dung @ 50 g were 9.33 and 7.98, respectively and were on par. *M. anisopliae* in talc @ 20 g l^{-1} and *M. anisopliae* @ 5×10^{10} spores ml^{-1} recorded same number of pests in rhizome (6.66) and were on par. When *M. anisopliae* @ 5×10^{12} spores ml^{-1} was applied the mean population of pests recorded were 5.98 and was on par with the treatment *B. bassiana* in neem cake @ 50 g (5.32). The population of *C. sordidus* grubs obtained were 4.00, 3.97, 3.65 and 2.96 in the treatments *M. anisopliae* in neem cake @ 50 g, *M. anisopliae* in talc @ 30 g l^{-1}, *B. bassiana* @ 5×10^{9} spores ml^{-1} and *B. bassiana* in talc @ 20g l^{-1} respectively and were on par. Relatively lower number of pests were observed in the treatments *B. bassiana* @ 5×10^{11} spores ml^{-1} , carbofuran 3G @ 20 g $plant^{-1}$, and *B. bassiana* in talc @ 30g l^{-1} which had 2.32, 1.31 and 0.91, respectively and were on par. The treatment with chemical pesticide chlorpyriphos 0.03 per cent recorded significantly lower pest count of 0.29 and differed from all the other treatments.

4.4.1.2 Population of C. sordidus in soil in the main crop

The population of pests around the treated plants were 4.13, 3.86, 3.62, 3.32, 2.65 and 2.61 in the treatments carbofuran 3G @ 20 g $plant^{-1}$, *M. anisopliae* in cow dung @ 50 g, control, *M. anisopliae* in neem cake @ 50 g, chlorpyriphos 0.03 per cent and *M. anisopliae* @ 5×10^{10} spores ml^{-1}, respectively, and showed statistical similarity (Table 61). The mean pest count in the treatments with

Table 61. Mean population of *C. sordidus* in soil and the yield in the various treatments during the first field trial in banana

Sl. No.	Treatments	No. of *C. sordidus* kg^{-1} soil	Yield Plant^{-1} (kg)	Yield (t ha^{-1})	B : C ratio
T1	*B. bassiana* @ 5×10^9 spores ml^{-1}	1.59 (1.61)	5.85 (2.62)	14.63 (3.82)	0.90
T2	*B. bassiana* @ 5×10^{11} spores ml^{-1}	0.91 (1.38)	9.68 (3.27)	24.20 (4.92)	1.52
T3	Talc based *B. bassiana* @ 20 g l^{-1}	0.29 (1.14)	7.33 (2.89)	18.33 (4.28)	1.14
T4	Talc based *B. bassiana* @ 30 g l^{-1}	0 (1.00)	10.01 (3.32)	25.03 (5.00)	1.57
T5	*M. anisopliae* @ 5×10^{10} spores ml^{-1}	2.61 (1.90)	5.57 (2.56)	13.93 (3.73)	0.85
T6	*M. anisopliae* @ 5×10^{12} spores ml^{-1}	1.59 (1.61)	5.86 (2.62)	14.65 (3.83)	0.90
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	0.99 (1.41)	5.28 (2.51)	13.20 (3.63)	0.81
T8	Talc based *M. anisopliae* @ 30g l^{-1}	0.63 (1.28)	8.08 (3.01)	20.20 (4.49)	1.26
T9	*B. bassiana* in cow dung @ 50 g	1.59 (1.61)	5.64 (2.58)	14.10 (3.75)	0.87
T10	*M. anisopliae* in cow dung @ 50 g	3.86 (2.20)	4.11 (2.26)	10.28 (3.21)	0.62
T11	*B. bassiana* in neem cake @ 50 g	1.64 (1.63)	5.26 (2.50)	13.15 (3.63)	0.81
T12	*M. anisopliae* in neem cake @ 50 g	3.32 (2.08)	4.59 (2.37)	11.48 (3.39)	0.69
T13	Carbofuran 3G @ 20 g plant^{-1}	4.13 (2.27)	8.45 (3.07)	21.13 (4.59)	0.79
T14	Chlorpyriphos 0.03 %	2.65 (1.91)	12.91 (3.73)	32.28 (5.68)	1.24
T15	Control (Untreated)	3.62 (2.15)	6.18 (2.68)	15.45 (3.93)	-
	C.D (0.05)	(0.477)	(0.189)	(0.957)	-

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

M. anisopliae @ 5 × 10^{12} spores ml^{-1}, *B. bassiana* in cow dung @ 50 g and *B. bassiana* @ 5 × 10^{9} spores ml^{-1} was 1.59 each and was on par with *B. bassiana* in neem cake @ 50 g (1.64). A mean population of 0.99, 0.91, 0.63 and 0.29 was recorded when applied with *M. anisopliae* in talc @ 20 g l^{-1}, *B. bassiana* @ 5 × 10^{11} spores ml^{-1}, *M. anisopliae* in talc @ 30 g l^{-1} and *B. bassiana* in talc @ 20 g l^{-1}, respectively and were on par. In the plots where *B. bassiana* in talc @ 30 g l^{-1} was applied, no pests could be collected from soil.

4.4.1.3 Yield

During the first crop, significantly higher yield of 12.91 kg was recorded in the plots treated with chlorpyriphos 0.03 per cent (Table 61). The yield obtained from the treatments *B. bassiana* in talc @ 30 g l^{-1} and *B. bassiana* @ 5 × 10^{11} spores ml^{-1} were 10.01 and 9.68 kg respectively and were on par. From the carbofuran treated plants the yield obtained was 8.45 kg and was on par with that in *M. anisopliae* in talc @ 30 g l^{-1} with a mean yield of 8.08 kg, which was on par with the plants treated with *B. bassiana* in talc @ 20 g l^{-1} where a mean yield of 7.33 kg was obtained. The yield of bunches recorded in control, *M. anisopliae* @ 5 × 10^{12} spores ml^{-1}, *B. bassiana* @ 5 × 10^{9} spores ml^{-1}, *B. bassiana* in cow dung @ 50g, *M. anisopliae* @ 5 × 10^{10} spores ml^{-1}, *M. anisopliae* talc @ 20 g l^{-1} and *B. bassiana* in neem cake @ 50 g were 6.18, 5.86, 5.85, 5.64, 5.57, 5.28 and 5.26 kg respectively and were on par. The least yield was obtained in the case of *M. anisopliae* in neem cake @ 50 g and *M. anisopliae* in cow dung @ 50 g which produced a mean yield of 4.59 and 4.11 kg respectively and were on par.

The average yield per hectare ranged from 32.28 to 10.28 tons in the various treatments.

4.4.1.4 B : C Ratio

The highest B : C ratio of 1.57 was obtained for the treatment with *B. bassiana* in talc @ 30 g l^{-1} followed by the spore suspension with *B. bassiana* @ 5 × 10^{11} spores ml^{-1} which recorded a B : C ratio of 1.52 (Table 61). The B : C ratio for the treatment with *M. anisopliae* in talc @ 30 g l^{-1} was 1.26 and

chlorpyriphos 0.03 per cent obtained a B : C ratio of 1.24. The B : C ratio ranged between 1.14 and 0.62 for the other treatments.

4.4.1.5 Population of C. sordidus in rhizome and intensity of damage

In the ratoon crop, significantly higher number of galleries were observed in control and the mean number was 10.94 galleries (Table 62). Relatively higher damage was recorded in the rhizomes treated with *M. anisopliae* in cow dung @ 50 g, *M. anisopliae* @ 5×10^{10} spores ml^{-1}, carbofuran @ 20 g $plant^{-1}$, chlorpyriphos 0.03 per cent and *M. anisopliae* @ 5×10^{12} spores ml^{-1} with a mean number of 4.66, 4.66, 4.29, 3.58 and 3.65 galleries respectively and were on par. A mean number of 2.65 galleries per rhizome each was observed in the treatments *M. anisopliae* in talc @ 20 g l^{-1} and *B. bassiana* in cow dung @ 50 g and was on par with that in *B. bassiana* @ 5×10^{9} spores ml^{-1}, *B. bassiana* in neem cake @ 50 g and *M. anisopliae* in neem cake @ 50 g which recorded 2.32 galleries each respectively. The treatments with *M. anisopliae* in talc @ 30 g l^{-1} and *B. bassiana* in talc @ 20 g l^{-1} recorded a mean number of 1.64 galleries each, respectively and was on par with the treatments *B. bassiana* in talc @ 30 g l^{-1} and *B. bassiana* @ 5 $\times 10^{11}$ spores ml^{-1} which produced the least number of 0.63 galleries each, respectively upon application of the treatment.

The mean number of the grubs in the control was significantly high and was 15.65, followed by *M. anisopliae* in cow dung @ 50 g which recorded 5.66 insects per rhizome and exhibited statistical significance. The mean pest count in the *M. anisopliae* @ 5×10^{10} spores ml^{-1}, *M. anisopliae* @ 5×10^{12} spores ml^{-1}, *M. anisopliae* in talc @ 20 g l^{-1} and *B. bassiana* in cow dung @ 50 g treatments were 4.29, 4.32, 3.32 and 3.32, respectively and had statistical similarity. A mean pest count of 2.65 each was recorded in the *B. bassiana* in neem cake @ 50 g, carbofuran @ 20 g $plant^{-1}$ and *M. anisopliae* in talc @ 30 g l^{-1} treatments, respectively and were on par. The *M. anisopliae* in neem cake @ 50 g applied plants recorded a mean pest population of 2.00 and it was on par with the treatment with *B. bassiana* @ 5×10^{9} spores ml^{-1} which had a mean pest count of

Table 62. Mean number of galleries and grubs in rhizome in banana in the various treatments during the succeeding crop

Sl. No.	Treatments	No. of galleries	No. of grubs
T1	*B. bassiana* @ 5×10^9 spores ml^{-1}	2.32 (1.82)	1.64 (1.63)
T2	*B. bassiana* @ 5×10^{11} spores ml^{-1}	0.63 (1.28)	0.91 (1.38)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	1.64 (1.63)	1.21 (1.49)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	0.63 (1.28)	0.29 (1.14)
T5	*M. anisopliae* @ 5×10^{10} spores ml^{-1}	4.66 (2.38)	4.29 (2.29)
T6	*M. anisopliae* @ 5×10^{12} spores ml^{-1}	3.65 (2.16)	4.32 (2.31)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	2.65 (1.91)	3.32 (2.08)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	1.64 (1.63)	2.65 (1.91)
T9	*B. bassiana* in cow dung @ 50 g	2.65 (1.91)	3.32 (2.08)
T10	*M. anisopliae* in cow dung @ 50 g	4.66 (2.38)	5.66 (2.58)
T11	*B. bassiana* in neem cake @ 50 g	2.32 (1.82)	2.65 (1.91)
T12	*M. anisopliae* in neem cake @ 50 g	2.32 (1.82)	2.00 (1.73)
T13	Carbofuran 3G @ 20 g $plant^{-1}$	4.29 (2.29)	2.65 (1.91)
T14	Chlorpyriphos 0.03 %	3.58 (2.14)	0.91 (1.38)
T15	Control (Untreated)	10.94 (3.46)	15.65 (4.08)
	C.D (0.05)	(0.343)	(0.371)

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

1.64. The treatments with *B. bassiana* in talc @ 20 g l^{-1}, chlorpyriphos 0.03 per cent and *B. bassiana* @ 5 × 10^{11} spores ml^{-1} application resulted in a mean pest count of 1.21, 0.91 and 0.91, respectively and showed statistical similarity. The treatment with *B. bassiana* in talc @ 30 g l^{-1} recorded the least population of *C. sordidus* with a mean count of 0.29.

4.4.1.6 Population of C. sordidus in soil in the succeeding crop

The pest population in soil around the plants treated with carbofuran @ 20 g $plant^{-1}$, *M. anisopliae* in cow dung @ 50 g, control and chlorpyriphos 0.03 per cent were 5.32, 4.29, 4.32 and 3.97 respectively and were on par (Table 63). A mean population of 3.97, 2.96, 2.65, 2.65 and 2.48 was recorded in the treatments involving *B. bassiana* @ 5 × 10^{9} spores ml^{-1}, *B. bassiana* in cow dung @ 50 g, *M. anisopliae* @ 5 × 10^{12} spores ml^{-1}, *B. bassiana* @ 5 × 10^{11} spores ml^{-1} and *M. anisopliae* @ 5 × 10^{10} spores ml^{-1} respectively and had statistical similarity. The population in the plants treated with *M. anisopliae* in neem cake @ 50 g, *B. bassiana* in neem cake @ 50 g, talc based *M. anisopliae* @ 20 g l^{-1}, talc based *M. anisopliae* @ 30 g l^{-1} and talc based *B. bassiana* @ 20 g l^{-1} was 2.22, 1.94, 1.94, 1.74 and 1.31, respectively and were on par. The plants treated with *B. bassiana* in talc @ 30 g l^{-1} recorded the least pest count of 0.91 per rhizome.

4.4.1.7 Yield

The plants treated with chlorpyriphos 0.03 per cent yielded significantly higher quantity of 10.88 kg banana when compared to the other treatments (Table 63). The yield obtained in the treatments with *B. bassiana* in talc @ 30 g l^{-1} and carbofuran @ 20 g $plant^{-1}$ were 8.29 and 7.98 kg and were on par. The application of *B. bassiana* @ 5 × 10^{11} spores ml^{-1} recorded a mean yield of 6.99 kg. The yield recorded after the application of treatments with *B. bassiana* in talc @ 20 g l^{-1}, *M. anisopliae* in talc @ 30 g l^{-1} and *B. bassiana* @ 5 × 10^{9} spores ml^{-1} were 5.83, 5.76 and 5.13 kg, respectively and were statistically on par. A mean yield of 4.75, 4.73, 4.50, 4.49 and 4.47 kg was recorded when treated with *B. bassiana* in neem cake @ 50 g, *M. anisopliae* @ 5 × 10^{10}

Table 63. Mean population of *C. sordidus* in soil and the yield in the various treatments during the succeeding crop of banana

Sl. No.	Treatments	No. of *C.sordidus* kg^{-1} soil	Yield Plant^{-1} (kg)	Yield (t ha^{-1})	B : C ratio
T1	*B. bassiana* @ 5×10^9 spores ml^{-1}	3.97 (2.23)	5.13 (2.48)	12.83 (3.58)	1.08
T2	*B. bassiana* @ 5×10^{11} spores ml^{-1}	2.65 (1.91)	6.99 (2.83)	17.48 (4.18)	1.49
T3	Talc based *B. bassiana* @ 20 g l^{-1}	1.31 (1.52)	5.83 (2.61)	14.58 (3.82)	1.23
T4	Talc based *B. bassiana* @ 30 g l^{-1}	0.91 (1.38)	8.29 (3.05)	20.73 (4.55)	1.78
T5	*M. anisopliae* @ 5×10^{10} spores ml^{-1}	2.48 (1.87)	4.73 (2.39)	11.83 (3.44)	0.99
T6	*M. anisopliae* @ 5×10^{12} spores ml^{-1}	2.65 (1.91)	4.36 (2.32)	10.90 (3.30)	0.91
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	1.94 (1.72)	4.49 (2.34)	11.23 (3.35)	0.94
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	1.74 (1.66)	5.76 (2.60)	14.40 (3.79)	1.22
T9	*B. bassiana* in cow dung @ 50 g	2.96 (1.99)	4.47 (2.34)	11.18 (3.34)	0.94
T10	*M. anisopliae* in cow dung @ 50 g	4.29 (2.29)	3.66 (2.16)	9.15 (3.02)	0.76
T11	*B. bassiana* in neem cake @ 50 g	1.94 (1.72)	4.75 (2.39)	11.88 (3.45)	0.99
T12	*M. anisopliae* in neem cake @ 50 g	2.22 (1.79)	3.99 (2.24)	9.98 (3.16)	0.82
T13	Carbofuran 3G @ 20 g plant^{-1}	5.32 (2.51)	7.98 (2.99)	19.95 (4.47)	1.03
T14	Chlorpyriphos 0.03 %	3.97 (2.23)	10.88 (3.45)	27.20 (5.22)	1.43
T15	Control (Untreated)	4.32 (2.31)	4.50 (2.35)	11.25 (3.35)	-
	C.D (0.05)	(0.538)	(0.145)	(0.597)	-

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

spores ml^{-1}, control, *M. anisopliae* in talc @ 20g l^{-1} and *B. bassiana* in cow dung @ 50 g respectively and were on par. Relatively lower yield of 4.36 and 3.99 kg was obtained when treated with *M. anisopliae* @ 5×10^{12} spores ml^{-1} and *M. anisopliae* in neem cake @ 50 g respectively and was on par with that received in *M. anisopliae* in cow dung @ 50 g treatment and recorded a mean yield of 3.66 kg.

The average yield of the crop ranged from 27.20 to 9.15 tons per hectare for the different treatments.

4.4.1.8 B : C Ratio

The maximum B : C ratio of 1.78 was recorded for the treatment with talc based *B. bassiana* @ 30 g l^{-1} followed by *B. bassiana* @ 5×10^{11} spores ml^{-1} and chlorpyriphos 0.03 per cent which had a B : C ratio of 1.49 and 1.43 respectively (Table 63). The B : C ratios ranged from 1.23 to 0.76 for the other treatments.

4.4.2 Sweet potato

4.4.2.1 First field trial with soil drenching

4.4.2.1.1 Population of weevils in the foliage

One week after the application of treatments, the plots treated with imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent had no weevils and were on par and differed significantly from others (Table 64). The maximum population of weevils was recorded from the treatments with *B. bassiana* and *M. anisopliae* in cow dung @ 50 g which had a mean population of 7.00 weevils each, respectively and was on par with the untreated control which recorded a mean population of 6.33. The mounds treated with *M. anisopliae* @ 10^{12} spores ml^{-1}, *M. anisopliae* @ 10^{10} spores ml^{-1}, *B. bassiana* @ 10^{9} spores ml^{-1}, *M. anisopliae* talc @ 20 g l^{-1} and *B. bassiana* @ 10^{11} spores ml^{-1} recorded a mean population of 5.00, 5.00, 4.60, 4.00 and 4.00 respectively and were on par. The talc based formulations of *M. anisopliae* @ 30 g l^{-1} and *B. bassiana* @ 20 g l^{-1} treated plots recorded a mean population of 3.33 each respectively and was on par

Table 64. Population of adult *C. formicarius* in the foliage of sweet potato during first field trial with soil drenching

Sl. No.	Treatments	Pre count	Number of adult weevils (in 0.19 m^2)							
			1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT
T1	*B. bassiana* @ 10^9 spores ml^{-1}	10.00 (3.14)	4.60 (2.15)	4.25 (2.06)	2.87 (1.69)	2.33 (1.53)	3.46 (1.86)	5.35 (2.31)	3.95 (1.99)	7.35 (2.71)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	6.33 (2.51)	4.00 (1.99)	3.79 (1.95)	3.41 (1.85)	2.67 (1.63)	3.23 (1.79)	4.81 (2.19)	4.21 (2.05)	6.11 (2.47)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	10.33 (3.21)	3.33 (1.79)	3.26 (1.81)	2.64 (1.62)	2.67 (1.63)	2.89 (1.70)	3.62 (1.90)	2.27 (1.51)	4.26 (2.06)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	6.33 (2.51)	3.00 (1.72)	2.53 (1.59)	1.38 (1.17)	2.00 (1.41)	2.00 (1.41)	3.33 (1.82)	2.39 (1.55)	3.19 (1.79)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	8.33 (2.85)	5.00 (2.23)	4.83 (2.19)	5.00 (2.24)	3.27 (1.81)	3.62 (1.90)	6.24 (2.49)	4.86 (2.20)	8.67 (2.94)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	5.67 (2.37)	5.00 (2.24)	7.00 (2.65)	3.27 (1.81)	3.33 (1.82)	3.33 (1.82)	7.13 (2.67)	5.49 (2.34)	7.91 (2.81)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	9.67 (3.11)	4.00 (1.99)	5.00 (2.24)	4.60 (2.14)	2.67 (1.63)	4.27 (2.07)	5.41 (2.33)	3.19 (1.79)	6.32 (2.51)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	7.67 (2.73)	3.33 (1.82)	5.00 (2.24)	3.33 (1.82)	2.00 (1.41)	3.33 (1.82)	4.99 (2.23)	3.23 (1.79)	5.00 (2.24)
T9	*B. bassiana* in cow dung @ 50 g	5.33 (2.31)	7.00 (2.64)	5.00 (2.24)	5.67 (2.38)	4.00 (2.00)	5.33 (2.31)	4.25 (2.06)	3.92 (1.98)	5.00 (2.24)
T10	*M. anisopliae* in cow dung @ 50 g	8.33 (2.87)	7.00 (2.63)	4.00 (2.00)	6.33 (2.52)	7.00 (2.65)	8.25 (2.87)	6.27 (2.50)	5.34 (2.31)	6.42 (2.53)
T11	*B. bassiana* in neem cake @ 50 g	5.00 (2.21)	2.00 (1.41)	3.25 (1.80)	3.13 (1.77)	5.00 (2.23)	3.63 (1.91)	3.98 (1.99)	4.26 (2.06)	4.33 (2.08)
T12	*M. anisopliae* in neem cake @ 50 g	6.00 (2.43)	2.33 (1.52)	6.11 (2.47)	5.00 (2.24)	5.00 (2.23)	5.00 (2.23)	5.00 (2.23)	6.47 (2.54)	7.67 (2.77)
T13	Imidacloprid 0.006 per cent	9.33 (3.05)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3.13 (1.77)	2.61 (1.62)	0.64 (0.80)
T14	Lambda cyhalothrin 0.025 per cent	6.33 (2.51)	0 (0)	0 (0)	2.33 (1.53)	2.33 (1.53)	3.33 (1.82)	5.46 (2.34)	3.95 (1.99)	3.63 (1.91)
T15	Control (Untreated)	5.33 (2.29)	6.33 (2.51)	9.34 (3.06)	13.42 (3.66)	8.19 (2.86)	9.67 (3.11)	12.38 (3.52)	10.08 (3.17)	11.89 (3.45)
	C.D (0.05)	(0.589)	(0.376)	(0.597)	(1.025)	(1.117)	(1.191)	(1.511)	(0.635)	(0.758)

Figures in parentheses are square root transformed values

with *B. bassiana* talc @ 30 g l^{-1} treatment which recorded a population of 3.00. Relatively lower population of weevils was observed in the mounds treated with *B. bassiana* and *M. anisopliae* cultured in neem cake and applied @ 50 g each per mound which recorded a mean population of 2.00 and 2.33 respectively and were on par.

There were no weevils in the imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent treated plots at two WAT and found to be superior when compared to the other treatments and were on par. The untreated control plot recorded significantly higher population of 9.34 weevils, followed by the treatment with *M. anisopliae* @ 10^{12} spores ml^{-1} (7.00) and *M. anisopliae* in neem cake @ 50 g (6.11) and were on par. The mean population of weevils ranged from 2.53 to 5.00 in the other treatments.

The imidacloprid 0.006 per cent treated plot did not record any weevil population even after three weeks from the treatment. The lowest number of weevils was recorded from the plots treated with *B. bassiana* in talc @ 30 g l^{-1} which had a mean population of 1.38 and was on par with lambda cyhalothrin 0.025 per cent treated plot (2.33). The control plot without any treatment recorded significantly higher number of weevils (13.42). The mean population of weevils in the other treatments were 2.64 to 6.33.

During the fourth week after treatment application the imidacloprid 0.006 per cent treated plot had no weevils. The lowest number of weevils was observed in the plots treated with *B. bassiana* in talc @ 30 g l^{-1} and *M. anisopliae* in talc @ 30 g l^{-1} which had a mean population of 2.00 weevils $mound^{-1}$ each respectively and were on par with lambda cyhalothrin 0.025 per cent, *B. bassiana* @ 10^{9} spores ml^{-1}, *M. anisopliae* in talc @ 20 g l^{-1}, *B. bassiana* @ 10^{11} spores ml^{-1} and *B. bassiana* in talc @ 20 g l^{-1} which recorded a mean population of 2.33, 2.33, 2.67, 2.67 and 2.67 each respectively. The weevil count ranged from 3.27 to 8.19 in the other treatments.

In the fifth week, as observed earlier, the population of weevils was not encountered in the mounds treated with imidacloprid 0.006 per cent and found to be superior among the other treatments. The mean population of weevils recorded from the plots treated with talc based formulations of *B. bassiana* @ 30 g l^{-1} and *B. bassiana* @ 20 g l^{-1} were 2.00 and 2.89, respectively and were on par. The untreated control recorded a higher mean population of 9.67 weevils as seen earlier. The population of weevils ranged between 3.23 to 8.25 in various treatments.

Weevil infestation was noticed during the sixth week in imidacloprid 0.006 per cent treated plot with a mean population of 3.13 weevils which recorded the lowest population and was on par with *B. bassiana* in talc @ 30 g l^{-1}, *B. bassiana* in talc @ 20 g l^{-1}, *B. bassiana* in neem cake @ 50 g, *B. bassiana* in cow dung @ 50 g and *B. bassiana* @ 10^{11} spores ml^{-1} which recorded a mean population of 3.33, 3.62, 3.98, 4.25 and 4.81, respectively. As observed during the other weeks, the highest population of weevils was recorded in the untreated control (12.38). The population of weevils in the different treatments ranged from 4.99 to 7.13.

During the seventh week after treatment, the lowest population of *C. formicarius* was observed in the plot treated with *B. bassiana* in talc @ 20 g l^{-1} with a mean population of 2.27 weevils, followed by *B. bassiana* in talc @ 30 g l^{-1} and imidacloprid 0.006 per cent with a mean population of 2.39 and 2.61, respectively and were on par. The control plot had the higher weevil count of 10.08 and the population in the other treatments ranged between 3.19 and 6.47.

Before harvesting, at eight WAT, the lowest mean population was observed in the treatment with imidacloprid 0.006 per cent with a population of 0.64 weevils and the highest weevil population was recorded in the control plot (11.89) as observed in the previous weeks. The mean population of weevils ranged from 3.19 to 8.67 in the different treatments.

4.4.2.1.2 Intensity of damage in tubers

When the intensity of damage in the tubers was assessed during the first field trial, the minimum number of galleries in the tubers was recorded in the two chemical treatments *viz.* imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent, which recorded a mean number of 2.33 and 4.67 galleries, respectively and were significantly superior to other treatments (Table 65). The tubers from the untreated control mounds recorded significantly higher number of galleries with a mean number of 18.67 galleries. The treatments with *M. anisopliae* @ 10^{10} spores ml^{-1}, *B. bassiana* @ 10^{9} spores ml^{-1}, *M. anisopliae* @ 10^{12} spores ml^{-1} and *B. bassiana* in cow dung @ 50 g recorded a mean number of 13.00, 12.00, 11.33 and 11.00 galleries, respectively and were on par. Moderate level of damage was noticed when treated with *M. anisopliae* in talc @ 20 g l^{-1}, *M. anisopliae* in cow dung @ 50 g, *B. bassiana* @ 10^{11} spores ml^{-1} and *B. bassiana* in neem cake @ 50 g where a mean number of 10.33, 9.67, 9.67 and 8.33 galleries, respectively and were statistically on par. The tubers obtained from mounds treated with *M. anisopliae* in talc @ 30 g l^{-1}, *M. anisopliae* in neem cake @ 50 g, *B. bassiana* in talc @ 20 g l^{-1} and *B. bassiana* in talc @ 30 g l^{-1} had relatively lower damage with a mean number of 7.33, 7.00, 7.00 and 5.33 galleries per three tubers and were on par.

4.4.2.1.3 Population of grubs in tubers

Considering the population of grubs within the tubers, the tubers obtained from imidacloprid treated mounds recorded a mean number of 1.33 grubs only per three tubers and was significantly low when compared to the other treatments (Table 65). The maximum number of grubs of *C. formicarius* was recorded from tubers in the control mounds (11.00) and was significantly higher when compared to other treatments. The mean population of grubs recorded in the tubers obtained from mounds treated with *M. anisopliae* @ 10^{10} spores ml^{-1}, *M. anisopliae* @ 10^{12} spores ml^{-1}, *M. anisopliae* in cow dung @ 50 g, *B. bassiana* @ 10^{9} spores ml^{-1} and *B. bassiana* in cow dung @ 50 g were 8.67, 7.67, 7.33, 7.00 and 7.00, respectively

Table 65. Mean number of galleries and grubs in tubers and the population of adult *C. formicarius* in soil in the first field trial with soil drenching

Sl. No.	Treatments	Number of galleries (3 tubers mound^{-1})	Number of grubs of *C.formicarius* (3 tubers mound^{-1})	Number of adult *C.formicarius* (kg^{-1} soil)
T1	*B. bassiana* @ 10^9 spores ml^{-1}	12.00 (3.46)	7.00 (2.64)	6.33 (2.51)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	9.67 (3.11)	5.00 (2.23)	5.00 (2.23)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	7.00 (2.64)	3.33 (1.82)	6.00 (2.44)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	5.33 (2.31)	2.33 (1.52)	4.33 (2.08)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	13.00 (3.59)	8.67 (2.94)	10.33 (3.21)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	11.33 (3.36)	7.67 (2.76)	9.67 (3.11)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	10.33 (3.21)	6.00 (2.44)	6.67 (2.57)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	7.33 (2.71)	5.00 (2.23)	6.33 (2.51)
T9	*B. bassiana* in cow dung @ 50 g	11.00 (3.31)	7.00 (2.64)	4.00 (1.99)
T10	*M. anisopliae* in cow dung @ 50 g	9.67 (3.11)	7.33 (2.69)	9.00 (2.99)
T11	*B. bassiana* in neem cake @ 50 g	8.33 (2.88)	4.00 (1.99)	4.00 (1.99)
T12	*M. anisopliae* in neem cake @ 50 g	7.00 (2.64)	4.33 (2.06)	8.00 (2.80)
T13	Imidacloprid 0.006 per cent	2.33 (1.52)	1.33 (1.14)	0.67 (0.67)
T14	Lambda cyhalothrin 0.025 per cent	4.67 (2.16)	2.67 (1.63)	4.33 (2.04)
T15	Control (Untreated)	18.67 (4.31)	11.00 (3.31)	16.33 (4.03)
	C.D (0.05)	(0.343)	(0.399)	(0.468)

Figures in parentheses are square root transformed values

and were on par. A mean population of 6.00, 5.00, 5.00 and 4.33 grubs were observed in the tubers from plots treated using *M. anisopliae* in talc @ 20 g l^{-1}, *M. anisopliae* in talc @ 30 g l^{-1}, *B. bassiana* @ 10^{11} spores ml^{-1} and *M. anisopliae* in neem cake @ 50 g respectively and were on par. When treated with *B. bassiana* in neem cake @ 50 g and *B. bassiana* in talc @ 20 g l^{-1} a mean number of 4.00 and 3.33 grubs per three tubers was noticed. The insecticide lambda cyhalothrin and *B. bassiana* in talc @ 30 g l^{-1} recorded relatively lower number of grubs within the tubers and were 2.67 and 2.33, respectively and were on par.

4.4.2.1.4 Population of weevils in soil

When the weevil population in soil were sampled, it was noted that the imidacloprid treated mound had the lowest load of weevils with a mean population of 0.67 only and differed significantly from the other treatments (Table 65). In the untreated control significantly higher number of weevils were recorded and it came to about 16.33 weevils kg^{-1} soil. A mean population of 10.33, 9.67 and 9.00 weevils were observed in the mounds treated with *M. anisopliae* @ 10^{10} spores ml^{-1}, *M. anisopliae* @ 10^{12} spores ml^{-1} and *M. anisopliae* in cow dung @ 50 g respectively and were on par. The treatments with *M. anisopliae* in neem cake @ 50 g, *M. anisopliae* in talc @ 20 g l^{-1}, *M. anisopliae* in talc @ 30 g l^{-1}, *B. bassiana* @ 10^{9} spores ml^{-1} and *B. bassiana* in talc @ 20 g l^{-1} recorded a mean population of 8.00, 6.67, 6.33, 6.33 and 6.00, respectively and were on par. *B. bassiana* when applied @ 10^{11} spores ml^{-1} a mean population of 5.00 weevils were found, which was on par with the treatments with *B. bassiana* in talc @ 30 g l^{-1} and lambda cyhalothrin 0.025 per cent, which recorded a mean population of 4.33 each respectively and were on par. When treated with *B. bassiana* cultured in substrates like cow dung and neem cake relatively lower number of pests in soil was observed and recorded a mean population of 4.00 each and were on par.

4.4.2.1.5 Population of natural enemies

The mean population of hymenopteran natural enemies and predatory spiders on the foliage of sweet potato during the first field trial is not significant and the data is presented in Tables 66 and 67.

4.4.2.1.6 Yield

Weight of tubers

The mean yield obtained after the application of imidacloprid 0.006 per cent was significantly high and recorded 3.79 kg when compared to the other treatments (Table 68). The total mean yield of tubers from the mounds in which *B. bassiana* in talc @ 30 g l^{-1}, lambda cyhalothrin 0.025 per cent and *M. anisopliae* in talc @ 30 g l^{-1} were 3.23, 3.08 and 3.07 kg, respectively and were on par. When the treatments with *B. bassiana* in cow dung @ 50 g, *B. bassiana* in neem cake @ 50 g, *M. anisopliae* @ 10^{12} spores ml^{-1}, *B. bassiana* @ 10^{11} spores ml^{-1}, *B. bassiana* in talc @ 20 g l^{-1}, *M. anisopliae* @ 10^{10} spores ml^{-1} and *M. anisopliae* in neem cake @ 50 g were applied, a mean yield of 2.67, 2.60, 2.58, 2.48, 2.33, 2.33 and 2.32 kg, respectively were obtained and were statistically on par. The treatments involving *B. bassiana* @ 10^{9} spores ml^{-1}, *M. anisopliae* in talc @ 20 g l^{-1} and *M. anisopliae* in cow dung @ 50 g yielded a mean of 2.15, 2.10 and 1.93 kg tubers respectively and were on par. The lowest yield (0.97 kg) was obtained from the mounds maintained as the control treatment with no spraying.

Marketable yield

The highest marketable yield of 3.50 kg was obtained for the treatment with imidacloprid 0.006 per cent followed by the treatment with talc based *B. bassiana* @ 30 g l^{-1} and lambda cyhalothrin 0.025 per cent which had 2.63 and 2.60 kg respectively and were on par (Table 68). The marketable yield for the other treatments ranged from 1.87 to 1.05 kg, respectively and the untreated control recorded the lowest marketable yield of 0.20 kg.

Table 66. Population of hymenopteran natural enemies in the foliage of sweet potato during the first field trial with soil drenching

Sl. No.	Treatments	Pre count	Post count after application of treatments (in 0.19 m^2)							
			1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT
T1	*B. bassiana* @ 10^9 spores ml^{-1}	0 (0)	1.13 (1.06)	0 (0)	0.64 (0.80)	0.69 (0.83)	0 (0)	1.27 (1.13)	1.00 (1.00)	0.93 (0.96)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	0 (0)	0 (0)	0 (0)	0 (0)	1.16 (1.08)	1.00 (1.00)	0 (0)	0 (0)	0 (0)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	1.24 (1.11)	0 (0)	2.14 (1.46)	0 (0)	1.00 (1.00)	0 (0)	1.00 (1.00)	0 (0)	1.27 (1.13)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	0.93 (0.96)	1.27 (1.13)	0 (0)	1.09 (1.04)	0 (0)	0 (0)	0 (0)	1.83 (1.35)	0 (0)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	0 (0)	0 (0)	0 (0)	0 (0)	0.54 (0.73)	0.65 (0.81)	0 (0)	0 (0)	0 (0)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	0 (0)	0 (0)	1.00 (1.00)	0 (0)	0 (0)	0 (0)	0.73 (0.85)	0 (0)	0.86 (0.93)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	1.43 (1.19)	0 (0)	0 (0)	1.27 (1.13)	0 (0)	1.37 (1.17)	0 (0)	1.21 (1.10)	0 (0)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	0 (0)	0.69 (0.83)	1.00 (1.00)	0 (0)	0 (0)	0.91 (0.95)	1.22 (1.10)	0 (0)	0 (0)
T9	*B. bassiana* in cow dung @ 50 g	2.16 (1.47)	0 (0)	0 (0)	0 (0)	1.34 (1.16)	0 (0)	0 (0)	0 (0)	1.39 (1.18)
T10	*M. anisopliae* in cow dung @ 50 g	0 (0)	0 (0)	0.68 (0.82)	1.00 (1.00)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
T11	*B. bassiana* in neem cake @ 50 g	0 (0)	0.82 (0.91)	0 (0)	0 (0)	1.67 (1.29)	0 (0)	2.16 (1.47)	0 (0)	0 (0)
T12	*M. anisopliae* in neem cake @ 50 g	1.72 (1.31)	0 (0)	0 (0)	0 (0)	0 (0)	1.00 (1.00)	0 (0)	0.91 (0.95)	0.54 (0.73)
T13	Imidacloprid 0.006 per cent	0 (0)	0 (0)	1.35 (1.16)	1.00 (1.00)	2.16 (1.47)	0 (0)	0 (0)	0 (0)	0 (0)
T14	Lambda cyhalothrin 0.025 per cent	0 (0)	1.53 (1.24)	0 (0)	0.91 (0.95)	0 (0)	0 (0)	1.19 (1.09)	0.37 (0.61)	1.55 (1.24)
T15	Control (Untreated)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.17 (1.08)	0 (0)	0 (0)	1.00 (1.00)
	C.D (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

Figures in parentheses are square root transformed values

Table 67. Population of predatory spiders in the foliage of sweet potato during the first field trial with soil drenching

Sl. No.	Treatments	Pre count	Post count after application of treatments (in 0.19 m^2)							
			1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT
T1	*B. bassiana* @ 10^9 spores ml^{-1}	0.67 (0.81)	2.00 (1.41)	0 (0)	1.42 (1.19)	0.67 (0.81)	0 (0)	0 (0)	0 (0)	1.23 (1.11)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	1.00 (1.00)	1.33 (1.15)	0 (0)	0 (0)	2.15 (1.47)	0 (0)	1.72 (1.31)	1.00 (1.00)	0 (0)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	0 (0)	0 (0)	1.23 (1.11)	0 (0)	0 (0)	0 (0)	2.67 (1.63)	0 (0)	0 (0)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	0.67 (0.81)	1.00 (1.00)	0 (0)	0 (0)	2.43 (1.56)	0 (0)	1.47 (1.21)	0 (0)	0 (0)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	1.33 (1.15)	0 (0)	1.00 (1.00)	0 (0)	0 (0)	1.64 (1.28)	0 (0)	2.33 (1.53)	1.67 (1.29)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	1.00 (1.00)	0 (0)	1.15 (1.07)	1.57 (1.25)	2.15 (1.47)	0 (0)	0 (0)	0 (0)	0 (0)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.28 (1.13)	1.00 (1.00)	0 (0)	1.46 (1.21)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	0 (0)	1.67 (1.29)	0 (0)	0 (0)	1.00 (1.00)	1.61 (1.27)	0 (0)	0 (0)	1.59 (1.26)
T9	*B. bassiana* in cow dung @ 50 g	2.00 (1.41)	2.67 (1.63)	2.23 (1.49)	1.84 (1.36)	0 (0)	1.33 (1.15)	1.58 (1.26)	0 (0)	2.61 (1.62)
T10	*M. anisopliae* in cow dung @ 50 g	1.33 (1.15)	2.00 (1.41)	1.22 (1.10)	1.84 (1.36)	0 (0)	2.36 (1.54)	0 (0)	1.15 (1.07)	0 (0)
T11	*B. bassiana* in neem cake @ 50 g	2.00 (1.41)	0 (0)	1.54 (1.24)	1.21 (1.10)	2.00 (1.41)	0 (0)	1.00 (1.00)	0 (0)	0 (0)
T12	*M. anisopliae* in neem cake @ 50 g	1.00 (1.00)	0 (0)	1.33 (1.15)	1.00 (1.00)	0 (0)	0 (0)	0 (0)	0 (0)	2.57 (1.60)
T13	Imidacloprid 0.006 per cent	2.00 (1.41)	0 (0)	0 (0)	0 (0)	0 (0)	1.62 (1.27)	1.49 (1.22)	2.31 (1.52)	1.47 (1.21)
T14	Lambda cyhalothrin 0.025 per cent	3.15 (1.77)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
T15	Control (Untreated)	0 (0)	1.33 (1.15)	2.57 (1.60)	0 (0)	0 (0)	3.16 (1.78)	1.33 (1.15)	1.00 (1.00)	2.47 (1.57)
	C.D (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 68. Mean weight of sweet potato tubers in the first field trial with soil drenching and benefit : cost ratio

Sl. No.	Treatments	Yield mound^{-1} (kg)	Marketable yield mound^{-1} (kg)	Yield (ha^{-1}) (tons)	Benefit : Cost ratio
T1	*B. bassiana* @ 10^9 spores ml^{-1}	2.15 (1.47)	1.27 (1.13)	21.59 (4.65)	4.02
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	2.48 (1.57)	1.70 (1.30)	28.90 (5.38)	6.17
T3	Talc based *B. bassiana* @ 20 g l^{-1}	2.33 (1.53)	1.62 (1.27)	27.54 (5.25)	5.84
T4	Talc based *B. bassiana* @ 30 g l^{-1}	3.23 (1.79)	2.63 (1.62)	44.71 (6.69)	10.80
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	2.33 (1.53)	1.47 (1.21)	24.99 (4.99)	5.02
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	2.58 (1.61)	1.87 (1.37)	31.79 (5.64)	7.02
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	2.10 (1.45)	1.20 (1.09)	20.40 (4.52)	3.74
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	3.07 (1.75)	1.82 (1.35)	30.94 (5.56)	6.75
T9	*B. bassiana* in cow dung @ 50 g	2.67 (1.63)	1.68 (1.29)	28.56 (5.34)	6.29
T10	*M. anisopliae* in cow dung @ 50 g	1.93 (1.39)	1.05 (1.02)	17.85 (4.22)	3.14
T11	*B. bassiana* in neem cake @ 50 g	2.60 (1.61)	1.72 (1.31)	29.24 (5.41)	6.31
T12	*M. anisopliae* in neem cake @ 50 g	2.32 (1.52)	1.52 (1.23)	25.84 (5.08)	5.31
T13	Imidacloprid 0.006 per cent	3.79 (1.95)	3.50 (1.87)	59.50 (7.71)	10.90
T14	Lambda cyhalothrin 0.025 per cent	3.08 (1.76)	2.60 (1.61)	44.20 (6.65)	7.42
T15	Control (Untreated)	0.97 (0.98)	0.20 (0.45)	3.40 (1.84)	-
	C.D (0.05)	(0.117)	(0.263)	(1.165)	-

Figures in parentheses are square root transformed values

The average per hectare yield computed for the various treatments ranged between 59.50 and 3.40 tons.

Benefit : Cost ratio

The B : C ratio was maximum for the treatment with imidacloprid 0.006 per cent which obtained 10.90 followed by talc based *B. bassiana* @ 30 g l^{-1} (Table 68). The treatments involving lambda cyhalothrin 0.025 per cent and *M. anisopliae* @ 10^{12} spores ml^{-1} recorded a B : C ratio of 7.42 and 7.02 respectively. The other treatments recorded B : C ratio ranging between 6.75 to 3.14.

4.4.2.1.7 Number of tubers and extent of damage

The total mean number of tubers obtained upon treatment with imidacloprid 0.006 per cent, *B. bassiana* in neem cake @ 50 g, *M. anisopliae* in talc @ 30 g l^{-1} and *B. bassiana* in cow dung @ 50 g were 12.67, 10.67, 10.67 and 10.33, respectively and were on par and were superior to the other treatments (Table 69). A mean number of 9.33, 9.00, 9.00, 8.67, 7.67 and 7.33 tubers were obtained from the mounds where the treatments with *B. bassiana* in talc @ 30 g l^{-1}, *M. anisopliae* @ 10^{10} spores ml^{-1}, *M. anisopliae* @ 10^{12} spores ml^{-1}, lambda cyhalothrin 0.025 per cent, *M. anisopliae* in neem cake @ 50 g and *B. bassiana* @ 10^{11} spores ml^{-1}, respectively and were statistically on par. The mean number of tubers produced when applied the talc formulation of *B. bassiana* and *M. anisopliae* at 20 g l^{-1} were 6.33 each, respectively and were on par. The application of *M. anisopliae* in cow dung @ 50 g and untreated control obtained a mean number of 6.00 tubers each respectively and on par with that of *B. bassiana* @ 10^{9} spores ml^{-1} treatment which recorded a mean number of 5.33 tubers.

Considering the extent of damage to the tubers, the treatment with imidacloprid 0.006 per cent recorded the minimum percentage of infested tubers, which was on par with the application of lambda cyhalothrin 0.025 per cent and *B. bassiana* @ 10^{11} spores ml^{-1} where a mean number of 12.90, 19.44 and 31.74 percentage tubers, respectively were found to be infested. The treatments with

Table 69. Mean number of tubers in the first field trial in sweet potato with soil drenching and the extent of damage in tubers

Sl. No.	Treatments	Total number of tubers	Percentage of infested tubers by number	Percentage weight of infested tubers
T1	*B. bassiana* @ 10^9 spores ml^{-1}	5.33 (2.29)	35.95 (5.99)	41.17 (6.42)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	7.33 (2.69)	31.74 (5.63)	30.84 (5.55)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	6.33 (2.51)	32.38 (5.69)	31.67 (5.63)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	9.33 (3.05)	32.70 (5.72)	18.31 (4.28)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	9.00 (2.96)	33.87 (5.82)	37.05 (6.09)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	9.00 (2.99)	37.62 (6.13)	27.52 (5.25)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	6.33 (2.51)	36.94 (6.08)	43.04 (6.56)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	10.67 (3.26)	38.40 (6.19)	40.79 (6.39)
T9	*B. bassiana* in cow dung @ 50 g	10.33 (3.21)	38.38 (6.19)	36.89 (6.07)
T10	*M. anisopliae* in cow dung @ 50 g	6.00 (2.44)	33.97 (5.83)	45.74 (6.76)
T11	*B. bassiana* in neem cake @ 50 g	10.67 (3.26)	37.78 (6.15)	33.84 (5.82)
T12	*M. anisopliae* in neem cake @ 50 g	7.67 (2.76)	43.39 (6.59)	33.91 (5.82)
T13	Imidacloprid 0.006 per cent	12.67 (3.55)	12.90 (3.59)	7.89 (2.81)
T14	Lambda cyhalothrin 0.025 per cent	8.67 (2.94)	19.44 (4.41)	15.70 (3.96)
T15	Control (Untreated)	6.00 (2.44)	79.37 (8.91)	81.67 (9.04)
	C.D (0.05)	(0.497)	(3.275)	(2.619)

Figures in parentheses are square root transformed values

B. bassiana in talc @ 20 g l^{-1} , *B. bassiana* in talc @ 30 g l^{-1} , *M. anisopliae* @ 10^{10} spores ml^{-1}, *M. anisopliae* in cow dung @ 50 g and *B. bassiana* @ 10^{9} spores ml^{-1} recorded a mean of 32.38, 32.70, 33.87, 33.97 and 35.95 percentage infested tubers and were on par with that of *M. anisopliae* in talc @ 20 g l^{-1} , *M. anisopliae* @ 10^{12} spores ml^{-1} , *B. bassiana* in neem cake @ 50 g, *B. bassiana* in cow dung @ 50 g, *M. anisopliae* in talc @ 30 g l^{-1} , *M. anisopliae* in neem cake @ 50 g and untreated control which recorded a mean percentage infested tubers of 36.94, 37.62, 37.78, 38.38, 38.40, 43.39 and 79.37, respectively.

The mean weight of infested tubers was maximum in the untreated control, which recorded 81.67 per cent infested tubers among the total and showed statistical similarity with the treatments with *M. anisopliae* in cow dung @ 50 g and *M. anisopliae* in talc @ 20 g l^{-1} which had a mean of 45.74 and 43.04 per cent infested tubers respectively. The mean percentage of infested tubers in the treatments involving *B. bassiana* @ 10^{9} spores ml^{-1} , *M. anisopliae* in talc @ 30 g l^{-1} , *M. anisopliae* @ 10^{10} spores ml^{-1}, *B. bassiana* in cow dung @ 50 g, *M. anisopliae* in neem cake @ 50 g , *B. bassiana* in neem cake @ 50 g, *B. bassiana* in talc @ 20 g l^{-1} , *B. bassiana* @ 10^{11} spores ml^{-1} , *M. anisopliae* @ 10^{12} spores ml^{-1} , *B. bassiana* in talc @ 30 g l^{-1} and lambda cyhalothrin 0.025 per cent were 41.17, 40.79, 37.05, 36.89, 33.91, 33.84, 31.67, 30.84, 27.52, 18.31 and 15.70, respectively and were statistically on par. The lowest mean percentage weight of infested tubers was recorded in the treatment with imidacloprid 0.006 per cent which had only 7.89 per cent among the total weight of harvested tubers and differed significantly from the other treatments.

4.4.2.2 Second field trial with soil drenching

4.4.2.2.1 *Population of weevils in the foliage*

The imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent treated plots had no weevils at one week after treatment and were statistically on par and significantly superior to other treatments during the second crop season (Table 70). The control plot without any treatment recorded a maximum mean

Table 70. Population of adult *C. formicarius* in the foliage of sweet potato during second field trial with soil drenching

Sl. No.	Treatments	Pre count	Number of adult weevils (in 0.19 m^2)							
			1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT
T1	*B. bassiana* @ 10^9 spores ml^{-1}	8.33 (2.89)	3.67 (1.91)	4.66 (2.16)	4.28 (2.07)	5.17 (2.27)	4.42 (2.10)	6.23 (2.49)	3.97 (1.99)	4.28 (2.07)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	7.67 (2.76)	3.33 (1.82)	2.65 (1.63)	3.29 (1.81)	2.85 (1.69)	4.62 (2.15)	5.87 (2.42)	4.55 (2.13)	3.23 (1.79)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	10.00 (3.16)	2.33 (1.52)	3.97 (1.99)	5.27 (2.29)	2.98 (1.73)	4.15 (2.04)	3.26 (1.81)	2.98 (1.73)	3.84 (1.96)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	7.67 (2.75)	2.33 (1.52)	3.17 (1.78)	3.52 (1.88)	2.85 (1.69)	3.92 (1.98)	3.74 (1.93)	2.27 (1.51)	3.55 (1.88)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	11.00 (3.31)	3.67 (1.91)	5.34 (2.31)	4.95 (2.22)	5.38 (2.32)	4.74 (2.18)	4.83 (2.19)	3.72 (1.93)	4.13 (2.03)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	7.67 (2.76)	2.67 (1.63)	4.35 (2.09)	3.89 (1.97)	5.72 (2.39)	3.75 (1.94)	3.88 (1.97)	4.28 (2.07)	4.69 (2.17)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	9.33 (3.05)	3.33 (1.82)	3.85 (1.6)	5.46 (2.34)	4.81 (2.19)	6.27 (2.50)	3.96 (1.99)	5.42 (2.33)	4.73 (2.17)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	6.33 (2.49)	3.00 (1.73)	4.26 (2.06)	5.18 (2.28)	3.66 (1.91)	4.28 (2.07)	4.00 (2.00)	5.09 (2.26)	3.86 (1.96)
T9	*B. bassiana* in cow dung @ 50 g	5.67 (2.37)	5.00 (2.24)	5.32 (2.31)	5.14 (2.27)	4.92 (2.22)	4.48 (2.17)	5.22 (2.28)	4.65 (2.16)	3.52 (1.88)
T10	*M. anisopliae* in cow dung @ 50 g	7.33 (2.69)	5.67 (2.37)	5.79 (2.41)	5.64 (2.37)	6.13 (2.48)	5.81 (2.41)	4.99 (2.23)	4.17 (2.04)	5.38 (2.32)
T11	*B. bassiana* in neem cake @ 50 g	3.67 (1.90)	2.33 (1.52)	6.18 (2.49)	4.93 (2.22)	4.52 (2.13)	5.34 (2.31)	5.62 (2.37)	4.38 (2.09)	3.76 (1.94)
T12	*M. anisopliae* in neem cake @ 50 g	5.00 (2.23)	4.00 (1.89)	5.82 (2.41)	5.22 (2.28)	4.93 (2.22)	6.28 (2.51)	5.94 (2.44)	5.35 (2.31)	6.27 (2.50)
T13	Imidacloprid 0.006 per cent	10.00 (3.16)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.83 (0.91)	1.14 (1.07)	1.69 (1.30)
T14	Lambda cyhalothrin 0.025 per cent	8.67 (2.92)	0 (0)	0 (0)	0 (0)	1.23 (1.11)	1.47 (1.21)	2.34 (1.53)	1.95 (1.39)	3.86 (1.96)
T15	Control (Untreated)	8.33 (2.83)	9.00 (2.99)	7.35 (2.71)	9.17 (3.03)	11.32 (3.36)	10.24 (3.20)	9.35 (3.06)	8.27 (2.88)	7.61 (2.76)
	C.D (0.05)	0.596	0.449	0.391	0.583	0.628	0.665	0.691	0.536	0.256

Figures in parentheses are square root transformed values

population of 9.00 weevils per mound and differed significantly from the other treatments. Both the fungi grown in cow dung when applied @ 50 g recorded relatively higher population of weevil and were 5.67 and 5.00 respectively and were on par. The mean population of weevils ranged from 2.33 to 3.67 in various treatments.

At two weeks after the application of different treatments, the mounds with imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent treatment were found to be weevil free and superior and statistically on par when compared to the other treatments. The untreated control plot recorded a maximum mean population of 7.35 weevils and was on par with the treatments with *B. bassiana* and *M. anisopliae* cultured in neem cake and applied @ 50 g each which recorded a mean population of 6.18 and 5.82 weevils respectively. The weevil population in the other treatments ranged between 2.65 and 5.34 weevils per mound.

As observed during the first and second weeks after treatment, in the third week also there was no weevils present in the mounds where imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent were applied and were on par and found to be superior over the other treatments. As seen in the earlier observations, the control plots recorded a maximum mean population of 9.17 weevils per mound and differed significantly from the other treatments. In the other treatments the population of weevils ranged from 3.29 to 5.64 weevils per mound.

In the imidacloprid 0.006 per cent treated mounds there was no weevils at four weeks after treatment and stood superior among the other treatments. The mounds treated with lambda cyhalothrin 0.025 per cent recorded the minimum number of 1.23 weevils and was on par with that found in the *B. bassiana* @ 10^{11} spores ml^{-1}, *B. bassiana* in talc @ 30 g l^{-1} and *B. bassiana* in talc @ 20 g l^{-1} which recorded a mean number of 2.85, 2.85 and 2.98 weevils, respectively. The other treatments recorded a mean population of 3.66 to 6.13 weevils per mound.

At five weeks after application of treatments, the imidacloprid 0.006 per cent treated mound remained weevil free as noticed in the previous weeks and

differed significantly from the other treatments. The lowest weevil population was observed in the lambda cyhalothrin 0.025 per cent and recorded a mean population of 1.47 weevils per mound. The control mounds recorded significantly higher mean population of 10.24 weevils and differed significantly from the other treatments. The mean population of weevils ranged from 3.75 to 6.28 in the other treatments.

The population of weevils on the mounds treated with imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent were 0.83 and 2.34 weevils per mound and were statistically on par at six weeks after treatment application. The untreated control plot recorded maximum mean population of weevils on foliage and was 9.35, which was on par with the mean population noticed in the mounds treated with *B. bassiana* @ 10^9 spores ml^{-1} and was 6.23 weevils. The population of weevils ranged from 3.26 to 5.94 in various treatments.

A mean population of 1.14 and 1.95 was observed in the mounds where imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent treatment was given and were the lowest among the different treatments and was on par with the mean population noticed in the mounds treated with *B. bassiana* in talc @ 30 g l^{-1} which recorded a mean of 2.27 weevils. As observed in the previous weeks, the untreated control recorded the highest weevil population of 8.27 weevils per mound and was on par with the weevil population observed in the mounds treated with *M. anisopliae* in talc @ 20 g l^{-1} (5.42). The population of weevils in the other treatments varied from 2.98 to 5.35.

At eight weeks after treatment application, just before harvesting of the crop, the lowest weevil population was observed in the mounds treated with imidacloprid 0.006 per cent and recorded a mean of 1.69 weevils per mound, which was significantly low and differed from the other treatments. Relatively lower weevil population was observed in the treatments with *B. bassiana* @ 10^{11} spores ml^{-1}, *B. bassiana* in cow dung @ 50 g and *B. bassiana* in talc @ 30 g l^{-1} and were 3.23, 3.52 and 3.55 weevils, respectively and were on par. The highest

weevil count was recorded in the untreated control with a mean population of 7.61 weevils and differed significantly from the other treatments. The mean population of weevils ranged from 3.76 to 6.27 in the other treatments.

4.4.2.2.2 Intensity of damage in tubers

During the second crop season, number of galleries recorded in the tubers obtained from the imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent treated mounds were 1.00 and 1.33 galleries respectively and recorded the lowest among the different treatments and found to be superior (Table 71). The talc formulation of *B. bassiana* @ 30 g l^{-1} treated mounds recorded a mean number of 3.67 galleries in the tubers and was relatively lower than the other treatments. The tubers from the untreated control plot were severely damaged with a maximum number of 15 galleries and was significantly higher when compared to all the other treatments. The treatments with *M. anisopliae* @ 10^{10} spores ml^{-1}, *B. bassiana* @ 10^{9} spores ml^{-1}, *B. bassiana* in cow dung @ 50 g and *B. bassiana* @ 10^{11} spores ml^{-1} recorded a mean number of 10.33, 9.67, 9.00 and 9.00 galleries and were on par. A mean number of 8.00 galleries was noticed in the tubers obtained from *M. anisopliae* in cow dung @ 50 g treated plot and was on par with the treatments with *M. anisopliae* in talc @ 20 g l^{-1} and *M. anisopliae* @ 10^{12} spores ml^{-1} which recorded a mean number of 7.67 galleries each, respectively. Moderately lower number of galleries was observed in tubers obtained from the mounds treated with *M. anisopliae* in talc @ 30 g l^{-1}, *M. anisopliae* in neem cake @ 50 g, *B. bassiana* in talc @ 20 g l^{-1} and *B. bassiana* in neem cake @ 50 g which recorded a mean number of 6.00, 5.67, 5.33 and 5.33, respectively and were on par.

4.4.2.2.3 Population of grubs in tubers

When the population of grubs in the tubers were sampled, the tubers obtained by the application of imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent recorded the lowest number of grubs of *C. formicarius* and were 0.67 and 1.67 grubs and were on par and found to be the superior treatments

Table 71. Mean number of galleries and grubs in tubers and the population of adult *C. formicarius* in soil in the second field trial with soil drenching

Sl. No.	Treatments	Number of galleries (3 tubers mound^{-1})	Number of grubs of *C.formicarius* (3 tubers mound^{-1})	Number of adult *C.formicarius* (kg^{-1} soil)
T1	*B. bassiana* @ 10^9 spores ml^{-1}	9.67 (3.11)	5.67 (2.38)	5.00 (2.24)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	9.00 (2.99)	4.33 (2.08)	4.33 (2.08)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	5.33 (2.31)	3.00 (1.72)	4.33 (2.08)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	3.67 (1.90)	2.33 (1.52)	3.33 (1.82)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	10.33 (3.21)	6.00 (2.44)	6.67 (2.58)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	7.67 (2.76)	5.67 (2.37)	6.00 (2.44)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	7.67 (2.77)	4.67 (2.16)	5.33 (2.31)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	6.00 (2.44)	4.00 (1.99)	4.67 (2.16)
T9	*B. bassiana* in cow dung @ 50 g	9.00 (2.99)	5.00 (2.24)	3.33 (1.82)
T10	*M. anisopliae* in cow dung @ 50 g	8.00 (2.83)	4.67 (2.16)	5.67 (2.38)
T11	*B. bassiana* in neem cake @ 50 g	5.33 (2.31)	3.33 (1.82)	3.00 (1.72)
T12	*M. anisopliae* in neem cake @ 50 g	5.67 (2.37)	4.00 (1.99)	4.00 (1.99)
T13	Imidacloprid 0.006 per cent	1.00 (1.00)	0.67 (0.67)	0.33 (0.33)
T14	Lambda cyhalothrin 0.025 per cent	1.33 (1.14)	1.67 (1.05)	2.33 (1.41)
T15	Control (Untreated)	15.00 (3.87)	8.67 (2.94)	11.67 (3.39)
	C.D (0.05)	(0.358)	(0.523)	(0.518)

Figures in parentheses are square root transformed values

(Table 71). The treatments where talc based formulations of *B. bassiana* @ 30 g l^{-1}, *B. bassiana* @ 20 g l^{-1} and *B. bassiana* in neem cake @ 50 g were applied, the population of grubs recorded were 2.33, 3.00 and 3.33 respectively and were on par. The tubers obtained from the untreated control and the mounds treated with *M. anisopliae* @ 10^{10} spores ml^{-1} recorded a men population of 8.67 and 6.00 grubs, respectively and were statistically on par. A mean number of 4.00, 4.00, 4.33, 4.67, 4.67, 5.00, 5.67 and 5.67 grubs were recorded from the tubers obtained from the application of *M. anisopliae* in talc @ 30 g l^{-1}, *M. anisopliae* in neem cake @ 50 g, *B. bassiana* @ 10^{11} spores ml^{-1}, *M. anisopliae* in talc @ 20 g l^{-1}, *M. anisopliae* in cow dung @ 50 g, *B. bassiana* in cow dung @ 50 g, *M. anisopliae* @ 10^{12} spores ml^{-1} and *B. bassiana* @ 10^{9} spores ml^{-1}, respectively and were on par.

4.4.2.2.4 Population of weevils in soil

The population of adult weevils of *C. formicarius* was the lowest in the imidacloprid 0.006 per cent treated plot with a mean number of 0.33, followed by the treatment with lambda cyhalothrin 0.025 per cent which recorded a mean number of 2.33 weevils per mound and found to be superior to the other treatments and significant difference was there when compared to the other treatments (Table 71). The mean number of weevils found in the mounds treated with *B. bassiana* in neem cake @ 50 g, *B. bassiana* in talc @ 30 g l^{-1}, *B. bassiana* in cow dung @ 50 g and *M. anisopliae* in neem cake @ 50 g were 3.00, 3.33, 3.33 and 4.00, respectively and were on par. The maximum number of weevils was recorded in the untreated control plot with a mean population of 11.67 weevils and differed significantly from the other treatments. The treatments with *M. anisopliae* @ 10^{10} spores ml^{-1}, *M. anisopliae* @ 10^{12} spores ml^{-1}, *M. anisopliae* in cow dung @ 50 g, *M. anisopliae* in talc @ 20 g l^{-1}, *B. bassiana* @ 10^{9} spores ml^{-1}, *M. anisopliae* in talc @ 30 g l^{-1}, *B. bassiana* @ 10^{11} spores ml^{-1} and *B. bassiana* in talc @ 20 g l^{-1} recorded a mean number of 6.67, 6.00, 5.67, 5.33, 5.00, 4.67, 4.33 and 4.33 weevils, respectively and were on par.

4.4.2.2.5 Population of natural enemies

During the second field trial the mean population of hymenopteran natural enemies and predatory spiders on the foliage of sweet potato is not significant and the data is presented in Tables 72 and 73.

4.4.2.2.6 Yield

Weight of tubers

The imidacloprid 0.006 per cent treated plots recorded the maximum yield of 4.15 kg tubers during the second crop season and was on par with that obtained when treated with lambda cyhalothrin 0.025 per cent which recorded a mean yield of 3.98 kg tubers (Table 74). The mean tubers yielded when treated with the talc formulation of *B. bassiana* @ 30 g l^{-1} was 3.70 kg. The yield obtained when treated with *M. anisopliae* in talc @ 30 g l^{-1} and *B. bassiana* in cow dung @ 50 g were 3.02 and 2.70 kg and were on par. The treatments with *B. bassiana* @ 10^{11} spores ml^{-1}, *M. anisopliae* in talc @ 20 g l^{-1}, *M. anisopliae* @ 10^{10} spores ml^{-1}, *B. bassiana* @ 10^{9} spores ml^{-1}, *B. bassiana* in neem cake @ 50 g, *B. bassiana* in talc @ 20 g l^{-1}, *M. anisopliae* @ 10^{12} spores ml^{-1} and *M. anisopliae* in neem cake @ 50 g recorded a mean yield of 2.65, 2.50, 2.48, 2.47, 2.47, 2.47, 2.47 and 2.37 kg respectively and were on par. The yield obtained by the application of *M. anisopliae* in cow dung @ 50 g and in the untreated control were 1.90 kg each, respectively and were on par.

Marketable yield

The maximum mean marketable yield of 3.83 kg tubers was recorded by the treatment with imidacloprid 0.006 per cent and was on par with lambda cyhalothrin 0.025 per cent which had a mean marketable yield of 3.47 kg (Table 74). The treatments using talc formulations of *B. bassiana* and *M. anisopliae* @ 30 g l^{-1} each recorded a mean marketable yield of 3.32 and 2.37 kg, respectively and differed significantly from the other treatments. A mean marketable yield of 1.92 kg tubers was observed in the case of treatment with

Table 72. Population of hymenopteran natural enemies in the foliage of sweet potato during the second field trial with soil drenching

Sl. No.	Treatments	Pre count	Post count after application of treatments (in 0.19 m^2)							
			1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT
T1	*B. bassiana* @ 10^9 spores ml^{-1}	0 (0)	0 (0)	0 (0)	0.91 (0.95)	0 (0)	0 (0)	0 (0)	0 (0)	1.00 (1.00)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	1.00 (1.00)	0 (0)	1.13 (1.06)	0 (0)	0 (0)	0.54 (0.73)	0 (0)	0.94 (0.97)	0.54 (0.73)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	0 (0)	0.64 (0.80)	0 (0)	2.11 (1.45)	0 (0)	0 (0)	2.09 (1.45)	0 (0)	0 (0)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	0 (0)	0.96 (0.98)	0 (0)	0 (0)	1.33 (1.15)	1.00 (1.00)	0 (0)	0.67 (0.82)	0 (0)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	0 (0)	1.00 (1.00)	0 (0)	0 (0)	2.13 (1.46)	0 (0)	1.00 (1.00)	0 (0)	0.67 (0.82)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	1.32 (1.15)	0 (0)	0 (0)	0.64 (0.80)	1.00 (1.00)	1.33 (1.15)	0 (0)	0 (0)	0.93 (0.96)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	0 (0)	0 (0)	0.94 (0.97)	0 (0)	0 (0)	0.67 (0.82)	0 (0)	0 (0)	0 (0)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	0 (0)	0.59 (0.77)	0 (0)	1.00 (1.00)	1.16 (1.08)	2.19 (1.48)	0 (0)	0.54 (0.73)	0 (0)
T9	*B. bassiana* in cow dung @ 50 g	1.13 (1.06)	0 (0)	0 (0)	1.33 (1.15)	0 (0)	0 (0)	1.82 (1.35)	0 (0)	1.00 (1.00)
T10	*M. anisopliae* in cow dung @ 50 g	0.92 (0.96)	0 (0)	0.54 (0.73)	2.13 (1.46)	0 (0)	1.00 (1.00)	0 (0)	1.36 (1.17)	0 (0)
T11	*B. bassiana* in neem cake @ 50 g	1.83 (1.35)	0 (0)	0 (0)	0 (0)	0 (0)	1.00 (1.00)	0 (0)	0 (0)	0 (0)
T12	*M. anisopliae* in neem cake @ 50 g	0 (0)	1.33 (1.15)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.12 (1.06)	0 (0)
T13	Imidacloprid 0.006 per cent	0 (0)	0 (0)	0 (0)	0 (0)	0.64 (0.80)	1.00 (1.00)	0.64 (0.80)	0 (0)	1.00 (1.00)
T14	Lambda cyhalothrin 0.025 per cent	0.67 (0.82)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.27 (1.13)	0 (0)	1.73 (1.32)
T15	Control (Untreated)	0 (0)	0 (0)	2.15 (1.47)	0 (0)	1.13 (1.06)	1.00 (1.00)	0.97 (0.98)	0 (0)	0 (0)
	C.D (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

Figures in parentheses are square root transformed values

Table 73. Population of predatory spiders in the foliage of sweet potato during the second field trial with soil drenching

Sl. No.	Treatments	Pre count	Post count after application of treatments (in 0.19 m^2)							
			1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT
T1	*B. bassiana* @ 10^9 spores ml^{-1}	1.00 (1.00)	0.54 (0.73)	0 (0)	0 (0)	0.96 (0.98)	0 (0)	0.59 (0.77)	0 (0)	0 (0)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	0.69 (0.83)	0 (0)	1.00 (1.00)	1.33 (1.15)	0 (0)	1.00 (1.00)	0 (0)	1.15 (1.07)	1.19 (1.09)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	0 (0)	1.00 (1.00)	0 (0)	0 (0)	1.00 (1.00)	0 (0)	1.33 (1.15)	0 (0)	0 (0)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	0 (0)	1.69 (1.30)	0.37 (0.61)	0.96 (0.98)	0 (0)	0 (0)	0 (0)	2.34 (1.53)	1.00 (1.00)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	0.96 (0.98)	0.64 (0.80)	1.33 (1.15)	0.67 (0.82)	1.33 (1.15)	0.69 (0.83)	1.00 (1.00)	0 (0)	0.54 (0.73)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	0 (0)	0 (0)	1.00 (1.00)	0 (0)	0.64 (0.80)	1.33 (1.15)	0 (0)	0.96 (0.98)	0 (0)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	1.33 (1.15)	1.00 (1.00)	0 (0)	1.00 (1.00)	0 (0)	1.00 (1.00)	0 (0)	0 (0)	0.37 (0.61)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	0 (0)	0.92 (0.96)	0.54 (0.73)	0 (0)	1.58 (1.26)	0 (0)	1.59 (1.26)	0.64 (0.80)	0 (0)
T9	*B. bassiana* in cow dung @ 50 g	1.67 (1.29)	0 (0)	0 (0)	2.13 (1.46)	0 (0)	1.33 (1.15)	0 (0)	0 (0)	2.19 (1.48)
T10	*M. anisopliae* in cow dung @ 50 g	0.69 (0.83)	1.00 (1.00)	1.33 (1.15)	0 (0)	1.00 (1.00)	0 (0)	0.37 (0.61)	1.00 (1.00)	0 (0)
T11	*B. bassiana* in neem cake @ 50 g	0 (0)	0.37 (0.61)	0 (0)	1.47 (1.21)	0 (0)	2.16 (1.47)	0 (0)	0 (0)	1.00 (1.00)
T12	*M. anisopliae* in neem cake @ 50 g	1.00 (1.00)	0 (0)	0.96 (0.98)	0 (0)	0.54 (0.73)	0 (0)	1.27 (1.13)	1.00 (1.00)	1.33 (1.15)
T13	Imidacloprid 0.006 per cent	2.13 (1.46)	1.00 (1.00)	0 (0)	0.64 (0.80)	0 (0)	0.54 (0.73)	0 (0)	1.33 (1.15)	0.64 (0.80)
T14	Lambda cyhalothrin 0.025 per cent	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.00 (1.00)	0 (0)	0 (0)
T15	Control (Untreated)	0.69 (0.83)	1.00 (1.00)	2.16 (1.47)	1.00 (1.00)	0 (0)	1.00 (1.00)	0 (0)	0.96 (0.98)	1.00 (1.00)
	C.D (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

Figures in parentheses are square root transformed values

Table 74. Mean weight of sweet potato tubers in the second field trial with soil drenching and benefit : cost ratio

Sl. No.	Treatments	Yield mound^{-1} (kg)	Marketable yield mound^{-1} (kg)	Yield (ha^{-1}) (tons)	Benefit : Cost ratio
T1	*B. bassiana* @ 10^9 spores ml^{-1}	2.47 (1.57)	1.65 (1.28)	28.05 (5.29)	1.25
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	2.65 (1.63)	1.77 (1.33)	30.09 (5.49)	1.37
T3	Talc based *B. bassiana* @ 20 g l^{-1}	2.47 (1.57)	1.90 (1.38)	32.30 (5.68)	1.52
T4	Talc based *B. bassiana* @ 30 g l^{-1}	3.70 (1.92)	3.32 (1.82)	56.44 (7.51)	3.00
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	2.48 (1.58)	1.32 (1.15)	22.44 (4.74)	0.89
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	2.47 (1.57)	1.50 (1.22)	25.50 (5.05)	1.09
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	2.50 (1.58)	1.77 (1.33)	30.09 (5.49)	1.39
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	3.02 (1.74)	2.37 (1.54)	40.29 (6.35)	2.00
T9	*B. bassiana* in cow dung @ 50 g	2.70 (1.64)	1.92 (1.39)	32.64 (5.71)	1.58
T10	*M. anisopliae* in cow dung @ 50 g	1.90 (1.34)	1.27 (1.13)	21.59 (4.65)	0.89
T11	*B. bassiana* in neem cake @ 50 g	2.47 (1.57)	1.75 (1.32)	29.75 (5.45)	1.36
T12	*M. anisopliae* in neem cake @ 50 g	2.37 (1.54)	1.38 (1.17)	23.46 (4.84)	0.97
T13	Imidacloprid 0.006 per cent	4.15 (2.04)	3.83 (1.96)	65.11 (8.07)	2.56
T14	Lambda cyhalothrin 0.025 per cent	3.98 (1.99)	3.47 (1.86)	58.99 (7.68)	2.25
T15	Control (Untreated)	1.90 (1.34)	0.95 (0.97)	16.15 (4.02)	-
	C.D (0.05)	(0.102)	(0.117)	(0.427)	-

Figures in parentheses are square root transformed values

B. bassiana in cow dung @ 50 g and was statistically on par with the treatments involving *B. bassiana* in talc @ 20 g l^{-1}, *B. bassiana* @ 10^{11} spores ml^{-1}, *M. anisopliae* in talc @ 20 g l^{-1}, *B. bassiana* in neem cake @ 50 g and *B. bassiana* @ 10^{9} spores ml^{-1} which had a mean marketable yield of 1.90, 1.77, 1.77, 1.75 and 1.65 kg respectively. Relatively lower marketable yield was recorded for *M. anisopliae* @ 10^{12} spores ml^{-1} , *M. anisopliae* in neem cake @ 50 g, *M. anisopliae* @ 10^{10} spores ml^{-1} and *M. anisopliae* in cow dung @ 50 g 1.50, 1.38, 1.32 and 1.27 kg respectively and were on par. The lowest mean marketable yield of 0.95 kg was noticed in the untreated control.

The mean average yield per hectare was maximum in the treatment with imidacloprid 0.006 per cent followed by lambda cyhalothrin 0.025 per cent which recorded 65.11 and 58.99 tons, respectively. In the treatment with *B. bassiana* in talc @ 30 g l^{-1} , a mean yield of 56.44 kg per hectare was obtained. The average yield for the other treatments ranged from 40.29 to 21.59 tons of tubers. The lowest was recorded in the case of untreated control, which had a mean yield of 16.15 tons per hectare.

Benefit : Cost ratio

The B : C ratio obtained was maximum in the case of treatment with *B. bassiana* in talc @ 30 g l^{-1} which had a ratio of 3.00, followed by the treatments with imidacloprid 0.006 per cent, lambda cyhalothrin 0.025 per cent and *M. anisopliae* in talc @ 30 g l^{-1} which recorded a B : C ratio of 2.56, 2.25 and 2.00 respectively (Table 74). The B : C ratio in the case of other treatments ranged between 1.58 and 0.89.

4.4.2.2.7 Number of tubers and extent of damage

Considering the total number of tubers, the maximum number of tubers was recorded for the treatment with imidacloprid 0.006 per cent with a mean of 12.67 tubers, followed by *M. anisopliae* in talc @ 30 g l^{-1} , *B. bassiana* in neem cake @ 50 g and *B. bassiana* in cow dung @ 50 g which had a mean number of 10.67, 10.67 and 10.33 tubers, respectively and were statistically on par (Table

75). The mean number of tubers observed in the treatments with talc formulation of *B. bassiana* @ 30 g l^{-1}, *M. anisopliae* @ 10^{12} spores ml^{-1}, *M. anisopliae* @ 10^{10} spores ml^{-1}, lambda cyhalothrin 0.025 per cent, *M. anisopliae* in neem cake @ 50 g and *B. bassiana* @ 10^{11} spores ml^{-1} were 9.33, 9.00, 9.00, 8.67, 7.67 and 7.33 respectively and showed statistical similarity. Relatively lower number of tubers was recorded in the treatments involving talc formulations of *B. bassiana* and *M. anisopliae* @ 20 g l^{-1}, *M. anisopliae* in cow dung @ 50 g, untreated control and *B. bassiana* @ 10^{9} spores ml^{-1} where a mean of 6.33, 6.33, 6.00, 6.00 and 5.33tubers was noticed and were statistically on par.

The untreated control recorded the highest mean number of infested tubers with 69.72 per cent infested tubers among the total tubers harvested and was on par with the treatments with *M. anisopliae* in talc @ 20 g l^{-1}, *M. anisopliae* in cow dung @ 50 g, *B. bassiana* @ 10^{9} spores ml^{-1}, *B. bassiana* in talc @ 20 g l^{-1}, *M. anisopliae* @ 10^{12} spores ml^{-1}, talc formulation of *B. bassiana* @ 30 g l^{-1}, *B. bassiana* @ 10^{11} spores ml^{-1}, *M. anisopliae* in neem cake @ 50 g, *M. anisopliae* @ 10^{10} spores ml^{-1} and *B. bassiana* in neem cake @ 50 g which had a mean percentage of 45.77, 44.81, 40.12, 36.94, 35.97, 35.93, 35.73, 34.34, 33.40 and 32.69 infested tubers respectively out of the total harvested. The treatments with *M. anisopliae* in talc @ 30 g l^{-1}, *B. bassiana* in cow dung @ 50 g and lambda cyhalothrin 0.025 per cent recorded relatively lower mean percentage of infested tubers and were 28.11, 27.07 and 13.93 per cent respectively and were on par with imidacloprid 0.006 per cent which recorded the lowest percentage of infested tubers with a mean of 11.05 per cent among the total tubers harvested.

The untreated control recorded a maximum of 50.19 per cent of infested tubers among the total harvested produce in the treatment and was on par with the treatment with *M. anisopliae* @ 10^{10} spores ml^{-1} which had a mean of 46.94 per cent infested tubers. A mean weight of 41.67 and 38.84 per cent infested tubers was noticed in the treatments with *M. anisopliae* in neem cake @ 50 g and *M. anisopliae* @ 10^{12} spores ml^{-1} and were statistically on par. The treatments with *M. anisopliae* in cow dung @ 50 g, *B. bassiana* @ 10^{11} spores ml^{-1} and

Table 75. Mean number of tubers in the second field trial in sweet potato with soil drenching and the extent of damage

Sl. No.	Treatments	Total number of tubers	Percentage of infested tubers by number	Percentage weight of infested tubers
T1	*B. bassiana* @ 10^9 spores ml^{-1}	5.33 (2.29)	40.12 (6.33)	33.11 (5.75)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	7.33 (2.69)	35.73 (5.98)	33.29 (5.77)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	6.33 (2.51)	36.94 (6.08)	22.94 (4.79)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	9.33 (3.05)	35.93 (5.99)	10.42 (3.23)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	9.00 (2.96)	33.40 (5.78)	46.94 (6.85)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	9.00 (2.99)	35.97 (5.99)	38.84 (6.23)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	6.33 (2.51)	45.77 (6.77)	29.46 (5.43)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	10.67 (3.26)	28.11 (5.30)	21.65 (4.65)
T9	*B. bassiana* in cow dung @ 50 g	10.33 (3.21)	27.07 (5.20)	28.94 (5.38)
T10	*M. anisopliae* in cow dung @ 50 g	6.00 (2.44)	44.81 (6.69)	33.77 (5.81)
T11	*B. bassiana* in neem cake @ 50 g	10.67 (3.26)	32.69 (5.72)	28.97 (5.38)
T12	*M. anisopliae* in neem cake @ 50 g	7.67 (2.76)	34.34 (5.86)	41.67 (6.46)
T13	Imidacloprid 0.006 per cent	12.67 (3.55)	11.05 (3.32)	7.63 (2.76)
T14	Lambda cyhalothrin 0.025 per cent	8.67 (2.94)	13.93 (3.73)	13.14 (3.62)
T15	Control (Untreated)	6.00 (2.44)	69.72 (8.35)	50.19 (7.08)
	C.D (0.05)	(0.497)	(2.716)	(0.358)

Figures in parentheses are square root transformed values

B. bassiana @ 10^9 spores ml^{-1} recorded a mean weight of 33.77, 33.29 and 33.11 per cent infested tubers respectively and were on par. The mean weight of infested tubers observed in the treatments with *M. anisopliae* in talc @ 20 g l^{-1}, *B. bassiana* in neem cake @ 50 g and *B. bassiana* in cow dung @ 50 g were statistically on par and were 29.46, 28.97 and 28.94 per cent respectively. Relatively lower quantity of infested tubers was recorded in the treatments with *B. bassiana* in talc @ 20 g l^{-1} and *M. anisopliae* in talc @ 30 g l^{-1} which had 22.94 and 21.65 per cent of infested tubers respectively and were on par. The treatments with lambda cyhalothrin 0.025 per cent and talc formulation of *B. bassiana* @ 30 g l^{-1} recorded a mean weight of 13.14 and 10.42 per cent infested tubers respectively among the total harvested. The least quantity of infested tubers was noticed in the treatment with imidacloprid 0.006 per cent which had a mean weight of 7.63 per cent.

4.4.2.3 First field trial with foliar spraying

4.4.2.3.1 Population of weevils in the foliage

One week after treatment there were no *C. formicarius* in the plots treated with imidacloprid 0.006 per cent and showed statistical significance among the different treatments (Table 76). The plots treated with *B. bassiana* in talc @ 20 g l^{-1} and *M. anisopliae* @ 10^{10} spores ml^{-1} recorded a mean number of 2.42 and 2.67 weevils respectively and were on par. The maximum mean number of 6.33 weevils was observed in the untreated control which was on par with the treatment with *M. anisopliae* @ 10^{12} spores ml^{-1} which recorded a mean number of 4.97 weevils. A mean number of weevils ranging from 4.33 to 2.97 were observed in the mounds treated with the other treatments and were statistically on par.

There were no weevils in the plots treated with imidacloprid 0.006 per cent at two weeks after treatment and had significant difference when compared to the other treatments. The mean population of weevils in the plots treated with *B. bassiana* in talc @ 30 g l^{-1}, *B. bassiana* @ 10^{11} spores ml^{-1} and

Table 76. Population of adult *C. formicarius* in the foliage of sweet potato during first field trial with foliar spraying

Sl. No.	Treatments	Pre count	Number of adult weevils (in 0.19 m^2)							
			1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT
T1	*B. bassiana* @ 10^9 spores ml^{-1}	6.35 (2.52)	4.33 (2.08)	5.37 (2.32)	3.66 (1.91)	4.27 (2.07)	3.92 (1.98)	4.13 (2.03)	5.92 (2.43)	4.13 (2.03)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	4.77 (2.18)	3.67 (1.92)	3.33 (1.82)	5.16 (2.27)	4.67 (2.16)	3.33 (1.82)	5.17 (2.27)	2.96 (1.72)	3.67 (1.92)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	5.16 (2.27)	2.42 (1.56)	3.67 (1.92)	2.98 (1.73)	2.63 (1.62)	3.79 (1.95)	4.15 (2.04)	5.27 (2.29)	3.33 (1.82)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	4.92 (2.22)	3.33 (1.82)	2.97 (1.72)	4.16 (2.04)	3.87 (1.97)	2.67 (1.63)	3.92 (1.98)	1.33 (1.15)	2.92 (1.71)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	3.79 (1.95)	2.67 (1.63)	3.43 (1.85)	2.97 (1.72)	4.63 (2.15)	3.67 (1.92)	5.48 (2.34)	6.17 (2.48)	3.85 (1.96)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	5.43 (2.33)	4.97 (2.23)	5.16 (2.27)	3.66 (1.91)	2.97 (1.72)	2.79 (1.67)	5.33 (2.31)	4.67 (2.16)	2.67 (1.63)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	6.33 (2.52)	3.83 (1.96)	3.81 (1.95)	2.47 (1.57)	4.92 (2.22)	3.54 (1.88)	4.37 (2.09)	5.11 (2.26)	3.86 (1.96)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	4.67 (2.16)	2.97 (1.72)	3.63 (1.91)	4.58 (2.14)	4.67 (2.16)	4.33 (2.08)	2.87 (1.69)	3.16 (1.78)	2.79 (1.67)
T9	Imidacloprid 0.006 per cent	5.33 (2.31)	0 (0)	0 (0)	0 (0)	0 (0)	1.33 (1.15)	0 (0)	1.67 (1.29)	2.33 (1.53)
T10	Control (Untreated)	4.97 (2.23)	6.33 (2.52)	5.35 (2.31)	4.82 (2.19)	4.69 (2.17)	7.62 (2.76)	6.13 (2.48)	6.47 (2.54)	5.67 (2.38)
	C.D (0.05)	(0.183)	(0.409)	(0.469)	(0.322)	(0.538)	(0.637)	(0.512)	(0.408)	(0.284)

Figures in parentheses are square root transformed values

M. anisopliae @ 10^{10} spores ml^{-1} were 2.97, 3.33 and 3.43, respectively and were statistically on par. The mean population of adult weevils ranged from 3.63 to 5.37 in the various treatments and were on par.

During the third week after the application of different treatments, in the imidacloprid 0.006 per cent treated plots there were no adult weevils and differed significantly from the other treatments. The lowest mean population of 2.47 weevils was recorded in the plots treated with *M. anisopliae* in talc @ 20 g l^{-1} and differed significantly from the other treatments. The mean population of weevils ranged from 2.97 to 5.16 in the different treatments.

In the imidacloprid 0.006 per cent treated plots there were no adult weevils at four weeks after the application of treatments and showed significant difference from the other treatments. The lowest mean population of 2.63 adult weevils was recorded in the plots treated with *B. bassiana* in talc @ 20 g l^{-1} and differed significantly from the other treatments. The mean population of adult weevils ranged between 2.97 to 4.92 in the various other treatments and were statistically on par.

At five weeks after the treatment, the plots treated with imidacloprid 0.006 per cent recorded the lowest mean population of 1.33 weevils and differed significantly from the other treatments. The mean population of weevils ranged from 2.67 to 4.33 weevils in the other treatments and were statistically on par. Significantly higher mean population of 7.62 weevils was observed in the untreated control plots.

During the sixth week after application of the treatments, no weevils were present in the imidacloprid 0.006 per cent treated plots and differed significantly from the other treatments. The lowest mean population of 2.87 adult weevils was observed in the plots treated with *M. anisopliae* in talc @ 30 g l^{-1} and showed significant difference. The mean population of weevils ranged between 3.92 to 6.13 in the various other treatments and were statistically on par.

The mean population of weevils in the talc formulation of *B. bassiana* @ 30 g l^{-1} treated plots were 1.33 at seven weeks after the application of treatments and was on par with the imidacloprid 0.006 per cent treated plots with a mean of 1.67 adult weevils. Relatively lower population of weevils were observed in the plots treated with *B. bassiana* @ 10^{11} spores ml^{-1} and *M. anisopliae* in talc @ 30 g l^{-1} with a mean of 2.96 and 3.16 adult weevils respectively and were statistically on par. The population of adult weevils ranged from 4.67 to 6.47 in the other treatments and were on par.

At eight weeks after application of treatments, the mean population of adult weevils in the plots treated with imidacloprid 0.006 per cent, *M. anisopliae* @ 10^{12} spores ml^{-1} , *M. anisopliae* in talc @ 30 g l^{-1} and *B. bassiana* in talc @ 30 g l^{-1} were 2.33, 2.67, 2.79 and 2.92 respectively and were on par. The highest mean population of 5.67 was observed in the untreated control plot which differed significantly from the other treatments. The mean population of weevils in the other treatments ranged from 3.33 to 4.13 and were statistically on par.

4.4.2.3.2 Intensity of damage in tubers

When the number of galleries in three tubers per mound was scored, the tubers from the mounds treated with imidacloprid 0.006 per cent recorded significantly lower number of galleries when compared to the other treatments and was found to be superior with a mean number of 5.33 galleries (Table 77). The mean number of galleries noticed in the treatments with *B. bassiana* in talc @ 30 g l^{-1} , *M. anisopliae* in talc @ 30 g l^{-1}, *B. bassiana* @ 10^{9} spores ml^{-1} , *M. anisopliae* in talc @ 20 g l^{-1} , *M. anisopliae* @ 10^{12} spores ml^{-1} , *B. bassiana* @ 10^{11} spores ml^{-1} , *M. anisopliae* @ 10^{10} spores ml^{-1} , *B. bassiana* in talc @ 20 g l^{-1} and untreated control were 9.66, 11.33, 11.54, 12.73, 13.09, 13.45, 14.66, 14.82 and 15.79 respectively and were statistically on par.

4.4.2.3.3 Population of grubs in tubers

The tubers obtained from the imidacloprid 0.006 per cent treated mounds recorded the lowest mean population of 3.67 grubs of *C. formicarius* and was

Table 77. Mean population of *C. formicarius* and extent of damage in sweet potato during the first field trial with foliar spray

Sl. No.	Treatments	Number of galleries (3 tubers mound^{-1})	Number of grubs (3 tubers mound^{-1})	Number of adult *C.formicarius* kg^{-1} soil
T1	*B. bassiana* @ 10^{9} spores ml^{-1}	11.54 (3.39)	9.33 (3.05)	7.67 (2.77)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	13.45 (3.67)	7.67 (2.77)	6.00 (2.45)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	14.82 (3.85)	8.33 (2.89)	5.37 (2.32)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	9.66 (3.11)	9.67 (3.11)	6.00 (2.43)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	14.66 (3.83)	9.00 (3.00)	9.33 (3.00)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	13.09 (3.62)	8.33 (2.89)	7.42 (2.72)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	12.73 (3.57)	11.63 (3.41)	8.67 (2.94)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	11.33 (3.37)	9.67 (3.11)	7.39 (2.72)
T9	Imidacloprid 0.006 per cent	5.33 (2.31)	3.67 (1.92)	2.67 (1.63)
T10	Control (Untreated)	15.79 (3.97)	16.67 (4.08)	9.67 (3.11)
	C.D (0.05)	(0.964)	(1.127)	(1.093)

Figures in parentheses are square root transformed values

found to be the superior treatment among the others and it was on par with *B. bassiana* @ 10^{11} spores ml^{-1}, *B. bassiana* in talc @ 20 g l^{-1} and *M. anisopliae* @ 10^{12} spores ml^{-1} which recorded a mean population of 7.67, 8.33 and 8.33 grubs respectively (Table 77). The mean population of grubs of *C. formicarius* in the tubers harvested from the mounds treated with *M. anisopliae* @ 10^{10} spores ml^{-1}, *B. bassiana* @ 10^{9} spores ml^{-1}, *B. bassiana* in talc @ 30 g l^{-1}, *M. anisopliae* in talc @ 30 g l^{-1}, *M. anisopliae* in talc @ 20 g l^{-1} and the untreated control were 9.00, 9.33, 9.67, 9.67, 11.63 and 16.67 respectively and were statistically on par.

4.4.2.3.4 Population of weevils in soil

The population of adult *C. formicarius* per kg soil in the mounds treated with imidacloprid 0.006 per cent was the lowest with a mean population of 2.67 weevils and differed significantly from the other treatments and was found to be the superior treatment (Table 77). The mean population of adult weevils in the soil in the mounds treated with *B. bassiana* in talc @ 20 g l^{-1}, *B. bassiana* in talc @ 30 g l^{-1}, *B. bassiana* @ 10^{11} spores ml^{-1}, *M. anisopliae* @ 10^{12} spores ml^{-1}, *M. anisopliae* in talc @ 30 g l^{-1}, *B. bassiana* @ 10^{9} spores ml^{-1}, *M. anisopliae* in talc @ 20 g l^{-1} and *M. anisopliae* @ 10^{10} spores ml^{-1} and in the untreated control were 5.37, 6.00, 6.00, 7.42, 7.39, 7.67, 8.67, 9.33 and 9.67 weevils respectively and were on par.

4.4.2.3.5 Population of natural enemies

The mean population of hymenopteran natural enemies and the predatory spiders present in the foliage of the sweet potato in the treated mounds was counted at weekly intervals and the data was insignificant and presented in Tables 78 and 79.

4.4.2.3.6 Yield

Weight of tubers

The mean yield obtained from the mounds treated with imidacloprid 0.006 per cent was 3.66 kg and was the maximum among the different treatments,

Table 78. Population of hymenopteran natural enemies in the foliage of sweet potato during first field trial with foliar spraying

Sl. No.	Treatments	Pre count	Number of hymenopteran natural enemies (in 0.19 m^2)							
			1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT
T1	*B. bassiana* @ 10^9 spores ml^{-1}	0.93 (0.96)	0 (0)	0 (0)	0.54 (0.73)	1.77 (1.33)	1.33 (1.15)	0 (0)	1.00 (1.00)	0 (0)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	0 (0)	0 (0)	1.32 (1.15)	0 (0)	0.92 (0.96)	0 (0)	0 (0)	0 (0)	0.54 (0.73)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	0 (0)	0.64 (0.80)	1.67 (1.29)	0 (0)	0 (0)	0 (0)	1.47 (1.21)	0 (0)	1.00 (1.00)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	1.00 (1.00)	0 (0)	0 (0)	0.33 (0.57)	0 (0)	0 (0)	1.00 (1.00)	0 (0)	0 (0)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	0 (0)	0 (0)	1.00 (1.00)	0 (0)	2.33 (1.53)	0 (0)	0 (0)	0 (0)	0 (0)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	0.54 (0.73)	1.00 (1.00)	0 (0)	0 (0)	0 (0)	1.67 (1.29)	0 (0)	0 (0)	1.79 (1.34)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	0.75 (0.87)	0 (0)	0 (0)	0.95 (0.97)	0 (0)	0.67 (0.82)	0 (0)	1.67 (1.29)	0 (0)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	0 (0)	0 (0)	0 (0)	1.92 (1.39)	0.66 (0.81)	0 (0)	0 (0)	1.00 (1.00)	0.67 (0.82)
T9	Imidacloprid 0.006 per cent	1.63 (1.28)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.54 (0.73)	0 (0)	1.33 (1.15)
T10	Control (Untreated)	0 (0)	0 (0)	0 (0)	2.16 (1.47)	0 (0)	1.00 (1.00)	1.47 (1.21)	0 (0)	1.00 (1.00)
	C.D (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

Figures in parentheses are square root transformed values

Table 79. Population of predatory spiders in the foliage of sweet potato during first field trial with foliar spraying

Sl. No.	Treatments	Pre count	Number of spiders (in 0.19 m^2)							
			1 WAT	2 WAT	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT
T1	*B. bassiana* @ 10^9 spores ml^{-1}	0 (0)	2.39 (1.55)	0 (0)	1.17 (1.08)	0 (0)	0 (0)	1.37 (1.17)	0 (0)	0 (0)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	0 (0)	0 (0)	0.33 (0.57)	0.63 (0.79)	0 (0)	0 (0)	0.92 (0.96)	1.00 (1.00)	1.00 (1.00)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	0 (0)	1.33 (1.15)	1.67 (1.29)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.33 (1.15)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	1.00 (1.00)	0 (0)	1.00 (1.00)	0 (0)	1.00 (1.00)	1.33 (1.15)	0 (0)	2.16 (1.47)	0 (0)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	0 (0)	0 (0)	0 (0)	1.21 (1.10)	0.67 (0.82)	1.00 (1.00)	1.00 (1.00)	0 (0)	0 (0)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	2.33 (1.53)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2.33 (1.53)	2.33 (1.53)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	0 (0)	2.67 (1.63)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.94 (1.39)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	1.67 (1.29)	0 (0)	0 (0)	0 (0)	0.33 (0.57)	0 (0)	0 (0)	0 (0)	0 (0)
T9	Imidacloprid 0.006 per cent	0 (0)	1.93 (1.39)	0 (0)	1.33 (1.15)	0 (0)	2.49 (1.58)	1.33 (1.15)	3.00 (1.73)	0 (0)
T10	Control (Untreated)	0 (0)	0 (0)	3.16 (1.78)	0 (0)	0 (0)	0 (0)	0.67 (0.82)	0 (0)	1.00 (1.00)
	C.D (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS

Figures in parentheses are square root transformed values

followed by *B. bassiana* in talc @ 30 g l^{-1} and *M. anisopliae* in talc @ 20 g l^{-1} treated mounds which yielded a mean of 2.57 and 2.33 kg respectively and were statistically on par (Table 80). The mounds treated with *M. anisopliae* in talc @ 30 g l^{-1}, *B. bassiana* @ 10^{11} spores ml^{-1} , *M. anisopliae* @ 10^{12} spores ml^{-1} , *B. bassiana* @ 10^{9} spores ml^{-1} , *M. anisopliae* @ 10^{10} spores ml^{-1} , *B. bassiana* in talc @ 20 g l^{-1} and untreated control yielded a mean of 2.27, 2.15, 1.97, 1.93, 1.84, 1.82 and 1.55 kg tubers respectively and were on par.

Marketable yield

The mean marketable yield of tubers recorded for the treatment with imidacloprid 0.006 per cent was maximum with a yield of 3.45 kg and was on par with the treatment with *B. bassiana* in talc @ 30 g l^{-1} which recorded a mean of 1.96 kg tubers (Table 80). The treatments with *M. anisopliae* in talc @ 20 g l^{-1} , *M. anisopliae* in talc @ 30 g l^{-1}, *B. bassiana* in talc @ 20 g l^{-1} , *B. bassiana* @ 10^{11} spores ml^{-1} , *M. anisopliae* @ 10^{12} spores ml^{-1} , *M. anisopliae* @ 10^{10} spores ml^{-1} and *B. bassiana* @ 10^{9} spores ml^{-1} yielded a mean marketable yield of 1.75, 1.64, 1.40, 1.35, 1.30, 1.25 and 1.12 kg tubers respectively and were statistically on par. The untreated control yielded the lowest mean of 0.67 kg marketable tubers.

The average per hectare yield was maximum in the treatment with imidacloprid 0.006 per cent with a mean of 58.65 tons of tubers and was statistically on par with the treatments involving *B. bassiana* in talc @ 30 g l^{-1} , *M. anisopliae* in talc @ 20 g l^{-1} and *M. anisopliae* in talc @ 30 g l^{-1} which recorded an average yield of 33.32, 29.75 and 27.88 tons of tubers respectively. The mean yield ranged between 23.80 to 11.39 tons of tubers in the other treatments and were on par.

Benefit : Cost ratio

The highest B : C ratio of 3.19 was recorded for the treatment with imidacloprid 0.006 per cent, which was followed by the treatment with *B. bassiana* in talc @ 30 g l^{-1} which had a B : C ratio of 2.23 (Table 80). The B : C ratio ranged from 1.94 to 0.98 among the other treatments.

Table 80. Mean weight of tubers in sweet potato in the first field trial with foliar spraying and benefit : cost ratio

Sl. No.	Treatments	Yield mound^{-1} (kg)	Marketable yield mound^{-1} (kg)	Yield ha^{-1} (tons)	Benefit : Cost ratio
T1	*B. bassiana* @ 10^9 spores ml^{-1}	1.93 (1.39)	1.12 (1.06)	19.04 (4.36)	0.98
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	2.15 (1.47)	1.35 (1.16)	22.95 (4.76)	1.32
T3	Talc based *B. bassiana* @ 20 g l^{-1}	1.82 (1.35)	1.40 (1.18)	23.80 (4.88)	1.42
T4	Talc based *B. bassiana* @ 30 g l^{-1}	2.57 (1.60)	1.96 (1.40)	33.32 (5.77)	2.23
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	1.84 (1.36)	1.25 (1.12)	21.25 (4.61)	1.17
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	1.97 (1.40)	1.30 (1.14)	22.10 (4.70)	1.24
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	2.33 (1.53)	1.75 (1.32)	29.75 (5.45)	1.94
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	2.27 (1.51)	1.64 (1.28)	27.88 (5.28)	1.75
T9	Imidacloprid 0.006 per cent	3.66 (1.91)	3.45 (1.86)	58.65 (7.66)	3.19
T10	Control (Untreated)	1.55 (1.24)	0.67 (0.82)	11.39 (3.37)	-
	C.D (0.05)	(0.397)	(0.431)	(2.408)	-

Figures in parentheses are square root transformed values

4.4.2.3.7 Number of tubers and extent of damage

The treatment with imidacloprid 0.006 per cent recorded significantly higher number of tubers with a mean of 9.67 and was the superior treatment among others (Table 81). The mean number of tubers obtained by the application of *B. bassiana* @ 10^{11} spores ml^{-1} , *M. anisopliae* @ 10^{10} spores ml^{-1} and *B. bassiana* in talc @ 20 g l^{-1} were 7.67, 7.33 and 6.33 tubers respectively and were statistically on par. The treatments with *B. bassiana* @ 10^{9} spores ml^{-1}, *B. bassiana* in talc @ 30 g l^{-1}, *M. anisopliae* in talc @ 30 g l^{-1} and the untreated control recorded a mean number of 6.00 tubers each respectively and was on par with *M. anisopliae* in talc @ 20 g l^{-1} which yielded a mean number of 5.00 tubers. The lowest number of tubers was obtained from the treatment with *M. anisopliae* @ 10^{12} spores ml^{-1} which recorded a mean number of 4.33 tubers.

The percentage of infested tubers was the lowest for the treatment with imidacloprid 0.006 per cent which recorded a mean of 20.20 per cent damaged tubers and differed significantly from the other treatments and was found to be the superior treatment. The mounds treated with *B. bassiana* @ 10^{9} spores ml^{-1} had 39.52 per cent infested tubers and showed significant difference from the other treatments. The mean percentage of infested tubers recorded for the treatments with *B. bassiana* @ 10^{11} spores ml^{-1} and *B. bassiana* in talc @ 30 g l^{-1} were 43.45 and 44.29 and were statistically on par. The talc based *B. bassiana* @ 20 g l^{-1} had a mean of 47.62 per cent infested tubers and was on par with the untreated control which had 50.95 per cent infested tubers. The treatments with *M. anisopliae* in talc @ 20 g l^{-1} , *M. anisopliae* @ 10^{12} spores ml^{-1} , *M. anisopliae* @ 10^{10} spores ml^{-1} and *M. anisopliae* in talc @ 30 g l^{-1} yielded a mean number of 53.33, 53.33, 54.76 and 55.71 per cent tubers and were statistically on par.

The mean weight of infested tubers obtained from the mounds treated with imidacloprid 0.006 per cent is 5.74 per cent and differed significantly from the other treatments and was the superior treatment. A mean weight of 23.08 and 23.74 per cent infested tubers was obtained for the treatments with talc based

Table 81. Mean number of tubers in the first field trial in sweet potato with foliar spraying and the extent of damage

Sl. No.	Treatments	Total number of tubers	Percentage of infested tubers by number	Percentage weight of infested tubers
T1	*B. bassiana* @ 10^9 spores ml^{-1}	6.00 (2.45)	39.52 (6.29)	41.97 (6.48)
T2	*B. bassiana* @ 10^{11} spores ml^{-1}	7.67 (2.77)	43.45 (6.59)	37.21 (6.10)
T3	Talc based *B. bassiana* @ 20 g l^{-1}	6.33 (2.52)	47.62 (6.90)	23.08 (4.80)
T4	Talc based *B. bassiana* @ 30 g l^{-1}	6.00 (2.45)	44.29 (6.66)	23.74 (4.87)
T5	*M. anisopliae* @ 10^{10} spores ml^{-1}	7.33 (2.71)	54.76 (7.40)	32.07 (5.66)
T6	*M. anisopliae* @ 10^{12} spores ml^{-1}	4.33 (2.08)	53.33 (7.30)	34.01 (5.83)
T7	Talc based *M. anisopliae* @ 20 g l^{-1}	5.00 (2.24)	53.33 (7.29)	24.89 (4.99)
T8	Talc based *M. anisopliae* @ 30 g l^{-1}	6.00 (2.45)	55.71 (7.46)	27.75 (5.27)
T9	Imidacloprid 0.006 per cent	9.67 (3.11)	20.20 (4.49)	5.74 (2.39)
T10	Control (Untreated)	6.00 (2.45)	50.95 (7.14)	56.77 (7.53)
	C.D (0.05)	(0.291)	(0.306)	(1.217)

Figures in parentheses are square root transformed values

B. bassiana @ 20 g l^{-1} and @ 30 g l^{-1} respectively and were statistically on par. The treatments with *M. anisopliae* in talc @ 20 g l^{-1} , *M. anisopliae* in talc @ 30 g l^{-1} , *M. anisopliae* @ 10^{10} spores ml^{-1} , *M. anisopliae* @ 10^{12} spores ml^{-1} and *B. bassiana* @ 10^{11} spores ml^{-1} produced a mean weight of 24.89, 27.75, 32.07, 34.01 and 37.21per cent infested tubers and were statistically on par. The highest percentage weight of infested tubers was recorded for the treatments with *B. bassiana* @ 10^{9} spores ml^{-1} and the untreated control which had a mean of 41.97 and 56.77 per cent among the total tubers harvested and were statistically on par.

4.5 COMPATIBILITY OF FUNGI WITH PESTICIDES

The compatibility of *B. bassiana* and *M. anisopliae* with insecticides and fungicides was studied using poison food technique and the results are presented in Tables 82 to 85.

4.5.1 Fungal growth

4.5.1.1 B. bassiana

When *B. bassiana* was cultured in poisoned food media, 2 DAI, the growth of the fungus in the control Petri plates without chemicals reached 1.54 cm and was on par with the mean radial growth of the fungus in imidacloprid poisoned media (1.36 cm) (Table 82). The carbofuran and carbosulfan treated media recorded a mean growth of 1.26 cm and 1.21 cm, respectively and were statistically on par. The growth of the fungus in the media poisoned with chlorpyriphos, lambda cyhalothrin and mancozeb were 0.93, 0.83 and 0.79 cm and these were on par. The least growth was observed in the case of carbendazim and malathion, which recorded a mean growth of 0.67 and 0.50 cm, respectively.

Five days after inoculation, the radial growth of the fungus in the control plate was significantly higher and recorded a growth of 4.24 cm, followed by imidacloprid poisoned media (2.93 cm) and these were statistically superior to other treatments. *B. bassiana* in carbosulfan and carbofuran treated PDA had a

Table 82. Mean diameter of *B. bassiana* grown in poisoned media at different intervals

Sl. No	Treatments	Growth (cm)				
		2DAI	5DAI	7DAI	10DAI	13DAI
1	Carbendazim 0.1 %	0.67 (0.82)	1.15 (1.07)	2.30 (1.52)	3.39 (1.84)	4.15 (2.04)
2	Mancozeb 0.2 %	0.79 (0.89)	1.12 (1.06)	1.84 (1.36)	2.51 (1.58)	2.84 (1.68)
3	Carbofuran 0.30 ppm	1.26 (1.12)	2.39 (1.55)	3.39 (1.84)	4.25 (2.06)	5.82 (2.41)
4	Carbosulfan 0.10 ppm	1.21 (1.10)	2.41 (1.55)	3.53 (1.88)	4.85 (2.20)	6.89 (2.62)
5	Imidacloprid 0.006 %	1.36 (1.17)	2.93 (1.71)	4.45 (2.11)	7.21 (2.68)	8.27 (2.88)
6	Chlorpyriphos 0.03 %	0.93 (0.96)	2.09 (1.45)	3.36 (1.83)	4.80 (2.19)	5.51 (2.35)
7	Lambda cyhalothrin 0.025 %	0.83 (0.91)	1.64 (1.28)	2.66 (1.63)	3.18 (1.78)	4.22 (2.05)
8	Malathion 0.15 %	0.50 (0.71)	0.87 (0.93)	1.34 (1.16)	1.64 (1.28)	2.72 (1.65)
9	Control	1.54 (1.24)	4.24 (2.06)	5.92 (2.43)	8.16 (2.86)	9.00 (3.00)
	C.D (0.05)	(0.075)	(0.054)	(0.095)	(0.064)	(0.052)

Figures in parentheses are square root transformed values

mean growth of 2.41 and 2.39 cm and were statistically on par. *B. bassiana* in chlorpyriphos poisoned media recorded a growth of 2.09 cm and that in lambda cyhalothrin had a mean growth of 1.64 cm and these were significantly higher from others. The growth of the fungus in the two fungicides, carbendazim and mancozeb mixed media were 1.15 and 1.12 cm, respectively and were on par. The fungus cultured in malathion had significantly lower growth (0.87 cm).

The growth of the fungus in the control was significantly higher and recorded 5.92 cm at 7 DAI, followed by 4.45 cm growth of the fungus in the imidacloprid mixed media. Carbosulfan, carbofuran and chlorpyriphos supported a mean growth of 3.53, 3.39 and 3.36 cm respectively and showed statistical similarity. The mean radial growth of *B. bassiana* in poisoned media with lambda cyhalothrin, carbendazim, mancozeb and malathion were 2.66, 2.30, 1.84 and 1.34 cm respectively and differed significantly from the others.

The growth of the fungus increased at ten days after inoculation and the control plate had significantly higher growth of 8.16 cm, followed by the one in imidacloprid treated PDA, which had a mean radial growth of 7.21 cm and showed statistical significance from among the other treatments. The mean growth of 4.85 and 4.80 cm of the fungus was observed in carbosulfan and chlorpyriphos respectively and were on par. Growth in carbofuran poisoned media was 4.25 cm. The fungicide carbendazim and the synthetic pyrethroid lambda cyhalothrin supported a mean radial growth of 3.39 and 3.18 cm respectively and were on par. The mean growth of the fungus in mancozeb was 2.51 cm and was on par with malathion (1.64 cm).

At 13 DAI, the fungus completely covered the nine cm Petri dish. Fungus grown in imidacloprid, carbosulfan, carbofuran and chlorpyriphos which recorded a mean radial growth of 8.27, 6.89, 5.82 and 5.51 cm respectively and were statistically significant to others. Lambda cyhalothrin and carbendazim poisoned media produced a mean growth of 4.22 and 4.15 cm, respectively and were on par. The growth of the fungus in the mancozeb and malathion mixed PDA were

the lowest and recorded a mean growth of 2.84 and 2.72 cm, respectively and showed statistical significance (Plate 15).

4.5.1.2 M. anisopliae

M. anisopliae recorded the highest mycelial growth in the control plate two days after inoculation and had a mean growth of 1.26 cm, followed by the growth in imidacloprid and carbofuran mixed media, which produced 1.12 and 1.09 cm respectively and showed statistical similarity (Table 83). *M. anisopliae* in carbosulfan recorded a mean growth of 1.05 cm, while that in chlorpyriphos and mancozeb recorded a mean radial growth of 0.69 and 0.63 cm respectively and were on par. Relatively lower growth was observed in the case of lambda cyhalothrin, carbendazim and malathion, in which the fungus had a mean growth of 0.57, 0.53 and 0.53 cm respectively and were on par.

At five days after inoculation of the fungus, significantly higher radial growth was observed in the control plate (3.84 cm) followed by imidacloprid (3.35 cm) and carbosulfan (2.86 cm) and showed statistical significance. The fungus radially covered 2.24, 1.99, 1.91 and 1.74 cm of the Petri plate when grown in carbofuran, mancozeb, lambda cyhalothrin and chlorpyriphos respectively and were on par. The lowest growth of the fungus was observed for carbendazim and malathion, in which a radial growth of 1.25 and 1.03 cm, respectively was observed which were on par.

M. anisopliae covered a diameter of 4.37 and 4.06 cm in the control and imidacloprid poisoned media respectively at seven days after inoculation and exhibited statistical similarity. The mean radial growth of 3.83 and 3.39 cm was observed in the case of fungus grown in carbofuran and carbosulfan respectively and differed significantly from the other treatments. *M. anisopliae* in mancozeb and lambda cyhalothrin poisoned PDA recorded a mean growth of 2.89 and 2.63 cm respectively and were on par and the latter was on par with chlorpyriphos (2.52 cm). Relatively lower mycelial growth was observed in carbendazim and

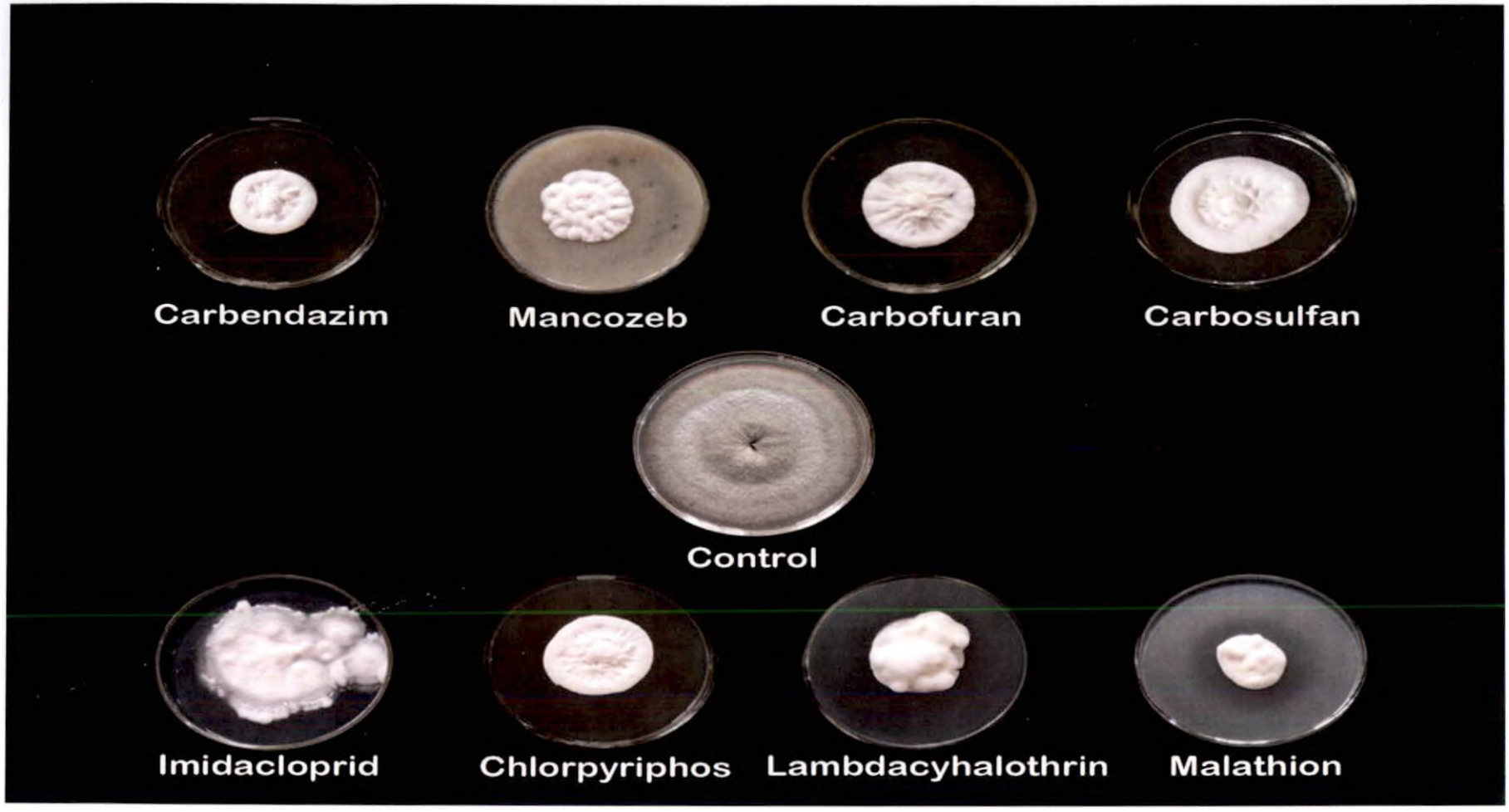


Plate 15. Mean growth of *B. bassiana* in different poison media

Table 83. Mean diameter of *M. anisopliae* grown in poisoned media at different intervals

Sl. No.	Treatments	Growth (cm)				
		2DAI	5DAI	7DAI	10DAI	15DAI
1	Carbendazim 0.1 %	0.53 (0.73)	1.25 (1.12)	1.93 (1.39)	2.39 (1.55)	3.31 (1.82)
2	Mancozeb 0.2 %	0.63 (0.79)	1.99 (1.41)	2.89 (1.70)	3.24 (1.79)	4.14 (2.03)
3	Carbofuran 0.30 ppm	1.09 (1.05)	2.24 (1.49)	3.83 (1.96)	4.27 (2.07)	5.46 (2.34)
4	Carbosulfan 0.10 ppm	1.05 (1.03)	2.86 (1.69)	3.39 (1.84)	4.23 (2.06)	5.63 (2.37)
5	Imidacloprid 0.006 %	1.12 (1.06)	3.35 (1.83)	4.06 (2.01)	5.44 (2.33)	6.37 (2.52)
6	Chlorpyriphos 0.03 %	0.69 (0.84)	1.74 (1.32)	2.52 (1.59)	3.14 (1.77)	4.33 (2.08)
7	Lambda cyhalothrin 0.025 %	0.57 (0.75)	1.91 (1.38)	2.63 (1.62)	3.16 (1.78)	4.89 (2.21)
8	Malathion 0.15 %	0.53 (0.73)	1.03 (1.01)	1.58 (1.26)	2.14 (1.46)	3.53 (1.89)
9	Control	1.26 (1.12)	3.84 (1.96)	4.37 (2.09)	6.17 (2.48)	9.00 (3.00)
	C.D (0.05)	(0.083)	(0.111)	(0.099)	(0.086)	(0.073)

Figures in parentheses are square root transformed values

malathion, which had a mean diameter of 1.93 and 1.58 cm, respectively and differed significantly.

The radial growth of *M. anisopliae* ten days after inoculation was highest in the control plate with a mean of 6.17 cm, followed by imidacloprid treated PDA with a mean of 5.44 cm and was statistically significant when compared to the other treatments. The fungus grown in carbofuran and carbosulfan recorded a mean growth of 4.27 and 4.23 cm respectively and were on par. Moderate growth of the fungus was seen in the case of mancozeb, lambda cyhalothrin and chlorpyriphos with a mean diameter of 3.24, 3.16 and 3.14 cm respectively and showed statistical similarity. *M. anisopliae* when grown on carbendazim and malathion had relatively lower growth of 2.39 and 2.14 cm, respectively.

The fungus fully covered the Petri plate at 15 DAI in control and recorded a mean diameter of 9 cm. This was followed by the growth in imidacloprid treated PDA with a mean growth of 6.37 cm and exhibited statistical significance from among the other treatments. Carbosulfan and carbofuran produced a mean radial growth of 5.63 and 5.46 cm of the fungus, respectively and were on par and this was followed by lambda cyhalothrin (4.89 cm). The growth of the fungus in the chlorpyriphos and mancozeb treated PDA was 4.33 and 4.14 cm, respectively and had statistical similarity. The fungus grown in malathion and carbendazim could grow only 3.53 and 3.31 cm, respectively of the culture plate and were statistically on par (Plate 16).

4.5.2 Sporulation

4.5.2.1 B. bassiana

B. bassiana grown without pesticide in PDA recorded a significantly higher spore count of 1108.31×10^8 spores ml^{-1} (Table 84). The media poisoned with imidacloprid, carbofuran, carbosulfan, chlorpyriphos, malathion, lambda cyhalothrin, carbendazim and mancozeb supported a mean sporulation of 35.45×10^8, 19.82×10^8, 7.65×10^8, 1.41×10^8, 0.27×10^8, 0.26×10^8, 0.05×10^8 and 0.04×10^8 spores ml^{-1} respectively and these were statistically on par.

Plate 16. Mean growth of *M. anisopliae* in different poison media

Table 84. Mean spore count and bioefficacy of *B. bassiana* grown in poisoned media

Sl. No.	Treatments	Spore count @ 13 DAI (spores ml^{-1}) $\times 10^8$	Mortality of *C. formicarius* at 14 DAT	
			Adult	Grub
1	Carbendazim 0.1 %	0.05 (0.22)	24.34 (4.93)	33.33 (5.77)
2	Mancozeb 0.2 %	0.04 (0.19)	6.67 (2.58)	28.81 (5.37)
3	Carbofuran 0.30 ppm	19.82 (4.45)	44.39 (6.66)	71.07 (8.43)
4	Carbosulfan 0.10 ppm	7.65 (2.76)	39.81 (6.31)	62.18 (7.89)
5	Imidacloprid 0.006 %	35.45 (5.95)	51.06 (7.15)	75.52 (8.69)
6	Chlorpyriphos 0.03 %	1.41 (1.19)	26.38 (5.14)	42.17 (6.49)
7	Lambda cyhalothrin 0.025 %	0.26 (0.51)	19.62 (4.43)	31.03 (5.57)
8	Malathion 0.15 %	0.27 (0.52)	10.86 (3.29)	28.81 (5.37)
9	Control	1108.31 (33.29)	77.74 (8.82)	100.00 (10.00)
	C.D (0.05)	(10.614)	(0.814)	(0.449)

Figures in parentheses are square root transformed values

4.5.2.2 M. anisopliae

M. anisopliae when cultured without pesticides yielded significantly higher spore concentration of 84.67 × 10^8 spores ml^{-1}, this was followed by imidacloprid cultured fungus with a spore count of 26.74 × 10^8 spores ml^{-1} and was statistically superior (Table 85). The spore load of the fungus present in the media cultured with carbosulfan, carbofuran, carbendazim, chlorpyriphos, mancozeb, lambda cyhalothrin and malathion were 1.47 × 10^8, 1.39 × 10^8, 0.14 × 10^8, 0.09 × 10^8, 0.06 × 10^8, 0.03 × 10^8 and 0.03 × 10^8 spores ml^{-1} respectively and were statistically on par.

4.5.3 Bioefficacy

4.5.3.1 B. bassiana

4.5.3.1.1 On adults of C. formicarius

When the bioefficacy of the fungi cultured in poisoned media was tested against the adults of *C. formicarius*, the highest mean mortality of 77.74 per cent at 14 DAT was recorded when treated with the fungus cultured without poison and was significantly superior when compared to the other treatments (Table 84). The mean mortality caused by the fungus cultured in imidacloprid was 51.06 per cent and was on par with the fungus grown in carbofuran poisoned media (44.39) and the latter had statistical similarity with the mean mortality caused by the fungus cultured in carbosulfan treated media (39.81 per cent). A mean mortality of 26.38, 24.34 and 19.62 per cent was observed when treated with the fungus in chlorpyriphos, carbendazim and lambda cyhalothrin poisoned media, respectively and these were on par. The lower mortality rates were observed when treated with the fungus cultured in malathion and mancozeb, which recorded a mean mortality of 10.86 and 6.67 per cent respectively and were statistically on par.

Table 85. Mean spore count and bioefficacy of *M. anisopliae* grown in poisoned media

Sl. No.	Treatments	Spore count @ 15 DAI (spores ml^{-1}) $\times 10^8$	Mortality of *C. formicarius* at 14 DAT	
			Adult	Grub
1	Carbendazim 0.1 %	0.14 (0.37)	19.62 (4.43)	31.03 (5.57)
2	Mancozeb 0.2 %	0.06 (0.25)	10.86 (3.29)	28.51 (5.34)
3	Carbofuran 0.30 ppm	1.39 (1.18)	22.12 (4.70)	35.49 (5.96)
4	Carbosulfan 0.10 ppm	1.47 (1.21)	26.67 (5.16)	44.39 (6.66)
5	Imidacloprid 0.006 %	26.74 (5.17)	35.49 (5.96)	44.39 (6.66)
6	Chlorpyriphos 0.03 %	0.09 (0.31)	15.40 (3.92)	31.03 (5.57)
7	Lambda cyhalothrin 0.025 %	0.03 (0.16)	13.33 (3.65)	24.34 (4.93)
8	Malathion 0.15 %	0.03 (0.16)	8.64 (2.94)	15.40 (3.92)
9	Control	84.67 (9.20)	42.17 (6.49)	51.06 (7.15)
	C.D (0.05)	(3.922)	(0.789)	(0.710)

Figures in parentheses are square root transformed values

4.5.3.1.2 On grubs of C. formicarius

With respect to the grubs of *C. formicarius*, cent per cent mortality was achieved when treated with the fungus cultured without pesticides and this differed significantly from the other treatments (Table 84). A mean mortality of 75.52 and 71.07 per cent was observed when treated with the fungus grown in imidacloprid and carbofuran treated media, respectively and were on par. The carbosulfan poisoned culture caused a mortality percentage of 62.18, it was followed by chlorpyriphos cultured one (42.17 per cent) and these were on par. Relatively lower mortality was recorded for the fungal cultures grown in carbendazim, lambda cyhalothrin, mancozeb and malathion which caused 33.33, 31.03, 28.81 and 28.81 per cent mortality, respectively and were on par.

4.5.3.2 M. anisopliae

4.5.3.2.1 On adults of C. formicarius

The maximum mortality of the adult *C. formicarius* at 14 DAT when treated with the fungus cultured with different pesticides was 42.17 per cent and it was observed in the control with no chemicals, this was followed by the fungal culture grown in imidacloprid (35.49) and these were on par (Table 85). The mean mortality obtained when treated with the fungus grown with carbosulfan, carbofuran and carbendazim were 26.67, 22.12 and 19.62 per cent, respectively and were on par. The fungus cultured with chlorpyriphos, lambda cyhalothrin and mancozeb caused moderate mortality of 15.40, 13.33 and 10.86 per cent respectively and showed statistical similarity. The lowest mortality was recorded for the fungal culture poisoned with malathion (8.64 per cent).

4.5.3.2.2 On grubs of C. formicarius

When the fungus cultured without poison was sprayed on the grubs of *C. formicarius*, a higher mortality of 51.06 per cent was observed and it was on par with the mean mortality of 44.39 per cent each recorded for carbosulfan and carbofuran respectively (Table 85). The percentage mortality of 35.49, 31.03,

31.03 and 28.51 was obtained when the grubs were treated with the fungus cultured with carbofuran, carbendazim, chlorpyriphos and mancozeb, respectively and showed statistical similarity. Lower rates of mortality was recorded in the case of lambda cyhalothrin and malathion poisoned media, which recorded a mean mortality of 24.34 and 15.40 per cent respectively and differed significantly from the other treatments.

4.6 DEVELOPMENT OF PESTICIDE TOLERANT STRAIN AND MOLECULAR CHARACTERISATION

4.6.1 Pesticide tolerance of fungi

B. bassiana and *M. anisopliae* grown continuously in varying doses of two fungicides *viz.* carbendazim and mancozeb and six insecticides *viz.* carbofuran, carbosulfan, imidacloprid, chlorpyriphos, lambda cyhalothrin and malathion showed that there was inhibition in growth of both the fungi but they grew on all the media mixed with pesticides. The results are presented in Tables 86 to 91.

4.6.1.1 Growth of the fungi

4.6.1.1.1 B. bassiana

B. bassiana recorded the maximum radial growth (9 cm) in 13 days, followed by the fungi grown in imidacloprid, carbosulfan, carbofuran and chlorpyriphos which recorded a mean radial growth of 8.27, 6.89, 5.82 and 5.51 cm respectively and showed statistical significance from the other treatments (Table 86). The growth of the fungus on lambda cyhalothrin mixed media was 4.22 cm and was on par with fungus cultured in carbendazim which produced a mean radial growth of 4.15 cm. When the fungus was grown on media mixed with the fungicide, mancozeb and the insecticide malathion the growth was very low and recorded 2.84 and 2.72 cm, respectively and were statistically on par.

In the second passage with varying dose of insecticides, the control plate with no chemical treatment recorded significantly higher radial growth (9 cm) in

Table 86. Mean growth of *B. bassiana* in poisoned food media for continuous ten passages

Sl. No.	Treatments	Growth of the fungus (cm)									
		I @ 13 DAI	II @ 13 DAI	III @ 15 DAI	IV @ 15 DAI	V @ 18 DAI	VI @ 16 DAI	VII @ 18 DAI	VIII @ 19 DAI	IX @ 21 DAI	X @ 21 DAI
T1	Carbendazim	4.15 (2.04)	3.89 (1.97)	4.12 (2.03)	3.10 (1.76)	3.27 (1.81)	3.62 (1.90)	3.21 (1.79)	3.36 (1.83)	3.53 (1.88)	3.20 (1.79)
T2	Mancozeb	2.84 (1.68)	2.67 (1.63)	2.05 (1.43)	2.41 (1.55)	2.49 (1.58)	2.89 (1.70)	2.96 (1.72)	2.18 (1.48)	2.34 (1.53)	2.44 (1.56)
T3	Carbofuran	5.82 (2.41)	5.81 (2.41)	5.59 (2.37)	5.65 (2.38)	5.74 (2.39)	5.17 (2.27)	5.31 (2.30)	5.53 (2.35)	5.24 (2.29)	5.51 (2.35)
T4	Carbosulfan	6.89 (2.63)	6.19 (2.49)	6.27 (2.51)	5.78 (2.40)	5.94 (2.44)	5.45 (2.34)	5.66 (2.38)	5.82 (2.41)	5.46 (2.34)	5.85 (2.42)
T5	Imidacloprid	8.27 (2.88)	8.49 (2.91)	8.37 (2.89)	7.46 (2.73)	8.28 (2.88)	7.87 (2.81)	8.10 (2.85)	7.79 (2.79)	8.18 (2.86)	8.29 (2.88)
T6	Chlorpyriphos	5.51 (2.35)	4.17 (2.04)	4.12 (2.03)	4.28 (2.07)	3.71 (1.93)	4.19 (2.05)	4.34 (2.08)	4.27 (2.07)	4.52 (2.13)	4.72 (2.17)
T7	Lambda cyhalothrin	4.22 (2.05)	3.17 (1.78)	3.06 (1.75)	3.35 (1.83)	2.71 (1.65)	2.76 (1.66)	2.96 (1.72)	2.34 (1.53)	2.56 (1.60)	2.97 (1.72)
T8	Malathion	2.72 (1.65)	2.63 (1.62)	1.95 (1.39)	2.11 (1.45)	2.39 (1.55)	2.72 (1.65)	2.45 (1.57)	2.73 (1.65)	2.23 (1.49)	2.64 (1.63)
T9	Untreated Control	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)
	C.D (0.05)	(0.052)	(0.061)	(0.048)	(0.066)	(0.098)	(0.047)	(0.044)	(0.047)	(0.045)	(0.038)

Figures in parentheses are square root transformed values

13 days, followed by imidacloprid, carbosulfan, carbofuran, chlorpyriphos, carbendazim and lambda cyhalothrin which recorded a mean radial growth of 8.49, 6.19, 5.81, 4.17, 3.89 and 3.17 cm respectively and differed significantly from the other treatments. Relatively lower growth was observed in the case of mancozeb mixed media (2.67 cm) and was on par with that grown in malathion which recorded the least radial growth of 2.63 cm.

During the third continuous passage of the fungi in varying concentrations of the chemicals, it was observed that the fungus in control treatment fully covered the Petri dish with a mean radial growth of 9 cm in 15 days and showed statistical similarity with that grown with imidacloprid, carbosulfan and carbofuran where a mean radial growth of 8.37, 6.27 and 5.59 cm respectively was recorded and which differed significantly from the other treatments. The fungal growth on chlorpyriphos mixed media was on par with carbendazim mixed media which had a mean radial growth of 4.12 cm each respectively. In lambda cyhalothrin mixed media *B. bassiana* had a mean growth of 3.06 cm. Relatively lower growth was observed in fungus cultured with mancozeb (2.05 cm) and was on par with malathion mixed media which recorded the lowest mean growth of 1.95 cm.

The control plate recorded higher mean growth of 9 cm in the fourth passage within 15 days followed by the fungus cultured with imidacloprid with a mean radial growth of 7.46 cm and differed significantly from others. Carbosulfan and carbofuran mixed media supported a mean growth of 5.78 and 5.65 cm respectively and were on par. The fungus cultured in media with chlorpyriphos, lambda cyhalothrin, carbendazim, mancozeb and malathion recorded a mean radial growth of 4.28, 3.35, 3.10, 2.41 and 2.11 cm, respectively and exhibited statistical significance between them.

In the fifth continuous passage of *B. bassiana*, the control plate recorded a mean growth of 9cm in 18 days and it was followed by the growth in imidacloprid poisoned media with a mean radial growth of 8.28 cm and it differed significantly

from the others. The growth of the fungus in carbosulfan and carbofuran mixed media were 5.94 and 5.74 cm respectively and were on par. The chlorpyriphos poisoned media recorded a mean radial growth of 3.71 cm and differed significantly from that cultured in carbendazim (3.27 cm). The mean growth of fungus in lambda cyhalothrin and mancozeb poisoned media were 2.71 and 2.49 cm respectively and were on par and the latter was on par with the malathion mixed media which recorded the lowest mean growth of 2.39 cm.

During the sixth passage, the mean radial growth in the control plate reached 9 cm in 16 days on plate followed by the poison media mixed with imidacloprid, carbosulfan, carbofuran, chlorpyriphos and carbendazim which recorded a mean growth of 7.87, 5.45, 5.17, 4.19 and 3.62 cm respectively and exhibited statistical significance. The growth of the fungus in mancozeb mixed media was 2.89 cm and was on par with the growth observed in lambda cyhalothrin poisoned media (2.76 cm) and the latter was on par with the malathion poisoned media which recorded a mean radial growth of 2.72 cm.

During the seventh passage, the growth of *B. bassiana* in control reached 9 cm in 18 days, was followed by the growth in media poisoned with imidacloprid, carbosulfan, carbofuran, chlorpyriphos and carbendazim with a mean radial growth of 8.10, 5.66, 5.31, 4.34 and 3.21 cm respectively and showed significant statistical difference between them. The mean growth of fungi cultured with lambda cyhalothrin and mancozeb were 2.96 each respectively and were on par. The fungi cultured with malathion recorded the lowest mean radial growth of 2.45 cm.

A similar trend was observed in the eighth passage, the growth in control reached 9 cm in 19 days and was the highest among the different treatments. The fungi grown with media poisoned with imidacloprid, carbosulfan, carbofuran, chlorpyriphos, carbendazim, malathion, lambda cyhalothrin and mancozeb recorded a mean radial growth of 7.79, 5.82, 5.53, 4.27, 3.36, 2.73, 2.34 and 2.18,

respectively and showed significant statistical difference between the different treatments.

When the fungi was subjected to the next passage, as in other observations, the growth in the control plate was superior and reached 9 cm in 21 days and it was followed by imidacloprid, carbosulfan, carbofuran, chlorpyriphos, carbendazim, and lambda cyhalothrin had a mean radial growth of 8.18, 5.46, 5.24, 4.52, 3.53 and 2.56 cm, respectively and exhibited significant statistical difference. The mean radial growth in mancozeb was 2.34 cm and was on par with that cultured in malathion which recorded the lowest growth of 2.23 cm.

Even in the tenth passage of the fungi, a similar trend was observed with a maximum of 9 cm growth recorded for the control within 21 days. Imidacloprid, carbosulfan, carbofuran, chlorpyriphos, carbendazim, lambda cyhalothrin, malathion and mancozeb poisoned media recorded a mean radial growth of 8.29, 5.85, 5.51, 4.72, 3.20, 2.97, 2.64 and 2.44 cm, respectively and exhibited significant statistical difference.

4.6.1.1.2 M. anisopliae

When *M. anisopliae* was cultured in poison mixed media, the maximum growth of 9 cm was recorded for the control treatment without pesticide exposure on fifteenth day and it was followed by the growth of the fungus in imidacloprid mixed media which recorded a mean radial growth of 6.37 cm and exhibited significant statistical difference from the other treatments (Table 87). The fungi cultured with carbosulfan and carbofuran recorded a mean growth of 5.63 and 5.46 cm respectively and were on par. Lambda cyhalothrin poisoned media had a mean growth of 4.89 cm of *M. anisopliae*. The media mixed with chlorpyriphos and mancozeb had recorded a mean growth of 4.33 and 4.14 cm respectively of the fungus and were on par. Relatively lower growth was observed in the media poisoned with malathion and recorded a mean radial growth of 3.53 cm and was

Table 87. Mean growth of *M. anisopliae* in poisoned food media for continuous ten passages

Sl. No.	Treatments	Growth of the fungus (cm)									
		I @ 15 DAI	II @ 15 DAI	III @ 17 DAI	IV @ 17 DAI	V @ 17 DAI	VI @ 23 DAI	VII @ 21 DAI	VIII @ 24 DAI	IX @ 23 DAI	X @ 26 DAI
T1	Carbendazim	3.31 (1.82)	3.12 (1.77)	2.99 (1.73)	3.13 (1.77)	3.28 (1.81)	3.43 (1.85)	3.26 (1.81)	3.48 (1.86)	3.83 (1.96)	3.99 (2.00)
T2	Mancozeb	4.14 (2.03)	4.26 (2.06)	4.18 (2.04)	4.24 (2.06)	4.22 (2.05)	4.27 (2.07)	4.31 (2.08)	4.29 (2.07)	4.37 (2.09)	4.26 (2.06)
T3	Carbofuran	5.46 (2.34)	5.15 (2.27)	5.23 (2.29)	5.24 (2.29)	5.26 (2.29)	5.25 (2.29)	5.20 (2.28)	5.37 (2.32)	5.39 (2.32)	5.31 (2.30)
T4	Carbosulfan	5.63 (2.37)	5.48 (2.34)	5.39 (2.32)	5.25 (2.29)	5.29 (2.30)	5.53 (2.35)	5.56 (2.36)	5.86 (2.42)	5.74 (2.39)	5.71 (2.39)
T5	Imidacloprid	6.37 (2.52)	6.35 (2.52)	6.44 (2.54)	6.48 (2.55)	6.38 (2.53)	6.63 (2.58)	6.73 (2.59)	6.67 (2.58)	6.63 (2.58)	6.97 (2.64)
T6	Chlorpyriphos	4.33 (2.08)	4.13 (2.03)	4.05 (2.01)	4.17 (2.04)	4.27 (2.07)	4.38 (2.09)	4.53 (2.13)	4.37 (2.09)	4.29 (2.07)	4.23 (2.06)
T7	Lambda cyhalothrin	4.89 (2.21)	4.51 (2.12)	4.43 (2.10)	4.37 (2.09)	4.23 (2.06)	4.08 (2.02)	4.06 (2.02)	4.12 (2.03)	3.92 (1.98)	3.84 (1.96)
T8	Malathion	3.53 (1.88)	3.33 (1.83)	3.26 (1.81)	3.12 (1.77)	3.09 (1.76)	3.09 (1.76)	3.29 (1.81)	3.25 (1.80)	3.12 (1.77)	3.09 (1.76)
T9	Untreated Control	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)	9.00 (3.00)
	C.D (0.05)	(0.073)	(0.059)	(0.057)	(0.069)	(0.063)	(0.046)	(0.058)	(0.059)	(0.067)	(0.040)

Figures in parentheses are square root transformed values

on par with that in carbendazim (3.31 cm) which had the lowest growth among the different treatments.

In the second passage when the fungi was grown in varying concentrations of pesticides, the maximum mycelial growth was observed in the control plate and covered 9 cm in 15 days, followed by that grown in imidacloprid, carbosulfan, carbofuran and lambda cyhalothrin which recorded a mean growth of 6.35, 5.48, 5.15 and 4.51 cm respectively and exhibited statistical significance from the other treatments. The fungi cultured in media mixed with mancozeb and chlorpyriphos recorded a mean radial growth of 4.26 and 4.13 cm respectively and were on par. The malathion poisoned culture had a mean growth of 3.33 cm followed by that grown with carbendazim poisoned media which recorded the least growth of 3.12 cm.

M. anisopliae cultured without pesticides completely covered the Petri plate in the third passage with a mean radial growth of 9 cm in 17 days followed by imidacloprid mixed media which had a mean growth of 6.44 cm. The fungi cultured with carbosulfan and carbofuran recorded a mean growth of 5.39 and 5.23 cm respectively and were on par. Lambda cyhalothrin poisoned media had a mean radial growth of 4.43 cm. A mean growth of 4.18 and 4.05 cm was observed in media treated with mancozeb and chlorpyriphos, respectively and were on par. Relatively lower growth of 3.26 cm was recorded for malathion and the least for carbendazim mixed media with a mean of 2.99 cm.

In the fourth passage the growth of *M. anisopliae* in the untreated control plate reached a mean of 9 cm within 17 days followed by that grown with imidacloprid poisoned media with a mean radial growth of 6.48 cm and differed significantly from the other treatments. The mean radial growth of the fungi grown with carbosulfan and carbofuran were 5.25 and 5.24 cm respectively and were statistically on par. The fungus cultured in media poisoned with lambda cyhalothrin, mancozeb and chlorpyriphos recorded a mean radial growth of 4.37, 4.24 and 4.17 cm respectively and were on par. The lowest fungal growth

was observed in the treatments with carbendazim and malathion, where a mean radial growth of 3.13 and 3.12 cm respectively was noticed and were statistically on par.

In the untreated control plate *M. anisopliae* recorded a mean radial growth of 9 cm within a period of 17 days during the fifth continuous passage and showed statistical significance among the different treatments. The imidacloprid treated media recorded a mean radial growth of 6.38 cm and differed significantly from the other treatments. The fungi grown with carbosulfan and carbofuran mixed media had a mean growth of 5.29 and 5.26 cm respectively and were on par. The growth of the fungi in the chlorpyriphos, lambda cyhalothrin and mancozeb poisoned media were 4.27, 4.23 and 4.22 cm respectively and were statistically on par. The mean radial growth of the fungi in the carbendazim mixed media was 3.28 cm and was on par with the lowest radial growth of the fungus with malathion mixed media which had a mean of 3.09 cm.

M. anisopliae completely covered the Petri plate in 23 days and recorded a mean radial growth of 9 cm in the untreated control during the sixth passage. The mean radial growth of the fungus grown in media poisoned with imidacloprid, carbosulfan and carbofuran were 6.63, 5.53 and 5.25 cm respectively and showed significant difference with the other treatments. A mean growth of 4.38 and 4.27 cm was obtained for the fungus grown with chlorpyriphos and mancozeb respectively and had statistical similarity. The treatments with lambda cyhalothrin, carbendazim and malathion recorded a mean radial growth of 4.08, 3.43 and 3.09 cm respectively and were statistically on par.

In the seventh continuous passage, *M. anisopliae* in the untreated control treatment took 21 days to completely cover the Petri plate and a mean radial growth of 9.00 cm was achieved and had significant difference among the different treatments. The mean radial growth of the fungus in the media treated with imidacloprid, carbosulfan and carbofuran were 6.73, 5.56 and 5.20 cm respectively and differed significantly from the other treatments. The fungi

cultured with chlorpyriphos and mancozeb had a mean radial growth of 4.53 and 4.31 cm respectively and were on par. Lambda cyhalothrin mixed media recorded a mean growth of 4.06 cm. Relatively lower growth was observed for the treatments with malathion and carbendazim which had a radial growth of 3.29 and 3.26 cm respectively and were statistically on par.

The mean radial growth of *M. anisopliae* in the untreated control plate reched 9.00 cm in 24 days during the eighth passage and exhibited significant difference from the other treatments. The fungus cultured with imidacloprid, carbosulfan and carbofuran had a mean radial growth of 6.67, 5.86 and 5.37 cm respectively and differed significantly from the other treatments. A mean radial growth of 4.37 and 4.29 cm was obtained for the chlorpyriphos and mancozeb poisoned media and were statistically on par. The treatments with lambda cyhalothrin, carbendazim and malathion recorded relatively lower growth of the fungus and were 4.12, 3.48 and 3.25 cm respectively.

In the ninth continuous passage, the mean radial growth of *M. anisopliae* in the untreated control plate reached 9.00 cm within 23 days and differed significantly from the other treatments. A mean radial growth of 6.63, 5.74 and 5.39 cm was obtained for the treatments with imidacloprid, carbosulfan and carbofuran respectively and showed significant difference among the different treatments. The treatments with mancozeb and chlorpyriphos recorded a mean radial growth of 4.37 and 4.29 cm respectively and were statistically on par. The fungus cultured with lambda cyhalothrin and carbendazim had a mean growth of 3.92 and 3.83 cm respectively and were on par. The lowest mean radial growth of 3.12 cm was recorded for the fungus grown with malathion.

During the tenth passage the maximum growth of 9.00 cm was recorded for the control treatment on 26 days followed by that grown in imidacloprid, carbosulfan and carbofuran poisoned media, which recorded a mean radial growth of 6.97, 5.71 and 5.31 cm respectively and had significant difference when compared to the other treatments. The fungi grown with mancozeb and

chlorpyriphos mixed media recorded a mean growth of 4.26 and 4.23 cm respectively and were on par. A mean growth of 3.99, 3.84 and 3.09 cm was observed for the fungi cultured with carbendazim, lambda cyhalothrin and malathion respectively.

4.6.1.2 Sporulation of the fungi

4.6.1.2.1 B. bassiana

The spore count of *B. bassiana* in the control treatment with no chemical treatment was significantly high and recorded a mean spore count of 1108.31 × 10^8 spores ml^{-1} and differed significantly from the other treatments (Table 88). A mean spore count of 35.45 × 10^8, 19.82 × 10^8, 7.65 × 10^8, 1.41 × 10^8, 0.27 × 10^8, 0.26 × 10^8, 0.04 × 10^8 and 0.03 × 10^8 spores ml^{-1} was observed in the media treated with imidacloprid, carbosulfan, carbofuran, chlorpyriphos, malathion, lambda cyhalothrin, carbendazim and mancozeb respectively and were on par.

During the second continuous passage the highest spore concentration was recorded for the untreated control plate with a mean of 441.59 × 10^8 spores ml^{-1} and it differed significantly from the other treatments. The mean sporulation of the fungus grown with imidacloprid, carbofuran and carbosulfan poisoned media were 33.57 × 10^8, 12.29 × 10^8 and 10.03 × 10^8 spores ml^{-1} respectively and were statistically on par. The spore load present in the fungal culture treated with chlorpyriphos, malathion, lambda cyhalothrin, carbendazim and mancozeb were 0.75 × 10^8, 0.24 × 10^8, 0.15 × 10^8, 0.03 × 10^8 and 0.02 × 10^8 spores ml^{-1} respectively and were on par.

The mean sporulation of *B. bassiana* was significantly high in the plates maintained as the control during the third passage which recorded a mean of 244.76 × 10^8 spores ml^{-1} followed by that grown in imidacloprid treated media which had a spore count of 41.43 × 10^8 spores ml^{-1} and differed significantly from the other treatments. The spore concentration obtained from the fungal culture treated with carbosulfan and carbofuran were 13.38 × 10^8 and 9.56 × 10^8 spores ml^{-1} respectively and were on par. The mean spore count of the fungus cultured

Table 88. Mean sporulation of *B. bassiana* in poisoned food media for continuous ten passages

Sl. No.	Treatments	Spore count (spores $ml^{-1} \times 10^8$)									
		I @ 13 DAI	II @ 13 DAI	III @ 15 DAI	IV @ 15 DAI	V @ 18 DAI	VI @ 16 DAI	VII @ 18 DAI	VIII @ 19 DAI	IX @ 21 DAI	X @ 21 DAI
T1	Carbendazim	0.04 (0.22)	0.03 (0.17)	0.01 (1.20)	0.03 (0.18)	0.06 (0.24)	0.06 (0.25)	0.02 (0.17)	0.02 (0.16)	0.01 (0.10)	0.009 (0.009)
T2	Mancozeb	0.03 (0.19)	0.02 (0.15)	0.01 (1.04)	0.008 (0.093)	0.02 (0.12)	0.01 (0.12)	0.01 (0.12)	0.01 (0.12)	0.008 (0.089)	0.008 (0.009)
T3	Carbofuran	19.82 (4.45)	12.29 (3.51)	9.56 (3.09)	11.27 (3.36)	13.11 (3.62)	26.39 (5.14)	19.87 (4.46)	14.20 (3.77)	11.05 (3.32)	11.39 (3.38)
T4	Carbosulfan	7.65 (2.77)	10.03 (3.17)	13.38 (3.66)	28.40 (5.33)	52.99 (7.28)	59.26 (7.69)	42.48 (6.52)	48.17 (6.94)	37.94 (6.16)	34.76 (5.89)
T5	Imidacloprid	35.45 (5.95)	33.57 (5.79)	41.43 (6.44)	68.88 (8.29)	69.67 (8.35)	84.33 (9.18)	70.99 (8.43)	62.69 (7.92)	57.87 (7.61)	56.31 (7.50)
T6	Chlorpyriphos	1.41 (1.19)	0.75 (0.87)	0.16 (0.40)	0.19 (0.43)	0.22 (0.47)	0.22 (0.47)	0.21 (0.45)	0.28 (0.53)	0.32 (0.57)	0.31 (0.56)
T7	Lambda cyhalothrin	0.26 (0.51)	0.15 (0.39)	0.16 (0.40)	0.37 (0.61)	0.54 (0.74)	0.39 (0.62)	0.43 (0.66)	0.48 (0.69)	0.44 (0.66)	0.39 (0.63)
T8	Malathion	0.27 (0.52)	0.24 (0.49)	0.13 (0.36)	0.06 (0.24)	0.10 (0.32)	0.13 (0.36)	0.10 (0.32)	0.09 (0.31)	0.08 (0.28)	0.08 (0.28)
T9	Untreated Control	1108.31 (33.29)	441.59 (21.01)	244.76 (15.64)	229.49 (15.15)	223.89 (14.96)	222.67 (14.92)	222.45 (14.92)	213.57 (14.61)	213.53 (14.61)	208.81 (14.45)
	C.D (0.05)	(10.614)	(3.054)	(1.373)	(2.422)	(1.607)	(1.753)	(1.906)	(2.044)	(2.265)	(6.348)

Figures in parentheses are square root transformed values

with lambda cyhalothrin, chlorpyriphos, malathion, carbendazim and mancozeb mixed media were 0.16×10^8 , 0.16×10^8 , 0.13×10^8 , 0.01×10^8 and 0.01×10^8 spores ml^{-1} and were statistically on par.

During the fourth passage the mean sporulation of the untreated control was the superior treatment with a mean sporulation of 229.49×10^8 spores ml^{-1} followed by the fungus grown with imidacloprid treated media which had a mean sporulation of 68.88×10^8 spores ml^{-1} and differed significantly from the other treatments. A mean spore count of 28.40 and 11.27×10^8 spores ml^{-1} was obtained in the fungal culture grown with carbosulfan and carbofuran and were statistically on par. The mean spore load of 0.37×10^8, 0.19×10^8, 0.06×10^8, 0.03×10^8 and 0.008×10^8 spores ml^{-1} was recorded for the treatments with lambda cyhalothrin, chlorpyriphos, malathion, carbendazim and mancozeb and were statistically on par.

The mean spore count of the fungus in the untreated control was 223.89×10^8 spores ml^{-1} during the fifth passage and differed significantly from the other treatments. The fungus cultured with imidacloprid and carbosulfan mixed media recorded a mean spore count of 69.67 and 52.99×10^8 spores ml^{-1} and were statistically on par. The mean spore count of the fungus cultured in carbofuran poisoned media was 13.11×10^8 spores ml^{-1} . The treatments with lambda cyhalothrin, chlorpyriphos, malathion, carbendazim and mancozeb mixed media yielded a mean spore load of 0.54×10^8, 0.22×10^8, 0.10×10^8, 0.06×10^8 and 0.02×10^8 spores ml^{-1} respectively and were on par.

The fungus cultured as the untreated control without any pesticides had high spore count of 222.67×10^8 spores ml^{-1} during the sixth passage and differed significantly from the other treatments. The mean sporulation of *B. bassiana* in media poisoned with imidacloprid and carbosulfan were 84.33×10^8 and 59.26×10^8 spores ml^{-1} respectively and were statistically on par. The fungus grown in media treated with carbofuran had a mean spore load of 26.39×10^8 spores ml^{-1} . The spore concentration of the fungus grown in the treatments with

lambda cyhalothrin, chlorpyriphos, malathion, carbendazim and mancozeb were 0.39×10^8, 0.22×10^8 , 0.13×10^8, 0.06×10^8 and 0.01×10^8 spores ml^{-1} respectively and were statistically on par.

In the seventh continuous passage the mean sporulation of *B. bassiana* in the untreated control was superior when compared to the other treatments and had 222.45×10^8 spores ml^{-1} and differed significantly from the other treatments. The media poisoned with imidacloprid, carbosulfan and carbofuran yielded a mean spore concentration of 70.99×10^8 , 42.48×10^8 and 19.87×10^8 spores ml^{-1} and exhibited statistical significance among the other treatments. A mean sporulation of 0.43×10^8, 0.21×10^8, 0.10×10^8, 0.02×10^8 and 0.01×10^8 spores ml^{-1} was recorded in the media mixed with lambda cyhalothrin, chlorpyriphos, malathion, carbendazim and mancozeb and were statistically on par.

The untreated control recorded significantly higher spore concentration of 213.57×10^8 spores ml^{-1} during the eighth continuous passage, followed by the treatments with imidacloprid, carbosulfan and carbofuran mixed media which had a mean spore load of 62.69×10^8, 48.17×10^8 and 14.20×10^8 spores ml^{-1} respectively and differed significantly from the other treatments. A mean spore count of 0.48×10^8, 0.28×10^8, 0.09×10^8, 0.02×10^8 and 0.01×10^8 spores ml^{-1} was obtained in the media poisoned with lambda cyhalothrin, chlorpyriphos, malathion, carbendazim and mancozeb respectively and were statistically on par.

During the ninth passage, the fungus grown without any pesticides in the untreated control recorded the maximum sporulation of 213.53×10^8 spores ml^{-1} and differed significantly from the other treatments. A mean spore count of 57.87 $\times 10^8$ and 37.94×10^8 spores ml^{-1} was obtained for the fungus grown in the media mixed with imidacloprid and carbosulfan respectively and were on par. The media poisoned with carbofuran had a mean spore load of 11.05×10^8 spores ml^{-1}. The fungi cultured with lambda cyhalothrin, chlorpyriphos, malathion, carbendazim and mancozeb had a mean spore concentration of 0.44×10^8, $0.32 \times$

10^8, 0.08 × 10^8, 0.01 × 10^8 and 0.008 × 10^8 spores ml^{-1} respectively and were statistically on par.

In the tenth continuous passage, the highest spore concentration was recorded in the control treatment with a spore load of 208.81 × 10^8 spores ml^{-1}, followed by that grown with imidacloprid, carbosulfan and carbofuran mixed media which had a concentration of 56.31 × 10^8, 34.76 × 10^8 and 11.39 × 10^8 spores ml^{-1} and differed significantly from the other treatments. The spore count obtained from the fungi cultured with lambda cyhalothrin, chlorpyriphos, malathion, carbendazim and mancozeb mixed media were 0.39 × 10^8, 0.31 × 10^8, 0.08 × 10^8, 0.009 × 10^8 and 0.008 × 10^8 spores $ml^{-1}$and were statistically on par.

4.6.1.2.2 M. anisopliae

The spore count of *M. anisopliae* in the pure culture without any chemical treatments in the first subculturing was significantly higher and recorded a concentration of 84.67 × 10^8 spores ml^{-1} , followed by that grown with imidacloprid mixed media which had a spore load of 26.73 × 10^8 spores ml^{-1} and differed significantly from the other treatments (Table 89). The concentration of spores present in the fungi cultured with carbosulfan, carbofuran, carbendazim, chlorpyriphos, mancozeb, lambda cyhalothrin and malathion were 1.47 × 10^8, 1.39 × 10^8, 0.14 × 10^8, 0.09 × 10^8, 0.06 × 10^8, 0.03 × 10^8 and 0.02 × 10^8 spores ml^{-1} respectively and were on par.

In the second continuous passage, the maximum sporulation of *M. anisopliae* was observed in the untreated control with a mean spore count of 75.31 × 10^8 spores ml^{-1} and it was statistically on par with that grown in imidacloprid poisoned media which recorded a mean spore concentration of 39.69 × 10^8 spores ml^{-1}. The mean spore load of the fungus present in the media mixed with carbosulfan, carbofuran, carbendazim, chlorpyriphos, mancozeb, lambda cyhalothrin and malathion were 2.61 × 10^8, 1.68 × 10^8, 0.17 × 10^8, 0.16 × 10^8, 0.10 × 10^8, 0.05 × 10^8 and 0.02 × 10^8 spores ml^{-1} respectively and were on par.

Table 89. Mean sporulation of *M. anisopliae* in poisoned food media for continuous ten passages

Sl. No.	Treatments	Spore count (spores $ml^{-1} \times 10^8$)									
		I @ 15 DAI	II @ 15 DAI	III @ 17 DAI	IV @ 17 DAI	V @ 17 DAI	VI @ 23 DAI	VII @ 21 DAI	VIII @ 24 DAI	IX @ 23 DAI	X @ 26 DAI
T1	Carbendazim	0.14 (0.37)	0.17 (0.41)	0.17 (0.41)	0.26 (0.51)	0.32 (0.57)	0.26 (0.51)	0.17 (0.41)	0.17 (0.41)	0.19 (0.43)	0.19 (0.44)
T2	Mancozeb	0.06 (0.25)	0.10 (0.32)	0.16 (0.40)	0.17 (0.41)	0.20 (0.45)	0.14 (0.38)	0.15 (0.39)	0.22 (0.47)	0.21 (0.45)	0.22 (0.47)
T3	Carbofuran	1.39 (1.18)	1.68 (1.29)	1.83 (1.35)	1.42 (1.19)	1.55 (1.24)	1.27 (1.13)	1.24 (1.11)	1.82 (1.35)	1.77 (1.33)	2.70 (1.64)
T4	Carbosulfan	1.47 (1.21)	2.61 (1.62)	6.45 (2.54)	6.73 (2.59)	6.72 (2.59)	4.66 (2.16)	4.42 (2.10)	5.08 (2.25)	6.17 (2.48)	5.62 (2.37)
T5	Imidacloprid	26.73 (5.17)	39.69 (6.30)	59.83 (7.74)	59.19 (7.69)	56.36 (7.51)	38.19 (6.18)	27.61 (5.25)	39.24 (6.26)	50.06 (7.08)	51.60 (7.18)
T6	Chlorpyriphos	0.09 (0.31)	0.16 (0.39)	0.17 (0.41)	0.23 (0.48)	0.45 (0.67)	0.57 (0.76)	0.34 (0.58)	0.22 (0.47)	0.27 (0.52)	0.25 (0.49)
T7	Lambda cyhalothrin	0.03 (0.16)	0.05 (0.22)	0.04 (0.21)	0.06 (0.24)	0.05 (0.22)	0.04 (0.20)	0.03 (0.16)	0.03 (0.16)	0.03 (0.17)	0.02 (0.15)
T8	Malathion	0.02 (0.16)	0.02 (0.16)	0.02 (0.14)	0.01 (0.11)	0.009 (0.094)	0.01 (0.11)	0.007 (0.009)	0.02 (0.15)	0.009 (0.099)	0.01 (0.11)
T9	Untreated Control	84.67 (9.20)	75.31 (8.68)	64.31 (8.02)	75.64 (8.69)	88.55 (9.41)	72.13 (8.49)	51.74 (7.19)	47.12 (6.86)	66.63 (8.16)	67.74 (8.23)
	C.D (0.05)	(3.922)	(2.422)	(1.123)	(0.754)	(0.591)	(0.903)	(1.483)	(0.997)	(1.020)	(1.200)

Figures in parentheses are square root transformed values

The mean sporulation of the fungus in the untreated control during the third passage was 64.31 × 10^8 spores ml^{-1} and was the maximum among different treatments followed by the fungi cultured in media poisoned with imidacloprid which had a mean spore count of 59.83 × 10^8 spores ml^{-1} and were statistically on par. The media poisoned with carbosulfan yielded a spore load of 6.45 × 10^8 spores ml^{-1}. A mean spore concentration of 1.83 × 10^8, 0.17 × 10^8, 0.17 × 10^8 and 0.16 × 10^8 spores ml^{-1} was observed in the media mixed with carbofuran, carbendazim, chlorpyriphos and mancozeb respectively and were statistically on par. The sporulation of the fungi cultured in media poisoned with lambda cyhalothrin and malathion were 0.04 × 10^8 and 0.02 × 10^8 spores ml^{-1} respectively and were on par.

During the fourth continuous passage the maximum sporulation of *M. anisopliae* was recorded in the untreated control with a mean spore count of 75.64 × 10^8 spores ml^{-1} followed by the fungi grown with imidacloprid and carbosulfan poisoned media which recorded a mean of 59.19 × 10^8 and 6.73 × 10^8 spores ml^{-1} respectively and differed significantly from the other treatments. The mean sporulation of the fungi in carbofuran, carbendazim and chlorpyriphos poisoned media were 1.42 × 10^8, 0.26 × 10^8 and 0.23 × 10^8 spores ml^{-1} respectively and were statistically on par. A mean spore concentration of 0.17 × 10^8, 0.06 × 10^8 and 0.01 × 10^8 spores ml^{-1} was obtained when the fungi was cultured with mancozeb, lambda cyhalothrin and malathion respectively and were statistically on par.

In the fifth passage the maximum sporulation was observed in the untreated control without any pesticides which had a mean spore count of 88.55 × 10^8 spores ml^{-1} followed by the treatments with imidacloprid and carbosulfan which had a mean sporulation of 56.36 × 10^8 and 6.72 × 10^8 spores ml^{-1} respectively and differed significantly from the other treatments. The mean sporulation of the fungi cultured with carbofuran and chlorpyriphos were 1.55 × 10^8 and 0.45 × 10^8 spores ml^{-1} respectively and were statistically on par. The treatments with carbendazim, mancozeb, lambda cyhalothrin and malathion had a

mean sporulation of 0.32×10^8, 0.20×10^8, 0.05×10^8 and 0.009×10^8 spores ml^{-1} respectively and were statistically on par.

The untreated control recorded a maximum spore count of 72.13×10^8 spores ml^{-1} in the sixth continuous passage, followed by the treatments with imidacloprid and carbosulfan which had a mean spore load of 38.19×10^8 and 4.66×10^8 spores ml^{-1} respectively and differed significantly from the other treatments. The mean sporulation of *M. anisopliae* in the media poisoned with carbofuran, chlorpyriphos, carbendazim and mancozeb were 1.27×10^8, 0.57×10^8, 0.26×10^8 and 0.14×10^8 spores ml^{-1} respectively and were statistically on par. The lowest mean spore count of 0.04×10^8 and 0.01×10^8 spores ml^{-1} was obtained for the treatments with lambda cyhalothrin and malathion, respectively and were statistically on par.

In the seventh continuous passage of *M. anisopliae* through poison food, the highest mean spore concentration was observed for the untreated control with a mean of 51.74×10^8 spores ml^{-1} followed by that grown with imidacloprid mixed media which had a spore count of 27.61×10^8 spores ml^{-1} and differed significantly from the other treatments. The treatments with carbosulfan and carbofuran had a mean spore load of 4.42×10^8 and 1.24×10^8 spores ml^{-1} respectively and were statistically on par. A mean spore concentration of 0.34×10^8, 0.17×10^8, 0.15×10^8, 0.03×10^8 and 0.007×10^8 spores ml^{-1} was recorded for the treatments with chlorpyriphos, carbendazim, mancozeb, lambda cyhalothrin and malathion respectively and were statistically on par.

The maximum sporulation was recorded in the untreated control during the eighth continuous passage and was 47.12×10^8 spores ml^{-1} and was statistically on par with the mean spore count of the fungi cultured with imidacloprid which had a spore concentration of 39.24×10^8 spores ml^{-1} and were statistically on par. The mean spore count of *M. anisopliae* in carbosulfan and carbofuran treated media were 5.08×10^8 and 1.82×10^8 spores ml^{-1} respectively and were statistically on par. The treatments with chlorpyriphos, mancozeb, carbendazim,

lambda cyhalothrin and malathion yielded a mean spore count of 0.22×10^8, 0.22×10^8, 0.17×10^8, 0.03×10^8 and 0.02×10^8 spores ml^{-1} respectively and were statistically on par.

Among the different treatments, the untreated control recorded a maximum spore count of 66.63×10^8 spores ml^{-1} during the ninth continuous passage, followed by the treatments with imidacloprid and carbosulfan which had a mean spore count of 50.06×10^8 and 6.17×10^8 spores ml^{-1} respectively and differed significantly from the other treatments. A mean spore load of 1.77×10^8, 0.27×10^8, 0.21×10^8 and 0.19×10^8 spores ml^{-1} was recorded for the treatments with carbofuran, chlorpyriphos, mancozeb and carbendazim respectively and were statistically on par. The treatments with lambda cyhalothrin and malathion had a mean spore concentration of 0.03×10^8 and 0.009×10^8 spores ml^{-1}, respectively and were statistically on par.

During the tenth subculturing of the fungi, the fungi grown as control recorded maximum concentration of spores and it was 67.74×10^8 spores ml^{-1} and was on par with that grown with imidacloprid poisoned media which had a mean spore count of 51.60×10^8 spores ml^{-1}. The spore count of fungi grown with carbosulfan and carbofuran mixed media were 5.62×10^8 and 2.70×10^8 spores ml^{-1} respectively and were statistically on par. A mean spore count of 0.25 $\times 10^8$, 0.22×10^8, 0.19×10^8, 0.02×10^8 and 0.01×10^8 spores ml^{-1} was recorded in the cultures grown with chlorpyriphos, mancozeb, carbendazim, lambda cyhalothrin and malathion mixed media respectively and were on par.

4.6.1.3 Bioefficacy of pesticide grown fungi

4.6.1.3.1 B. bassiana

When the newly emerged adults of *C. formicarius* were treated with the pure culture of *B. bassiana* and the chemical mixed cultures, the pure culture of the fungi was found to be superior over the other treatments with a maximum mortality of 77.74 per cent at 14 DAT during the first subculture (Table 90). The mean mortality caused by the fungi cultured with imidacloprid was 51.06 per cent

Table 90. Bioefficacy of *B. bassiana* cultured in poisoned food media for continuous ten passages

Sl. No.	Treatments	Mortality of adult *C. formicarius* at 14 DAT (per cent)									
		I	II	III	IV	V	VI	VII	VIII	IX	X
T1	Carbendazim	24.34 (4.93)	22.12 (4.70)	26.67 (5.16)	22.12 (4.70)	26.67 (5.16)	28.81 (5.37)	19.62 (4.43)	26.38 (5.14)	17.63 (4.19)	19.62 (4.43)
T2	Mancozeb	6.67 (2.58)	2.96 (1.72)	8.63 (2.94)	10.86 (3.29)	10.86 (3.29)	2.96 (1.72)	2.96 (1.72)	8.64 (2.94)	2.96 (1.72)	0.74 (0.86)
T3	Carbofuran	44.39 (6.66)	39.81 (6.31)	46.67 (6.83)	42.17 (6.49)	39.81 (6.31)	46.51 (6.82)	39.81 (6.31)	35.49 (5.96)	42.17 (6.49)	37.71 (6.14)
T4	Carbosulfan	39.81 (6.31)	37.71 (6.14)	41.97 (6.48)	37.71 (6.14)	40.00 (6.33)	42.17 (6.49)	35.49 (5.96)	37.53 (6.13)	35.49 (5.96)	37.71 (6.14)
T5	Imidacloprid	51.06 (7.15)	53.33 (7.30)	48.84 (6.99)	46.51 (6.82)	48.67 (6.98)	53.19 (7.29)	46.51 (6.82)	48.84 (6.99)	44.39 (6.66)	48.84 (6.99)
T6	Chlorpyriphos	26.38 (5.14)	22.12 (4.70)	28.81 (5.37)	31.03 (5.57)	28.51 (5.34)	22.12 (4.70)	24.07 (4.91)	22.12 (4.70)	22.12 (4.70)	24.07 (4.91)
T7	Lambda cyhalothrin	19.62 (4.43)	22.12 (4.70)	17.63 (4.19)	15.40 (3.93)	22.12 (4.70)	17.63 (4.19)	17.63 (4.19)	20.00 (4.47)	15.40 (3.93)	17.63 (4.19)
T8	Malathion	10.86 (3.29)	13.33 (3.65)	8.64 (2.94)	12.73 (3.57)	13.33 (3.65)	8.64 (2.94)	8.64 (2.94)	12.73 (3.57)	8.64 (2.94)	6.67 (2.58)
T9	Untreated Control	77.74 (8.82)	82.19 (9.07)	75.52 (8.69)	79.91 (8.94)	73.23 (8.56)	75.52 (8.69)	71.07 (8.43)	75.52 (8.69)	75.52 (8.69)	71.07 (8.43)
	C.D (0.05)	(0.814)	(1.011)	(0.724)	(0.877)	(0.779)	(1.099)	(1.232)	(0.897)	(1.075)	(1.132)

Figures in parentheses are square root transformed values DAT: Days after treatment

and was on par with that obtained by the application of carbofuran mixed media with a mean mortality of 44.39 per cent and the latter had statistical similarity with that of carbosulfan, which recorded a mean mortality of 39.81 per cent. The fungi cultured with chlorpyriphos, carbendazim and lambda cyhalothrin mixed media recorded a mean mortality of 26.38, 24.34 and 19.62 per cent, respectively and were on par. A mean mortality of 10.86 and 6.67 per cent was observed when the fungi grown in media mixed with malathion and mancozeb, respectively was applied.

During the second passage maximum mortality of the adults of *C. formicarius* was caused by the fungi cultured as the untreated control which caused a mean mortality of 82.19 per cent followed by the fungi cultured in imidacloprid mixed media which had a mean mortality of 53.33 per cent and differed significantly from the other treatments. The treatments with carbofuran and carbosulfan caused a mean mortality of 39.81 and 37.71 per cent and were statistically on par. The fungi cultured with chlorpyriphos, lambda cyhalothrin and carbendazim poisoned media caused a mean mortality of 22.12 per cent each respectively and showed statistical similarity. The mortality caused by malathion mixed media was 13.33 per cent and the lowest rate of mortality was observed in the case of mancozeb which had a mean mortality of 2.96 per cent.

The highest morality of the adults of *C. formicarius* when treated with the fungi cultured during the third passage was recorded for the untreated control which caused a mean mortality of 75.52 per cent and differed significantly from the other treatments. The fungi grown with imidacloprid, carbofuran and carbosulfan caused a mean mortality of 48.84, 46.67 and 41.97 per cent respectively and were statistically on par. A mean per cent mortality of 28.81 and 26.67 was recorded for the fungi grown with chlorpyriphos and carbendazim and were on par. The fungi cultured with lambda cyhalothrin caused a mean mortality of 17.63 per cent. The treatments with malathion and mancozeb caused a mean per cent mortality of 8.64 and 8.63 respectively and were on par.

In the fourth continuous passage *B. bassiana* in the untreated control caused significantly higher mean mortality of the newly emerged adults of *C. formicarius* and recorded 79.91 per cent. The mean mortality of 46.51, 42.17 and 37.71 per cent was observed in the case of fungi cultured with imidacloprid, carbofuran and carbosulfan respectively and were on par. The fungi grown in media mixed with chlorpyriphos recorded a mean mortality of 31.03 per cent. A mean mortality of 22.12 and 1.40 per cent was caused by the fungi cultured with carbendazim and lambda cyhalothrin respectively and were statistically on par. Relatively lower per cent mortality of 12.73 was observed for the fungi cultured with malathion and was statistically on par with the lowest percentage mortality caused by the fungus grown with mancozeb poisoned media which had a mean of 10.86 per cent.

The fungi cultured as the untreated control during the fifth continuous passage caused significantly higher mean mortality of 73.23 per cent when applied on to the newly emerged adults of sweet potato weevil. The fungi grown in media poisoned with imidacloprid, carbosulfan and carbofuran caused a mean mortality of 48.67, 40.00 and 39.81 per cent respectively and were statistically on par. A mean mortality of 28.51, 26.67 and 22.12 per cent was recorded for the fungi cultured with chlorpyriphos, carbendazim and lambda cyhalothrin mixed media respectively and were on par. The treatments with malathion and mancozeb caused a mean mortality of 13.33 and 10.86 per cent, respectively and were statistically on par.

During the sixth continuous passage the fungi cultured as the untreated control recorded significantly higher mean mortality of 75.52 per cent on the adults of *C. formicarius*. The treatments with imidacloprid, carbofuran and carbosulfan recorded a mean mortality of 53.19, 46.51 and 42.17 per cent, respectively and were statistically on par. The fungi grown in media mixed with carbendazim and chlorpyriphos caused a mean mortality of 28.81 and 22.12 per cent respectively and were statistically on par. The mean mortality caused by the fungi cultured with lambda cyhalothrin, malathion and mancozeb were 17.63,

8.64 and 2.96 per cent respectively and differed significantly from the other treatments.

The mortality caused by the fungus cultured as the untreated control during the seventh continuous passage was 71.07 per cent and differed significantly from the other treatments. The treatments with imidacloprid, carbofuran and carbosulfan caused a mean mortality of 46.51, 39.81 and 35.49 per cent respectively and were statistically on par. The mean percentage mortality of 24.07, 19.62 and 17.63 was observed for the fungi grown in media mixed with chlorpyriphos, carbendazim and lambda cyhalothrin respectively and were statistically on par. The treatments with malathion and mancozeb caused a mean per cent mortality of 8.64 and 2.96 and were on par.

The fungi cultured as the untreated control without any pesticides during the eighth continuous passage caused a mean mortality of 75.52 per cent and differed significantly from the other treatments. The mean mortality caused by the fungus grown with imidacloprid and carbosulfan were 48.84 and 37.53 per cent respectively and were statistically on par. The fungi cultured in carbofuran recorded a mean mortality of 35.49 per cent. A mean per cent mortality of 26.38, 22.12 and 20.00 was recorded for the fungi grown in media mixed with carbendazim, chlorpyriphos and lambda cyhalothrin respectively and were on par. The treatments with malathion and mancozeb caused relatively lower mean mortality of 12.73 and 8.64 per cent respectively and were statistically on par.

During the ninth continuous passage the fungi cultured as the untreated control recorded significantly higher mean per cent mortality of 75.52 and differed significantly from the other treatments. The treatments with imidacloprid, carbofuran and carbosulfan caused a mean mortality of 44.39, 42.17 and 35.49 per cent respectively and were statistically on par. The fungi cultured in media poisoned with chlorpyriphos, carbendazim and lambda cyhalothrin caused a mean mortality of 22.12, 17.63 and 15.40 per cent respectively and were

statistically on par. The mean mortality of the fungus grown in media mixed with malathion and mancozeb were 8.64 and 2.96 per cent respectively and differed significantly from the other treatments.

When the tenth subcultured fungi was applied on the adults of *C. formicarius*, significantly higher mortality of 71.07 per cent was noticed in control. The mortality caused by the fungi grown in media mixed with imidacloprid, carbosulfan and carbofuran were 48.84, 37.71 and 37.71 per cent respectively and were on par. Moderate mortality was observed in the case of fungi cultured with chlorpyriphos, carbendazim and lambda cyhalothrin mixed media and it comes to about 24.07, 19.62 and 17.63 per cent, respectively and were on par. The fungus cultured with malathion caused a mean mortality of 6.67 per cent, followed by the lowest mortality caused by that grown in mancozeb which recorded only 0.74 per cent.

4.6.1.3.2 M. anisopliae

During the first passage, the maximum mortality of the adult *C. formicarius* at 14 DAT was 42.17 per cent when treated with the fungus in control, this was followed by the fungal culture grown in imidacloprid (35.49) and these were on par (Table 91). The mean mortality obtained when treated with the fungus grown with carbosulfan, carbofuran and carbendazim were 26.67, 22.12 and 19.62 per cent respectively and were on par. The fungus cultured with chlorpyriphos, lambda cyhalothrin and mancozeb caused moderate mortality of 15.40, 13.33 and 10.86 per cent respectively and showed statistical similarity. The lowest mortality was recorded for the fungal culture poisoned with malathion (8.64 per cent).

The fungi cultured as the untreated control recorded the maximum mortality of 44.39 per cent to the newly emerged adults of *C. formicarius* during the second continuous passage followed by the treatment with imidacloprid which recorded a mean mortality of 37.71 per cent and were statistically on par. The fungi cultured in carbosulfan and chlorpyriphos caused a mean mortality of 28.81

Table 91. Bioefficacy of *M. anisopliae* cultured in poisoned food media for continuous ten passages

Sl. No.	Treatments	Mortality of adult *C. formicarius* at 14 DAT (per cent)									
		I	II	III	IV	V	VI	VII	VIII	IX	X
T1	Carbendazim	19.62 (4.43)	15.40 (3.93)	22.12 (4.70)	24.34 (4.93)	22.12 (4.70)	24.34 (4.93)	22.12 (4.70)	12.73 (3.57)	15.40 (3.93)	22.12 (4.70)
T2	Mancozeb	10.86 (3.29)	6.67 (2.58)	13.33 (3.65)	12.73 (3.57)	15.40 (3.93)	8.64 (2.94)	15.40 (3.93)	12.73 (3.57)	15.40 (3.93)	8.64 (2.94)
T3	Carbofuran	22.12 (4.70)	15.40 (3.93)	19.62 (4.43)	24.34 (4.93)	28.81 (5.37)	24.34 (4.93)	26.67 (5.16)	26.38 (5.14)	26.38 (5.14)	21.71 (4.66)
T4	Carbosulfan	26.67 (5.16)	28.81 (5.37)	24.34 (4.93)	28.81 (5.37)	28.81 (5.37)	28.81 (5.37)	31.03 (5.57)	28.81 (5.37)	31.03 (5.57)	28.81 (5.37)
T5	Imidacloprid	35.49 (5.96)	37.71 (6.14)	39.81 (6.31)	37.71 (6.14)	37.71 (6.14)	42.17 (6.49)	39.81 (6.31)	42.17 (6.49)	39.81 (6.31)	40.00 (6.33)
T6	Chlorpyriphos	15.40 (3.93)	17.63 (4.19)	12.73 (3.57)	19.62 (4.43)	19.62 (4.43)	12.73 (3.57)	17.63 (4.19)	12.73 (3.57)	17.63 (4.19)	19.62 (4.43)
T7	Lambda cyhalothrin	13.33 (3.65)	10.86 (3.29)	8.64 (2.94)	15.40 (3.93)	15.40 (3.93)	12.73 (3.57)	10.86 (3.29)	8.64 (2.94)	13.33 (3.65)	15.40 (3.93)
T8	Malathion	8.64 (2.94)	4.32 (2.08)	10.86 (3.29)	10.86 (3.29)	12.73 (3.57)	10.86 (3.29)	13.33 (3.65)	10.86 (3.29)	13.33 (3.65)	10.86 (3.29)
T9	Untreated Control	42.17 (6.49)	44.39 (6.66)	46.51 (6.82)	48.84 (6.99)	51.06 (7.15)	50.93 (7.14)	46.51 (6.82)	48.84 (6.99)	53.19 (7.29)	50.93 (7.14)
	C.D (0.05)	(0.789)	(1.264)	(1.002)	(0.939)	(0.905)	(1.049)	(0.731)	(1.166)	(0.748)	(0.971)

Figures in parentheses are square root transformed values DAT: Days after treatment

and 17.63 per cent respectively and were on par. The treatments involving carbendazim, carbofuran and lambda cyhalothrin caused a mean mortality of 15.40, 15.40 and 10.86 per cent respectively and were statistically on par. Relatively lower mortality was caused when treated with the fungi grown in mancozeb and malathion which recorded a mean of 6.67 and 4.32 per cent mortality respectively and were statistically on par.

During the third continuous passage the fungi cultured as the untreated control caused the maximum mean mortality of the adults of *C. formicarius* and it was 46.51 per cent and was on par with that grown in imidacloprid mixed media which had caused a mean mortality of 39.81 per cent. The treatments with carbosulfan, carbendazim and carbofuran mixed media caused a mean mortality of 24.34, 22.12 and 19.62 per cent respectively and were on par. The mean mortality caused by the fungi grown with mancozeb, chlorpyriphos, malathion and lambda cyhalothrin were 13.33, 12.73, 10.86 and 8.64 per cent respectively and were statistically on par.

When the adults of *C. formicarius* was treated with the fungi cultured during the fourth continuous passage, the fungi grown as the untreated control recorded the maximum mean mortality of 48.84 per cent and was on par with that grown in imidacloprid poisoned media which recorded a mean mortality of 37.71 per cent. The treatments with carbosulfan, carbofuran, carbendazim and chlorpyriphos caused a mean mortality of 28.81, 24.34, 24.34 and 19.62 per cent respectively and were statistically on par. A mean mortality of 15.40, 12.73 and 10.86 per cent was recorded for the fungi cultured with lambda cyhalothrin, mancozeb and malathion poisoned media and were statistically on par.

During the fifth continuous passage the fungi grown as the untreated control caused significantly higher mean mortality of 51.06 per cent and differed significantly from the other treatments. The treatments with imidacloprid, carbofuran and carbosulfan caused a mean mortality of 37.71, 28.81 and 28.81 per cent respectively and were statistically on par. The fungi cultured in media

mixed with carbendazim, chlorpyriphos, lambda cyhalothrin and mancozeb recorded a mean mortality of 22.12, 19.62, 15.40 and 15.40 per cent, respectively and were statistically on par. The lowest mean mortality of 12.73 per cent was observed for the fungi cultured with malathion.

The fungi grown as the untreated control without any pesticides caused the maximum mortality of the adults of *C. formicarius* during the sixth continuous passage and recorded a mean mortality of 50.93 per cent and was on par with the fungi grown with imidacloprid which caused a mean mortality of 42.17 per cent. The mean mortality caused by the fungi cultured in media mixed with carbosulfan, carbofuran and carbendazim were 28.81, 24.34 and 24.34 per cent respectively and were on par. The treatments with lambda cyhalothrin, chlorpyriphos, malathion and mancozeb caused a mean mortality of 12.73, 12.73, 10.86 and 8.64 per cent, respectively and were statistically on par.

During the seventh continuous passage the fungi cultured as the untreated control caused the maximum mortality of 46.51 per cent and was on par with the fungi grown in media mixed with imidacloprid which recorded a mean mortality of 39.81 per cent. The fungi cultured with carbosulfan and carbofuran poisoned media caused a mean mortality of 31.03 and 26.67 per cent respectively and were statistically on par. The treatments with carbendazim and chlorpyriphos recorded a mean mortality of 22.12 and 17.63 per cent respectively and were on par. The mean mortality caused by the fungi grown in media poisoned with mancozeb, malathion and lambda cyhalothrin were 15.40, 13.33 and 10.86 per cent respectively and were statistically on par.

The maximum mean mortality in the eighth passage was caused by the fungi grown as the untreated control and was 48.84 per cent followed by the fungi cultured in imidacloprid poisoned media which recorded a mean mortality of 42.17 per cent and were statistically on par. The treatments with carbosulfan and carbofuran mixed media recorded a men mortality of 28.81 and 26.38 per cent respectively and were statistically on par. The mean mortality caused by the fungi

Plate 17. Agarose gel electrophoresis profile of DNA extracted from the different samples of *B. bassiana* and *M. anisopliae*

1 - *M. anisopliae* in Carbendazim 2 - *M. anisopliae* in Mancozeb 3 - *M. anisopliae* in Carbofuran 4 - *M. anisopliae* in Carbosulfan 5 - *M. anisopliae* in Imidacloprid 6 - *M. anisopliae* in Chlorpyriphos 7 - *M. anisopliae* in Lambda cyhalothrin 8 - *M. anisopliae* in Malathion 9 – Untreated control 10 - *B.bassiana* in Carbendazim 11 - *B.bassiana* in Mancozeb 12 - *B.bassiana* in Carbofuran 13 - *B.bassiana* in Carbosulfan 14 - *B.bassiana* in Imidacloprid 15 - *B.bassiana* in Chlorpyriphos 16 - *B.bassiana* in Lambda cyhalothrin 17 - *B.bassiana* in Malathion 18 – Untreated control

grown on carbendazim, chlorpyriphos, mancozeb, malathion and lambda cyhalothrin were 12.73, 12.73, 12.73, 10.86 and 8.64 per cent, respectively and were statistically on par.

The fungi cultured as the untreated control caused significantly higher mean mortality of 53.19 per cent during the ninth continuous passage and differed significantly from the other treatments. The fungi grown in imidacloprid mixed media recorded a mean mortality of 39.81 per cent and was on par with the mortality caused by the carbosulfan which had a mean of 31.03 per cent. The treatment with carbofuran caused a mean mortality of 26.38 per cent. A mean mortality of 17.63, 15.40, 15.40, 13.33 and 13.33 per cent was caused by the treatments with chlorpyriphos, mancozeb, carbendazim, mancozeb and lambda cyhalothrin, respectively and were statistically on par.

In the tenth continuous passage, maximum mortality was recorded when applied with spore suspension from the control as in the other cases and recorded a mean mortality of 50.93 per cent and was on par with that grown with imidacloprid which caused a mean mortality of 40 per cent. The carbosulfan mixed culture produced 28.81 per cent mortality of adults of *C. formicarius*. A mean mortality of 22.12, 21.71 and 19.62 was observed when treated with the fungi cultured in media mixed with carbendazim, carbofuran and chlorpyriphos respectively and were on par. The mortality recorded for lambda cyhalothrin and malathion mixed cultures were 15.40 and 10.86 per cent respectively and were on par. The lowest mortality was recorded for the fungal culture grown with mancozeb with a mean mortality of 8.64 per cent.

4.6.2 Molecular characterisation

4.6.2.1 DNA Isolation

The wet mycelium of all the samples of the fungus yielded good quality DNA as shown in Plate 17.

4.6.2.2 DNA Fingerprinting

4.6.2.2.1 PCR and molecular analysis of the amplified products

The number of bands resolved per amplification was primer dependant for both the fungi and varied from 1 to 15. The results of the molecular analysis is given in Tables 92 and 93.

4.6.2.2.2 Molecular analysis of B. bassiana amplified products

The primer RFu-1 generated 13 scorable bands, out of which eight were polymorphic and five were monomorphic (Table 92). *B. bassiana* cultured in carbofuran, carbosulfan, imidacloprid, chlorpyriphos, lambda cyhalothrin and malathion developed specific bands of 3 kb and 1.5 kb (Plate 18 a) and the bands at 950bp and 800bp were specific to the fungus cultured in mancozeb. The polymorphic bands developed at 600bp correspond to the fungus grown on carbendazim and mancozeb, respectively. The bands developed at 500bp and 700 bp positions were specific to the fungus cultured in carbendazim and showed about 61.54 per cent polymorphism.

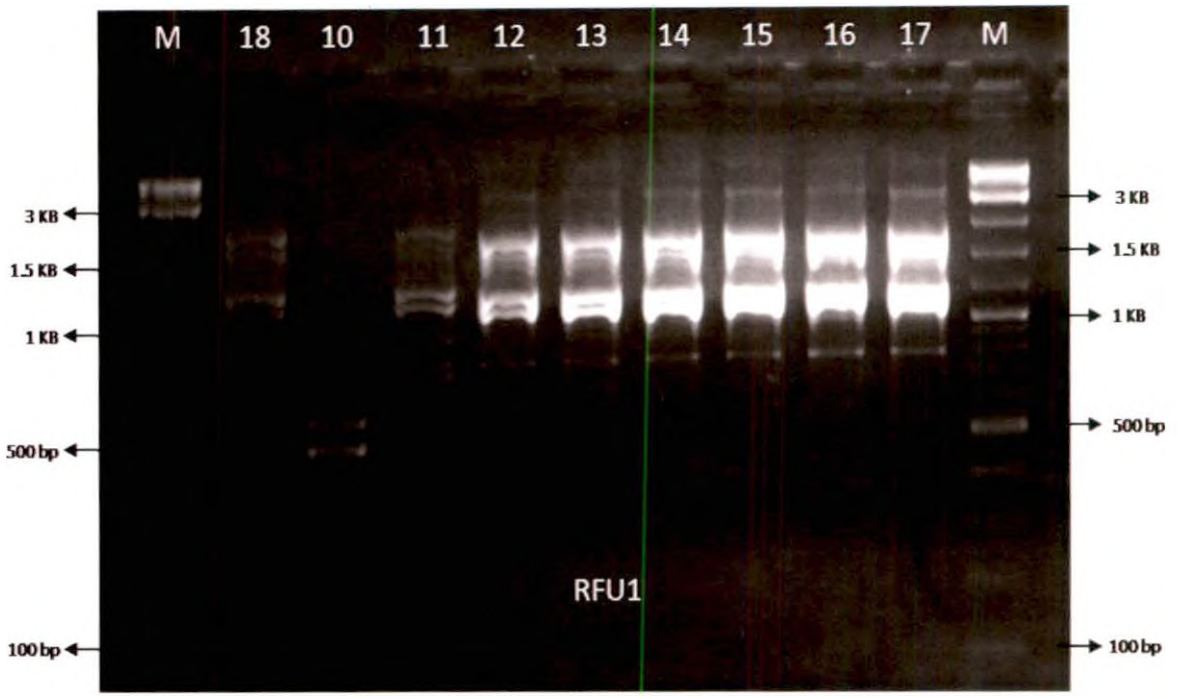
Out of the 12 scorable bands developed by the primer RFu -2, only one was monomorphic and 11 bands were polymorphic. The bands developed at 600 bp, 750 bp and 900 bp was specific for the fungi grown on carbendazim (Plate 18 b). The polymorphism exhibited was 91.67 per cent.

The primer RFu-3 produced 10 scorable bands, of which two were monomorphic and eight were polymorphic (Plate 19 a). The bands developed at 1.25 kb, 1.60 kb, 700 bp and 750 bp were specific to the fungus cultured in carbendazim. The per cent polymorphism recorded was 80.

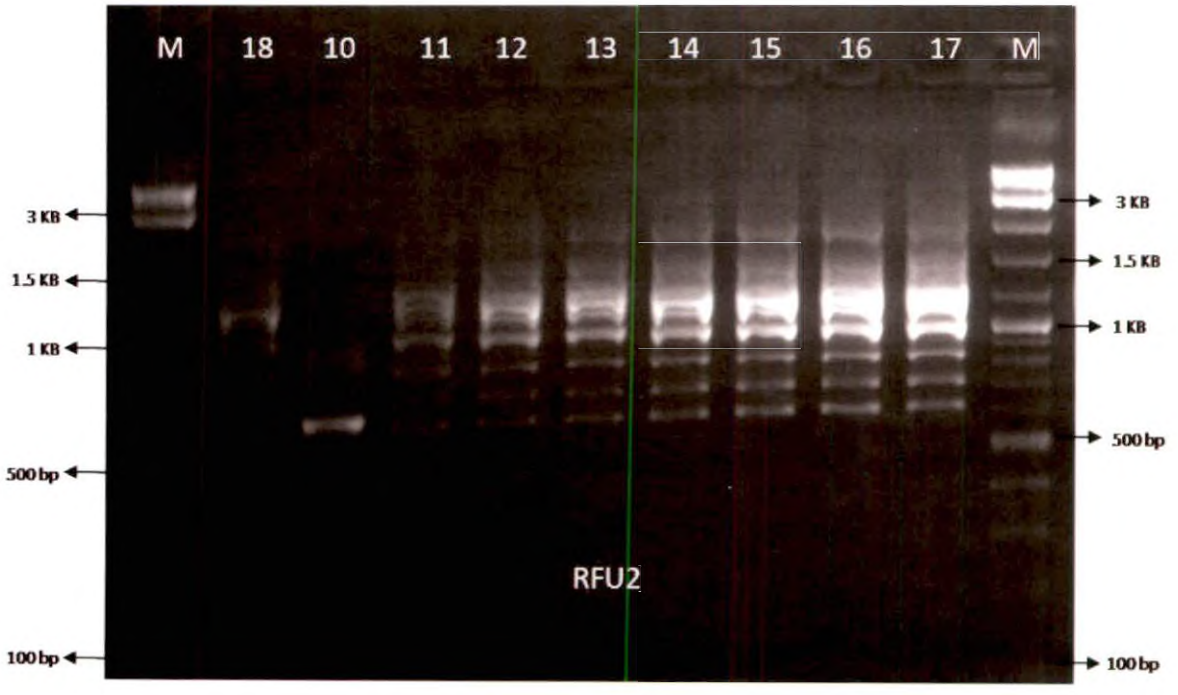
The number of scorable bands developed by the primer RFu-4 was 15 (Plate 19 b). Only one monomorphic band was there and the rest were polymorphic bands and it could produce 93.33 per cent polymorphism. The bands developed at 600 bp, 750 bp, 1 kb, 1.4 kb and 2 kb were specific for the fungus grown with carbendazim.

Table 92. DNA fingerprinting using ten primers and the polymorphism exhibited by *B. bassiana* grown on eight chemicals for ten passages

Primers	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	% of polymorphism
RFu-1	17	5	12	70.59
RFu-2	12	3	9	75.00
RFu-3	13	3	10	76.92
RFu-4	15	2	13	86.67
RFu-5	13	3	10	76.92
RFu-6	13	1	12	92.31
RFu-7	1	1	0	0
RFu-8	9	1	8	88.89
RFu-9	13	1	12	92.31
RFu-10	13	0	13	100
Total	119	20	99	83.19

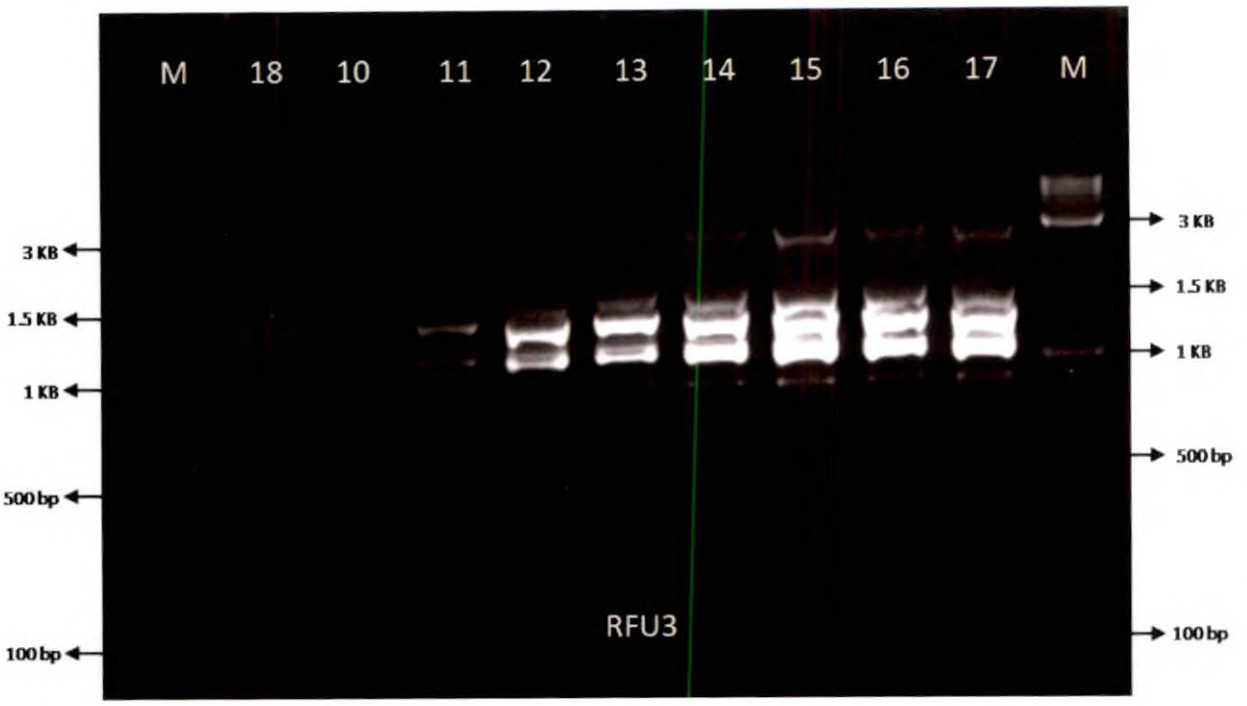


a. RAPD profile of *B. bassiana* using primer RFu 1

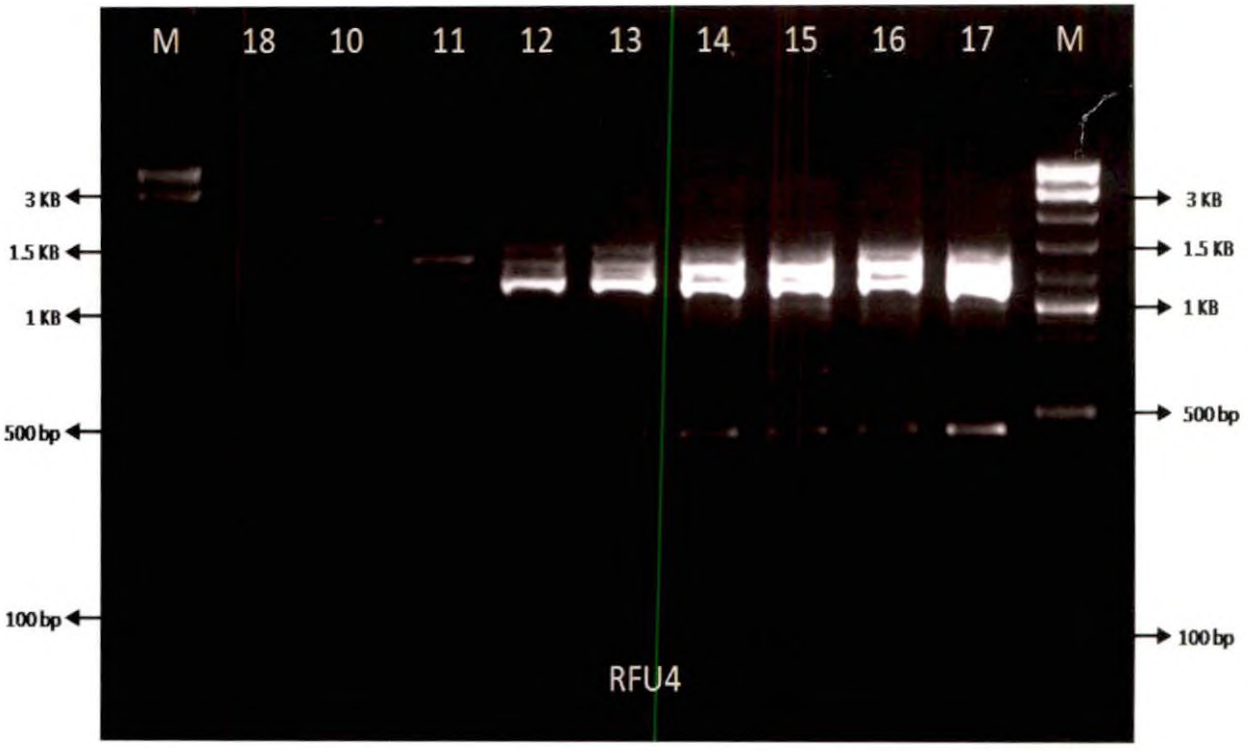


b. RAPD profile of *B. bassiana* using primer RFu 2

Plate 18. RAPD profile of *B. bassiana*



a. RAPD profile of *B. bassiana* using primer RFu 3



b. RAPD profile of *B. bassiana* using primer RFu 4

Plate 19. RAPD profile of *B. bassiana*

The primer RFu-5 developed 13 scorable bands (Plate 20 a). Among them, three were monomorphic and ten were polymorphic bands. The bands developed at 2 kb, 1.3 kb and 1 kb were specific for the fungus cultured in carbendazim. The specific band developed by the fungus cultured in mancozeb, carbofuran, carbosulfan, imidacloprid, chlorpyriphos, lambda cyhalothrin and malathion was at 800 bp. The polymorphism exhibited was 76.92 per cent.

Among the 12 scorable bands developed by the primer RFu-6 (Plate 20 b), only one was monomorphic and 11 were polymorphic and the per cent polymorphism exhibited was 91.67. The fungus cultured in carbendazim produced specific bands at 1.5 kb and at 750 bp.

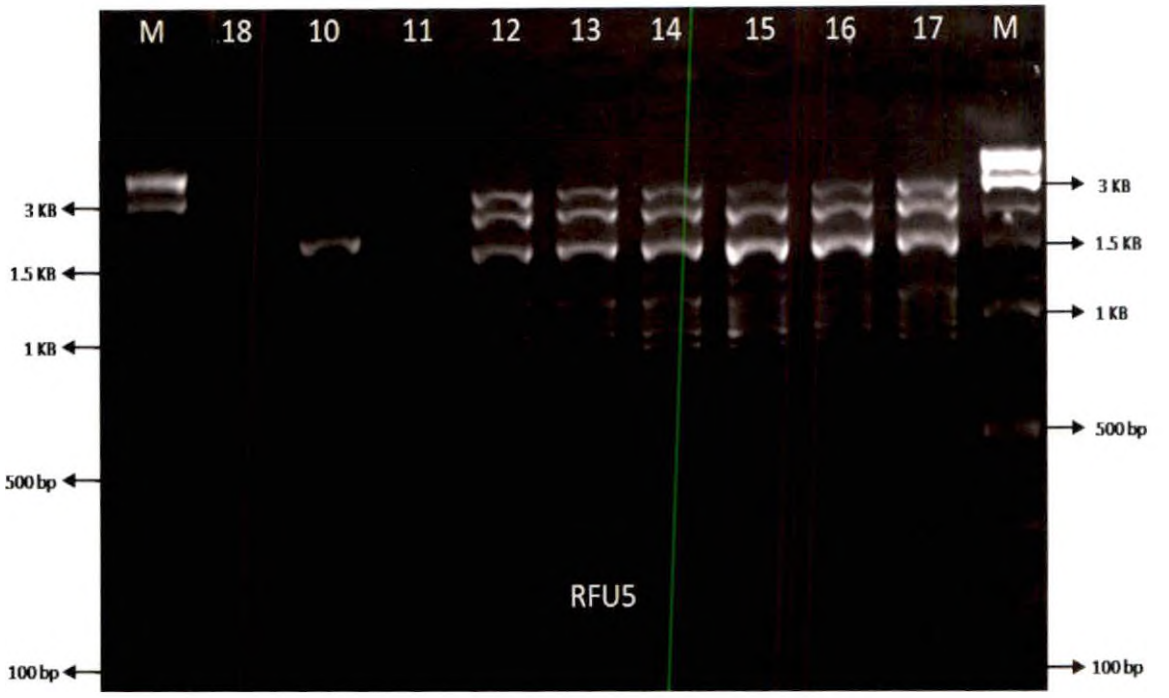
The primer RFu-7 produced only one scorable band and it was monomorphic and exhibited no polymorphism (Plate 21 a).

Out of the eight scorable bands produced by the primer RFu-8, seven were polymorphic and one was monomorphic and showed 87.50 per cent polymorphism (Plate 21 b). The bands at 1.1 kb were specific to the pure culture of *B. bassiana* and the fungus cultured in carbendazim.

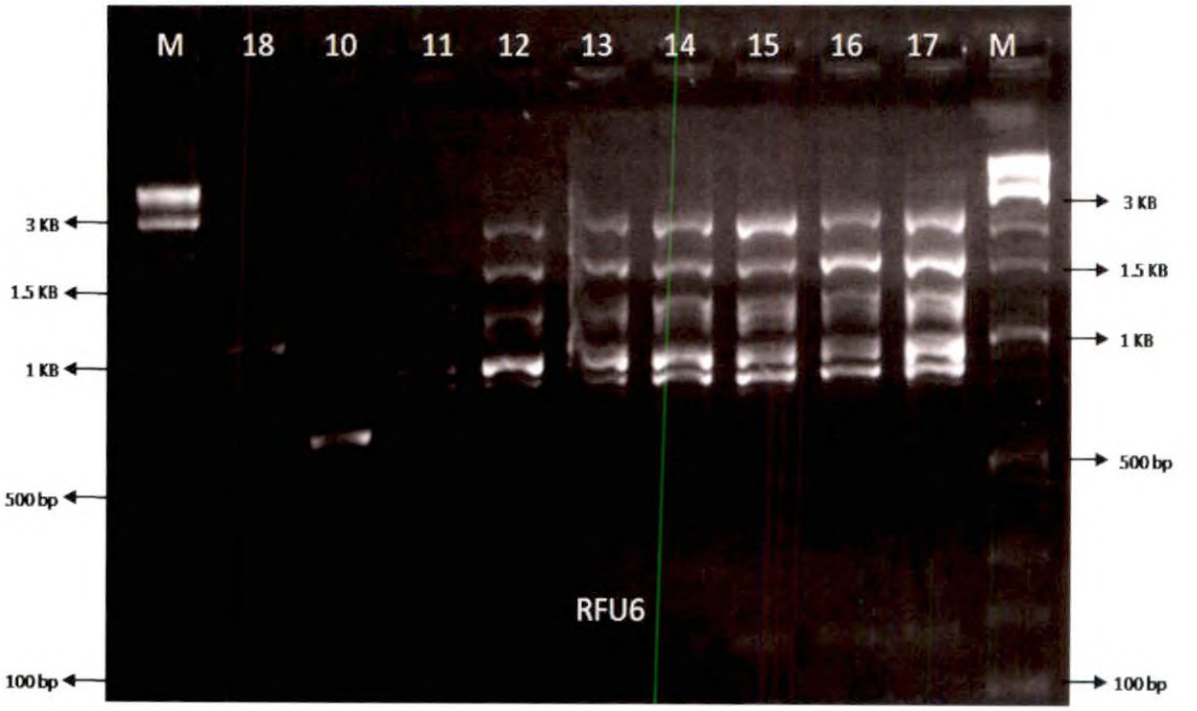
The primer RFu-9 developed 12 scorable bands and out of which 11 were polymorphic and one was monomorphic (Plate 22 a). The polymorphism exhibited was 91.67 per cent. The fungus grown in carbendazim developed specific bands at 2 kb, 1.5 kb and at 1.1 kb, respectively.

The primer RFu-10 produced 12 scorable bands and all were polymorphic bands and exhibited 100 per cent polymorphism (Plate 22 b). There was no amplification for the fungus cultured in carbendazim.

Among the ten primers tested, the primer RFu-10 was found to give maximum polymorphism of 100 per cent. The primer RFu-4 produced maximum number of scorable bands (15), whereas the primer RFu-7 obtained only one scorable band. *B. bassiana* exhibited a polymorphism of 82.30 per cent.

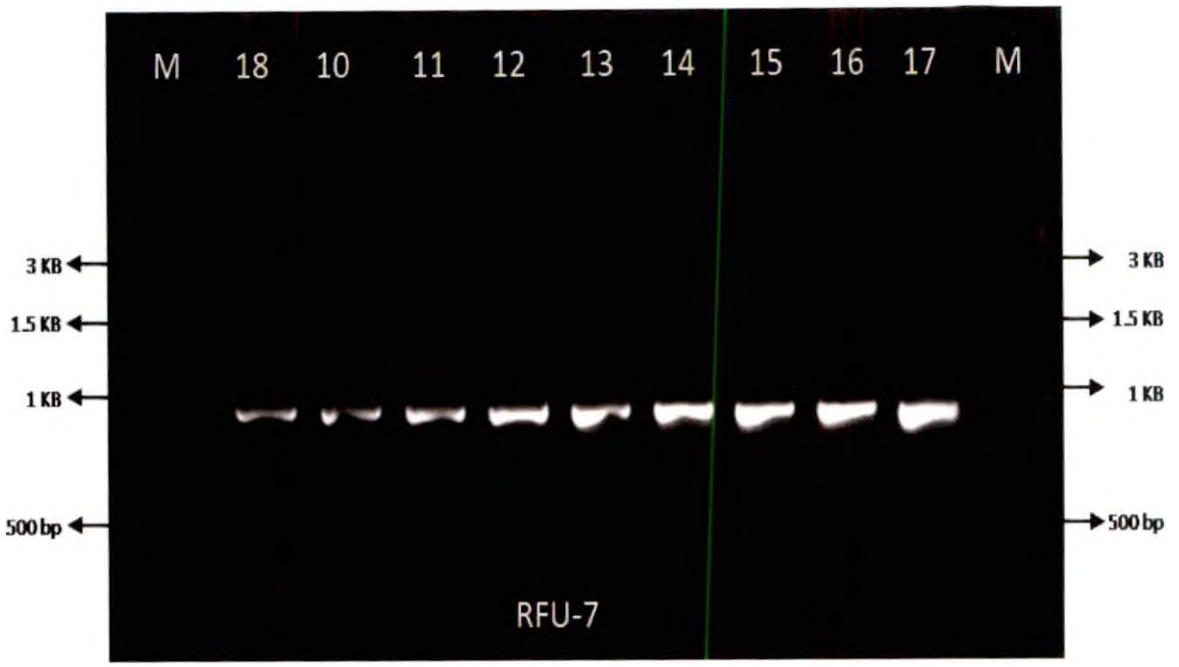


a. RAPD profile of *B. bassiana* using primer RFu 5

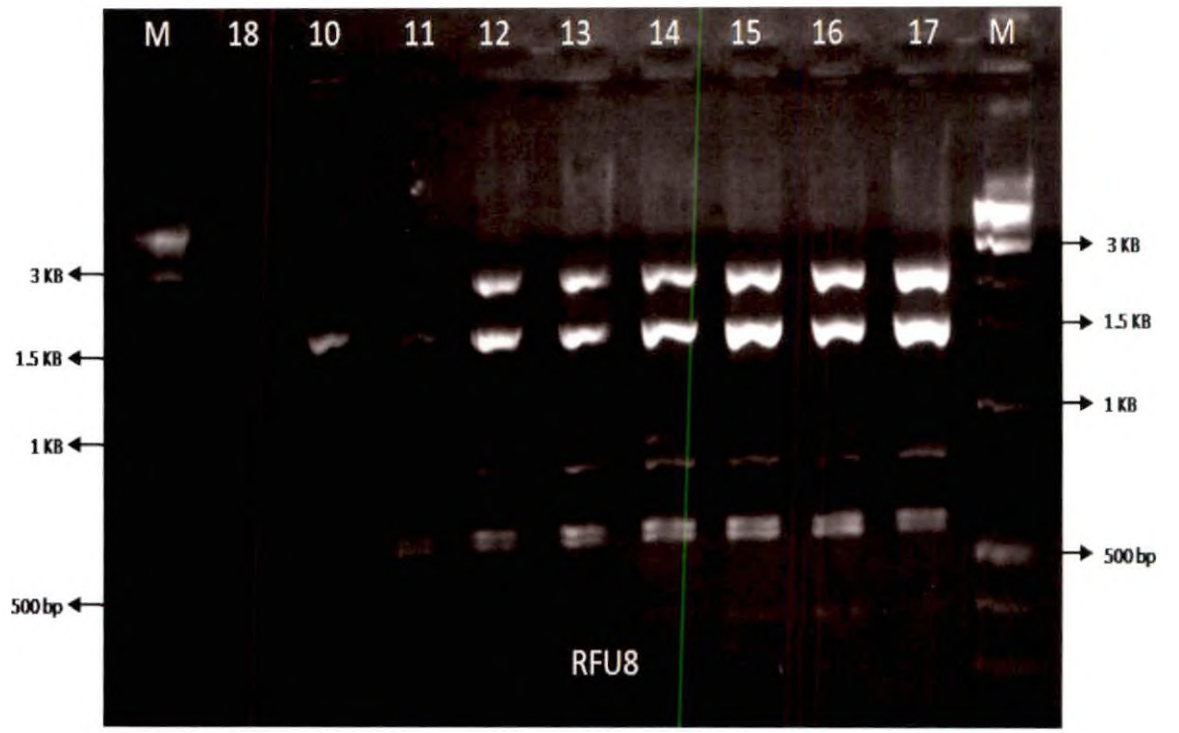


b. RAPD profile of *B. bassiana* using primer RFu 6

Plate 20. RAPD profile of *B. bassiana*

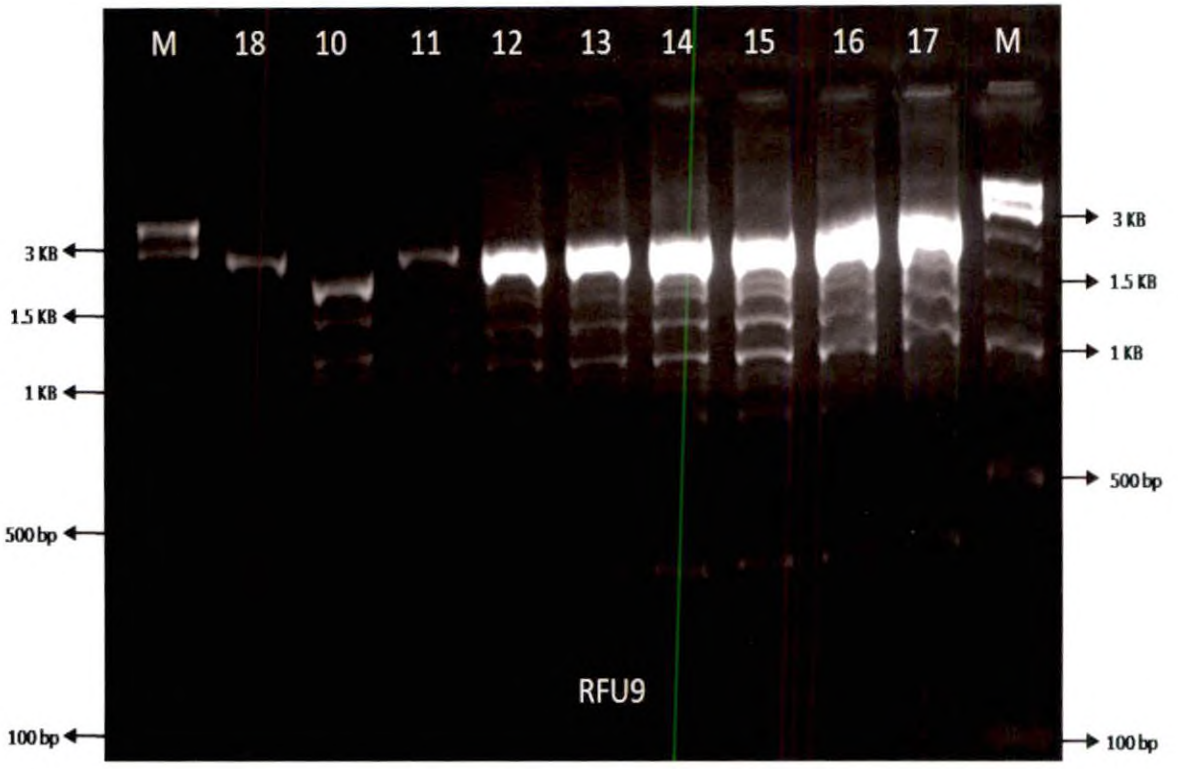


a. RAPD profile of *B. bassiana* using primer RFu 7

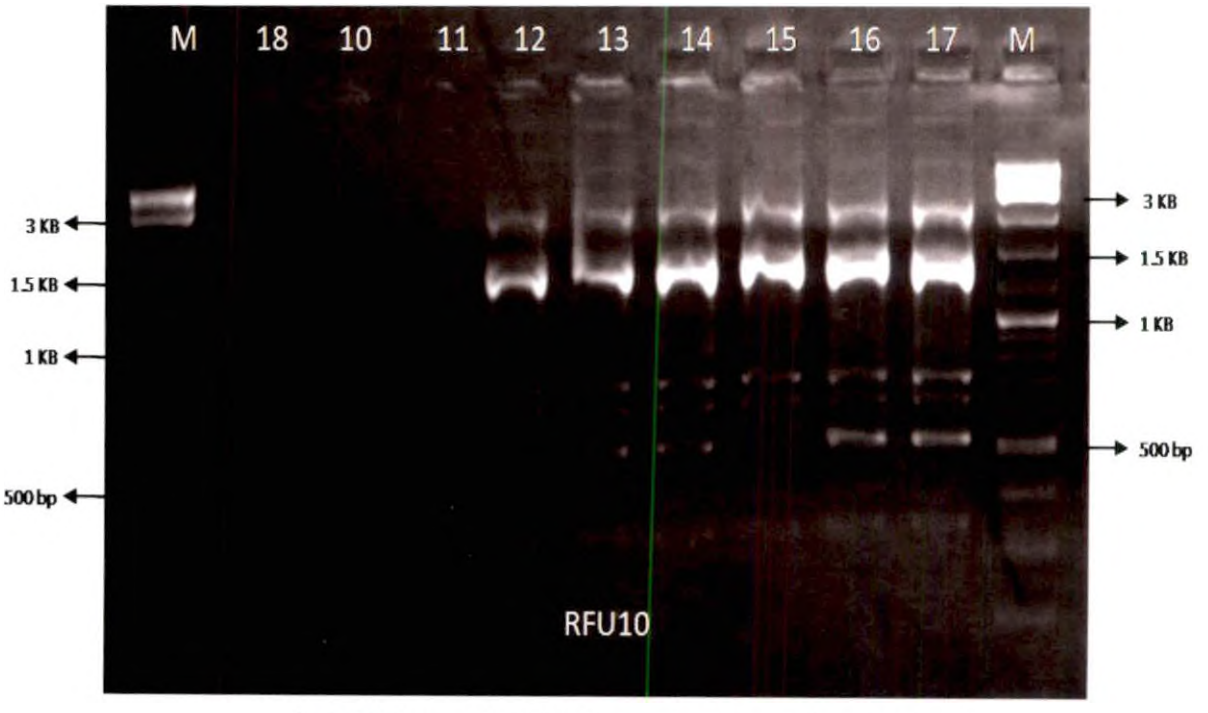


b. RAPD profile of *B. bassiana* using primer RFu 8

Plate 21. RAPD profile of *B. bassiana*



a. RAPD profile of *B. bassiana* using primer RFu 9



b. RAPD profile of *B. bassiana* using primer RFu 10

Plate 22. RAPD profile of *B. bassiana*

4.6.2.2.3 Molecular analysis of M. anisopliae amplified products

The primer RFu-1 produced seven scorable bands and among them five were monomorphic and two were polymorphic and the polymorphism exhibited was 28.57 per cent (Plate 23 a). The fungus cultured with imidacloprid, chlorpyriphos and lambda cyhalothrin developed specific bands at 1.4 kb (Table 93).

Out of the 11 scorable bands produced by the primer RFu-2, seven were monomorphic and four were polymorphic and exhibited 36.36 per cent polymorphism (Plate 23 b).

The primer RFu-3 produced seven scorable bands and out of which six were monomorphic and one was polymorphic and exhibited 14.28 per cent polymorphism (Plate 24 a).

The total number of scorable bands developed by the primer RFu-4 was nine and among them six were monomorphic and three were polymorphic and the polymorphism exhibited was 33.33 per cent (Plate 24 b). The pure culture of the fungus developed a specific band at 1 kb.

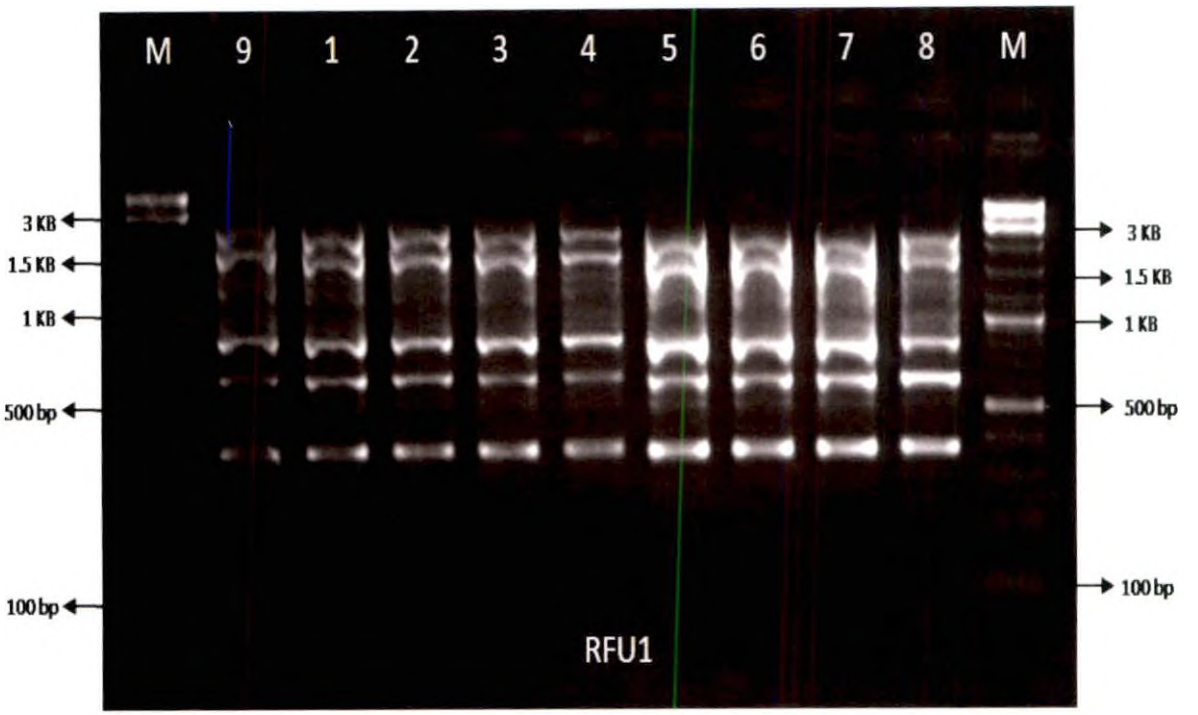
The primer RFu-5 produced eight scorable bands. Six of them were monomorphic and two were polymorphic and the polymorphism exhibited was 25.00 per cent (Plate 25 a).

Out of the five scorable bands developed by the primer RFu-6, four were monomorphic and one was polymorphic and the polymorphism exhibited was 20.00 per cent (Plate 25 b). A specific band at 400 bp was developed for the pure culture of the fungus.

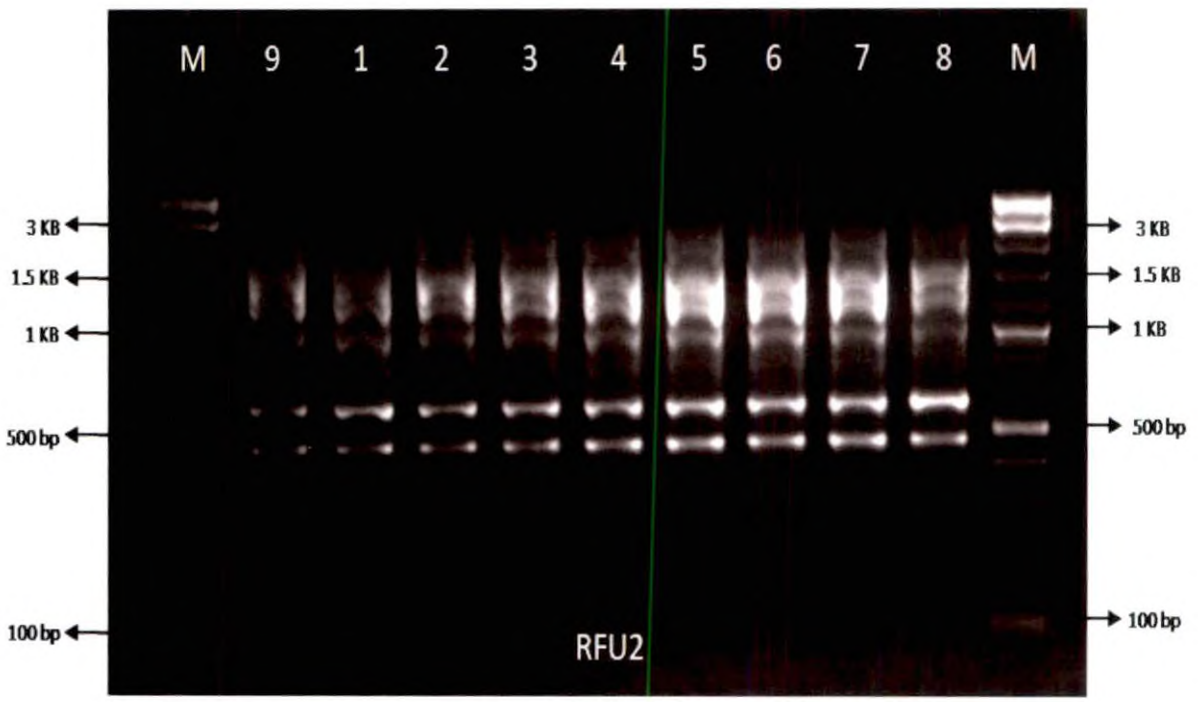
The primer RFu-7 produced ten scorable bands during the first reaction and the monomorphic bands came to about six and four were polymorphic and the polymorphism exhibited was 40.00 per cent (Plate 26 a). At 500 bp a specific band was obtained for the fungus grown in chlorpyriphos and carbofuran. Also a

Table 93. DNA fingerprinting using ten primers and the polymorphism exhibited by *M. anisopliae* grown on eight chemicals for ten passages

Primers	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	% of polymorphism
RFu-1	10	5	5	50.00
RFu-2	11	8	3	27.27
RFu-3	7	5	2	28.57
RFu-4	9	6	3	33.33
RFu-5	9	5	4	44.44
RFu-6	5	4	1	20.00
RFu-7	12	5	7	58.33
RFu-8	10	8	2	20.00
RFu-9	9	6	3	33.33
RFu-10	9	4	5	55.56
Total	91	56	35	38.46

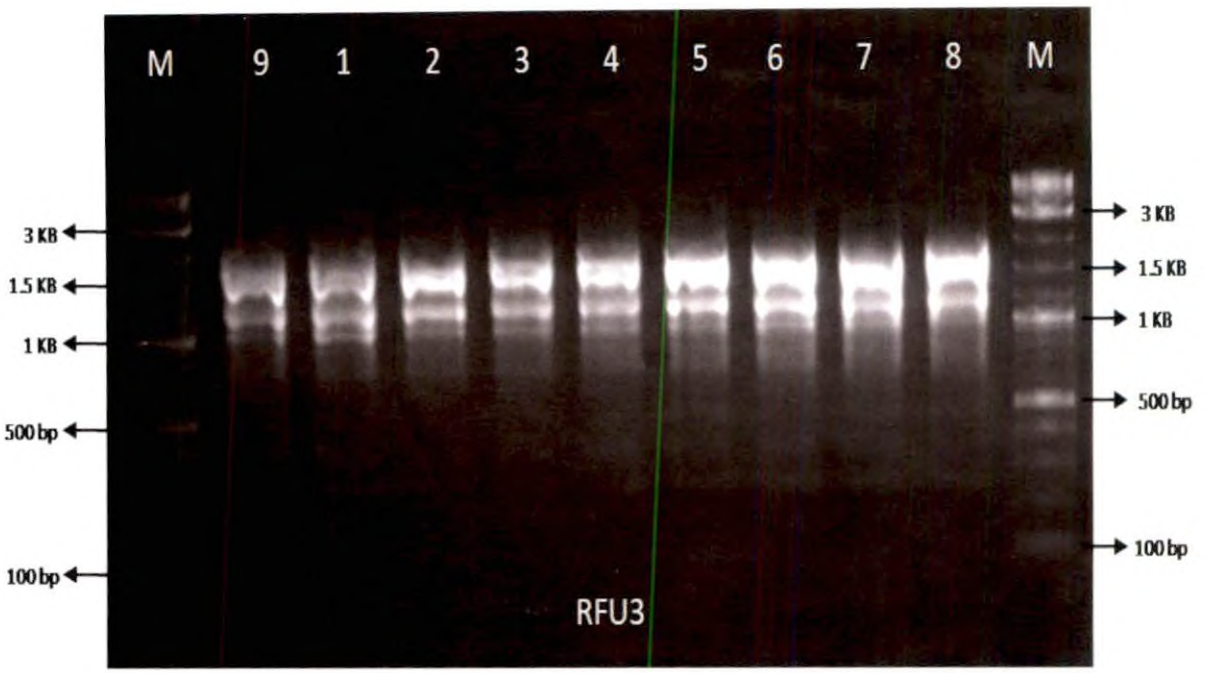


a. RAPD profile of *M. anisopliae* using primer RFu 1

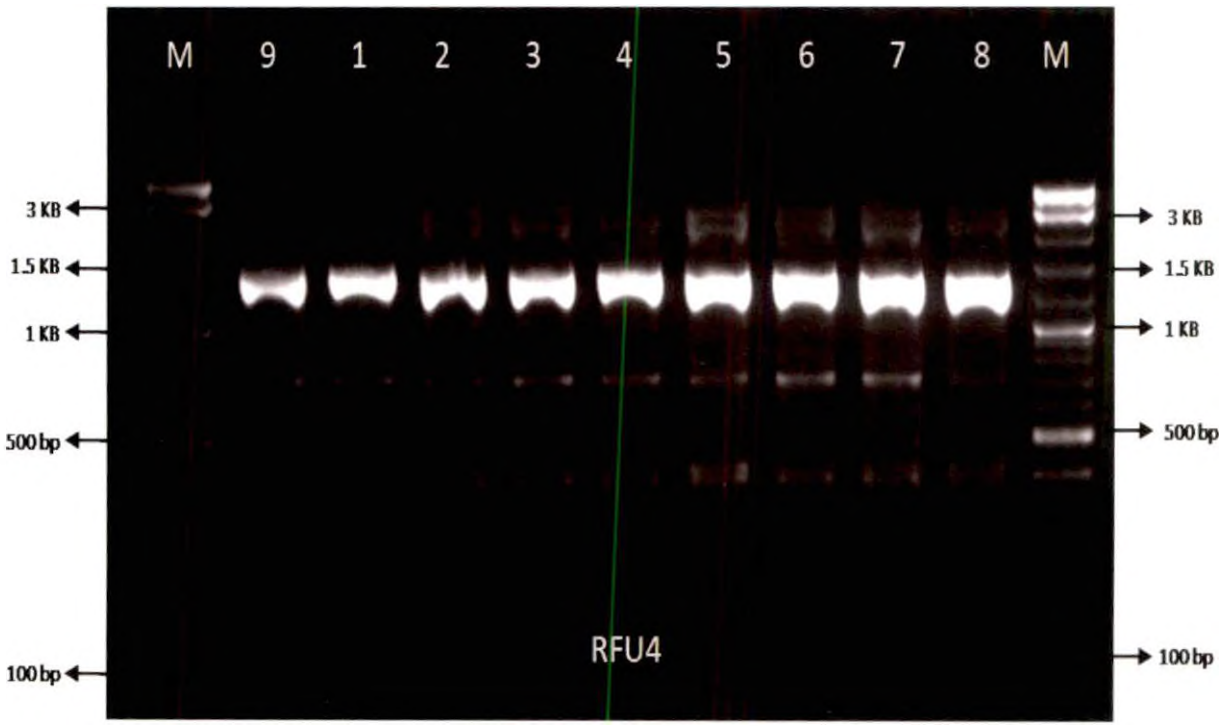


b. RAPD profile of *M. anisopliae* using primer RFu 2

Plate 23. RAPD profile of *M. anisopliae*

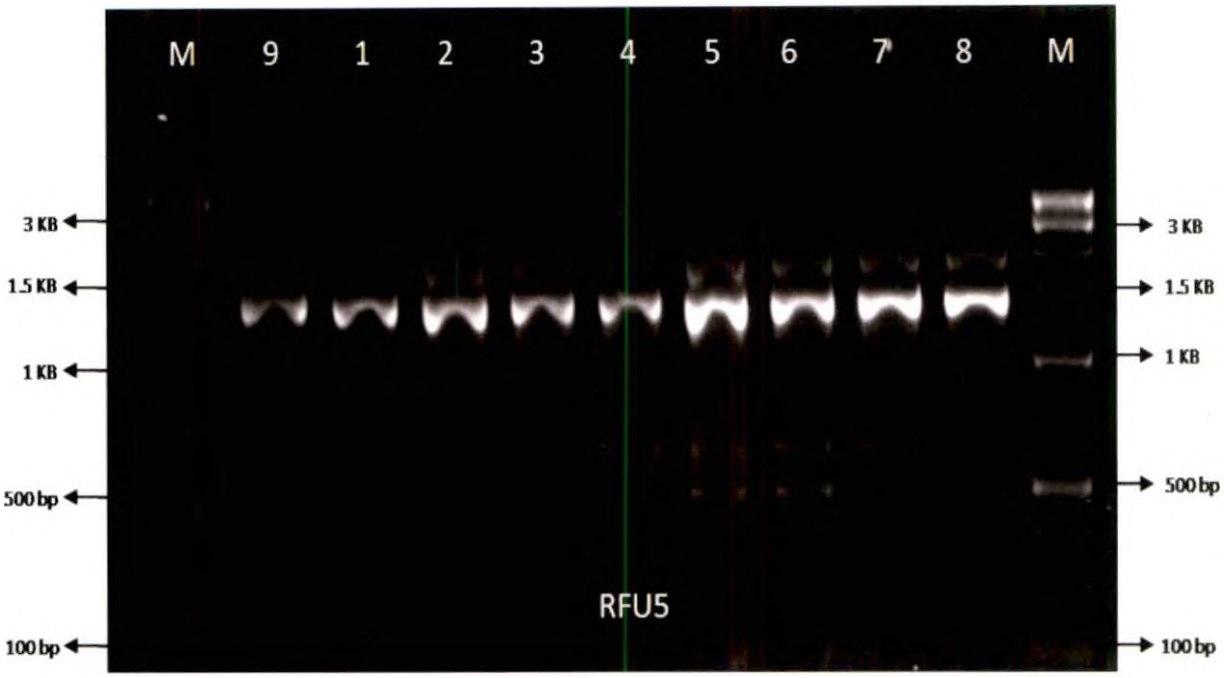


a. RAPD profile of *M. anisopliae* using primer RFu 3

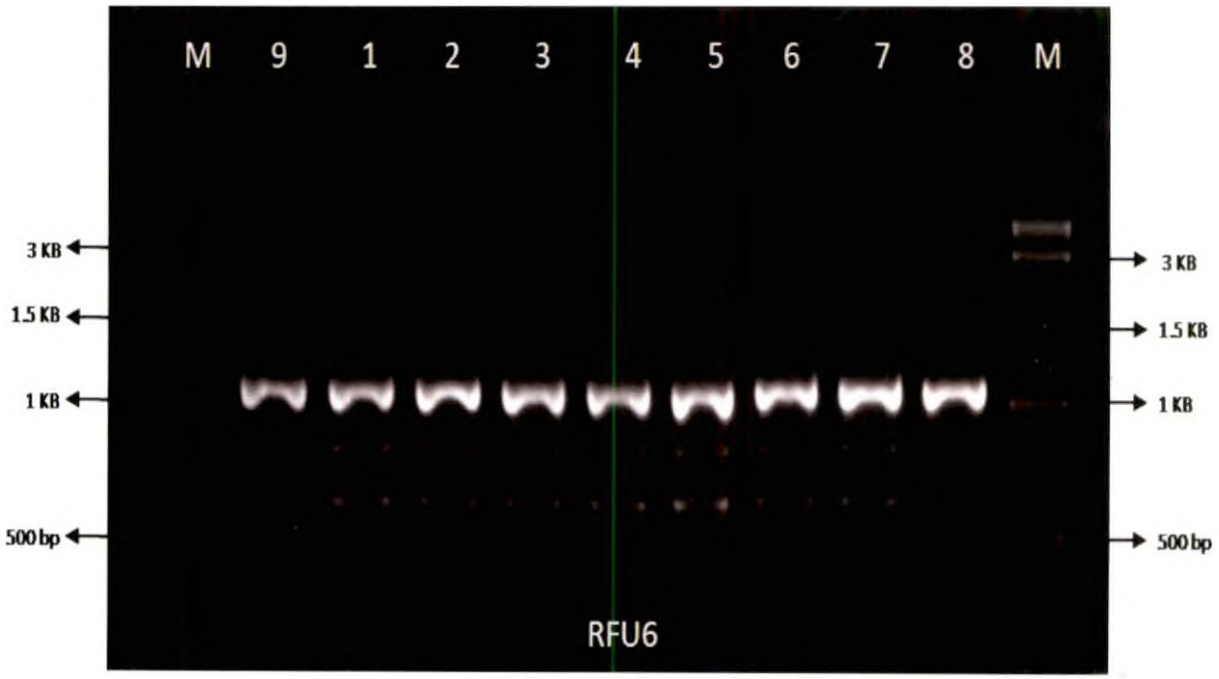


b. RAPD profile of *M. anisopliae* using primer RFu 4

Plate 24. RAPD profile of *M. anisopliae*



a. RAPD profile of *M. anisopliae* using primer RFu 5



b. RAPD profile of *M. anisopliae* using primer RFu 6

Plate 25. RAPD profile of *M. anisopliae*

specific band for carbofuran and carbosulfan poisoned fungus was developed at 350 bp.

Out of the nine scorable bands obtained for the primer RFu-8, eight were monomorphic and one was polymorphic and the polymorphism exhibited was about 11.11 per cent (Plate 26 b).

The primer RFu-9 produced seven scorable bands (Plate 27 a). Among them five were monomorphic and two were polymorphic and the polymorphism exhibited was 28.57 per cent.

Out of the six scorable bands developed by the primer RFu-10, five were monomorphic and one was polymorphic (Plate 27 b). The polymorphism exhibited was 16.67 per cent.

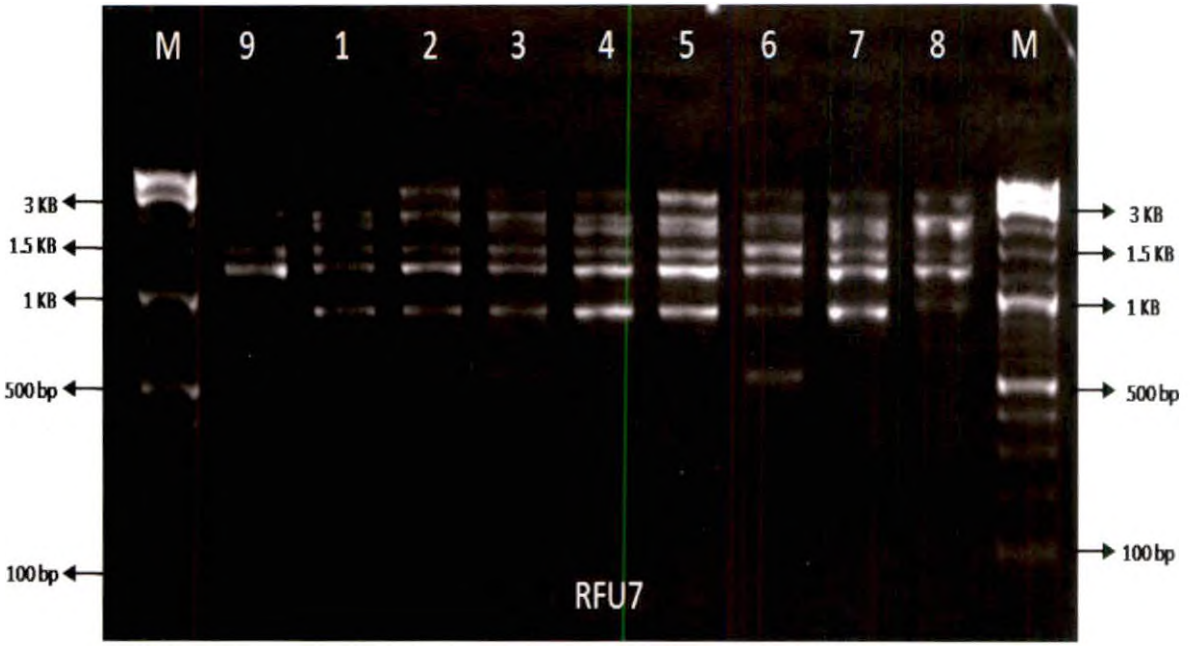
The primer RFu-7 induced maximum polymorphism of 40.00 per cent. Out of the 10 primers evaluated, the primer RFu-2 produced the maximum scorable bands (11 Nos.) and the primer RFu-6 developed the minimum number of scorable bands (5 Nos.). The polymorphism exhibited by the fungus is 26.58 per cent.

4.6.3 Statistical analysis

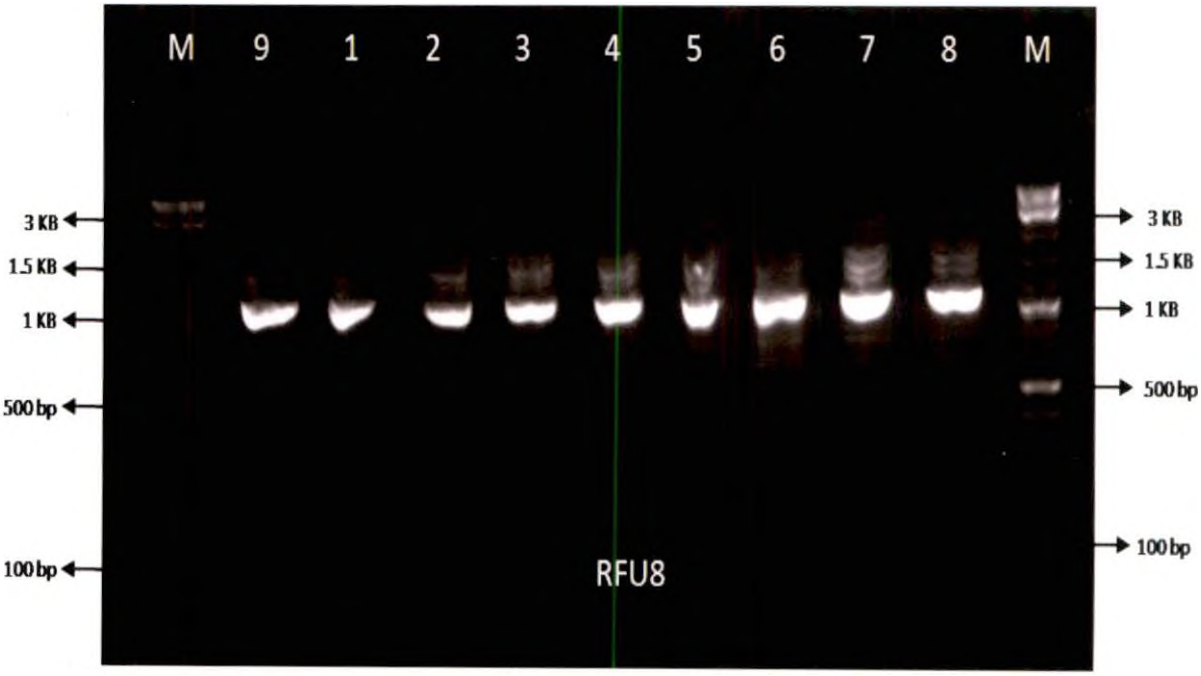
Jaccard's similarity coefficient values for each pair wise comparison between both the fungi cultured in different chemicals were calculated and a similarity coefficient matrix was constructed. The matrix was subjected to un-weighted pair-group method for arithmetic average analysis (UPGMA) to generate dendrogram using average linkage procedure. All these computations were carried out using NTSYS-pc version 2.02 software.

4.5.3.1 B.bassiana

In the dendrogram generated for the fungus, *B. bassiana* and the poison treated cultures (Fig 26), at 78 per cent dissimilarity the fungicide carbendazim treated fungal culture and the pure culture of the fungus along with the other

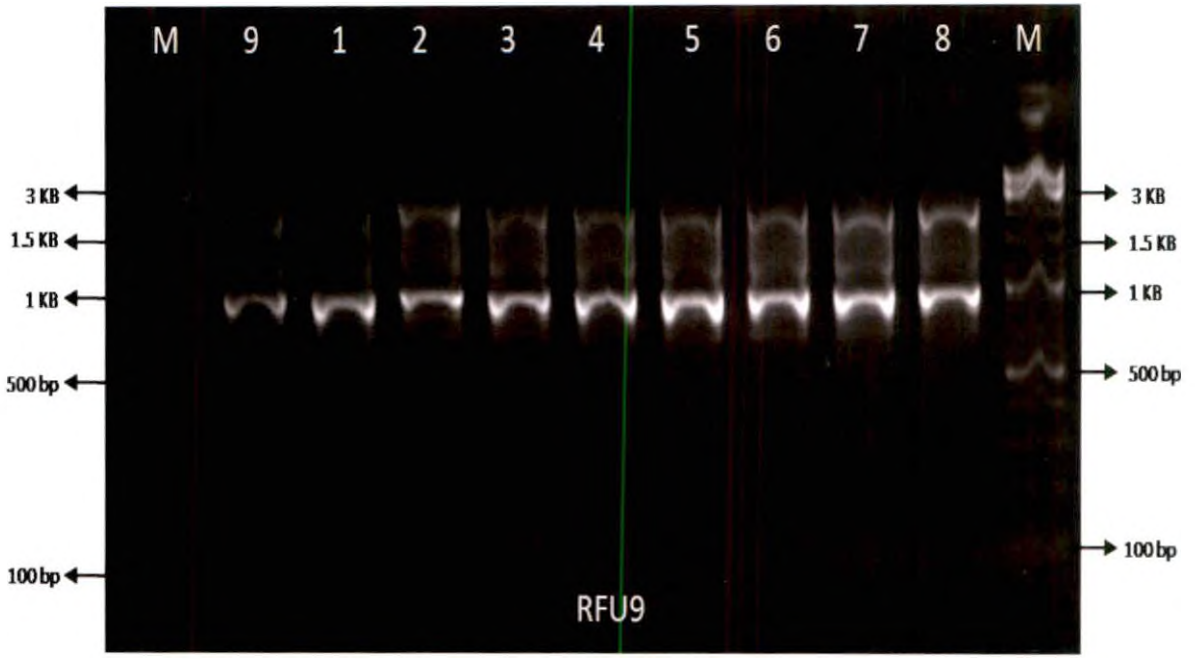


a. RAPD profile of *M. anisopliae* using primer RFu 7

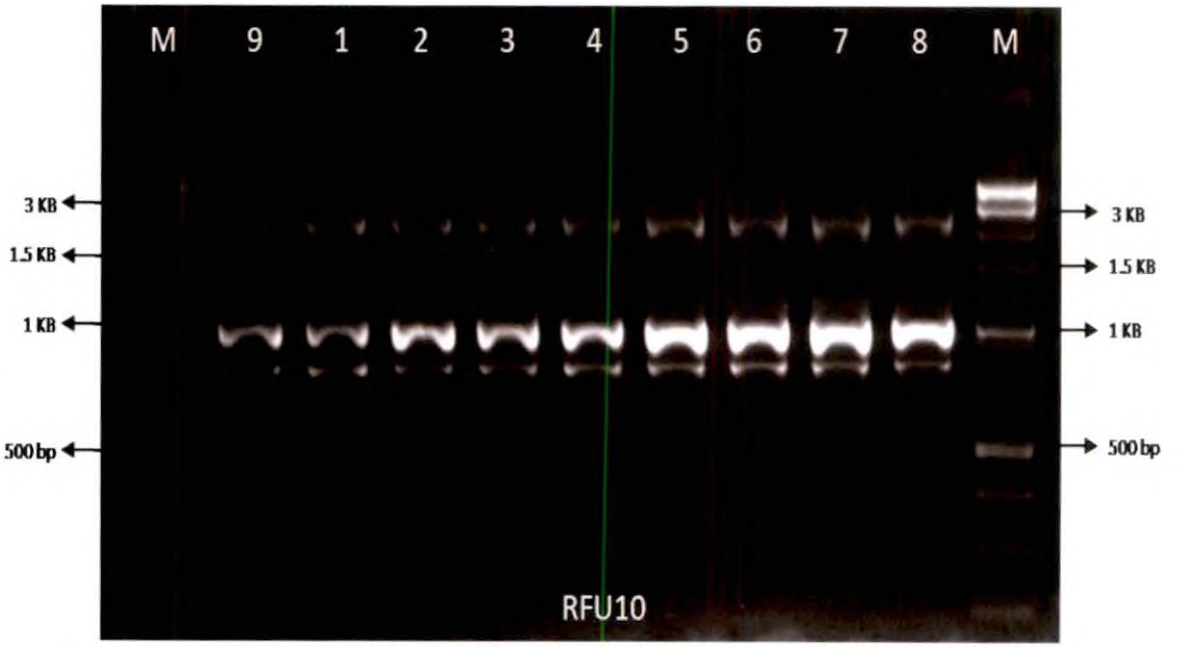


b. RAPD profile of *M. anisopliae* using primer RFu 8

Plate 26. RAPD profile of *M. anisopliae*



a. RAPD profile of *M. anisopliae* using primer RFu 9



b. RAPD profile of *M. anisopliae* using primer RFu 10

Plate 27. RAPD profile of *M. anisopliae*

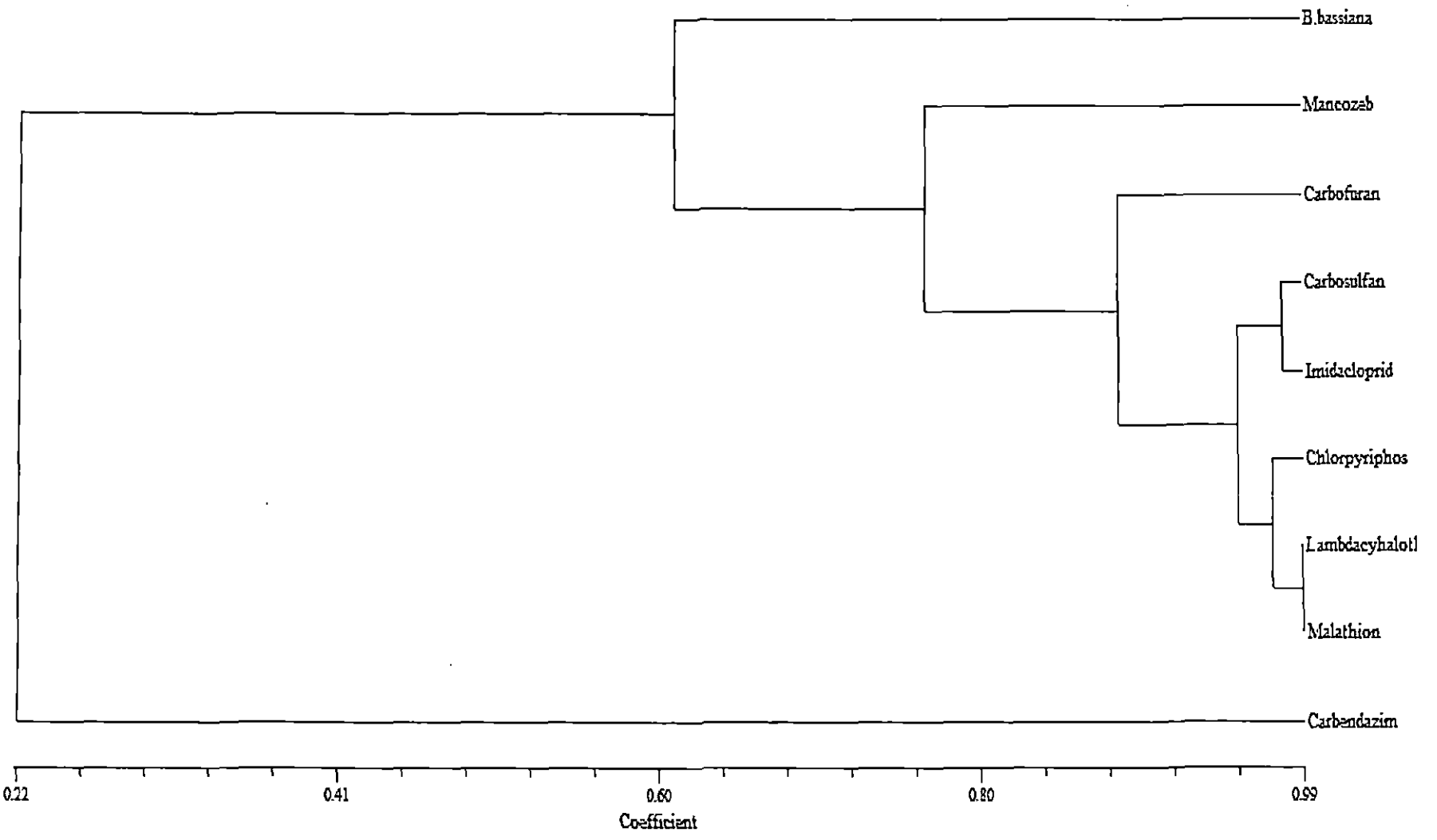


Fig. 26. Dendrogram generated from molecular analysis of *B. bassiana* cultured in poison media for ten passages

chemical treated cultures were grouped into two major clusters indicating significantly higher dissimilarity. The pure culture of *B. bassiana* and the other chemical cultured fungal cultures again cleaved into two groups at the similarity index 0.61 showing 39 percentage dissimilarity. At the similarity index 0.76, the fungicide grown fungus was separated from all the insecticide poisoned fungus. The carbofuran cultured *B. bassiana* cleaved from the other insecticide cultured fungal samples at the similarity index 0.89 showing 11 per cent dissimilarity. At the similarity index 0.96, the fungal cultures grown with carbosulfan and imidacloprid formed one hand of the tree matrix and the rest formed the other hand. The fungus grown with chlorpyriphos had 2 per cent dissimilarity from the others, whereas lambda cyhalothrin and malathion recorded same dissimilarity at 0.99 index with only one per cent dissimilarity.

4.5.3.2 M. anisopliae

At the similarity index at 0.77, the pure culture of *M. anisopliae* cleaved separately from all the poison cultured fungal samples, recording 23 per cent dissimilarity of the poison treated samples with the pure culture (Fig 27). The fungicide cultured *M. anisopliae* and insecticide cultured *M. anisopliae* were grouped into two major clusters at the similarity index 0.81 recording 19 per cent dissimilarity. The insecticides carbofuran, carbosulfan and malathion formed a new group at 0.85 index, from the others, which included imidacloprid, lambda cyhalothrin and chlorpyriphos cultured fungus. Malathion cultured fungus cleaved to another group at the similarity index 0.87 from the carbofuran and carbosulfan cultured fungus recording 13 per cent dissimilarity. carbofuran and carbosulfan poisoned fungi separated into two groups at 0.93 similarity index showing 7 per cent dissimilarity. At the similarity index of 0.96, imidcloprid and lambda cyhalothrin cultured samples cleaved into another group from chlorpyriphos, which were closely placed in the matrix, recording 4 per cent dissimilarity. Whereas imidacloprid and lambda cyhalothrin poisoned cultures showed a similarity coefficient of 0.98 with only 2 percentage dissimilarity.

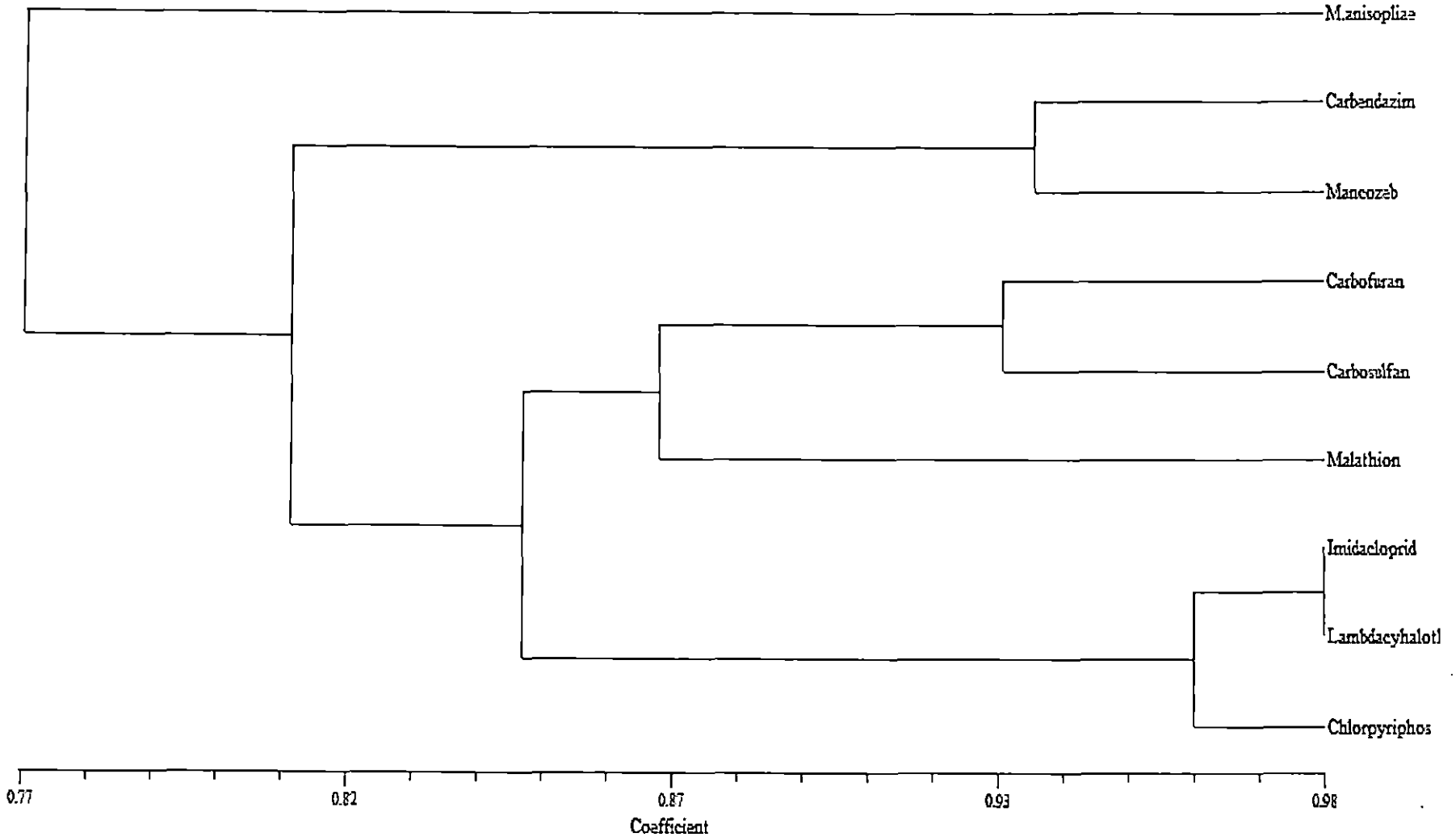


Fig. 27. Dendrogram generated from molecular analysis of *M. anisopliae* cultured in poison media for ten passages

4.7 IDENTIFICATION AND ITS SEQUENCING OF NEW ISOLATES

The nine new natural isolates obtained were subjected to ITS (Internal Transcribed Sequence) sequencing and BLAST (Basic Local Alignment Search Tool) and identified the cultures (Table 94). The natural isolate obtained from the adults of *B. fulvicorne* was identified as *B. bassiana* and the fungi isolated from the grubs of *B. fulvicorne* was *Metarhizium* sp. The entomopathogenic fungi *Paecilomyces* sp. was obtained from the grubs of *C. sordidus*. The naturally infected adults of *C. formicarius, H. vigintioctopunctata* and *M. circumdata* were identified as the pathogenesis of *Beauveria* sp., *Fusarium moniliformae* and *B. brongniartii* respectively. The natural isolates obtained from the grubs of *O. rhinoceros* were *Metarhizium album, Metarhizium anisopliae* var *majus* and *Metarhizium* sp. The details of the sequences obtained is presented below.

4.7.1 Isolate 1

Host insect : *B. fulvicorne*

Organism identified : *B. bassiana*

Sequence:

TACATACGCTGGTACCTCCGACCTCCAGCTCGAGCGTATGAACGTGTA
CTTCAACGAGGTGTGTGATGACCACACTGAAATATTTATTATTTATCGT
TCCTAATCCCATAAGCTACAGGCTTCCGGTAAGAAATACGTGCCTCGT
GCCGTCCTCGTCGATCTCGAACCTGGTACCATGGATGCCGTCCGCGCC
GGTCCTTTCGGACAGCTCTTCCGCCCCGACAACTTCGTTTTCGGACA
GTCTGGTGCCGGAAACAACTGGGCCAAGGG TCACTACACT GAGGGTA
TGTTTACAATGGCACTTCTGAGCTTCAGCTCGAGCGCATGAATGTCTACTTCA
ACGAGGTTTGTTGTGCCCTCCCAACGCGTTGCTTGATTTCGTTGTGGATACTG
ACCGCGATTTTCCAAAGGCCTCCGGCAACAAGTATGTTCCTCGCGCCGTCCTC
GTCGATCTTGAGCCCGGTACCATGGATGCTGTCCGTGCCGGTCCCTTCGGTCA
GCTCTTCCGTCCCGACAACTTCGTTTTCGGTCAGTCCGGTGCCGGCAACAACT
GGGCCAAGGGTCACTACACTGAGGGTATAGACTCCATAATTTGTTGTTTCTTG
TTGGTGGGGATAGTCGGGTCCTGTTGGCAGGACTACGCCGGCTAGTCGACAT

Table 94. New isolates of fungi isolated from coleopteran insects collected from Thiruvananthapuram and Idukki districts

Sl.No	Host insect	Stage of the Insect	Isolates
1	*B. fulvicorne*	A	*B. bassiana*
2	*B. fulvicorne*	G	*Metarhizium* sp.
3	*C. sordidus*	G	*Paecilomyces* sp.
4	*C. formicarius*	A	*Beauveria* sp.
5	*H. vigintioctopunctata*	A	*Fusarium moniliformae*
6	*M. circumdata*	A	*B. brongniartii*
7	*O. rhinoceros*	G	*Metarhizium album*
8	*O. rhinoceros*	G	*Metarhizium anisopliae* var *majus*
9	*O. rhinoceros*	G	*Metarhizium* sp.

A : Adult G : Grub

GACATACGCTGCAGGTGTCATGCGGCGACACAACCTGGATCGGGGAAGGCTA
ATGGCCTACGGGCCTATGCTAATCCCGAGTGCAGTCCTGGTAGAGTGATCTTC
CAGGACGCATGTAGAGCGCGGAAAGGTGTGGGTGACTCTTCTGGGTACGCCT
AGAAGGTTGCTTAAGGGACGTGCCAGACCCACGGGAAACCGTGCCGGATGCG
AAGGACCTGCAGTCCAGATCATCCGGGTGGCTCCGAGGCCGGGAGGAAATGC
CCGGAAGAGCCTGGTATACTATACCTACATGGTATTCGAATAGGGAATACAT
ACGCTGGTACCTCCGACCTCCAGCTCGAGCGTATGAACGTGTACTTCA
ACGAGGTGTGTGATGACCACACTGAAATATTTATTATTTATCGTTCCTA
ATCCCATAAGCTACAGGCTTCCGGTAAGAAATACGTGCCTCGTGCCGT
CCTCGTCGATCTCGAACCTGGTACCATGGATGCCGTCCGCGCCGGTCC
TTTCGGACAGCTCTTCCGCCCCGACAACTTCGTTTTCGGACAGTCTGGT
GCCGGAAACAACTGGGCCAAGGGTCACTACACTGAGGGTATGTTTACA
ATGGCACTTCTGAGCTTCAGCTCGAGCGCATGAATGTCTACTTCAACGAGGTT
TGTTGTGCCCTCCCAACGCGTTGCTTGATTTCGTTGTGGATACTGACCGCGAT
TTTCCAAAGGCCTCCGGCAACAAGTATGTTCCTCGCGCCGTCCTCGTCGATCT
TGAGCCCGGTACCATGGATGCTGTCCGTGCCGGTCCCTTCGGTCAGCTCTTCC
GTCCCGACAACTTCGTTTTCGGTCAGTCCG

4.7.2 Isolate 2

Host insect : *B. fulvicorne*

Organism identified : *Metarhizium* sp.

Sequence:

CGGCGGGAGTAACTATGACTCTCTTAAGGTAGCCAAATGCCTCGTCAT
CTAATTAGTGACGCGCATGAATGGATTAACGAGATTCCCACTGTCCCT
ATCTACTATCTAGCGAAACCACAGCCAAGGGAACGGGCTTGGCAGAA
TCAGCGGGGAAAGAAGACCCTGTTGAGCTTGACTCTAGTTTGACATT
GTGAAAAGACATAGGAGGTGTAGAATAGGTGGGAGCTTCGGCGCCGG
TGAAATACCACTACTCCTATTGTTTTTTTACTTATTCAATGAAGCGGGG
CTGGATTTTCGTCCAACTTCTGGTCTTAAGGTCCTTCGCGGGCTGACCC

GGGTTGAAGACATTGTCAGGTGGGGAGTTTGGCTGGGGCGGCACATCT
GTTAAACCATAACGCAGGTGTCCTAAGGGGGGCTCATGGAGAACAGA
AATCTCCAGTAGAACAAAAGGGTAAAAGTCCCCTTGATTTTGATTTTC
AGTGTGAATACAAACCATGAAAGTGTGGCCTATCGATCCTTTAGTCCC
TCGACATTTGAGGCTAGAGGTGCCAGAAAAGTTACCACAGGGATAACT
GGCTTGTGGCGGCCAAGCGTTCATAGCGACGTCGCTTTTTGATCCTTCG
ATGTCCGCTCTTCCTATCATACCGAAGCAGAATTCGGTAAGCGTTGGA
TTGTTCACCCACTAATAGGGAACGTGAGCTGGGTTTAGACCTCTCCGTT
GGTGAACCAGCGGAGGGATCATTACCGAGTTATCCAACTCCCAACCCC
TGTGAATTATACCTTTAATTGTTGCTTCGGCGGGACTTCGCGCCCGCCG
GGGACCCAAACCTTCTGAATTTTTTAATAAGTATCTTCTGAGTGGTTAA
AAAAAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCA
TCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
TTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGTCAGTATTC
TGGCGGGCATGCCTGTTCGAGCGTCATTACGCCCCTCAAGTCCCCTGC
GGACTTGGTGTTGGGGATCGGCGAGGCTGGTTTTCCAGCACAGCCGTC
CCTTAAATTAATTGGCGGTCTCGCCGTGGCCCTCCTCTGCGCAGTAGTA
AAGCACTCGCAACAGGAGCCCGGCGCGGTCCACTGCCGTAAAACCCC
CCAACTTTTTATAGTTGACCTCGAATCAGGTAGGACTACCCGCTGAAC
TTAA

4.7.3 Isolate 3

Host insect : *C. sordidus*

Organism identified : *Paecilomyces* sp.

Sequence:

AGGGTCCATCGCAATGTGTTTTTTTTTTTTTTTCGGTCTCCCTCTCTAC
AGGTAGCCTATATAAATTACGAAGAAGTTCCCCCTCTCCCATAGTCCT
CGATCTCGTAAACTGGTGGCAGTTCCCCTGCTTCTGTCCGGCCAGTCAT
CGCTGCACGCAGCCAACGACACCATGAGGATCAGCGGGTGGCAGGTT
ACCCTGGCCGCCTTGTCAGGCGTCTATGGCCAGGAAGCTTACTCGC

CGCCGAAATACCCCTCACCATGGGCCAACGGAGAGGGTGATTGGGCG
ATAGCGTATCAGAAAGCCGTCCAATTTGTTTCGCAGCTGAACCTGGCG
GAGAAGGTCAATCTGACCACGGGGACTGGCTGGCAACTAGGGCAATG
CGTTGGTGAGACTGGCAGCGTTCCTCGGCTGAACTTTCGTGGCCTCTG
CTTGCAAGATGGCCCGCTGGGCATTCGCTTCGCCGATTACATCTCCGC
ATTCCCGGCCGGTATTAACGTCGGTGCCACCTGGGACCGAAAGCTGTC
GTACCTGCGCGGAAAGGCCATGGGCGAGGAGAGCCGCGACAAGGGTA
TCGATGTCTTACTGGGCCCCTCAGCCGGGCCCCTGGGCAGATTCCCCG
ATGGTGGCCGCAACTGGGAGGGCTATTCACCGGATCCTGAGGGTCCAT
CGCAATGTGTTTTTTTTTTTTTTTTCGGTCTCCCTCTCTACAGGTAGCCT
ATATAAATTACGAAGAAGTTCCCCCTCTCCCATAGTCCTCGATCTCGTA
AACTGGTGGCAGTTCCCCTGCTTCTGTCCGGCCAGTCATCGCTGCACGC
AGCCAACGACACCATGAGGATCAGCGGGTGGCAGGTTACCCTGGCCG
CCTTGTCAGGCGTCTATGGCCAGGAAGCTTACTCGCCGCCGAAATACC
CCTCACCATGGGCCAACGGAGAGGGTGATTGGGCGATAGCGTATCAG
AAAGCCGTCCAATTTGTTTCGCAGCTGAACCTGGCGGAGAAGGTCAAT
CTGACCACGGGGACTGGCTGGCAACTAGGGCAATGCGTTGGTGAGACT
GGCAGCGTTCCTCGGCTGAACTTTCGTGGCCTCTGCTTGCAAGATGGC
CCGCTGGGCATTCGCTTCGCCGATTACATCTCCGCATTCCCGGCCGGTA
TTAACGTCGGTGCCACCTGGGACCGAAAGCTGTCGTACCTGCGCGGAA
AGGCCATGGGCGAGGAGAGCCGCGACAAGGGTATCGATGTCTTACTG
GGCCCCTCAGCCGGGCCCCTGGGCAGATTCCCCGATGGTGGCCGCAAC
TGGGAGGGCTATTCACCGGATCCTG

4.7.4 Isolate 4

Host insect : *C. formicarius*

Organism identified : *Beauveria* sp.

Sequence:

AGGGATCATTACCGAGTTTTCAACTCCCTAACCCTTCTGTGAACCTACC
TATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACTGGACCA
GCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCTTCTGAAT
ACGCCGCAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCT
CTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
TGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACATTGCG
CCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT
CGACCTCCCCTGGGGGAGGTCGGCGTTGGGGACCGGCAGCACACCGC
CGGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGCGACCTCTGCGTAGT
AATACAGCTCGCACCGGAACCCCGACGCGGCCACGCCGTAAAACACC
CAACTTCTGAACGTGACCTCGAATAGACTCCATAATTTGTTGTTTCTTG
TTGGTGGGGATAGTCGGGTCCTGTTGGCAGGACTACGCCGGCTAGTCG
ACATGACATACGCTGCAGGTGTCATGCGGCGACACAACCTGGATCGGG
GAAGGCTAATGGCCTACGGGCCTATGCTAATCCCGAGTGCAGTCCTGG
TAGAGTGATCTTCCAGGACGCATGTAGAGCGCGGAAAGGTGTGGGTG
ACTCTTCTGGGTACGCCTAGAAGGTTGCTTAAGGGACGTGCCAGACCC
ACGGGAAACCGTGCCGGATGCGAAGGACCTGCAGTCCAGATCATCCG
GGTGGCTCCGAGGCCGGGAGGAAATGCCCGGAAGAGCCTGGTATACT
ATACCTACATGGTATTCGAATAGGGAA

4.7.5 Isolate 5

Host insect : *H. vigintioctopunctata*

Organism identified : *Fusarium moniliformae*

Sequence:

AGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCA
ATTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCC
GCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAA
CCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCAT

CGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAAT
TCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCT
GGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCCCGGGT
TTGGTGTTGGGGATCGGCGAGCCCTTGCGGCAAGCCGGCCCCGAAATC
TAGTGGCGGTCTCGCTGCAGCTTCCATTGCGTAGTAGTAAAACCCTCG
CAACTGGTACGCGGCGCGGCCAAGCCGTTAAACCCCCAACTTCTGAAT
GTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAACATTTGCGA
CGTACCAACACCCCCATCGGACGAGATGGTAAGCTGGCCAAACCTCGT
CAGCTACACAACACCCATTGGGGTTTGGTGTGTCCTGCAGAAACGCCT
GAGGGACAGGCTTGTGGTCTGGTCAAGAACCTGTCTCTGATGTGTTAC
GTGAGTGTGGGCTCTCCTGCTGATCCTCTGATTGACTTCATGATCCACA
GAGGTATGGAAGTGGTTGAGGAGTATGAGCCAACAAGATACCCACAC
GCTACCAAGATTTTCGTCAACGGTAGCTGGGTTGGTGTTCACTCTGACC
CCAAGCATCTTGTGCACCAAGTTTTGTCCACCCGACGAAAGAATGTCG
TTCAATTCGAAGTGTCACTTGTTCGTGATATTCGAGACCGAGAATTCA
AGATCTTCTCTGATGCAGGCAGAGTCATGAGACCGGTCTTTACAGTAC
AGCAGGAGGATGACGACGAGACTGGTGTTCAGAAGGGACAGCTTATA
CTGACCAAGGAGCTGGTAACCAAGCTCGCCCAAGAGCAGGCGGAGCC
ATCTGATGATCCATCAGAGAAGCTCGGCTGGGAGGGTCTTGTTCGCGC
TGGAGTTATCGAGTATCTCGATGCCGAGGAAGAAGAAACGGCCATGA
TCTGCATGACGCCGGAAGATCTTGAACTTTACCGCGAGCAAAAGAATG
ACGAGGCGACCCTCACAGAGGAAGAGAGGCGGGCTAAGCAAGAGGC
GGAGAAGAGAGAACAAGAGGAGGAACGCAACAAGAGATTGAAGA
CAAAGGTCAATCCTACGACTCATGTGTACACACATTGTGAGATTCATC
CCAGTATGATTCTTGGTATCTGTGCCAGTATCATTCCCTTCCCCGATCA
CAACCAGGTATGTCAGGACGCTTAA

4.7.6 Isolate 6

Host insect : *M. circumdata*

Organism identified : *B.brongniartii*

Sequence:

CCTGCGGAGGGATCATTACCGAGTTTTCAACTCCCTAACCCTTCTGTGA
ACCTACCTATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGAC
TGGACCAGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCT
TCTGAATACGCCGCAAGGCAAAACAAATGAATCAAAACTTTCAACAA
CGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCA
CATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTT
CAACCCTCGACCTCCCCTTGGGGAGGTCGGCGTTGGGGACCGGCAGCA
CACCGCCGGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGCGACCTCTG
CGTAGTAATACAGCTCGCACCGGAACC CCGACGCGGC CACGCCGTAA
AACACCCAACTTCTGAACGTTGACCTCGAATCAGGTAGGACTACCCGC
TGAACTTAAGCATATCCCTGCGGAGGGATCATTACCGAGTTTTCAACT
CCCTAACCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCC
CAGCCCGGACGCGGACTGGACCAGCGGCCCGCCGGGGACCTCAAACT
CTTGTATTCCAGCATCTTCTGAATACGCCGCAAGGCAAAACAAATGAA
TCAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGAAGAAC
GCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATCCAGTGAATCAT
CGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGC
CTGTTCGAGCGTCATTTCAACCCTCGACCTCCCCTTGGGGAGGTCGGC
GTTGGGGACCGGCAGCACACCGCCGGCCCTGAAATGGAGTGGCGGCC
CGTCCGCGGCGACCTCTGCGTAGTAATACAGCTCGCACCGGAACCCCG
ACGCGGCACGCCGTAAAACACCCAACTTCTGAACGTTGACCTCGAATC
AGGTAGGACTACCCGCTGAACTTAAGCATATC

4.7.7 Isolate 7

Host insect : *O. rhinoceros*

Organism identified : *Metarhizium album*

Sequence:

CGAGTTACTACAACTCCCAAACCCCCTTGTGAACGTATACCTTTCCAGT
TGCTTCGGCGGGTATAGCCCCGGGGTCAGGTTCGCAAGAGCCTGTCCG
GAACCAGGCGCCTGCCGGGGGACCAAAACTCTTGTATTTCTGTACGAT
AAGGAATGTCTGAGTGGTTTATAGAAGAAAATGAATCAAAACTTTCAA
CAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC
GCACATTGCGCCCGCTAGTATTCTAGCGGGCATGCCTGTTCGAGCGTC
ATTTCAACCCTCAAGCCCCGGCGGTTTGGTGTTGGGGGCCGGAATGGT
TGTTGGGGGCGATCTCTTCGTCCCGCGCGCCGCCCCCGAAATAAAT
TGGCGGCCTCGCCGCGGCTCCTCTGCGTAGTAACATGTTGCCCTTCC
AACAGGAGCCGGCGCGGCACTGCCGTAAACCACCACTTTTTCACAAGT
TGACCCAATCAGGAAGAATCCCCTACTTAGATTCGAGTTACTACAACT
CCCAAACCCCCTTGTGAACGTATACCTTTCCAGTTGCTTCGGCGGGTAT
AGCCCCGGGGTCAGGTTCGCAAGAGCCTGTCCGGAACCAGGCGCCTGC
CGGGGGACCAAAACTCTTGTATTTCTGTACGATAAGGAATGTCTGAGT
GGTTTATAGAAGAAAATGAATCAAAACTTTCAACAACGGATCTCTTGG
TTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAAT
TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC
TAGTATTCTAGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGC
CCCGGCGGTTTGGTGTTGGGGGCCGGAATGGTTGTTGGGGGCGATCTC
TTCGTCCCGCGCGCGCCGCCCCCGAAATAAATTGGCGGCCTCGCCGCG
GCTCCTCTGCGTAGTAACATGTTGCCCTTCCAACAGGAGCCGGCGCGG
CACTGCCGTAAACCACCACTTTTTCACAAGTTGACCCAATCAGGAAGA
ATCCC CTACTTAGAT T

4.7.8 Isolate 8

Host insect : *O. rhinoceros*

Organism identified : *Metarhizium anisopliae* var *majus*

Sequence:

ATGCATCTGTCTGCTCTTCTCACTCTTCTCCCAGCCGTTCTGGCTGCCCC
TGCCACTATTGGCCGGCGCGCTGAGCCAGCTCCTCTCTTCACTCCTCAG
GCTGAGAGCATCATTGCCGACAAGTATATTGTCAAGTTCAAGGATGAT
ATTGCCCGTATCGCTACCGATGATACGGTGAGCGCTCTTACCTCCAAA
GCCGACTTCGTTTACGAGCACGCCTTCCATGGGTTTGCAGGCTCCCTCA
CCAAGGAGGAGCTGAAGATGCTTCGTGAGCACCCCGGTGTAAGCAC
CCCCTCCCACTTACCTAGGTAGTCAAAGGAGACATGTAGTTGTTTGTTC
CTGACCCGCTACGCCATAGGTTGATTTCATTGAGAAGGACGCTGTGAT
GCGTATCAGCGGCCTCACTGAGCAGAGCGGTGCTCCCTGGGGTCTTGG
GCGCATCTCTCACCGCAATAGGGGAAGCACCACCTATCGCTACGATGA
TAGTGCTGGTGAGGGTACTTGCGTATATATCATTGACACTGGTATTG
AGGCCTCCCACCCCGTAAGTTGTGCCGCCAAAACTCCATAGGGCGGAG
TAGGAAATTTAACAATATCATCCAGGAGTTTGAGGGTCGCGCCACTTT
TCTTAGGAGCTTCATCAGCGGTCAAGAAACTGATGGCCACGGCCATGG
GACTCA CTGCGCTGGT ACCATTGGTA GCAAAAGCTA CGGTGTTGCC
AAAAAGGCTAAGCTCTATGGTGTCAAGGTTCTTGACAACCAGGGCAGT
GGTTCCTACTCCGGTATCATCAGTGGCATGGACTACGTTGCCAGTGAC
TCCAAGACCCGCGGCTGCCCCAAAGGCGCCATTGCTTCCATGAGCCTG
GGAGGTGGCTACTCGGCGTCCGTCAACCAAGGTGCTGCTGCTTTGGTG
AATTCGGGTGTCTTCCTTGCCGTCGCCG CTGGCAACGA TAACCGGGAT
GCCCAGAACACCTCTCCCGCTTCCGAGCCTTCTGCCTGCACTGTTGGTG
CCACTGATTCAAGTGACAGACGATCTTCCTTCTCCAACTTCGGCAGAG
TTGTCGATATTTTCGCTCCTGGTACCGGTGTTCTTTCCACCTGGATTGG
TGGCAGCACTGTAAGTATTGTACCTACCTCGATAAGCTTAGAGACAGG
CTTTTG CTTCAGAACC AGCTCAAAAG GTTTAGAACA CCATCTCTGG
TACCTCCATGGCTACTCCCCATATTGCCGGTCTGGCTGCCTACCTCAGT
GCGCTCCAAGGCAAGACTACCCCTGCCGCTCTTTGCAAGAAGATCCAG
GACACTGCTACCAAGAACGCGCTCACCGGTGTTCCCTCTGGCACTGTC
AACTA CCTTGCCTAC AACGGCAACG GTGCCTAA

4.7.9 Isolate 9

Host insect : *O. rhinoceros*

Organism identified : *Metarhizium* sp.

Sequence:

AGGGATCATTACCGAGTTATCCAACTCCCAACCCCTGTGAATTATACC
TTTAATTGTTGCTTCGGCGGGACTTCGCGCCCGCCGGGGACCCAAACC
TTCTGAATTTTTTAATAAGTATCTTCTGAGTGGTTAAAAAAAAAATGAT
CAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACG
CAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC
GAATCTTTGAACGCACATTGCGCCCGTCAGTATTCTGGCGGGCATGCC
TGTTCGAGCGTCATTACGCCCCTCAAGTCCCCTGTGGACTTGGTGTTGG
GGATCGGCGAGGCTGGTTTTCCAGCACAGCCGTCCCTTAAATTAATTG
GCGGTCTCGCCGTGGCCCTCCTCTGCGCAGTAGTAAAGCACTCGCAAC
AGGAGCCCGGCGCGGTCCACTGCCGTAAAACCCCCCAACTTTTTATAG
TTGACCTCGAATCAGGTAGGACTACCCGCTGAACTTATCTCCGTTGGT
GAACCAGCGGAGGGATCATTACCGAGTTATCCAACTCCCAACCCCTGT
GAATTATACCTTTAATTGTTGCTTCGGCGGGACTTCGCGCCCGCCGGG
GACCCAAACCTTCTGAATTTTTTAATAAGTATCTTCTGAGTGGTTAAAA
AAAAAATGAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATC
GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT
TCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGTCAGTATTCT
GGCGGGCATGCCTGTTCGAGCGTCATTACGCCCCTCAAGTCCCCTGCG
GACTTGGTGTTGGGGATCGGCGAGGCTGGTTTTCCAGCACAGCCGTCC
CTTAAATTAATTGGCGGTCTCGCCGTGGCCCTCCTCTGCGCAGTAGTAA
AGCACTCGCAACAGGAGCCCGGCGCGGTCCACTGCCGTAAAACCCC
CCAACTTTTTATAGTTGACCTCGAATCAGGTAGGACTACCCGCTGAAC
TTAA

Discussion

5. DISCUSSION

Many beetles and weevils in the order Coleoptera are pests of important crops in Kerala as well as in other parts of the world. Some of the ruinous pests are banana rhizome weevil, *Cosmopolites sordidus* (Germar), cardamom root grub, *Basilepta fulvicorne* (Jacoby); coconut rhinoceros beetle, *Oryctes rhinoceros* L.; epilachna beetle, *Henosepilachna vigintioctopunctata* F.; pepper pollu beetle, *Lanka ramakrishnai* Prathapan and Viraktamath; red palm weevil, *Rhynchophorus ferrugineus* F.; red pumpkin beetle, *Aulacophora foveicollis* Lucas; sweet potato weevil, *Cylas formicarius* F. and sweet potato tortoise beetle, *Metriona circumdata* H. These pests take a heavy toll of the crop yield, 10 to 25, 10 to 50, 10 to 70 and 6 to 40 per cent loss were reported for red palm weevil, rhinoceros beetle, pepper pollu beetle and cardamom root grub and respectively (Murphy and Briscoe, 1999; Sathiamma *et al.*, 2001; Krishanamoorthy *et al.*, 2010; Varadarasan, 2013).

As with coevolution in insects and their host plants, pest problems in crops and their management strategies also coevolved. With the introduction of high yielding varieties and intensification of agriculture, pest problems also aggravated and to contain them miraculous pesticides were developed. As desired, spectacular control was achieved with these hard chemicals but today the concerns over the problems caused by these insecticides such as pest resurgence, secondary pest outbreaks, pesticide resistance and toxic residues in harvested produce are widely seen. So a paradigm shift in pest management strategy focusing on sustainable protection of crops with environment friendly tools such as botanicals, parasitoids, predators and microbial pesticides is gaining momentum all over the world.

Entomopathogenic fungi are now considered as an important group among the diverse groups of microorganisms for pest management. The fungal pathogens *viz. Beauveria bassiana* (Balsamo) Vuillimen and *Metarhizium anisopliae* (Metschnikoff) Sorokin are known to effectively suppress

pest populations in the field (Feng *et al.*, 1994; Ambethgar, 1997; Hiromori and Nishigaki, 2001 ; 1998; Singh, 2001).

Prior to translation of new strategies in pest management, detailed investigations on various aspects of the organism and their suitability to the different agroecosystems are absolutely essential to avoid pitfalls. Inglis *et al.*, (2001) opined that the factors responsible for the initiation and development of epizootic infection of Hyphomycetous fungi in insect populations are extremely complex, involving interactions among the pathogen(s), insect host, environment and time. An understanding of this interaction is important to achieve efficacious control of insect pests.

Moreover, the knowledge on the existence of isolates in entomopathogenic fungi *B. bassiana* and *M. anisopliae* and variations in their infectivity to different crop pests, lack of information on pathogenicity to the important coleopteran pests infesting crops of economic importance in Kerala, virulence of the pathogens and the bottlenecks in mass production and formulations of the fungi, instigated to undertake the present investigation entitled "Evaluation of entomopathogenic fungi for the management of major coleopteran pests and characterisation of pesticide tolerant strains" with the objectives: assessment of the pathogenicity of the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* to nine important coleopteran pests infesting crops in Kerala, determination of the LC_{50}, LC_{90} and LT_{50} of the pathogens against the pests acquiring infection through bioassay, identification of cost effective substrates for the mass production of *B. bassiana* and *M. anisopliae*, development of formulations of the fungi, evaluation of the efficacy of the fungi against two selected coleopteran pests under field conditions, assessment of the compatibility of fungi with pesticides and development and characterisation of pesticide tolerant strains of *B. bassiana* and *M. anisopliae*.

Any pathogen that is intended to be employed in biological control programmes needs to be evaluated for their pathogenicity and virulence initially. According to Alves (1998) pathogenicity is a genetic characteristic of an organism

which helps the organism to penetrate and cause disease whereas virulence is the degree of pathogenicity of the isolates to a specific host.

In the present research work, one isolate of *B. bassiana*, PDBC Bb 5 and one isolate of *M. anisopliae*, PDBC Ma 4 obtained from Project Directorate of Biological Control (PDBC), now known as National Bureau of Agriculturally Important Insects, Bengaluru, were evaluated for their pathogenicity and virulence against nine important coleopteran pests infesting crops in Kerala. Initial trials conducted by applying spore suspensions from fourteen day old cultures showed that both the fungi were pathogenic to all the nine insects tested *viz. A. foveicollis, B. fulvicorne, C. formicarius, C. sordidus, H. vigintioctopunctata, L. ramakrishnai, M. circumdata, O. rhinoceros* and *R. ferrugineus*. The grubs as well as adults of all these insects were susceptible to infection by these fungi. The pathogenicity to the pupal stages of all these insects except to that of *A. foveicollis* and *L. ramakrishnai* were evaluated. The pupa of *R. ferrugineus* treated with *M. anisopliae* alone was not infected while all other pupae treated with *B. bassiana* and *M. anisopliae* were infected. The pathogenicity to eggs were evaluated only for three insects *viz. H. vigintioctopunctata, M. circumdata* and *O. rhinoceros*. The egg stages of all these insects were also susceptible to the infection of the fungi.

The infectivity of *B. bassiana* and *M. anisopliae* to *A. foveicollis, L. ramakrishnai* and *M. circumdata* has not been documented earlier and the present reports are new. However, there are publications on the pathogenicity of *B. bassiana* to *B. fulvicorne* (Varadarasan, 2013), *C. sordidus* (Diaz *et al.*, 1986; Akello *et al.*, 2008; Fancelli *et al.*, 2013), *C. formicarius* (Pena *et al.*, 1995; Ondiaka, 2008), *H. vigintioctopuncta* (Jiji *et al.*, 2008; Swaminathan *et al.*, 2010), *O. rhinoceros* (Hochberg and Waage,1991; Moslim *et al.*, 2006; Latifian and Rad, 2012) and other coleopteran pests (Nehru and Jayarathnam, 1993; Rosa *et al.*, 1997; Todorova *et al.*, 2000; Haraprasad *et al.*, 2001).

The infectivity of *M. anisopliae* to *O. rhinoceros* (Moslim *et al.*, 2006) and other coleopterans (Anitha, 2004; Beegum and Anitha, 2008; Karthikeyan and Jacob, 2010) has also been documented earlier. Pathogenicity of *B. bassiana* and

M. anisopliae to other groups of pests are also evident from the earlier reports in Kerala (Simon, 2002; Meena, 2007; Sudharma and Archana 2009; Amala 2010) elsewhere in India (Singh, 2001) and abroad (Loc and Chi, 2007; Rodriguez *et al.*, 2009 a and 2009 b; Boudjelida and Soltani, 2011).

It is noteworthy that *B. bassiana* and *M. anisopliae* infected all the stages including the eggs of *H. vigintioctopunctata*, *M. circumdata* and *O. rhinoceros*. A tool that can achieve pest regulation even before the pest attaining the injurious stage should be preferred for inclusion in management programmes. In this context, *B. bassiana* and *M. anisopliae* seems ideal candidates. The present findings and earlier publications indicate the broad spectrum infectivity of *B. bassiana* and *M. anisopliae* and the feasibility of employing them for management of coleopteran pests.

The symptoms displayed in the test insects due to *B. bassiana* infection were all the same except minor variations but the period taken for symptom development and death varied with the insects. The infected adults of the test insects were actively flying and moving around for two to four days. As pathogenesis advanced food uptake and movement of infected insects reduced considerably. On losing their flashy sheen the adults were hideous and the size of the infected grubs was reduced. Dull beige spots developed on the intersegmental regions of the body of the grubs later that coalesced to form enlarged patches. The cadavers, both adults and grubs initially were soft and later hardened, ultimately white fungal mycelia covered all over the body of the dead insects. The infected pupae of *B. fulvicorne, C. sordidus* and *H. vigintioctopunctata* became dark and brittle and white mycelial growth appeared on the body within four days The plates (1 to 13) given in results recalls the adage that "A picture is worth a thousand words".

The infected grub of *B. fulvicorne* and *O. rhinoceros* remained on the surface of soil / cowdung without burrowing deep into the soil / breeding media. A unique behaviour noticed in *B. bassiana* infected adults of *B. fulvicorne* is that they remained in an upside down position for most of the time on the surface of top most leaves used for feeding the insects (Plate 14 a). The infected adults of

M. circumdata also exhibited a similar behaviour of resting on the top leaves (Plate 14 b). Host altered behaviour by some fungi such as summit disease has been demonstrated in diseased insects, in which the infected insects exhibit climbing behaviour (Roy *et al.*, 2006). He further stated that there are considerably fewer examples with hypocrealean infected insects. According to Inglis *et al.* (2001) the behaviour of an insect can influence epizootic development and can affect the dispersal of an entomopathogen, for example the insects infected with entomophthoralean fungi often climb to the top of plants just prior to death (summit disease syndrome) where they firmly clasp the plant. Such adaptations ensure that spores contact potential hosts within and beneath the plant canopy, and that such behaviour has not been observed for insects infected with Hyphomycetous fungi. In the light of the comments of Inglis *et al.* (2001), the present observations that the adults of *B. fulvicorne* and *M. circumdata* climb to the top of leaves on acquiring infection of *B. bassiana* besides being a new observation of summit disease syndrome in Hyphomycetous fungi adds to the suitability of the fungus in the management of *B. fulvicorne* and *M. circumdata.*

Adult *C. formicarius* infected by *B. bassiana* was found to die within the shortest period of four days. The adults *of A. foveicollis, B. fulvicorne, H. vigintioctopunctata, L. ramakrishnai* and *M. circumdata* were dead on the sixth day whereas slightly longer duration of seven days were required for the death of adult *C. sordidus.* Much longer period of fifteen and eighteen days were necessary to cause mortality in adults of *O. rhinoceros* and *R. ferrugineus,* respectively when sprayed with spore suspension from fourteen day old culture.

As in the case of adults, the mortality in grubs of *C. formicarius* was recorded in the shortest period of three days, in other insects excepting *O. rhinoceros* and *R. ferrugineus* the mortality occurred within four to five days. The period required in the case of grubs of *O. rhinoceros* was eleven days but only six days were enough to bring mortality of *R. ferrugineus* when sprayed with spore suspension from fourteen day old culture.

Upon acquiring infection of *M. anisopliae*, the symptoms exhibited by all the adult coleopterans as well as the immature stages were more or less similar to

that exhibited in the case of *B. bassiana* infection. Sluggishness, reduced feeding, loss of colour and development of darkened spots and patches on the body were seen. Nonetheless, the colour of the mycelia produced in *M. anisopliae* infection was initially white, which changed to olive green and ultimately dark green. It was seen that the *B. bassiana* isolate Bb 5 produced symptoms and brought about death of test insects earlier than *M. anisopliae* in all the test insects except *O. rhinoceros*.

Appearance of dark spots on the integument and loss of motion of *M. anisopliae* infected insects were noticed in earlier studies also. (Leucona *et al.*, 1986 and 2001; Alves, 1998). According to Hidalgo (2001) the dark brown stains on the integument and brown or blackish granules in the body of the larva is due to the accumulation of chitin around the points of penetration of the pathogen and the encapsulation of the fungus on the cuticle. Melanisation of the cuticle at the infection site has been stated as a defense mechanism in insect which prevents the penetration and growth of the fungus (Sandhu *et al.*, 2012). The statement of Inglis *et al.* (2001) that even if germination of spore occurs, fungus may not penetrate the cuticle of insects due to factors such as inappropriate environment or the presence of inhibitory factors such as melanin or fatty acids underlines the statement of Hidalgo (2001). Eventhough melanisation occurred in the present study also, the isolate Ma 4 could bring about mortality in all the test insects except pupa of *R. feruugineus*. A pathogen that is able to penetrate the cuticle and reach the haemocoel for the production of hyphal bodies and that can resist the defense mechanisms of the insects alone are able to cause death. Hence, it is inferred that Ma 4 is a virulent isolate as it could resist the melanistion and could bring about death of the insects.

B. bassiana and *M. anisopliae* were reported to have wide host ranges although there is considerable genetic diversity within species and some clades show a high degree of specificity, as exemplified by *M. anisopliae* var. *acridum* that is effective only against acridids (Driver *et al.*, 2000). In the present investigation it was seen that both the isolates, Bb 5 and Ma 4 were not specific in their activity, but had a relatively broad activity as they were able to infect all the

nine coleopterans tested belonging to five different families *viz.* Apionidae, Coccinellidae, Chrysomelidae, Curculionidae and Scarabaeidae.

An entomopathogenic fungi unless virulent will be sidelined in pest management programmes. Henceforth, the isolates Bb 5 and Ma 4 once identified to produce symptoms in the coleopteran test insects, these fungi were further evaluated for their virulence. Virulence is the quantitative amount of disease that a pathogen can incite in a group of insects. Since a highly virulent pathogen will require only few propagules to incite disease, selection of virulent genotypes has obvious consequences for efficacious microbial control of insects (Inglis *et al.*, 2001).

In the present investigation, the virulence of *B. bassiana,* Bb 5 and *M. anisopliae,* Ma 4 was assessed by spraying spore concentrations of the respective fungi @ 10^8 spores ml^{-1} on adults and immature stages of all the nine test insects. Seven days after treatment with *B. bassiana,* in the case of adults the highest mortality of 35.52 per cent was seen for *H. vigintioctopunctata* which was on par with the mortality observed for *C. formicarius*. Significantly lower virulence was shown to the adults of other insects. 28.84 per cent mortality was recorded for *L. ramakrishnai* and it was followed by *A. foveicollis* and *M. circumdata* each with 24.37 per cent mortality. *B. fulvicorne* and *C. sordidus* recorded 17.66 and 13.33 per cent mortality respectively. At the dose tested Bb 5 did not produce any infection on *R. ferrugineus* and *O. rhinoceros*. It was seen from the results that the virulence of the isolate Bb 5 to the different coleopterans tested varied (Fig 1 and 2). This observations is in tune with that of Mc Coy *et al.,* (1988) and Ferron *et al.,* (1991) that *B. bassiana* has a wide host range but there are differences in host specificity and virulence.

Substantial increase in mortality was seen on the fourteenth day after treatment. The virulence, assessed from the mortality rates, followed more or less the same trend as that noted on the seventh day after treatment except for *L. ramakrishnai,* which recorded the maximum mortality (78.15) but it was on par with that for *C. formicarius* and *H. vigintioctopunctata.* In other insects, the mortality rates varied from 33.22 to 78.15 per cent. As observed on the seventh

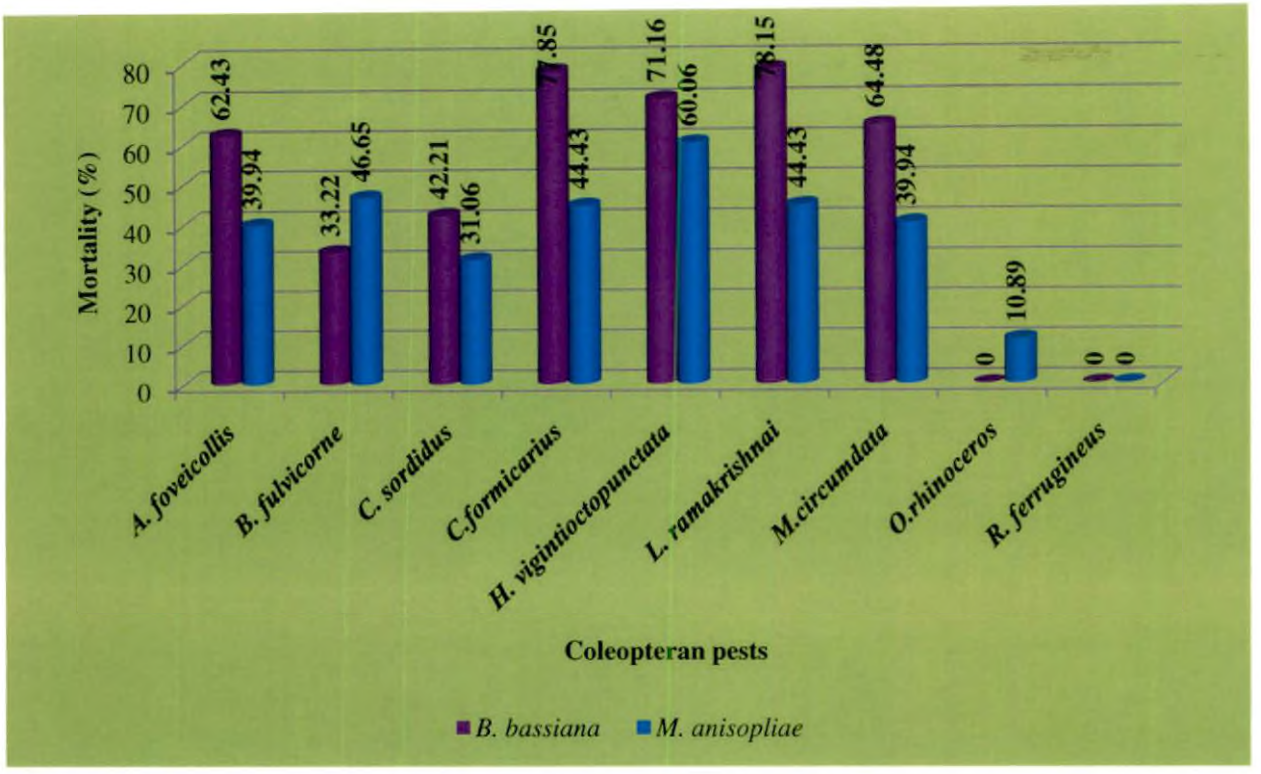


Fig. 1. Mortality of adult of coleopterans when treated with *B. bassiana* and *M. anisopliae* at 14 DAT

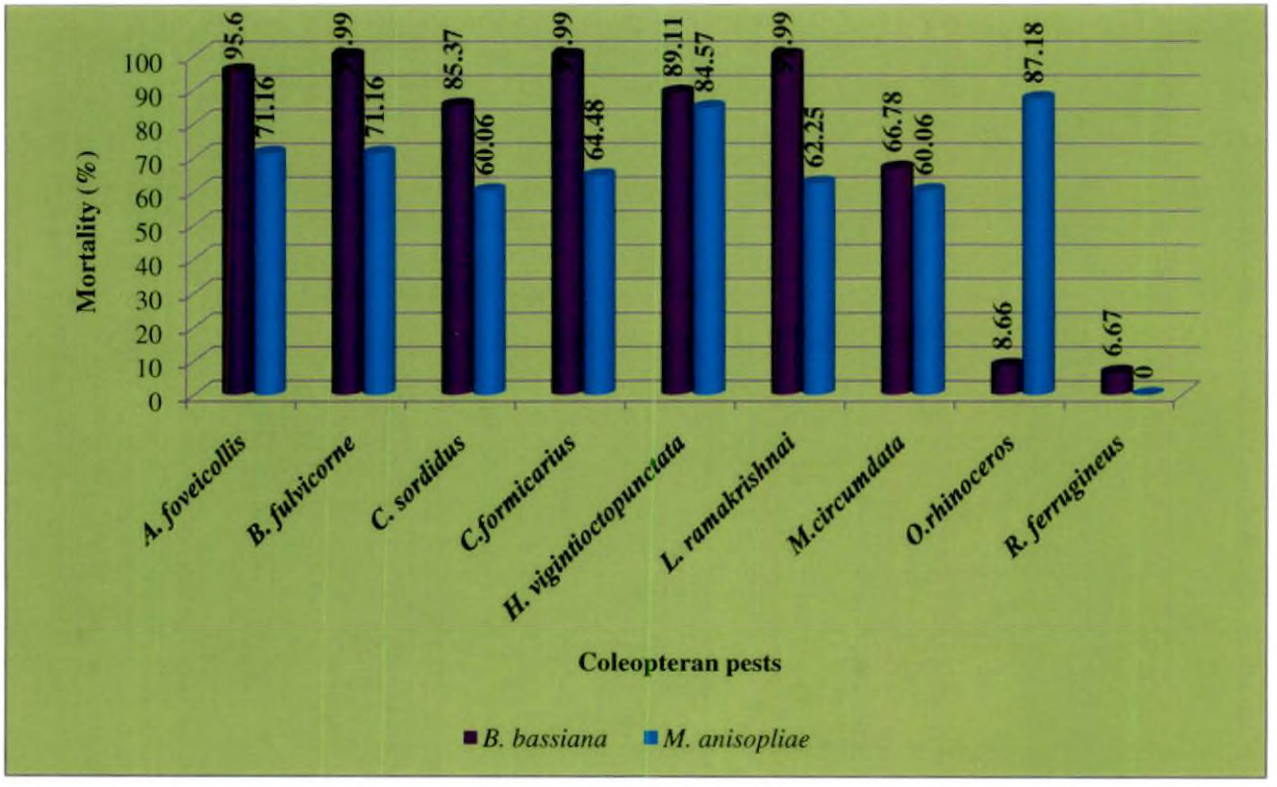


Fig. 2. Mortality of grubs of coleopterans when treated with *B. bassiana* and *M. anisopliae* at 14 DAT

* DAT – Days after treatment

day, *B. bassiana* did not produce any infection on *R. ferrugineus* and *O. rhinoceros* at the concentration of 10^8 spores ml^{-1}.

Unlike the adults, fairly good mortality rates were observed seven days after treatment in the grubs treated with *B. bassiana.* The fungus was most virulent to *B. fulvicorne* in which 84.75 per cent mortality was recorded and it was followed by *L. ramakrishnai*, *A. foveicollis*, *H. vigintioctopunctata* and *C. formicarius.* The mortality in these grubs were 80.29, 66.78, 64.48 and 57.79 respectively. The fungus though infected the grubs of *R. ferrugineus* and *O. rhinoceros* the mortality rate was very low (0.76) at the concentration of 10^8 spores ml^{-1}. There was considerable increase in mortality on the fourteenth day with 99.99 per cent mortality to *B. fulvicorne, C. formicarius* and *L. ramakrishnai.* In others, the mortality ranged from 66.78 to 96.6 per cent. According to Inglis *et al.* (2001) one of the host factors that has been demonstrated to play an important role in the success of entomopathogen is the developmental stage of an insect. Not all stages in an insect's life cycle are equally susceptible to infection by entomopathogenic Hyphomycetous. In some situations immature stages are more susceptible to infection. The higher mortality observed for the grubs of the test insects compared to the adults substantiates the views of Inglis *et al.* (2001). The time taken to achieve such high mortality in the grubs except to that for *O. rhinoceros* and *R. ferrugineuus* was also short, this further increases the quality of the Bb 5 isolate of *B. bassiana.*

At the comparable dose of 10^8 spores ml^{-1} Ma 4 proved inferior in its ability to infect the adults of all the test insects except that of *O. rhinoceros* and *B. fulvicorne* in both cases observations taken at 7 DAT as well as at14 DAT. The cumulative percentage of mortality on the seventh day ranged from 0.76 in *O. rhinoceros* to 22.15 in *B. fulvicorne*. The mortality further increased on the fourteenth day and the mortality percentage ranged from 10.89 per cent in *O. rhinoceros* to 60.06 in *H. vigintioctopunctata*. At the concentration of 10^8 spores ml^{-1} Ma 4 did not produce infection in *R. ferrugineus* adults.

Except in *O. rhinoceros* a similar trend in infection of *M. anisopliae* was noted in the grubs of the test insects also, Bb 5 was superior to Ma 4. At 7 DAT,

Ma 4 caused mortality of 66.78 per cent that later increased to 87.18 on the fourteenth day after treatment in the grubs of *O. rhinoceros*. In all other insects relatively less mortality was recorded. Ma 4 did not cause infection in in the grubs of *R. ferrugineus* at this dose as in the case of adult even after two weeks. As noted earlier, insect specificity was noted and Ma 4 was identified as a virulent pathogen of *O. rhinoceros*.

The infectivity to eggs were evaluated only for three insects *viz. H. vigintioctopunctata, M. circumdata* and *O. rhinoceros*. The egg stages of all these three insects were also susceptible to infection of *B. bassiana* and *M. anisopliae* @ 10^8 spores ml^{-1} (Fig. 3). The mortality in eggs was higher in *B. bassiana* infection. It ranged from 95.6 to 100 per cent in *B. bassiana* infection where as it ranged from 42.00 to 100 per cent in *M. anisopliae* infection.

Pupal stages of all the insects infected by *B. bassiana* succumbed to the infection earlier than the pupal stages infected by *M. anisopliae* (Fig. 4). The superior performance of *B. bassiana* was evident from the shorter duration to acquire infection and higher percentage mortality of 44.43 to 86.67 per cent compared to 24.37 to 55.57 per cent in *M. anisopliae* infection.

Further, bioassay of *B. bassiana* and *M. anisopliae* was conducted against the adults and grubs of all the nine test insects using varying concentrations, prepared from fourteen day old culture of the fungi grown in potato dextrose broth. The results revealed that to bring about 50 per cent mortality in adult *A. foveicollis,* at the highest spore concentration of 2.9×10^8 spores ml^{-1} tested, a period of 9.494 days was required. From the probit analysis, it was seen that to achieve 50 per cent mortality in a shorter period of five days a higher concentration of 5.27×10^8 spores ml^{-1} was essential and for the corresponding day the LC_{90} value was 10.22×10^8 spores ml^{-1}. With respect to the grubs, the LC_{50} value on the fifth day was 2.15×10^7 spores ml^{-1} and that was a lower concentration when compared to the concentration required for the adult *A. foveicollis*.

Death of *B. fulvicorne* treated with *B. bassiana* was observed from the seventh day onwards. It was found from the probit analysis, that a spore

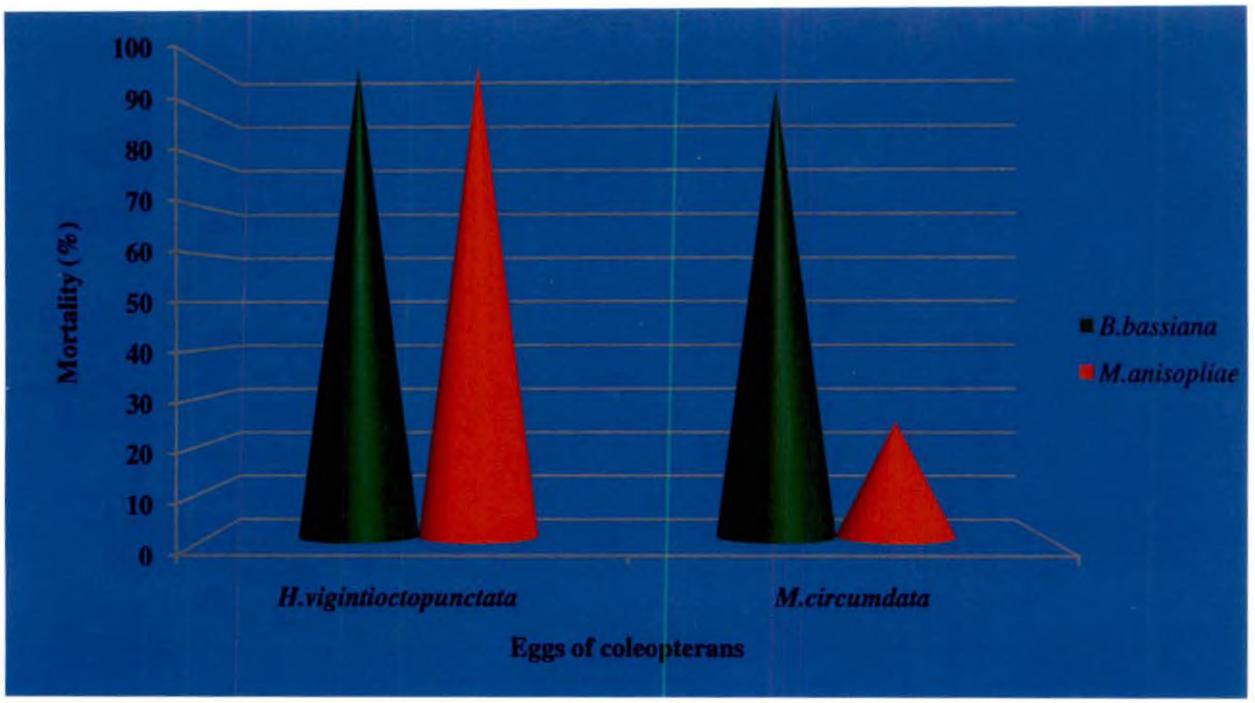


Fig. 3. Mortality of eggs treated with *B. bassiana* and *M. anisopliae* at 14 DAT

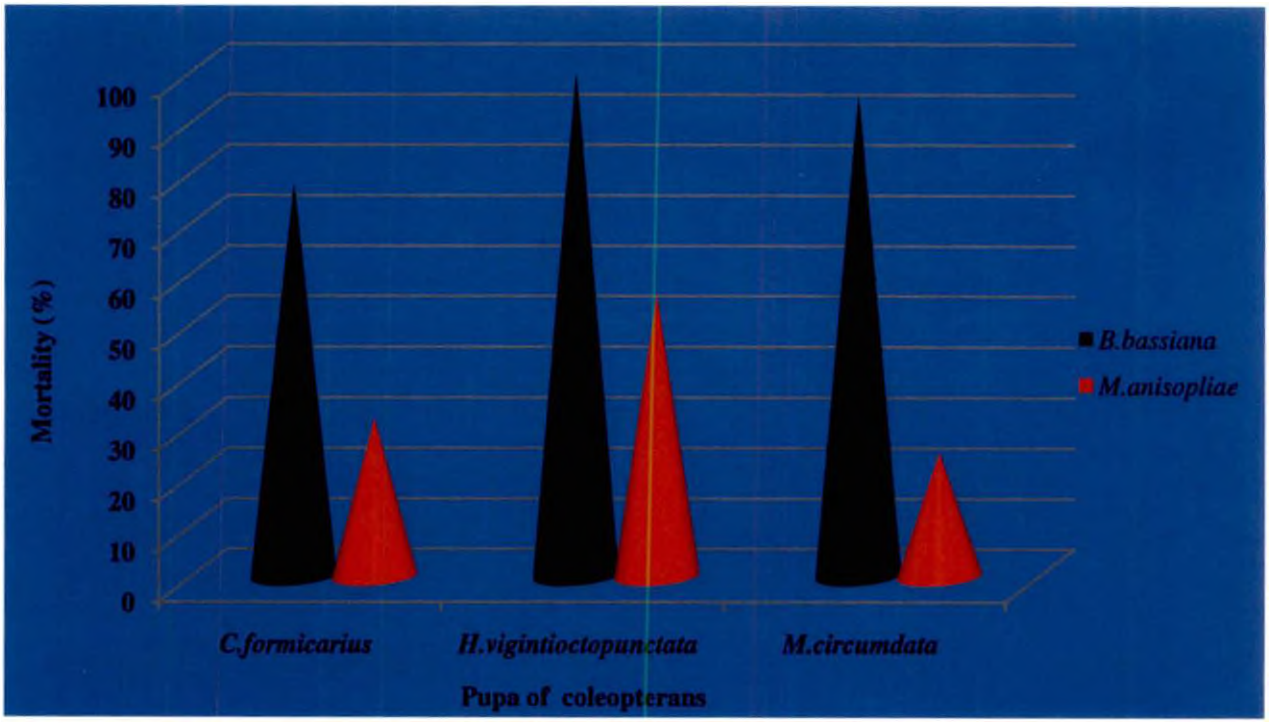


Fig. 4. Mortality of pupa treated with *B. bassiana* and *M. anisopliae* at 14 DAT

* DAT – Days after treatment

concentration of 6.53×10^{8} spores ml^{-1} was required to cause fifty per cent mortality of the adults and a concentration of 12.65×10^{8} spores ml^{-1} was required to bring 90 per cent mortality. The grubs of *B. fulvicorne* were highly susceptible to the infection of *B. bassiana* as it was evident from the mortality observed from the third day after treatment and the lower dose of 1.87×10^{6} spores ml^{-1} required for the grubs when compared to the adults.

To achieve fifty per cent mortality in *L. ramakrishnai* adults on the fifth day after treatment a spore concentration of 6.76×10^{8} spores ml^{-1} was required. On the same day the corresponding concentration for grubs was only 5.72×10^{6} spores ml^{-1}. The concentration to achieve 90 per cent mortality of adults and grubs five days after treatment were 12.65×10^{8} spores ml^{-1} and 11.02×10^{6} spores ml^{-1} respectively.

Bioassay of *B. bassiana* against adult *M. circumdata* showed that a period of 10. 23 days was required to bring fifty per cent mortality at the highest spore concentration of 2.52×10^{9} spores ml^{-1} tested. It was seen from the probit analysis of log dose - mortality responses that a concentration of 7.26×10^{9} spores ml^{-1} was required to bring fifty per cent mortality in five days, whereas to achieve LC_{90} concentration 14.04×10^{9} spores ml^{-1} was required. In the case of *M. circumdata* also a much lower concentration was enough to bring mortality in grubs, the LC_{50} was only 4.96×10^{8}. The LC_{90} values on the fifth day for adults and grubs were 14.04×10^{9} spores ml^{-1} and 10.76×10^{8} spores ml^{-1} respectively. A perusal of the literature revealed that no work has been carried out so far to assess the LC_{50} and LC_{90} of *B. bassiana* and *M. anisopliae* against *A. foveicollis*, *B. fulvicorne*, *L. ramakrishnai* and *M. circumdata* other than the present study.

Computation of LC_{50} values of *B. bassiana* against the curculionid, banana rhizome weevil, *C. sordidus* revealed that a concentration of 4.24×10^{9} spores ml^{-1} was required to bring mortality in adults and the corresponding LC_{90} value was 7.76×10^{9} spores ml^{-1} on the seventh day after treatment. The effect of the pathogen was evident in a very short period of three days in the grubs of *C. sordidus* and the concentration of the pathogen required to bring LC_{50} and LC_{90} were 3.83×10^{8} and 6.82×10^{8} spores ml^{-1}, respectively. The results are in

agreement with that of Brenes and Carballo (1994) who determined LC_{50} and LC_{90} values of A4 strain of *B. bassiana* against *C. sordidus* as 7.89×10^{7} and 2.67×10^{9} spores ml^{-1} respectively. The bioefficacy of *B. bassiana* to *C. sordidus* was revealed in an earlier study by Fancelli *et al.* (2013). In the attempt to select isolates of *B. bassiana* suitable for managing *C. sordidus* he observed that the isolates produced 14 to 96 per cent mortality in the adults.

The LC_{50} and LC_{90} values obtained from the probit analysis of the dose – mortality responses of the adults of the apionid, *C. formicarius* five days after treatment were 5.62×10^{7} and 11.54×10^{7} spores ml^{-1} respectively, that was much lower than that recorded for other coleopterans. A similar trend in the dose requirement was exhibited in the case of the grubs also, the LC_{50} and LT_{90} two days after treatment were 1.27×10^{7} and 6.02×10^{7} spores ml^{-1} respectively. Ondiaka *et al.* (2008) found variations in the virulence of isolates of *B. bassiana*, and he observed that among the different isolates, the isolate ICIPE 275 was the most active one with LC_{50} value 0.7×10^{6} conidia ml^{-1} in a related species of the sweet potato weevil, *Cylas puncticollis* (Boheman).

With respect to the adult *H. vigintioctopunctata,* the concentration of *B. bassiana* needed to bring fifty and ninety per cent mortalities were 3.99×10^{8} and 7.91×10^{8} spores ml^{-1} respectively on the fifth day after inoculation with the fungus. The grubs of *H. vigintioctopunctata* was also highly susceptible to the infection of *B. bassiana.* The mortality initiated on the third day after treatment and a concentration of 2.79×10^{7} and 5.04×10^{7} spores ml^{-1} was essential to achieve fifty and ninety percent mortalities. Jiji *et al.* (2008) had previously assessed the bioefficacy of *B. bassiana* to *H. vigintioctopunctata* and they have determined the LC_{50} value for the isolate ITCC 6063 as 3.8×10 spores ml^{-1} on the fifth day, which was a much lower concentration than noted in the present study and this may be due to the differences in the virulence of the isolates.

A longer duration was taken to express symptoms in both adults and grubs of *O. rhinoceros* and *R. ferrugineus* compared to the other coleopteran test insects. The mortality of the adult *O. rhinoceros* initiated only after thirty days but in the grubs, it initiated fourteen days after treatment. The LC_{50} values for the

adult and grub were 1.56×10^{15} and 6.22×10^{11} spores ml^{-1} respectively. The LC_{90} values were 2.67×10^{15} and 10.39×10^{11} spores ml^{-1} for the adults and grubs respectively thirty days after treatment.

Maximum duration for development of symptoms of *B.bassiana* was seen in the case of *R. ferrugineus*. To achieve fifty percent and ninety percent mortalities on the thirty fifth day after treatment 3.76×10^{13} and 8.79×10^{13} spores ml^{-1} respectively were needed for the adults and 4.64×10^{13} and 8.56×10^{13} spores ml^{-1} were required for the grubs of *R. ferrugineus*.

It is seen from the LC_{50} values and the time required for the initiation of symptoms, that the isolate Bb 5 of *B. bassiana* is an ideal candidate for the management of the coleopteran pests tested. At the concentration tested it took a longer time to bring mortality in *O. rhinoceros* and *R. ferrugineus* and hence it is presumed that much higher concentrations of the pathogens are required to bring an equal mortality in *O. rhinoceros* and *R. ferrugineus* in a shorter period. In the case of *O. rhinoceros*, even if it is taking a longer time to achieve mortality at this dose, it is beneficial as the grubs are not directly injurious and as the grub stage lasts 70 to 130 days. Since they are seen in cow dung and decaying organic matter they will be dead due to the infection even before reaching the pupal stage. Though reports on bioassay related to the insects in the present study are meager, reports on other coleopterans are available (Fabio *et al.*, 2003; Verghese *et al.*, 2003; Beron *et al.*, 2005; Karthikeyan and Jacob , 2010)

LC_{50}, LC_{90} and LT_{50} of *M. anisopliae* were also determined for these nine beetles. The computed LC_{50} for the adults of *A. foveicollis* at 7 DAT was 11.42×10^{10} spores ml^{-1} for *B. fulvicorne* it was 3.99×10^{8} at 7 DAT, for *C. sordidus* 8.86×10^{10} spores ml^{-1} at 10DAT, for *C. formicarius* 6.27×10^{9} spores ml^{-1} at 10 DAT, for *H. vigintioctopuncta* 6.96×10^{8} spores ml^{-1} at 5 DAT, for *L. ramakrishnai* 3.66×10^{11} spores ml^{-1} at 10 DAT, and *M. circumdata* 6.13×10^{10} spores ml^{-1} at10 DAT, for *O. rhinoceros* 2.58×10^{13} spores ml^{-1} at 30 DAT and for *R. ferrugineus* 2.73×10^{15} spores ml^{-1} at 50 DAT.

The grubs of these insects succumbed to the infection of *M. anisopliae* at a lower dose compared to the adults as in the case of *B. bassiana* infection. The

LC_{50} computed for the grubs *of A. foveicollis* at 7 DAT was 4.91×10^{7} spores ml^{-1}, for *B. fulvicorne* 6.09×10^{8} spores ml^{-1} at 5 DAT, for *C. sordidus* 4.81×10^{8} spores ml^{-1} at 7 DAT, for *C. formicarius* 5.83×10^{8} spores ml^{-1} at 5 DAT, for *H. vigintioctopunctata* 4.95×10^{7} spores ml^{-1} at 5 DAT, for *L. ramakrishnai* 2.42×10^{8} spores ml^{-1} at 10 DAT, for *M. circumdata* 4.64×10^{9} spores ml^{-1} at 5 DAT, for *O. rhinoceros* 3.79×10^{8} spores ml^{-1} at 10 DAT and for *R. ferrugineus* 10.29×10^{13} spores ml^{-1} at 30 DAT. A longer duration of 26.8 days to obtain fifty per cent mortality in *R. ferrugineus* and a comparatively shorter period of three weeks in *O. rhinoceros* and 4.2 days in *C. sordidus* was observed in earlier studies also (Sundarababu *et al.*, 1983 ; Lopez *et al.*, 2013)

When compared to *B. bassiana* isolate Bb 5, the isolate Ma 4 of *M. anisopliae* was inferior in its ability to infect the coleopteran test insects except to the adults and grubs of *O. rhinoceros*. *M. anisopliae* was less infective to the grubs of *B. fulvicorne* when compared to *B. bassiana* but its performance was better towards the adults of *B. fulvicorne,* as evident from the lower dose (3.99×10^{8} spores ml^{-1}) required for fifty percent mortality on the seventh day after treatment. Contrary to the present observations on the infectivity of the two fungal pathogens, Gindin *et al.* (2006) while examining the susceptibility of *M. anisopliae* and *B. bassiana* strains to *R. ferrugineus* found that *M. anisopliae* strains were more virulent than that of *B. bassiana* and that 100 per cent larval mortality was achieved within six to seven days. It is inferred that such differences in the observations may be due to the differences in the virulence of the isolates or may be due to the physiological state, nutritional aspects etc. of the test insects, considering the remarks of Inglis *et al.* (2001) that a complex array of physiological and morphological factors of the insect influence the susceptibility of insect pests to entomopatogenic fungi.

The importance of *M. anisopliae* as a pathogen of *O. rhinoceros* has been recognized by Hockberg and Waage (1991). Another encouraging side of *M. anisopliae* is that the fungus is harmless to the beneficial earthworm *Eudrillus euginae* (Kinberg) used in vermicomposting (Gopal *et al.*, 2006). Grubs of rhinoceros beetles often breed enormously in vemicomposting yards and are

aggravating the problems caused by the beetle to coconut in Kerala. In such situations *M. anisopliae* can be effectively exploited for the management of rhinoceros beetle.

A. foveicollis, *B. fulvicorne*, *L. ramakrishnai* and *M. circumdata* belong to the family Chrysomelidae, *H. vigintioctopunctata* belongs to the family Coccinellidae and *C. formicarius* belong to the family Apionidae. Both adults and grubs of these insects are injurious. The adult beetles of all these insects feed on the foliage and since a higher dose is required for managing the adults when compared to the grubs, the dose corresponding to LC_{90} for the adults needs to be recommended for foliar spray in the field. It may also be noted that if application of the fungus can be done at the initiation of grub stage itself, the dose corresponding to the LC_{90} values for the grubs would be sufficient to manage them. Unlike the grubs of *L. ramakrishnai*, *M. circumdata* and *H. vigintoctopunctata* that inhabit the aerial parts of the plants, the grubs of *A. foveicollis* and *B. fulvicorne* inhabit the soil. So for managing the grubs in the root / soil, the dose corresponding to the LC_{90} values for the grubs can be recommended, but for *C. formicarius* the adults and grubs are present both in the aerial parts as well as in tubers. In this case the LC_{90} for the adults (Tables 21, 22, 39 and 40) needs to be recommended as the field dose.

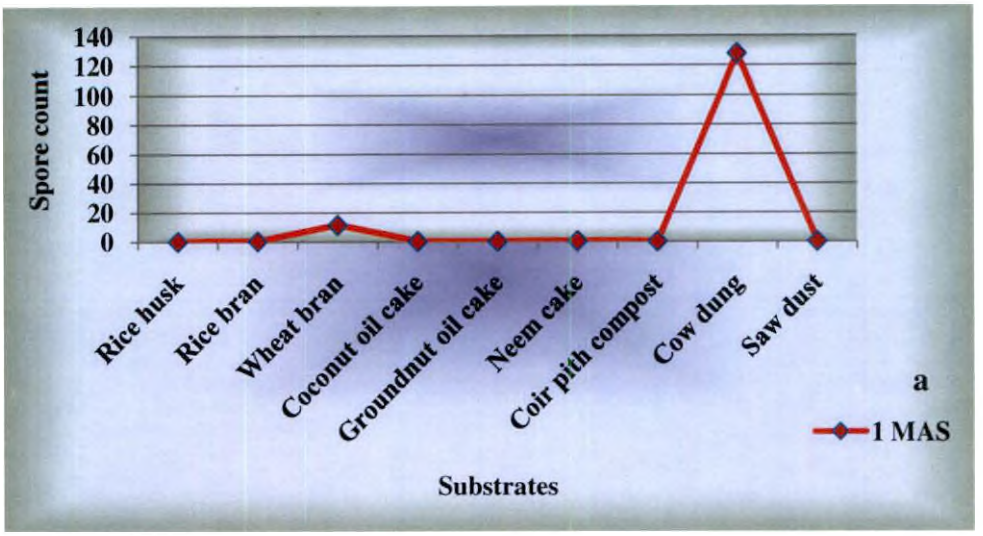
To be commercially successful, the bioagent should be amenable for easy mass multiplication in cost effective substrates. So inorder to identify such substrates *B. bassiana* and *M. anisopliae*, were multiplied in nine substrates *viz.* rice bran, rice husk, wheat bran, coconut oil cake, groundnut oil cake, neem cake, coir pith compost, cow dung and saw dust. Sporulation and colony forming units in the different substrates were determined at monthly intervals. In addition, visual scoring on the growth pattern of the fungus was also done. Profuse growth of *B. bassiana* was seen in cow dung, neem cake, rice bran and wheat bran whereas profuse growth of *M. anisopliae* was seen in cow dung, wheat bran and groundnut oil cake. In coir pith compost, saw dust and rice husk there was only slight growth. The feasibility of multiplying *M. anisopliae* in bran (Goettel,

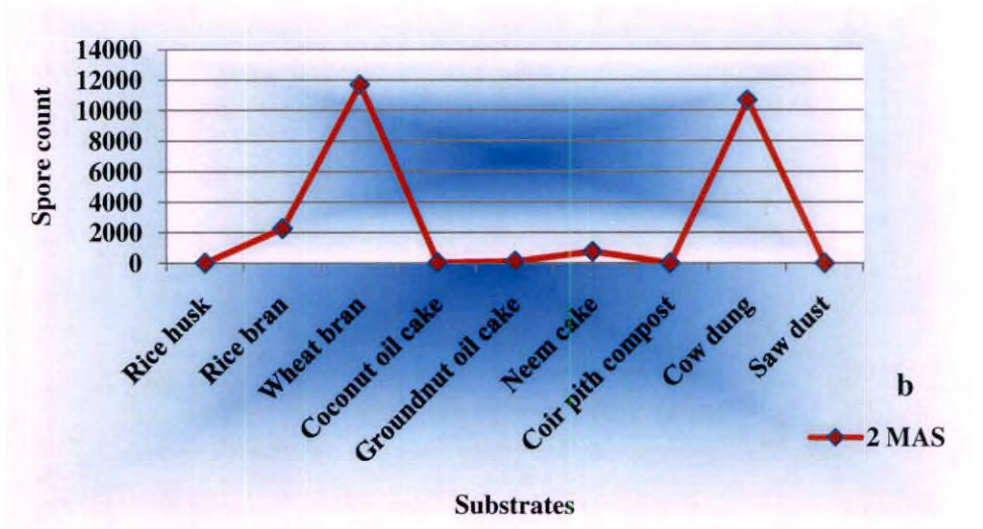
1984), rice husk and compost (Hussey and Tinsley, 1981) has been elucidated earlier.

It is also important that the substrates support good spore production. Estimation of spore count one month after storage revealed that the spore count was highest in cow dung (127.69×10^5 spores ml^{-1}) and this was significantly higher than in other substrates (Fig. 5 a). The peak sporulation was observed in the samples drawn two months after storage (Fig. 5 b). During this period the highest sporulation was noted in wheat bran which recorded a spore count of 11676.96×10^5 spores ml^{-1} but this was on par with that observed in cow dung and both were significantly higher than that noted in other substrates. During the third month after storage, a decline in sporulation was noted (Fig. 5 c). According to Sahayaraj and Namasivayam, 2008; Haraprasad *et al.*, 2001) sporulation of *B. bassiana* was maximum when wheat was used as the substrate for multiplication of the fungus. While attempting mass production of *B. bassiana* in solid substrates, Nelson *et al.* (1996) found that the spore production was highest in rice and it was 4.38×10^9 g^{-1} rice during the third week after inoculation at 23^0 C. It is seen from the results and the related literature that substrates influence the production of spores and the duration that it takes to reach the peak varies with the conditions of incubation of the fungus.

A similar trend in sporulation in these different substrates was noted with *M. anisopliae* also (Fig. 6 a, b and c). However, the sporulation of *M. anisopliae* was much lower when compared to *B. bassiana*. The spore load in cow dung and wheat bran were 8573.66×10^5 and 8447.06×10^5 spores ml^{-1}, respectively. In the observations taken three months after storage a decline in spore count was seen. Saw dust as a weak supporter for the fungal growth of *M. anisopliae* was evident from the studies of Chauhan *et al.* (2013) also who observed that the biomass production of *M. anisopliae* in rice was 16.22 g while it was only 0.62 g in saw dust when cultured in 250 ml of the substrates.

The determination of cfu at different intervals after storage revealed that during the first month after storage, in general the viable units were also low in the different substrates as in the case of spore count, thereafter there was





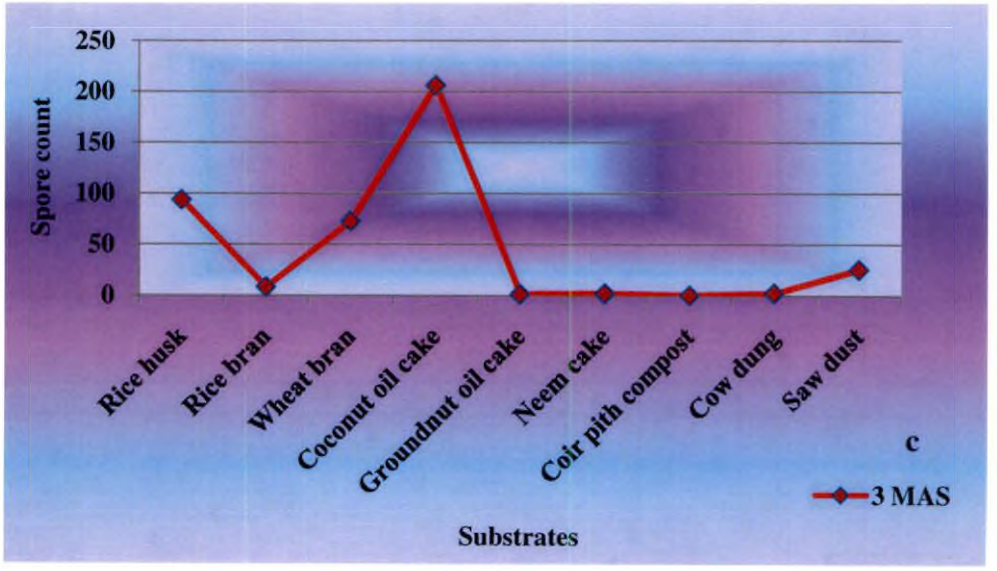
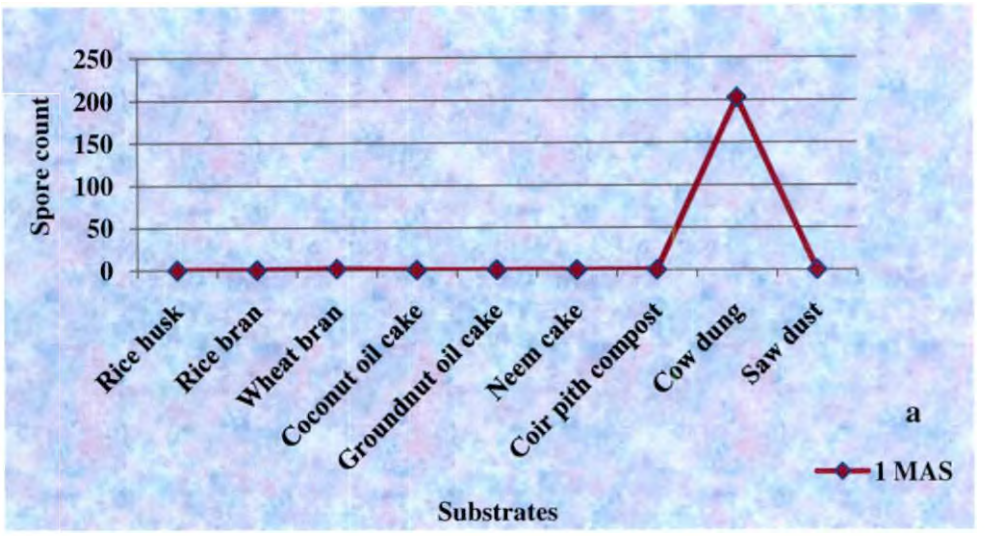
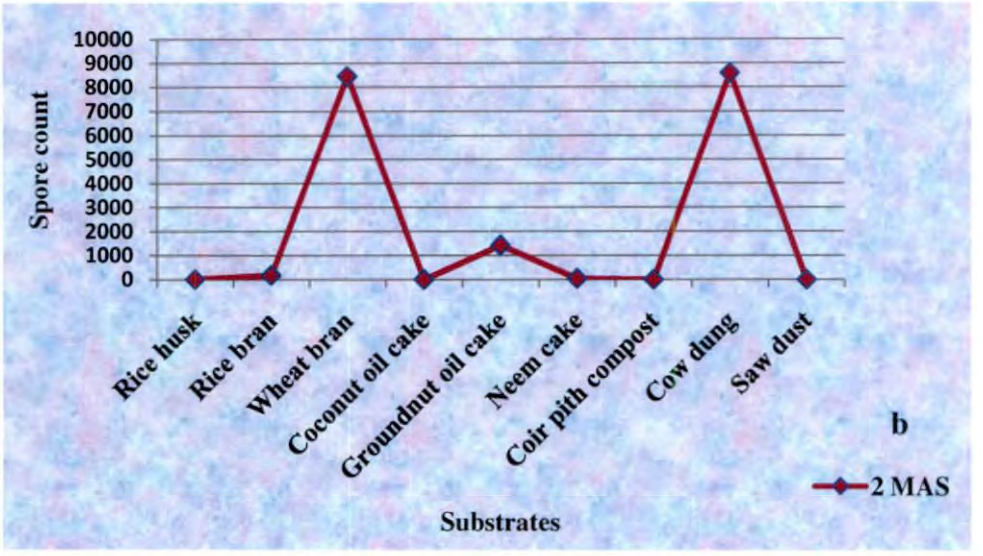


Fig. 5. Spore count ($\times 10^5$ spores ml^{-1}) of *B. bassiana* in various substrates at different intervals after storage

* MAS - Month after storage





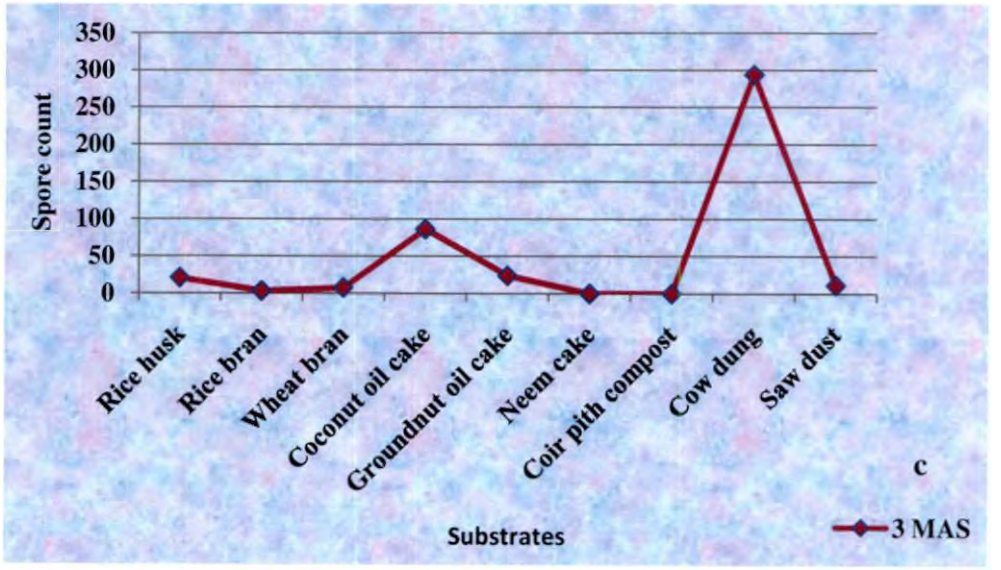


Fig. 6. Spore count ($\times 10^5$ spores ml^{-1}) of *M. anisopliae* in various substrates at different intervals after storage

* MAS – Months after storage

substantial increase and during the second month the colony forming units of *B. bassiana* was as high as 7717. 42 $\times 10^{5}$ spores ml^{-1} in cow dung and it was significantly superior than in other treatments. The count of the colony forming units in wheat bran, rice bran and neem cake were also significantly higher than in other substrates (Fig. 7 a, b and c). There was a drastic decline in the cfu during the third month in all the substrates except in saw dust which showed a slight increase. From the results it was seen that the ideal substrates that maintained the viability of *B. bassiana* were cow dung, wheat bran, rice bran and neem cake.

With respect to *M. anisopliae* cow dung and wheat bran supported maximum cfu. Significantly superior counts were noted in cow dung during the first and third months after storage compared to other substrates, the cfu in cow dung during these months were 23. 98 $\times$ 10^{5} and 7.29 $\times$ 10^{5} respectively. However, during the second month after storage maximum cfu was noted in wheat bran. It was also seen that the cfu reached its peak during the second month in these substrates, the values being 266.98 $\times$ 10^{5} and 658.24 $\times$ 10^{5} in cow dung and wheat bran respectively (Fig. 8 a, b and c). In earlier studies substrates such as humid rice (Alvarez *et al.*,1997; Narvaez *et al.*, 1997; Jenkin *et al.*, 1998) and coconut water (Danger *et al.*,1991) were found ideal for the multiplication of *M. anisopliae* .

The bioefficacy of fungi cultured in different substrates and stored for different months was also evaluated against the grubs and adults of *C. formicarius*. With respect to bioefficacy also, the fungi cultured in cow dung and wheat bran proved better. When sprayed with spore suspensions of *B. bassiana* cultured in cow dung and wheat bran and stored for one month, the mean mortality percentages of the adult weevil were 38.84 and 31.45, respectively at 14 DAT. Moderate efficacy of the fungus cultured in neem cake, groundnut oil cake, rice bran, coconut oil cake and rice husk and lowest efficacy of fungus cultured in saw dust was also evident. A similar trend in efficacy was seen for the spore suspensions prepared from different substrates during the second month also. *B. bassiana* cultured in cow dung and wheat bran maintained their superiority during the third month also (Fig. 9). However, there was mortality

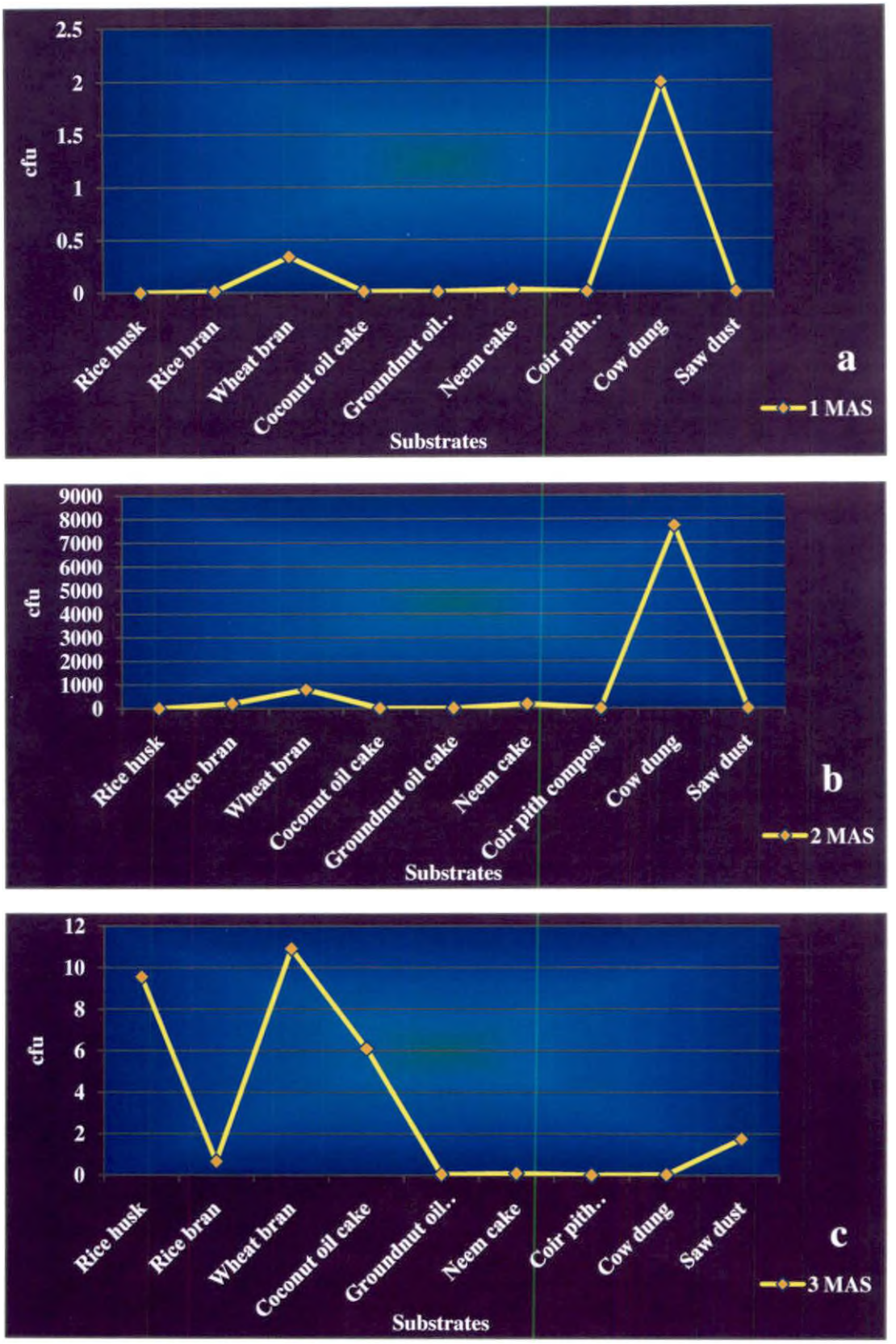


Fig.7. Colony forming units ($\times 10^5$) of *B. bassiana* in various substrates when stored for three months

*MAS – Months after storage

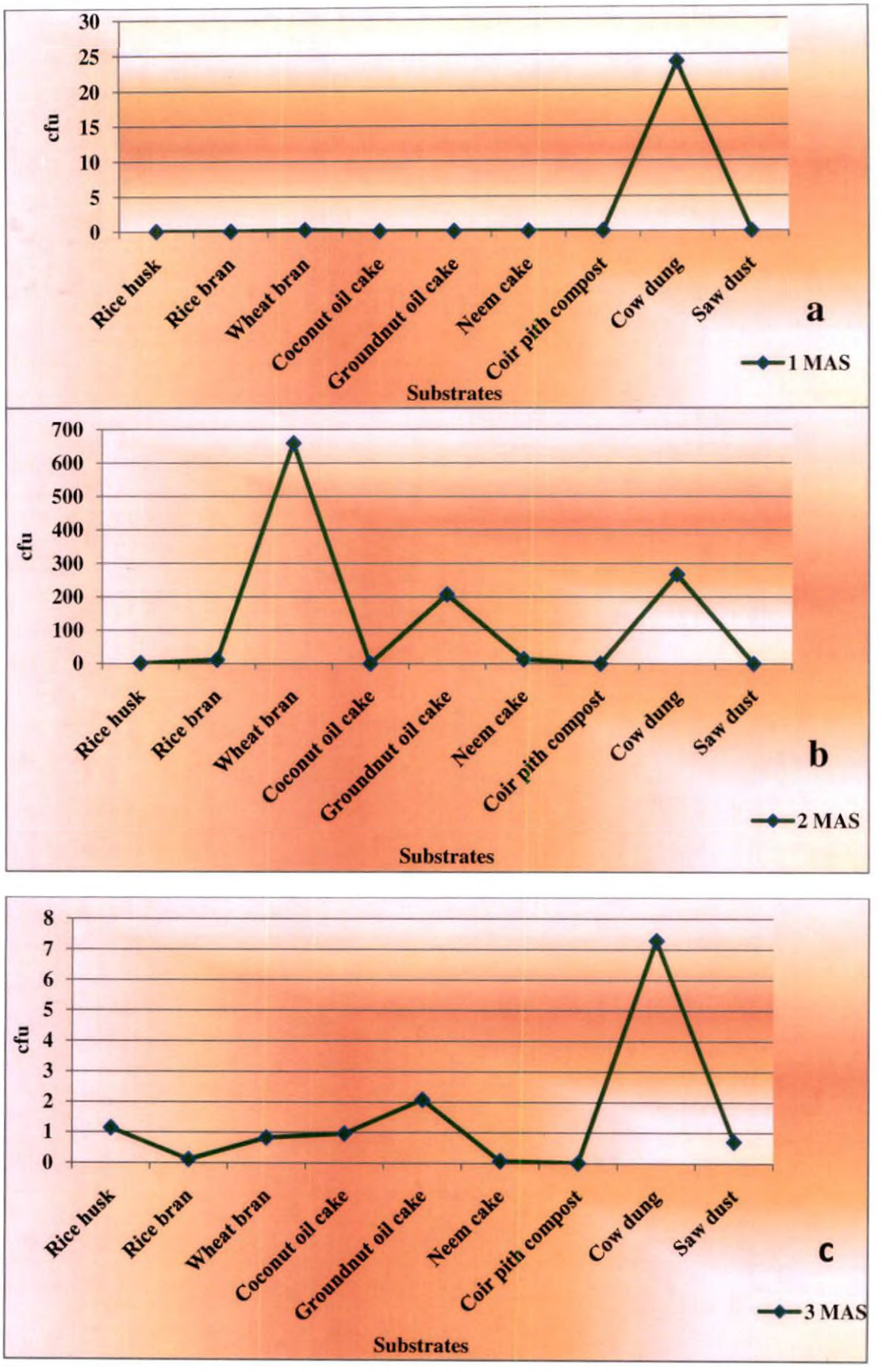


Fig.8. Colony forming units ($\times 10^5$) of *M. anisopliae* in various substrates when stored for three months

*MAS – Months after storage

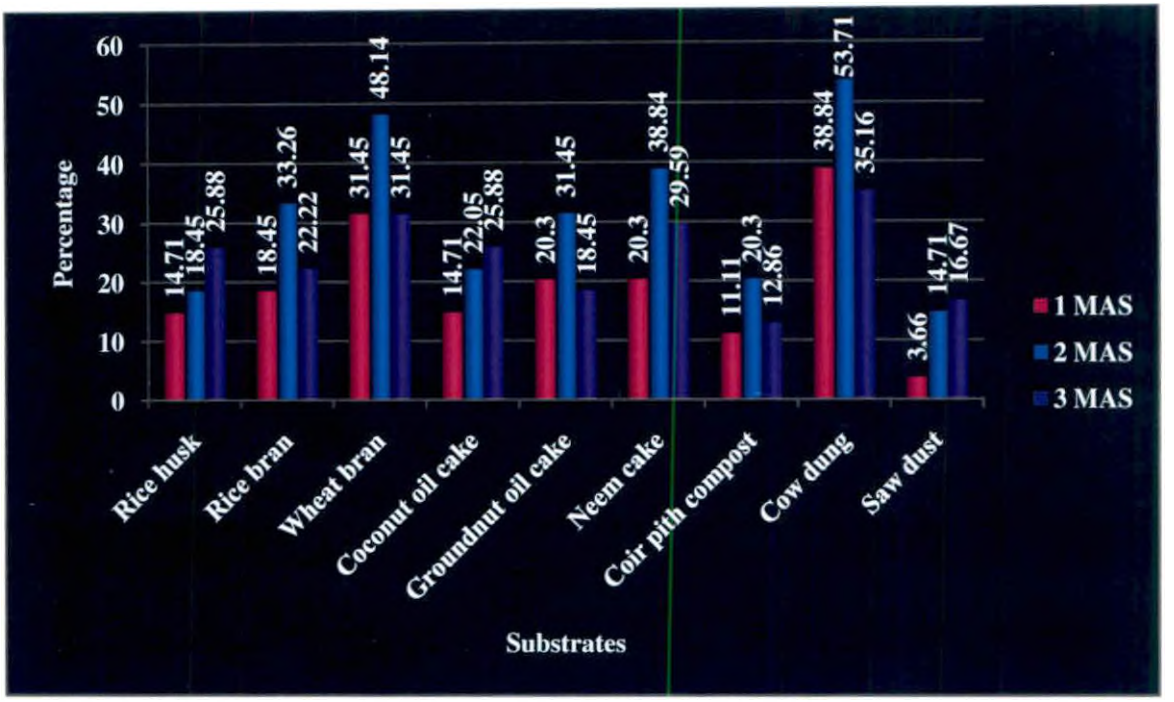


Fig. 9. Mortality of adults of *C. formicarius* when treated with *B. bassiana* grown in various substrates and stored for different months

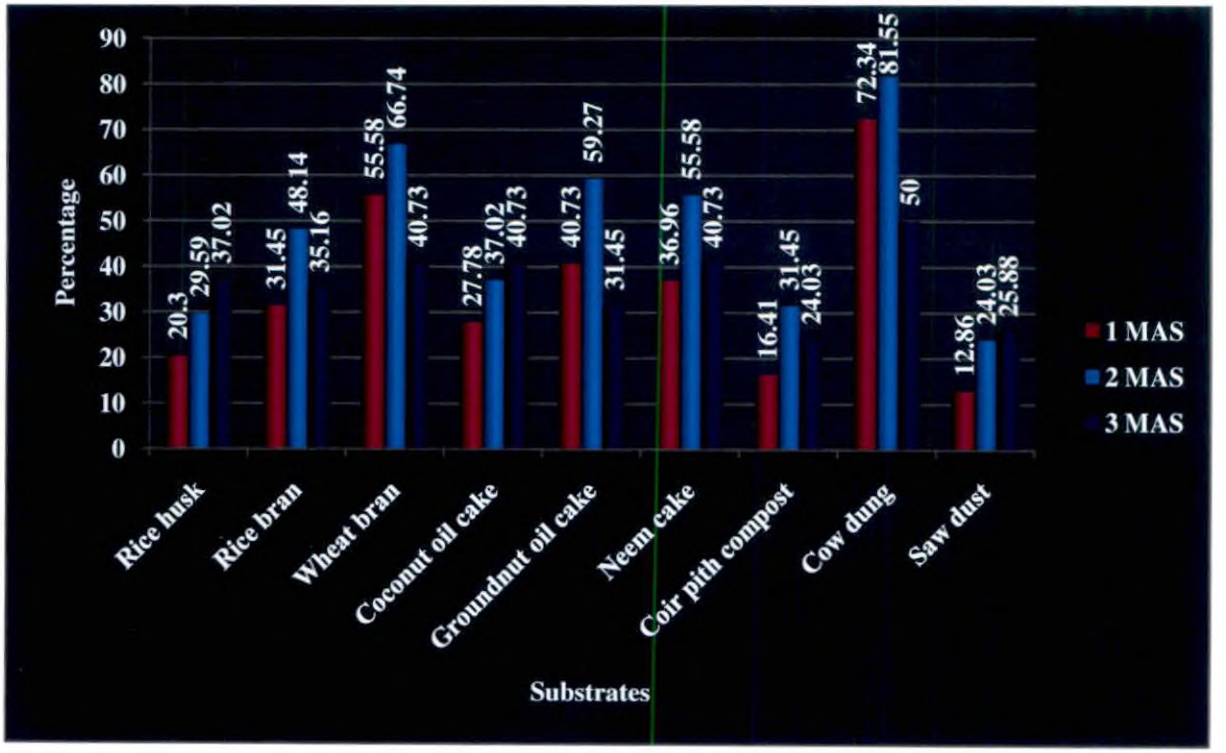


Fig. 10. Mortality of grubs of *C. formicarius* when treated with *B. bassiana* grown in various substrates and stored for different months

* MAS – Months after storage

when sprayed with fungal cultures from other substrates also and it ranged from 12.86 in coir pith compost to 29.59 in fungus cultured in neem cake during the third month. Among the fungi in different substrates, the effect of fungus cultured in cow dung and stored for one, two and three months was the maximum against the grubs of *C. formicarius* (Fig. 10). Effect of fungus grown in coir pith compost was also seen from the mortality of 24.03 percentage caused to the grubs, though it was the least among the different treatments.

Assessment of the bioefficacy of *M. anisopliae* cultured in different substrates revealed that the fungus grown in cow dung and wheat bran were superior in their efficacy to the adults and grubs of *C. formicarius* and that the effect was maximum in the spore suspension prepared and stored for two months though the mortality percentages were lower when compared to *B. bassiana* cultured in cow dung and wheat bran (Fig. 11 and 12). This superiority can be attributed to the higher cfu present in these substrates. After three months of storage also fungus cultured in cow dung performed best, and caused a mortality of 53.71 in the grubs but the effect of fungus from wheat bran was significantly lower and it was on par with that of neem cake with 38.84 per cent mortality. The fungus cultured in saw dust was not much effective as it could cause only 7.22 per cent mortality in the grubs which was the least mortality noted in the different treatments.

Estimation of the spore count and cfu of *B. bassiana* in the talc based formulation of the fungus stored for different periods showed that these were highest when stored for one month, thereafter declined. During the first month 21.05×10^9 spores ml^{-1} (Fig. 13) and 3.43×10^9 cfu g^{-1} (Fig. 14) were seen. When sprayed with this formulation at a concentration of 20 g l^{-1} mortality caused to the adults and grubs of *C. formicarius* was 88. 83 and 100 per cent respectively. Though the cfu declined during the third month to 0.2×10^9 g^{-1} high mortality of 85.16 and 100 per cent mortality was caused to the adults and grubs respectively (Fig. 15).

In the talc based formulation of *M. anisopliae,* the spore count was less when compared to *B. bassiana*, and the values were 1.86×10^9, 0.33×10^9, 0.29

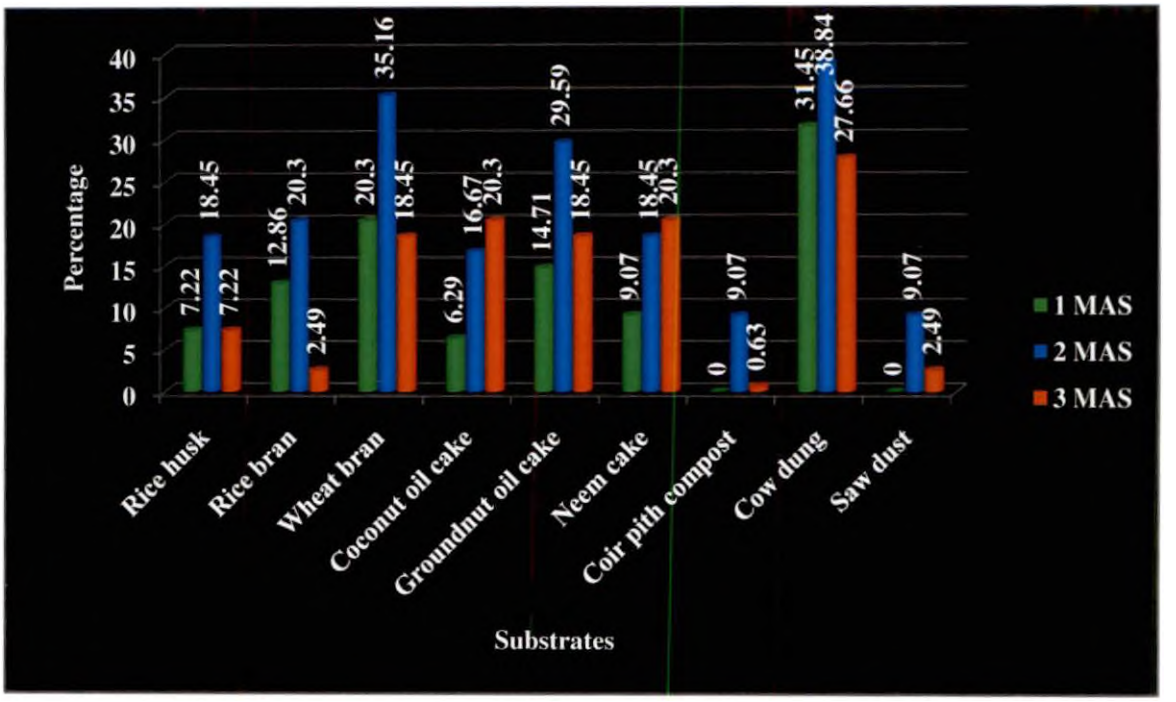


Fig. 11. Mortality of adults of *C. formicarius* when treated with *M. anisopliae* grown in various substrates and stored for different months

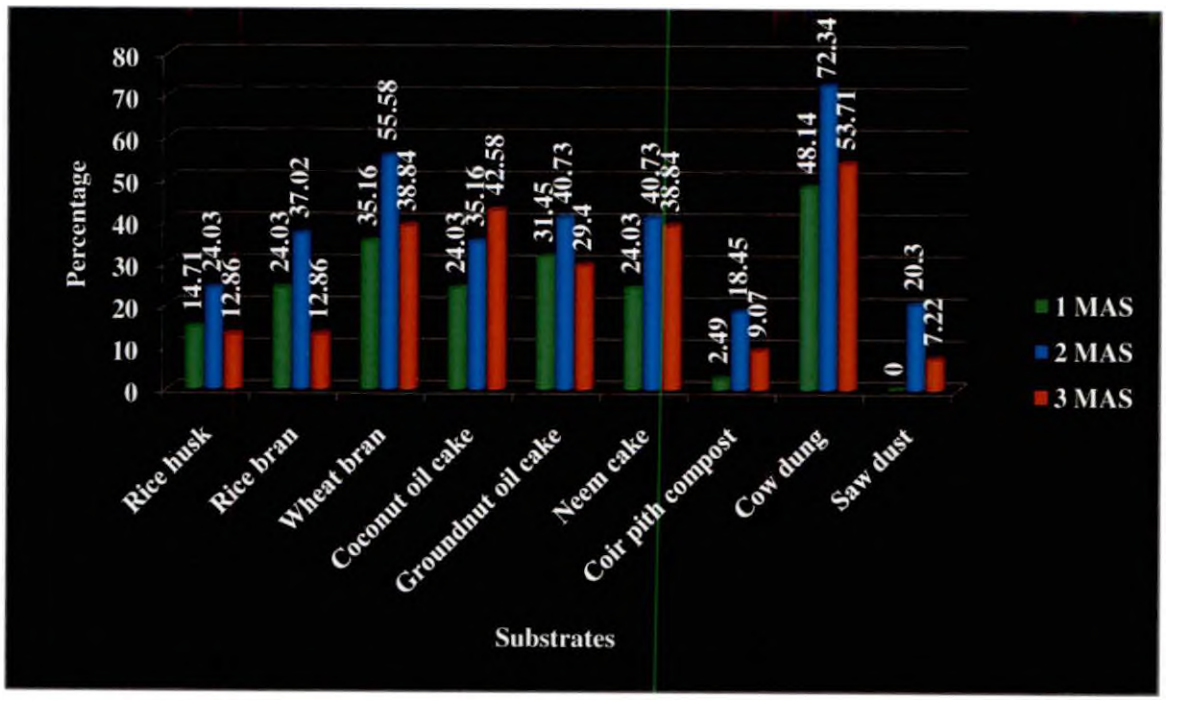


Fig. 12. Mortality of grubs of *C. formicarius* when treated with *M. anisopliae* grown in various substrates and stored for different months

*MAS – Months after storage

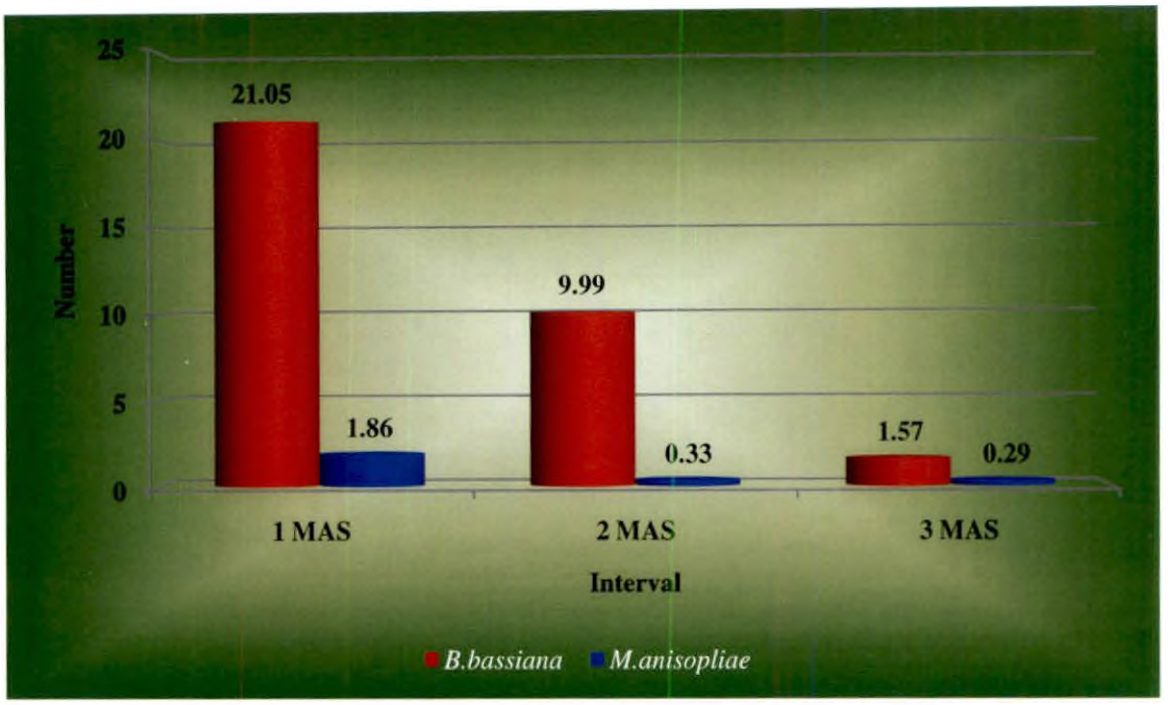


Fig. 13. Spore count (× 10^9 spores ml^{-1}) of talc based formulation of *B. bassiana* and *M. anisopliae* at different intervals after storage

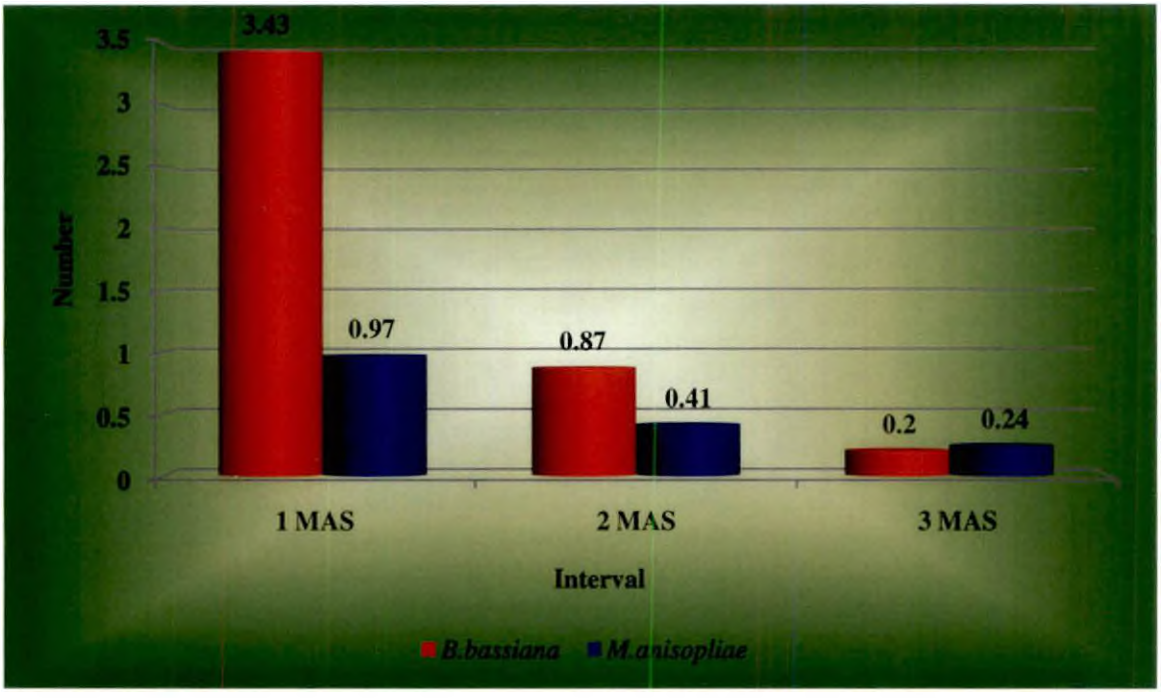


Fig. 14. Colony forming units (× 10^9 spores ml^{-1}) of talc based formulation of *B. bassiana* and *M. anisopliae* at different intervals after storage

* MAS - Months after storage

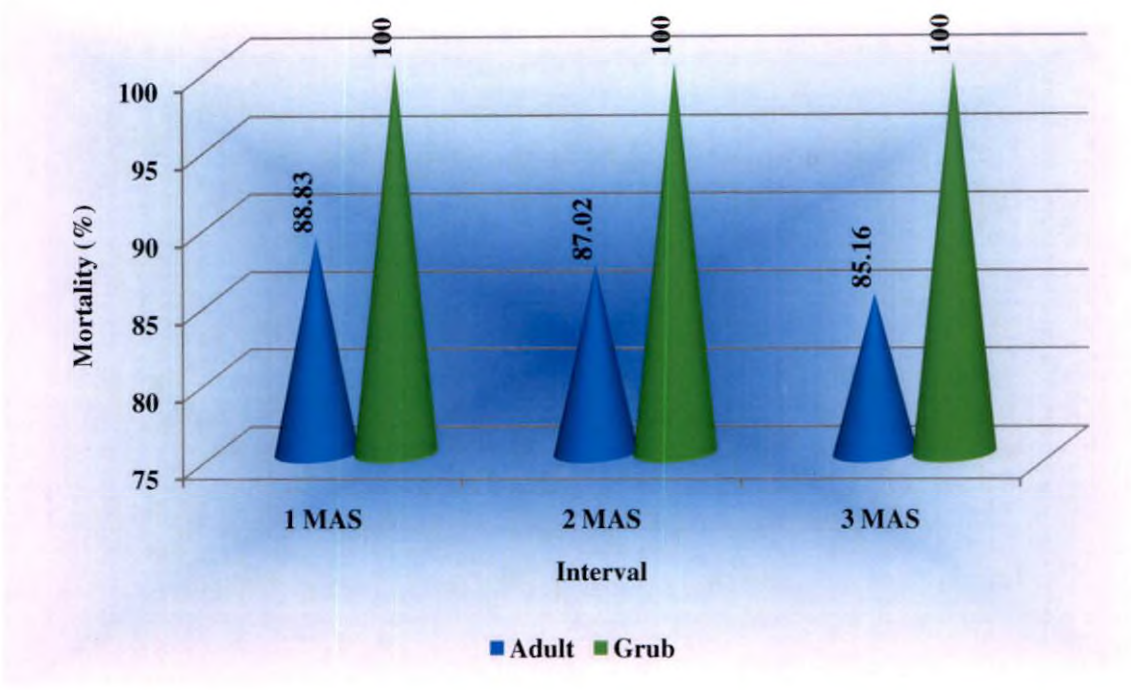


Fig. 15. Mortality of adults and grubs of *C. formicarius* when treated with *B. bassiana* talc formulation stored for different intervals

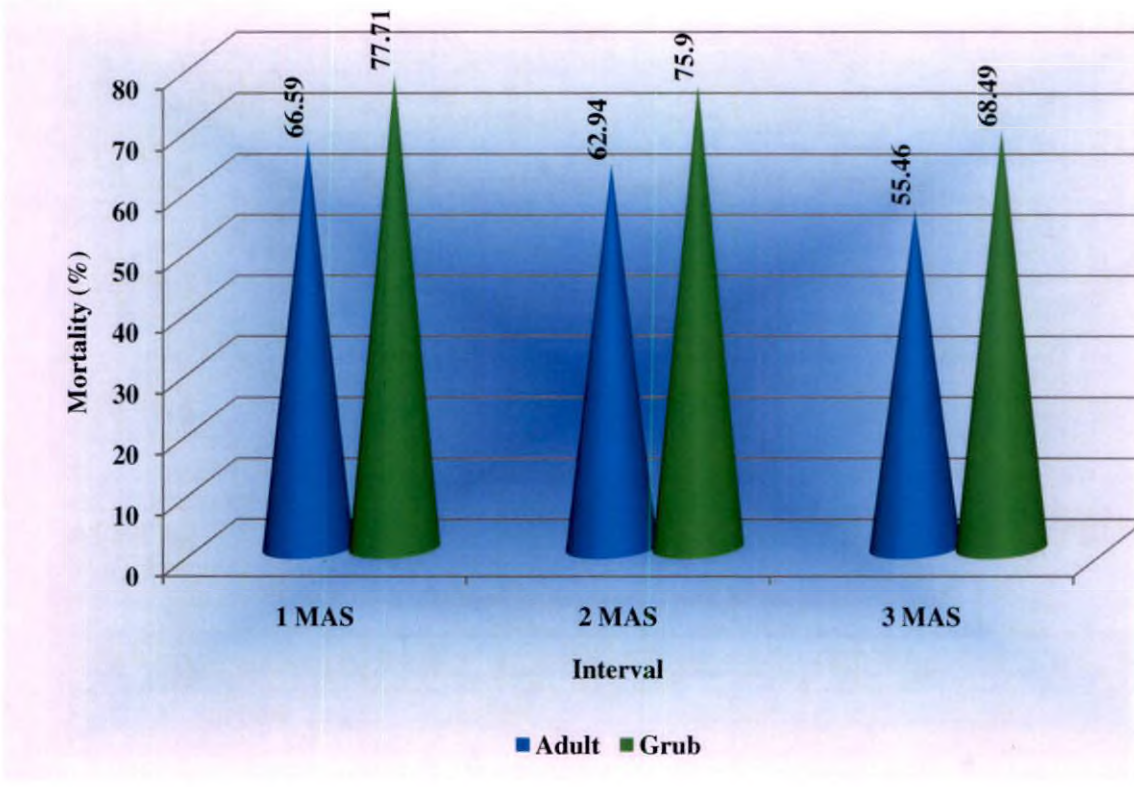


Fig. 16. Mortality of adults and grubs of *C. formicarius* when treated with *M. anisopliae* talc formulation stored for different intervals

* MAS - Months after storage

$\times 10^9$ spores ml^{-1} for the first, second and third month respectively. Good viability of the spores was also seen during the period and this brought about 55.46 per cent mortality to the adults and 68.49 percent in the grubs of *C. formicarius* respectively (Fig. 16).

Formulation is one of the challenges in the development of fungi based biopesticides. Unless sufficient quantities of infective propagules are present, the performance of the formulation will be hampered. It is mandatory that a standard formulation of fungus based biopesticide should contain cfu @10^8 g^{-1}. Carrier materials are inert in the sense that they do not have pest control capabilities but they can profoundly affect the shelf life (Burges, 1998). In the talc based formulation prepared in the study, cfu to the tune of 0.2×10^9 g^{-1} of *B. bassiana* and 0.24×10^9 g^{-1} of *M. anisopliae* were seen three months after storage and hence such formulations should find a place at least in the niche markets to cater to the needs of the local farmers.

Further, field experiments were conducted to assess the effect of *B. bassiana* Bb 5 and *M. anisopliae* Ma 4 for the management of *C. sordidus* in banana and *C.formicarius* in sweet potato. Spore suspensions of the fungi, fungal culture in cow dung and neem cake and the talc based formulations were evaluated in comparison with insecticides and untreated control. *B. bassiana* and *M. anisopliae* in neem cake were selected as treatments considering the soil amendment properties of neem.

Experiment in banana was conducted using the variety Nendran. Two trials, one in main crop and another in the succeeding crop were undertaken. The treatments were given at three months after planting. The results of the field experiments revealed that talc based *B.bassiana* @ 30 g l^{-1} was the superior treatment for the management of weevil in the main crop. The yield obtained from this treatment was also significantly higher (10.01 kg) excepting the insecticide check chlorpyriphos 0.03 per cent. In the succeeding crop the effect of talc based formulation of *B. bassiana* was found even superior to chlorpyriphos 0.03 per cent. It is remarkable that the best treatment during the succeeding crop was *B. bassiana* @ 30 g l^{-1} which produced the least number of galleries (0.63)

and that recorded the least number of grubs in the rhizomes (0.29) besides the lowest number of adult *C. sordidus* in soil samples.

Strikingly, the results from the succeeding crop indicate the ability of these fungi to self perpetuate and bring about long lasting effect in the treated area. When there was an increase in the number of galleries and number of grubs in the untreated control of the succeeding crop compared to the main crop, in fact, a reduction in these parameters were also noted in talc based *B. bassiana* @ 30 g l^{-1} treated plants in the succeeding crop. Analysis of the population of *C. sordidus* in the soil samples revealed that the soil around the plants treated with *B. bassiana* talc based formulation @ 30 g $plant^{-1}$was free from the weevils.

All the other treatments also effectively checked the incidence of *C. sordidus*. The number of galleries observed in the rhizome and the number of grubs in the rhizome in untreated control during the main crop were 8.33 and 12.27 and corresponding values for the succeeding crop were 10.94 and 15.65 respectively, which were significantly higher than in all other treatments. The effect of spore suspension as well as talc based formulation of *B. bassiana* were better than the talc based formulation of *M. anisopliae* as well as its spore suspension and came on par with the granular application of carbofuran 3G @ 20 g $plant^{-1}$ in the main crop. The mean number of galleries noted were 2.32, 1.31, 0.99 and 0.91 in talc based *B. bassiana* @ 20 g l^{-1}, *B. bassiana* @ 5×10^{11} spores ml^{-1}, carbofuran 3G @ 20 g $plant^{-1}$ and talc based *B. bassiana* @ 30 g l^{-1} respectively. The effect of application of *B. bassiana* and *M. anisopliae* in cow dung and neem based substrates showed moderate effect with respect to the number of galleries and number of grubs. The mean number of galleries observed were 6.64 and 6.67 when treated with *B. bassiana* in cow dung and *M. anisopliae* in cow dung, respectively in the main crop and 2.65 to 4.66 in the succeeding crop.

The percentage reduction over control with respect to the number of galleries were 89.08 and 82.47 per cent in talc based formulation of *B. bassiana* @ 30 g l^{-1} and *B. bassiana* spore suspension 5×10^{11} spores ml^{-1}, respectively (Fig. 17) in the first field trial in banana and in the succeeding crop, talc based

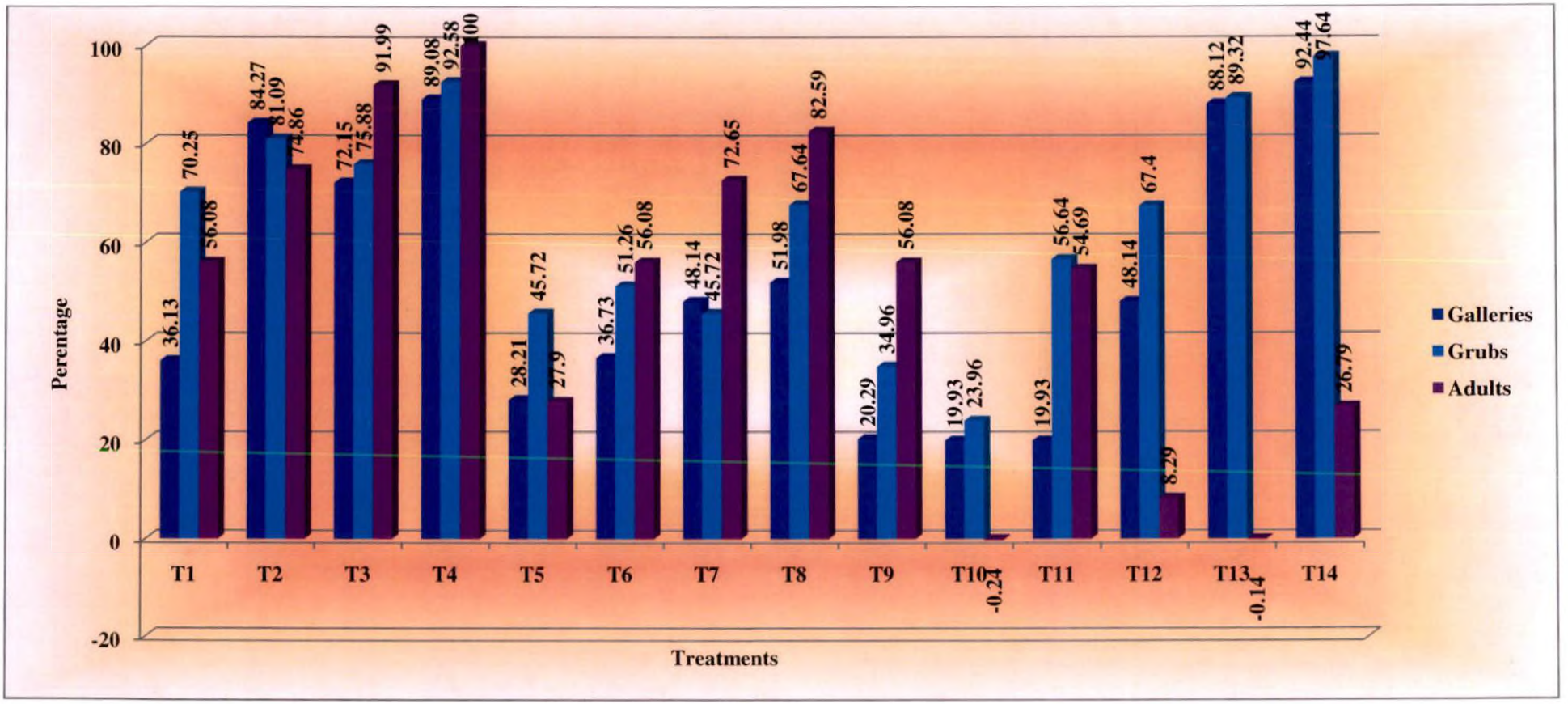


Fig. 17. Percentage reduction in the number of galleries and grubs in rhizome and adult *C. sordidus* in soil over control in the first field trial in banana

T1: *B.bassiana* @ 5×10^9 spores ml^{-1} T2: *B.bassiana* @ 5×10^{11} spores ml^{-1} T3:Talc based formulation of *B.bassiana* @ 20 g l^{-1} T4:Talc based formulation of *B.bassiana* @ 30 g l^{-1}
T5: *M.anisopliae* @ 5×10^{10} spores ml^{-1} T6: *M.anisopliae* @ 5×10^{12} spores ml^{-1} T7: Talc based formulation of *M.anisopliae* @ 20 g l^{-1} T8: Talc based formulation of *M.anisopliae* @ 30 g l^{-1}
T9: *B.bassiana* in cow dung @ 50 g T10: *M.anisopliae* in cow dung @ 50 g T11: *B.bassiana* in neem cake @ 50 g T12: *M.anisopliae* in neem cake @ 50 g T13:Carbofuran 3G @ 20 g $plant^{-1}$
T14: Chlorpyriphos 0.03 per cent

B. bassiana @ 30 g l^{-1} and spore suspension of *B. bassiana* 5×10^{11} spores ml^{-1} ranked top with 94.24 per cent reduction in both the treatments. This was followed by talc based *B. bassiana* @ 20g l^{-1} and *M. anisopliae* @ 30 g l^{-1} each with 85.01 percentage reduction over control. The insecticide check chlorpyriphos 0.03 per cent recorded 92.44 and 94.19 per cent reduction over control in the main crop and in the succeeding crop, respectively (Fig. 18).

Considering the premium price and higher demand for the organically produced banana in the market the B : C ratio of 1.57 calculated for *B. bassiana* talc based formulation @ 30 g $plant^{-1}$ was also higher compared to the B : C ratio 1.24 for chlorpyriphos 0.03 per cent in the main crop. Similarly the B : C ratios for the treatments with *B. bassiana* spore suspension @ 5×10^{11} spores ml^{-1} and talc based formulation of *M. anisopliae* @ 30 g l^{-1} were also higher than chlorpyriphos 0.03 per cent treatment, the values being 1.52 and 1.26, respectively. In all other treatments the B : C ratios were less than in chlorpyriphos 0.03 per cent.

The B : C ratios calculated for the treatments talc based *B. bassiana* @ 30 g l^{-1} and spore suspension of *B. bassiana* @ 5×10^{11} spores ml^{-1} in the succeeding crop of banana were 1.78 and 1.49 which were higher than that for chlorpyriphos 0.03 per cent.

B. bassiana is one of the most studied fungi worldwide aiming at *C. sordidus* control. Many researchers (Filho *et al.,* 1995; Godonou *et al,*; 2000, Nankinga *et al.,* 1998; Gold *et al.*, 2001) have also recognized the importance of *B.bassiana* in the population reduction of *C. sordidus* with different strategies and formulations of this fungus. In a trial to screen isolates of *B. bassiana* suitable for the management of *C. sordidus,* Fancelli *et al.* (2013) found that the application of the strain, CNPMF 218 of *B. bassiana* led to 40 per cent reduction in population size. Variations in infectivity of isolates of *B. bassiana* and *M. anisopliae* to *C. sordidus* are evident from the reports of Lopes *et al.* (2013) who found that among the different isolates tested, two insect derived isolates caused more than eighty per cent mortalities. In addition to reducing the population of *C. sordidus* in the treated plants by these fungi, it is probable that

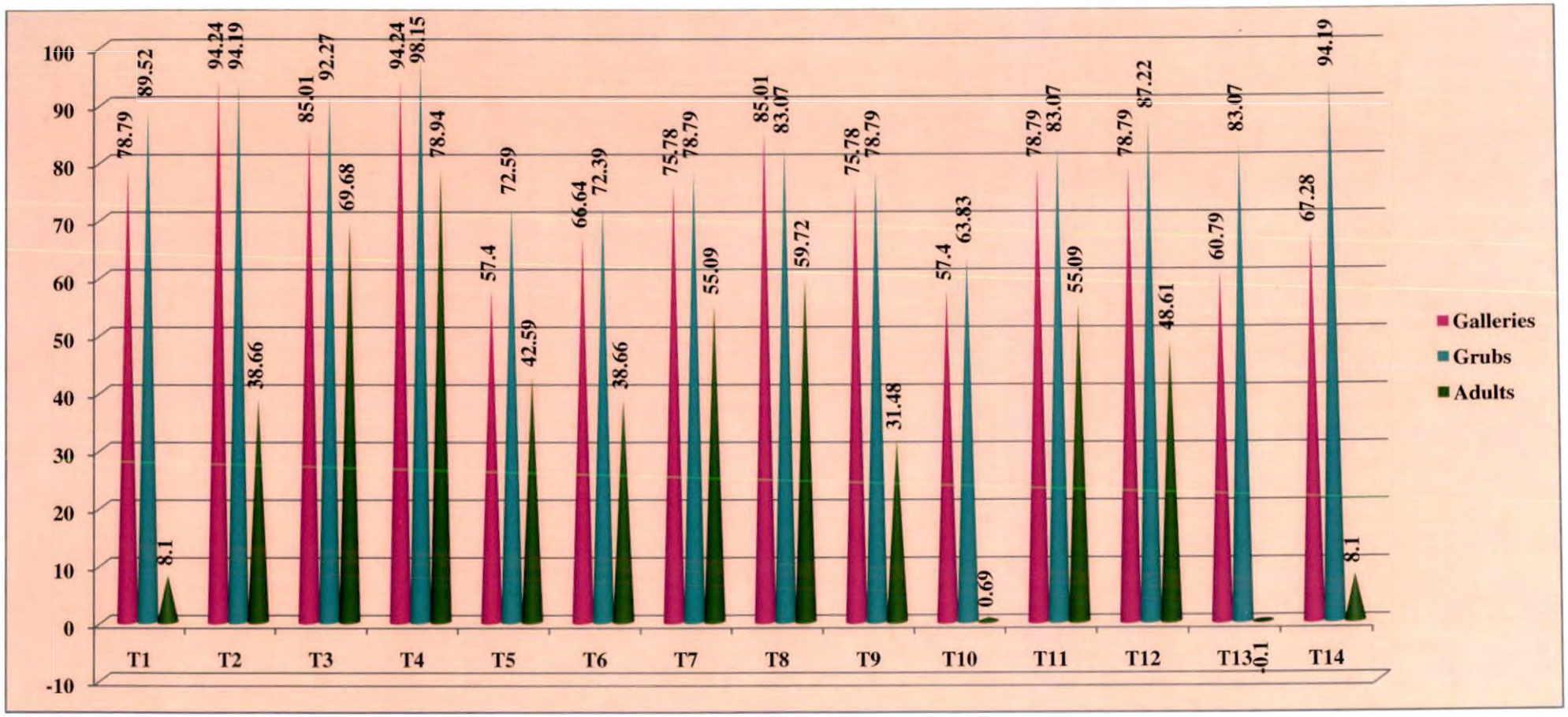


Fig. 18. Percentage reduction in the number of galleries and grubs in rhizome and adult *C. sordidus* in soil over control in the succeeding crop of banana

T1: *B.bassiana* @ 5×10^9 spores ml^{-1} T2: *B.bassiana* @ 5×10^{11} spores ml^{-1} T3:Talc based formulation of *B.bassiana* @ 20 g l^{-1} T4:Talc based formulation of *B.bassiana* @ 30 g l^{-1}
T5: *M.anisopliae* @ 5×10^{10} spores ml^{-1} T6: *M.anisopliae* @ 5×10^{12} spores ml^{-1} T7: Talc based formulation of *M.anisopliae* @ 20 g l^{-1} T8: Talc based formulation of *M.anisopliae* @ 30 g l^{-1}
T9: *B.bassiana* in cow dung @ 50 g T10: *M.anisopliae* in cow dung @ 50 g T11: *B.bassiana* in neem cake @ 50 g T12: *M.anisopliae* in neem cake @ 50 g T13:Carbofuran 3G @ 20 g $plant^{-1}$
T14: Chlorpyriphos 0.03 per cent

some of the infected ones remain alive for a while and dissiminate the spores to other non infected areas also and thus ensure management in a wider area for a longer period. The observations of Godonou *et al.*, (2000) that *B. bassiana* infected insects can move up to 18 m underlines this probability which further enhances the superiority of these fungi.

Field experiments were also conducted in sweet potato to evaluate the fungal pathogens in comparison with synthetic chemicals using the variety Sree Bhadra during 2008. Separate experiments were conducted to evaluate the effect of two methods of application *i.e.* drenching and foliar application.

The effect of soil drenching of *B. bassiana* and *M. anisopliae* spore suspensions, talc based formulations and the fungal culture in cow dung and neem substrates in comparison with the insecticides, imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent was assessed. The effect of the treatments given one month after planting showed the superiority and consistency of the talc based formulations of *B. bassiana* in the trials on sweet potato also. The effect of talc based *B. bassiana* @ 30 g l^{-1} on the population of weevils in the foliage came on par with lambda cyhalothrin 0.025 per cent during the third week and even found superior to this insecticide in the later observations. All the treatments were found to significantly check the population build up of *C. formicarius* when compared to control. The population ranged from 3.19 to 8.67 in the different treatments while a mean population of 11. 89 weevils were recorded in control. The results from the field experiment once again emphasizes the superior efficacy of the isolate Bb 5 of *B. bassiana* to Ma 4. Throughout the experiment imidacloprid 0.006 per cent recorded the least number of weevils in the foliage.

During the first field trial in sweet potato, with respect to the number of galleries and population of grubs present in the treated tubers talc based formulation of *B. bassiana* @ 30 g l^{-1} was superior, 71.45 and 78.82 per cent reduction in the number of galleries and population of grubs in tubers respectively over untreated control was seen. The corresponding reduction in imidacloprid 0.006 per cent was 87.52 and 87.91 respectively. Lambda cyhalothrin 0.025 per cent followed this treatment (Fig. 19 and 20).

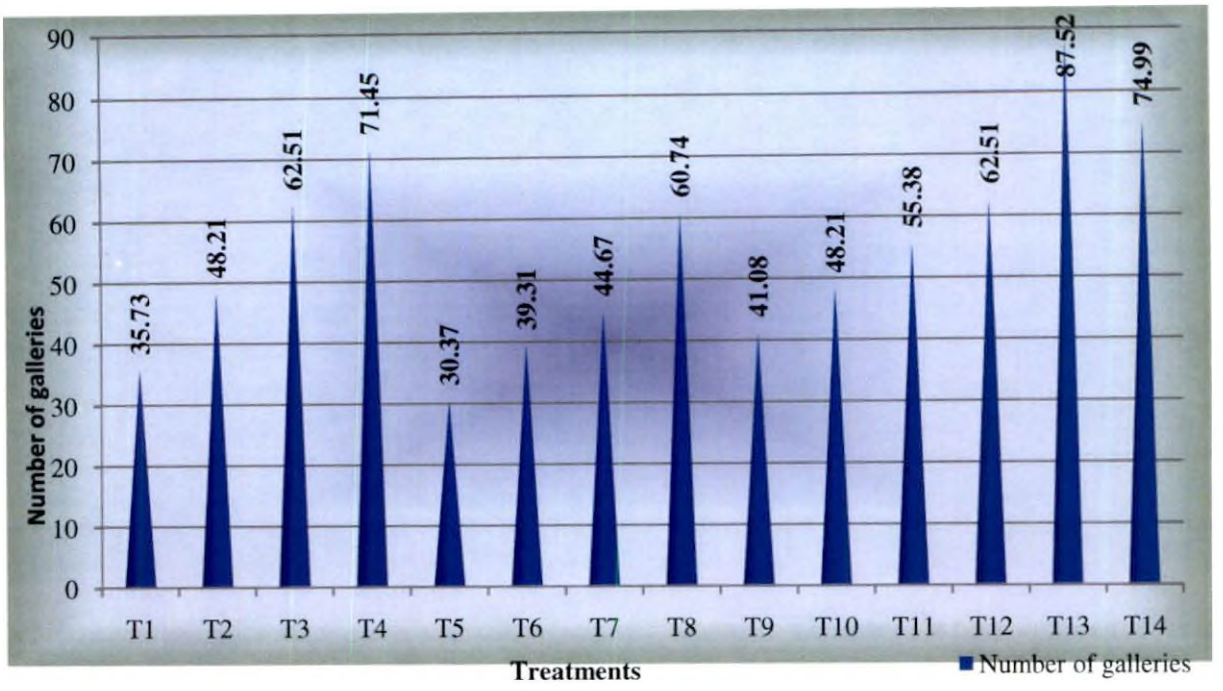


Fig. 19. Percentage reduction in number of galleries in different treatments over control in the first field trial on sweet potato (Soil drenching)

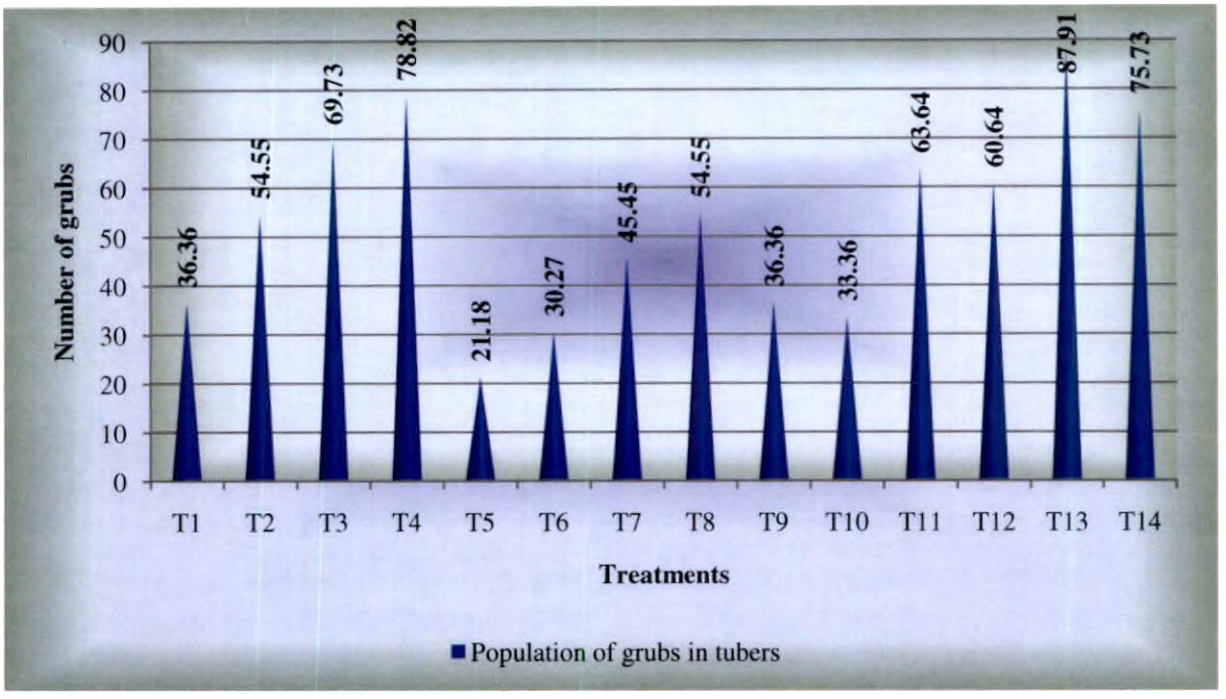


Fig. 20. Percentage reduction in population of grubs in tubers in different treatments over control in the first field trial on sweet potato (Soil drenching)

T1: *B.bassiana* @ 10^9 spores ml^{-1} T2: *B.bassiana* @ 10^{11} spores ml^{-1} T3:Talc based formulation of *B.bassiana* @ 20 g l^{-1} T4:Talc based formulation of *B.bassiana* @ 30 g l^{-1} T5: *M.anisopliae* @ 10^{10} spores ml^{-1} T6: *M.anisopliae* @ 10^{12} spores ml^{-1} T7: Talc based formulation of *M.anisopliae* @ 20 g l^{-1} T8: Talc based formulation of *M.anisopliae* @ 30 g l^{-1}

T9: *B.bassiana* in cow dung @ 50 g T10: *M.anisopliae* in cow dung @ 50 g T11: *B.bassiana* in neem cake @ 50 g

T12: *M.anisopliae* in neem cake @ 50 g T13: Imidacloprid 0.006 per cent T14: Lambda cyhalothrin 0.025 per cent

During the second trial the neem based *B. bassiana* @ 50 g was on par with that of the talc based formulation of *B. bassiana* @ 30 g l^{-1} with respect to the number of grubs present in tubers. Neem based *M. anisopliae* proved better and was on par with the talc based formulations of *B. bassiana* and *M. anisopliae* with respect to the number of galleries. The effect of neem based *B. bassiana* on soil population of weevils was higher and it recorded the least number of weevils kg^{-1} soil (Fig. 21).

During the first field trial the marketable yield (2.63 kg) in talc based formulation of *B. bassiana* @ 30 g l^{-1} was on par with imidacloprid 0.006 per cent (3.5 kg). The B : C ratios being 10.80 and 10.90 respectively in these treatments. This higher ratio obtained for talc based formulation of *B. bassiana* @ 30 g l^{-1} was due to the very low marketable yield in control and also due to the higher price of organic sweet potato. Though more or less a similar trend in marketable yield was noted in the second field trail also, the B : C ratio of the treatment *B. bassiana* talc @ 30 g l^{-1} was higher, the value being 3.0 whereas in imidacloprid 0.006 per cent it was 2.56 and in lambda cyhalothrin 0.025 per cent it was 2.25 (Fig. 22).

In the field trial to evaluate the foliar application of spore suspensions and talc based formulations of fungi and the insecticide imidacloprid 0.006 per cent it was seen that talc based formulation of *B. bassiana* @ 30 g l^{-1} was on par with imidacloprid 0.006 per cent in reducing the population of the weevils in the foliage, number of galleries in tubers and population of weevils in soil. The B : C ratios were 2.23 in *B. bassiana* in talc @ 30 g l^{-1} and 3.19 in imidacloprid 0.006 per cent. From the B : C ratios calculated for talc based formulation of *B. bassiana* @ 30 g l^{-1} in the three field experiments it is inferred that drenching is better than the foliar treatment. The population of natural enemies was low during the period and there was no significant difference in the different treatments.

Though reports are scanty, the effectiveness of both *B. bassiana* and *M. anisopliae* for sweet potato weevil management has been documented earlier. Field application of *M. anisopliae* was reported effective, either as conidial suspension at four weeks after planting or as conidia and mycelia on palay

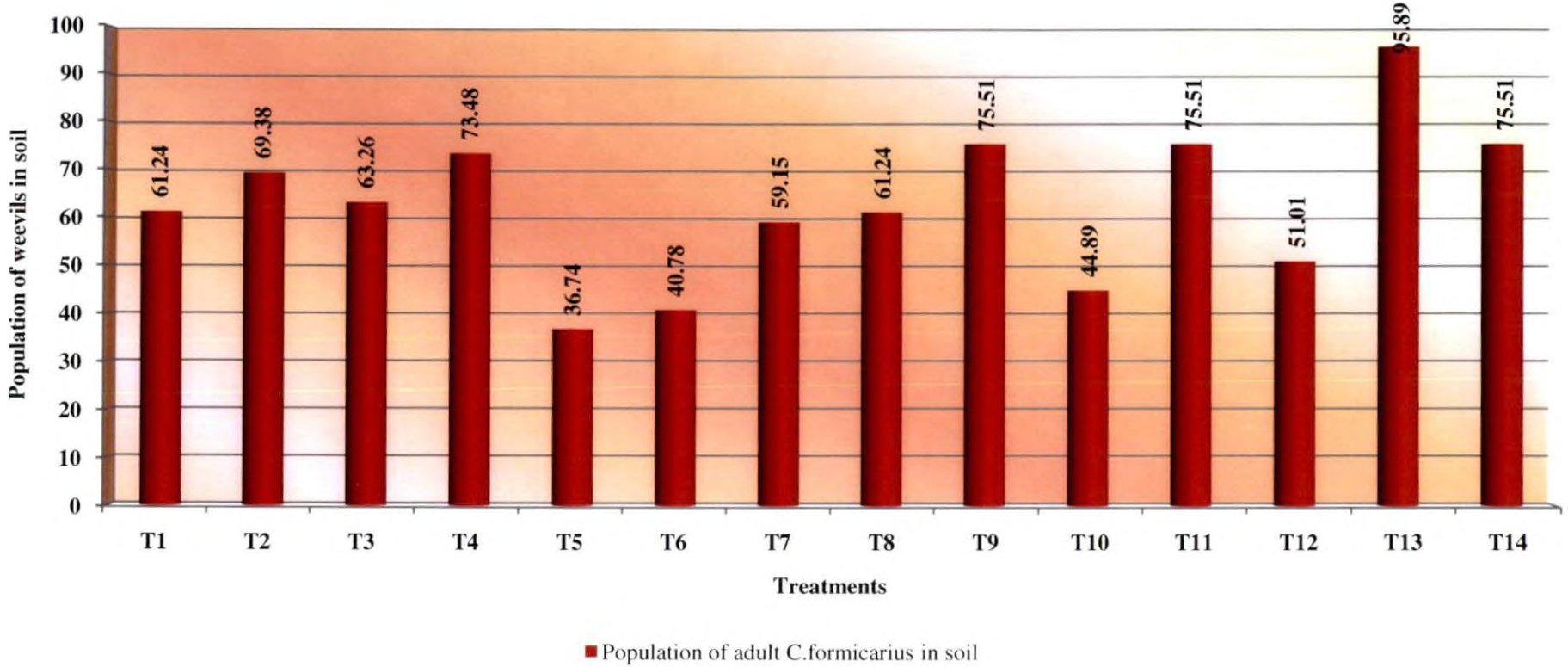
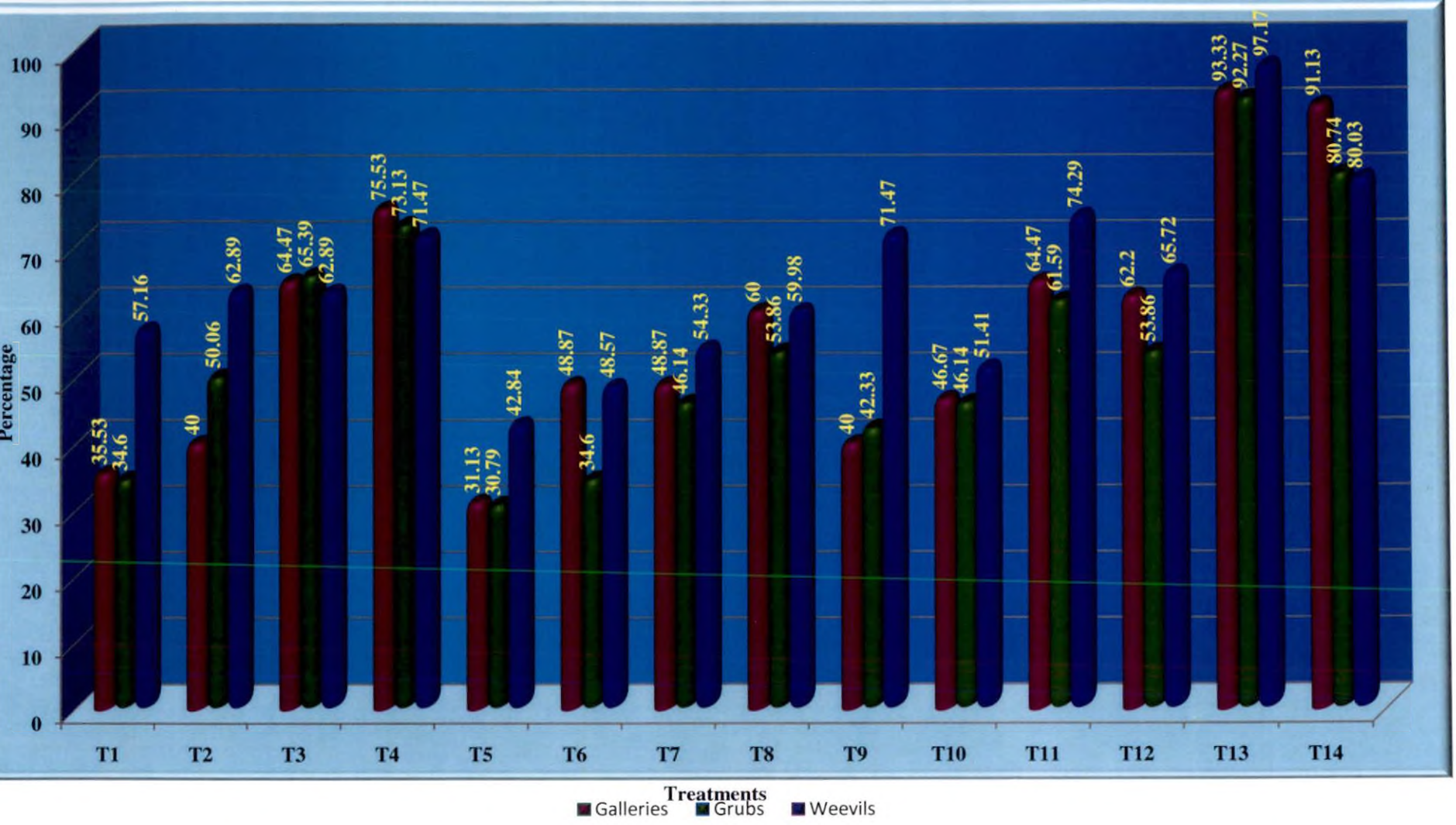


Fig. 21. Percentage reduction in population of adult *C. formicarius* in soil in different treatments over control in the first field trial in sweet potato (Soil drenching)

T1: *B.bassiana* @ 10^9 spores ml^{-1} T2: *B.bassiana* @ 10^{11} spores ml^{-1} T3:Talc based formulation of *B.bassiana* @ 20 g l^{-1} T4:Talc based formulation of *B.bassiana* @ 30 g l^{-1}
T5: *M.anisopliae* @ 10^{10} spores ml^{-1} T6: *M.anisopliae* @ 10^{12} spores ml^{-1} T7: Talc based formulation of *M.anisopliae* @ 20 g l^{-1} T8: Talc based formulation of *M.anisopliae* @ 30 g l^{-1}
T9: *B.bassiana* in cow dung @ 50 g T10: *M.anisopliae* in cow dung @ 50 g T11: *B.bassiana* in neem cake @ 50 g T12: *M.anisopliae* in neem cake @ 50 g T13: Imidacloprid 0.006 per cent
T14: Lambda cyhalothrin 0.025 per cent



22. Percentage reduction in the number of galleries, population of grubs in rhizome and weevils in soil over control in the different treatments during the nd field trial in sweet potato (Soil drenching)

bassiana @ 10^9 spores ml^{-1} T2: *B.bassiana* @ 10^{11} spores ml^{-1} T3:Talc based formulation of *B.bassiana* @ 20 g l^{-1} T4:Talc based formulation of *B.bassiana* @ 30 g l^{-1}

.anisopliae @ 10^{10} spores ml^{-1} T6: *M.anisopliae* @ 10^{12} spores ml^{-1} T7: Talc based formulation of *M.anisopliae* @ 20 g l^{-1} T8: Talc based formulation of *M.anisopliae* @ 30 g l^{-1}

bassiana in cow dung @ 50 g T10: *M.anisopliae* in cow dung @ 50 g T11: *B.bassiana* in neem cake @ 50 g

M.anisopliae in neem cake @ 50 g T13: Imidacloprid 0.006 per cent T14: Lambda cyhalothrin 0.025 per cent

substrate at three weeks after planting which reduced infested roots by nine per cent (Villacarlos and Polo, 1989). Su (1991 a) observed that *B. bassiana* effectively checked the weevil infestation in field with application of conidial suspension as well as broadcasting of soybean containing *B. bassiana* into the rows at planting. Korada *et al.* (2010) are of the opinion that the most important entomopathogenic fungus for sweet potato weevil management is *B. bassiana.*

According to Inglis *et al.* (2001) application of Hyphomycetous fungi has not always provided consistent suppression of insect pests. This may be true but interestingly, the results of the present studies shows that the effects are consistent and reproducible in the field also. Some of the notable observations in this regard are the superior performance of Bb 5 over Ma 4, superior effect of talc based formulations of *B. bassiana* @ 30 g l^{-1} throughout the studies and the moderate effects exhibited by the fungi in cow dung and neem substrates in both the experiments conducted in banana as well as sweet potato.

Today, the most sought after strategy for pest management is IPM wherein different methods, biological, chemical, cultural, mechanical methods etc. are adopted for the management of the noxious pests. It is a fact that in pest management we cannot totally supplant chemical pesticides with bioagents in the immediate future. The role of fungicides in maintaining crop health also cannot be overlooked. Henceforth, the compatibility of the entomopathogenic fungi with pesticides needs elucidation in order to harness their full potential in pest management programmes. So studies were taken up in this line also. The compatibility of *B. bassiana* and *M. anisopliae* with two fungicides *viz.* carbendazim 0.1 per cent and mancozeb 0.2 per cent and six insecticides *viz.* carbofuran 0.3 ppm, carbosulfan 0.1 ppm, imidacloprid 0.006 per cent chlorpyriphos 0.03 per cent lambda cyhalothrin 0.025 per cent and malathion 0.15 per cent were studied using poison food technique. The growth of the fungi in poisoned media, sporulation and the bioefficacy of the fungi cultured in the poisoned media were assessed.

Good compatibility of the insecticide, imidacloprid 0.006 per cent with *B. bassiana* and *M. anisopliae* was evident from the radial growth of these fungi

in the media poisoned with imidacloprid 0.006 per cent, which did not differ significantly from that in control in the observations taken two and five days after inoculation, though significant difference in growth was seen in the observations taken later. When compared to other insecticides and fungicides, the growth of the fungi was superior in imidacloprid 0.006 per cent mixed media. Among all the pesticides tested, malathion 0.15 per cent was the most inhibitory to the growth of *B. bassiana* and *M. anisopliae,* the mean radial growth of *B. bassiana* was 2.72 cm at 13 DAI and that of *M. anisopliae* was 3.53cm at 15 DAI (Fig. 23 and 24). Growth of *B. bassiana* and *M. anisopliae* in carbofuran and carbosulfan treated media was significantly superior than that in chlorpyriphos mixed media in the initial observations taken upto five days, but afterwards there was no significant difference between these three insecticides. The growth of both the fungi in carbendazim 0.1 per cent and mancozeb 0.2 per cent was significantly lower throughout, carbendazim was more inhibitory to the growth of *B. bassiana* whereas mancozeb was more inhibitory to the growth of *M. anisopliae.*

Considerable research has focused on the influence of various agrochemicals on the growth of entomopathogenic fungi *in vitro.* All classes of chemicals were inhibitory to entomopathogenic Hyphomycetes including insecticides and fungicides (Inglis *et al.,* 2001). The present findings corroborates these observations. According to Benz (1987) some insecticides are known to antogonise while some others are known to synergise the activities of these fungi. Ambethgar (2009) found that fungicides in general were antagonistic to *B. bassiana* at varying intensities. He further noted that carbendazim caused total inhibition of the mycelial growth of *B bassiana* even at concentrations of 0.1 x.

Inglis *et al.* (2001) stated that the inhibitory properties of insecticides and fungicides vary both between and within chemical classes. However, a similarity in performance of the fungi cultured in carbofuran and carbosulfan, both belonging to the carbamate group was observed in the present study.

Though imidacloprid 0.006 per cent was equally good in supporting the growth of these fungi as that of non poisoned media sporulation and bioefficacy of the fungi cultured in imidacloprid 0.006 per cent was found reduced when

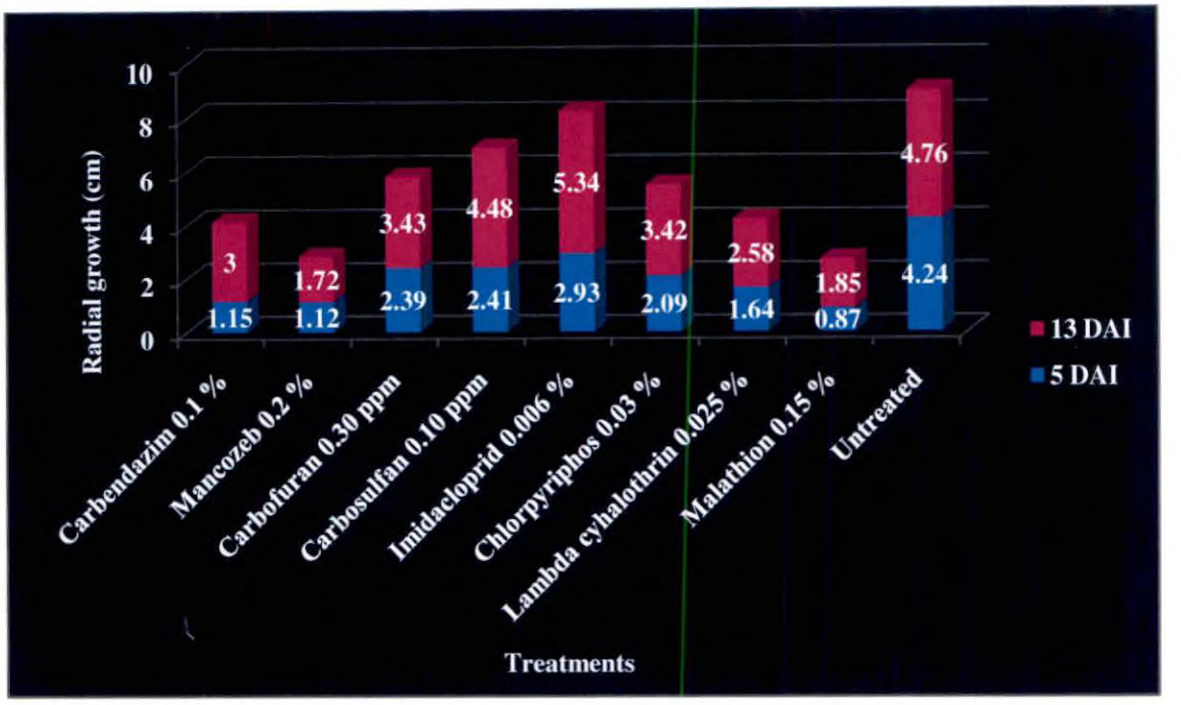


Fig. 23. Radial growth of *B. bassiana* in pesticide amended and untreated media at different intervals after inoculation

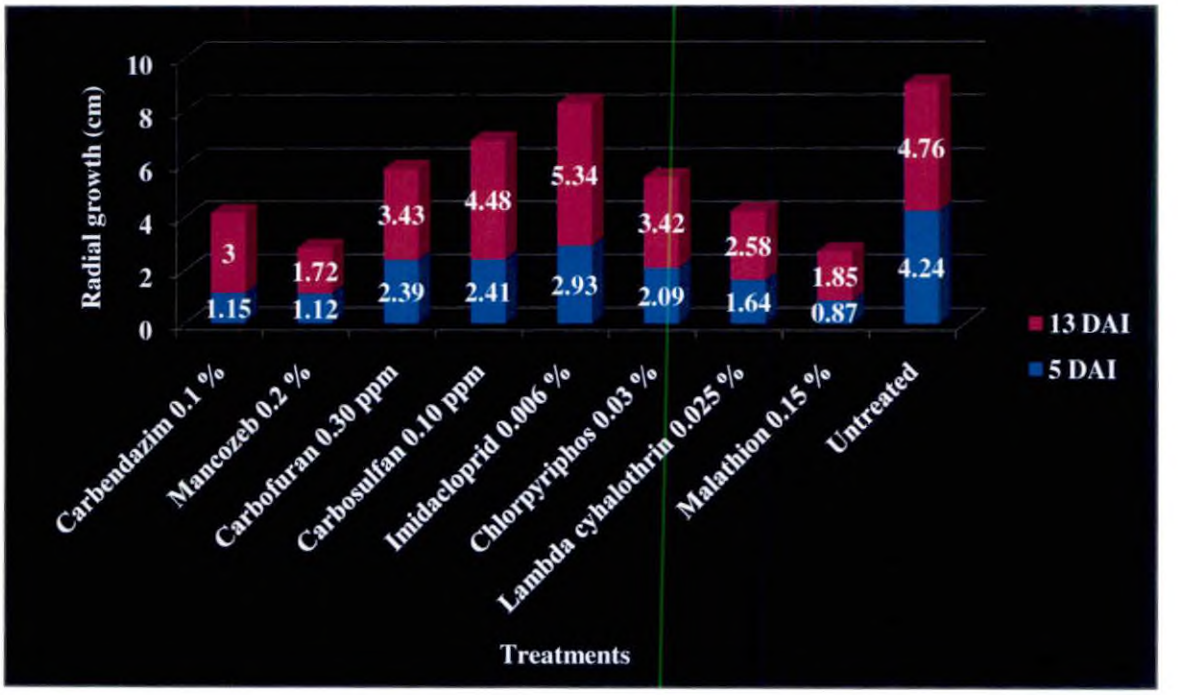


Fig. 24. Radial growth of *M. anisopliae* in pesticide amended and untreated media at different intervals after inoculation

* DAI- Days after inoculation

compared to the fungi grown in nonpoisoned media. Sporulation of *B. bassiana* in media mixed with all the insecticides were on par and it varied from 0.04×10^8 spores ml^{-1} in mancozeb 0.2 per cent treated media to 35.45×10^8 spores ml^{-1} in imidacloprid 0.006 per cent mixed media. However, the sporulation of *M. anisopliae* in imidacloprid amended media (26.74×10^8 spores ml^{-1}) was superior while that in all the other pesticides were on par and it ranged from 1.47×10^8 spores ml^{-1} in carbosulfan 0.1 ppm to 0.03×10^8 spores ml^{-1} in malathion 0.15 per cent.

With respect to the bioefficacy both *B. bassiana* and *M. anisopliae* cultured in imidacloprid 0.006 per cent mixed media indicated higher mortality among the different pesticides, the mortality percentages being 51.16 and 75.52 per cent for the adults and grubs of *B. bassiana* respectively and 35.49 and 44.39 per cent for the adults and grubs of *M. anisopliae* respectively. Bioefficacy of *M. anisopliae* cultured in malathion 0.15 per cent mixed media was also the least when tested against the grubs and adults of *C. formicarius*. Even when cultured in malation mixed media, Bb 5 performed better than Ma 4, 10.86 and 28.81 percentage mortality was recorded for adults and grubs of *C. fomicarius* respectively compared to 8.64 and 15.4 per cent mortality by *M. anisopliae* for the adults and grubs, respectively. Variations among taxa and strains of entomopathogenic Hyphomycetous fungi with respect to the inhibitory effects of agrochemicals on their growth has been recognised earlier (Vanninen and Hokkanen, 1988; Anderson *et al.*, 1988).

A pesticide tolerant strain of the fungus if available could be better utilized in integrated pest management programmes. So with a view to develop pesticide tolerant strains of *B. bassiana* and *M. anisopliae,* they were grown continuously in media poisoned with pesticides. A total of ten passages through poison food media was made. It was seen that both the fungi tolerated high doses of pesticides though there was inhibiton in growth, sporulation and bioefficacy. *B. bassiana* and *M. anisopliae* tolerated even 32 times higher the recommended field dose of imidacloprid whereas the fungus tolerated 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. This indicates the suitability of integrating *B. bassiana* with these chemicals in pest management programmes.

A general trend in the growth, sporulation and bioefficacy of *B. bassiana* was seen in all the ten passages through poisoned media. The fungus showed highest tolerance to imidacloprid. At the tenth passage also the growth of imidacloprid was on par with control and it was followed by that grown in carbosulfan and carbofuran. Perfomance of *M. anisopliae* was also similar to that of *B. bassiana*. The sporulation and bioefficacy of *M. anisopliae* in imidacloprid was on par with control even after ten passages. Next to imidacloprid, the carbamate insecticides, carbosulfan and carbofuran were tolerated by *B. bassiana* and *M. anisopliae* as evident from the data on growth after ten passages. Malathion and mancozeb arrested growth of *B. bassiana* substantially. For *M. anisopliae* the growth was least in malathion. The insecticide, malathion and the two fungicides carbendazim and mancozeb supported less sporulation of *B. bassiana*. So also was the support of malathion for the sporulation of *M. anisopliae*.

Spore production was found to decrease with subculturing in both the fungi. Notable reports in this context are that isolates of *M. anisopliae* suffer pleomorphic deterioration *i.e.* an irreversible loss of the ability to produce conidia in subcultures, influenced by fungal strain, media composition and number of subcultures (Kamp and Bidochka, 2002; Srikant *et al.*, 2011), loss of virulence due to rapid changes in the surface properties of conidia (Shah *et al.*, 2007) and that *M. anisopliae* showed degradation in thirty subcultures, nonetheless in only one of the two strains tested (Kamp and Bidochka, 2002). In the present study comparatively *M. anisopliae* showed more stability than *B. bassiana* during subculture.

With respect to bioefficacy towards adult *C. formicarius*, the fungus cultured in imidacloprid, carbosulfan and carbofuran was on par but significantly less than in control. Moderate mortality was seen with respect to the fungus cultured in chlorpyriphos, carbendazim and lambda cyhalothrin, the mortality

percentages were 24.07, 19.62 and 17.63, respectively. *B. bassiana* cultured in malathion caused only 6.67 mortality after ten passages and that cultured in mancozeb caused only 0.74 per cent. *M. anisopliae* in malathion and mancozeb amended media also showed low mortality, this being 19.86 and 8.64 per cent respectively. The bioefficacy of *B. bassiana* cultured in malathion was less, however the least virulence to the adult of *C. formicarius* was exhibited by the one grown in mancozeb.

Simple approaches such as artificial selection can be depended upon for improving the potential of biocontrol agents (Hoy, 1986). A positive note stating that artificial selection resulted in enhanced fungicide resistance in GHA, a commercial strain of *B. bassiana* was seen in the publication of Shapiro-Ilan *et al.* (2002) and Liu and Bauer (2008). Though such research findings pertaining to fungicide resistance are available, information related to entomopathogenic fungi for insecticide resistance is meager.

The results from an earlier work conducted by Sudharma (2006) revealed the possibilities of developing improved pesticide tolerant strains of the entomopathogenic fungus *Fusarium pallidoroseum* (Cooke) Sacc. through artificial selection. Moreover, variations in the genetic profile of the fungus was seen after ten passages through potato dextrose agar media poisoned with high and varying concentrations of insecticides belonging to different groups. Interestingly, in the study it was observed that variations in the genetic makeup of the fungus were induced by insecticides of the synthetic pyrethroid group. The results of this study set the stage for the present investigations on the development of pesticide tolerance of *B. bassiana and M. anisopliae.*

Adoption of PCR based tools for characterization of fungi has led to the understanding of the different species / isolates in *Beauveria* and *Metarhizium.* Random amplified polymorphic DNA (RAPD) that is based on the use of short general primers that anneal to unspecified regions on the template DNA has been used in many studies (Sandhu *et al.*, 2012).

In the two experiments conducted, to analyse the variations induced in *B. bassiana* and *M. anisopliae* after ten passages through poisoned media

RAPD was followed and similarity coefficients were worked out. Obviously, in both the reactions consistent results could be garnered on the polymorphism, that has developed in *B. bassiana* and *M. anisopliae* subjected to artificial selection. Among the two fungi, polymorphism was higher for *B. bassiana* while 82.3 per cent polymorphism was evident in *B. bassiana*, only 26.58 per cent was seen for *M. anisopliae*. Among the ten universal fungal primers tested (Rfu 1-10), Rfu - 10 was found to give maximum polymorphism of 100 per cent in *B. bassiana* whereas Rfu - 7 produced the maximum polymorphism of 40 per cent in *M. anisopliae* (Fig. 25).

From the dendrogram generated for *B. bassiana* it was seen that at the similarity index 0.12 the nine fungal cultures formed two clusters (Fig. 26). *B. bassiana* treated with the fungicide, carbendazim alone was seen separately with 78 per cent dissimilarity while all the others including the untreated control formed the second cluster. Further, at the similarity index 0.61 a dissimilarity of 39 per cent was noted for the poison treated fungi other than carbendazim, from the fungi in untreated control, which formed the second cluster. The dissimilarity for carbosulfan and imidacloprid was four per cent only and that for chlorpyriphos was two per cent. Fungal culture treated with lambda cyhalothrin and malathion recorded the same dissimilarity and it was only one per cent.

Obviously, the similarity coefficient indicates that the genetic makeup of *B.bassiana* can be altered highly even with ten passages of the fungus through carbendazim and that pesticides vary in their ability to induce such changes.

In the case of *M. anisopliae* at the similarity index of 0.77, the culture from media without poison alone cleaved separately and all the fungal cultures from poisoned media clustered in another group with 23 per cent dissimilarity (Fig. 27). Another striking observation was that at the similarity index of 0. 81 the fungus cultured in fungicides and those cultured in insecticides clustered separately and recorded 19 per cent dissimilarity. The effect imparted by the insecticides were also found to vary slightly with the fungus. Seven per cent dissimilarity was seen between the fungus cultured in media poisoned with carbofuran and carbosulfan and a dissimilarty of two per cent was observed

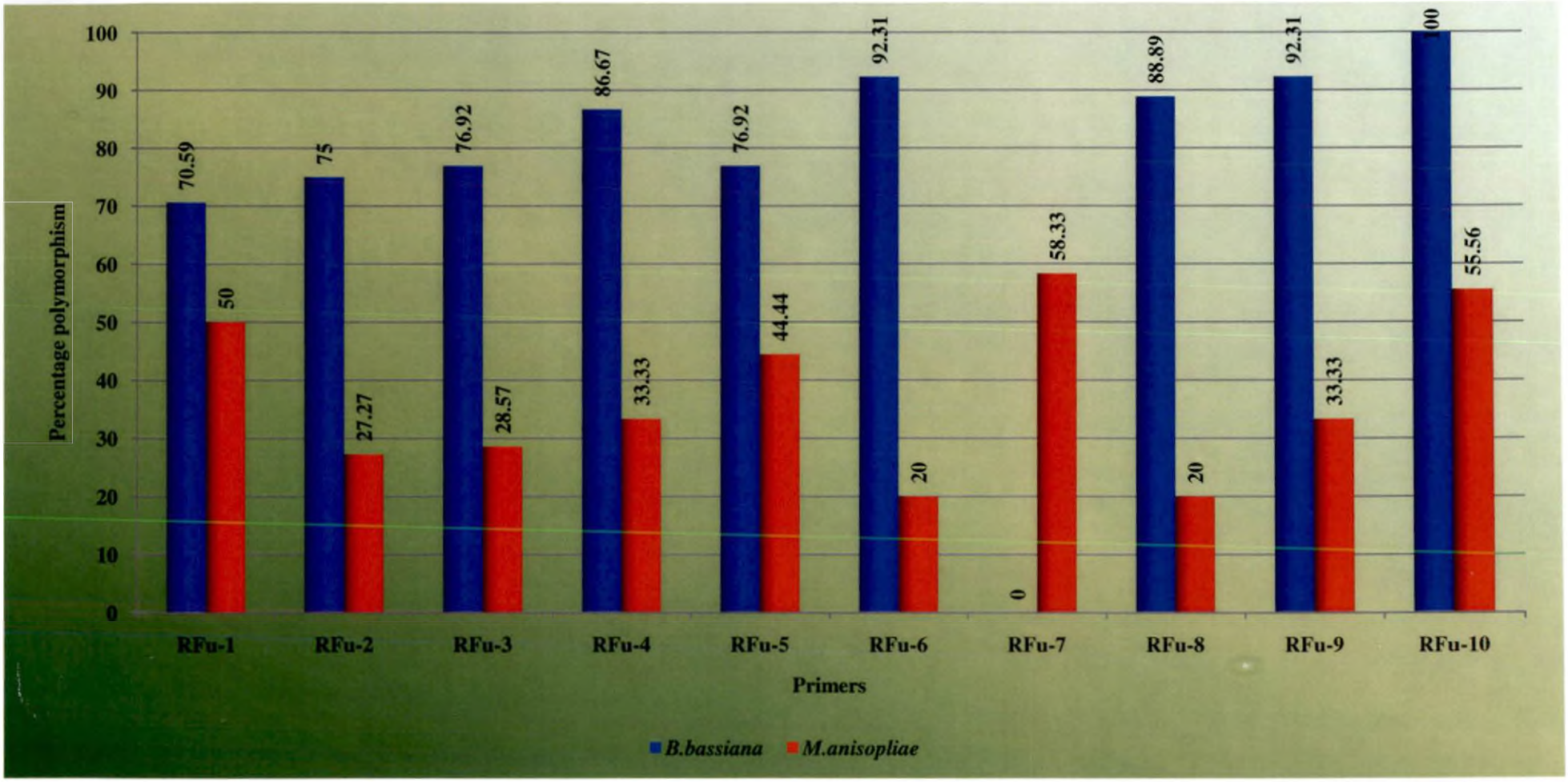


Fig. 25. Polymorphism exhibited by *B. bassiana* and *M. anisopliae* in the ten primers

between the culture in imidacloprid and lambda cyhalothrin. A comparison of the changes induced in the two fungi reveal that changes are higher for *B. bassiana* when compared to *M. anisopliae*.

The data garnered explicitly indicate that chemicals induced changes in the genetic make up of the fungal pathogens, *B. bassiana* and *M. anisoplae* and that it is possible to develop new strains. Hoy (1986) and Shapiro-Ilan *et al.* (1996) has the opinion that enhanced or superior beneficial traits in biological control agents can be lost or reduced when selection pressure is removed. Considering their views, the stability aspects of these induced changes, a toss-up, warrants further studies.

Another aspect that should not be over looked is the fact elucidated by Tinline and Noveillo (1971) and Messias and Azevedo (1980) that parasexual cycle, the main alternative for asexual fungi to legitimately exchange genetic material is present for the entomopathogen, *M. anisopliae*. Single spore isolation and successive subculturing of the wild type culture of *M. anisopliae* strain V275, led to the discovery of mutants with distinct morphological, biochemical, and genetic characteristics. Moreover, the presence of new genotypes indicated that mitotic recombination events had occurred.

From the present observations and related literature it can be concluded that *M. anisopliae* and *B. bassiana* are pathogens, the genetic makeup of which can be changed by the media or the environmental conditions and this may be the reasons for the existence of the numerous isolates reported from the different parts of the world.

Species such as *B. bassiana* and *M. anisopliae* have wide host ranges, spanning numerous orders within the Phylum Arthropoda. It is now recognized that *B. bassiana* and *M. anisopliae* contain a diverse assemblage of genotypes and probably comprise "species complexes" and therefore, it is not surprising that within these taxa, grouped on morphological characters individual isolates can exhibit a substantially restricted host range (Inglis *et al* ., 2001). Considering such aspects of the fungi, collection of different isolates and their evaluation is of utmost importance for their development into a commercially viable biopesticide.

Different diagnostic tools have been adopted for molecular characterization of fungi such as rDNA (Glare and Inwood, 1998), ISSR and AFLP (Muro *et al.*, 2003; Lopes *et al.*, 2013).

During the course of study nine isolates were collected and identified as *Beauveria* sp., *B. bassiana, B. brongniartii* , *Metarhizium* sp., *Metarhizium* sp., *Metarhizium album, Metarhizium anisopliae* var *majus, Fusarium moniliformae* and *Paecilomyces* sp. through ITS sequencing. These internal transcribed spacer sequences have been widely used in fungal systematics (Driver *et al.*, 2000). It has long been recognised that many isolates of *Metarhizium* are specific and they have been assigned variety status but recently they have been assigned as new *Metarhizium* species and nine distinct species have now been assigned to the well known *M. anisopliae* (Bischoff *et al.*, 2009), of which one is *M. majus* stat.nov (= *M. anisopliae* var. major). In the present study *M. majus* was collected from *O. rhinoceros*. *F. moniliformae* was isolated from *H. vigintioctopunctata*, which is in accordance with the earlier observations made by Jacob *et al.* (1978) and Beevi and Jacob (1982).

Considering the various information garnered in the present study the following inferences are made. Suggestions are also given to keep the coleopteran pests at bay.

- The isolate Bb 5 of *B. bassiana* and the isolate Ma 4 of *M. anisopliae* were pathogenic to *A. foveicollis, B. fulvicorne, C. formicarius, C. sordidus, H.vigintioctopunctata, L. ramakrishnai, M. circumdata O. rhinoceros* and *R. ferrugineus*. The grubs as well as adults of all these insects were susceptible to infection by these fungi. The grubs of these insects succumbed to the infection at a lower dose compared to the adults.
- The reports on the pathogenicity of *B. bassiana* and *M. anisopliae* to *A. foveicollis, L. ramakrishnai* and *M. circumdata* are new.

- Of the two fungi tested Bb 5 was more virulent than Ma 4 to all the test insects except *O. rhinoceros*.
- The LC_{90} values fixed for the nine coleopterans form a basis for fixing the field dose of *B. bassiana* and *M. anisopliae*.
- Cow dung, wheat bran, rice bran and neem cake are ideal substrates for the multiplication of *B. bassiana*. Cow dung and wheat bran are ideal for *M. anisopliae*.
- Talc based formulation of *B. bassiana* and *M. anisopliae* maintained cfu as per standards upto three months.
- Soil drenching of *B. bassiana* talc based formulation @ 30 g $plant^{-1}$ was the best treatment for the management of *C. sordidus*.
- Soil drenching of *B. bassiana* talc based formulation @ 30 g $plant^{-1}$ and *B. bassiana* spore suspension @ 5×10^{11} spores ml^{-1} are superior treatments for the management of sweet potato weevil.
- *B. bassiana* and *M. anisopliae* tolerated high doses of pesticides though there was inhibition in growth, sporulation and bioefficacy and both the fungi tolerated even 32 times higher the recommended field dose of imidacloprid.
- Subculturing reduced spore count of *B. bassiana* and *M. anisopliae* and this rate of reduction was greater for *B. bassiana*.
- *B. bassiana* exhibited cent per cent polymorphism in the primer RFu-10 and the primer RFu-7 recorded 58.33 per cent polymorphism for *M. anisopliae*.
- Among the two fungi, polymorphism was higher for *B. bassiana*. The genetic makeup of *B. bassiana* can be altered highly even with ten passages of the fungus through carbendazim and that pesticides varied in their ability to induce such changes. It is possible to develop

pesticide tolerant strains through artificial selection. Stability aspects of the induced changes needs further investigations.

- Nine isolates of the fungi identified through ITS sequencing could be exploited in insect specific management programmes.

Summary

6. SUMMARY

The mounting problems created by the abuse of synthetic pesticides and the growing concerns on such issues ingeminates the need for identification of safe alternatives in pest management. In this context, biological control of crop pests has gained popularity all over the world. Entomopathogenic fungi are now recognized as important tools as they regulate the pest population without affecting the non targets. Considering this, the present research work entitled 'Evaluation of entomopathogenic fungi for the management of coleopteran pests and characterisation of pesticide tolerant strains' was undertaken at College of Agriculture, Vellayani during 2007-2014. The fungi selected for the study were the white muscardine fungus, *Beauveria bassiana* (Balsamo) Vuillimen and the green muscardine fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin. The objectives were assessment of the pathogenicity and virulence of *B. bassiana* and *M. anisopliae*, bioassay to fix LC_{50}, LC_{90} and LT_{50}, assessment of the field efficacy of the fungi and determination of the compatibility with pesticides. Besides this, development of pesticide tolerant strains and their characterization was also attempted. The isolate PDBC Bb 5 of *B. bassiana* and the isolate PDBC Ma 4 of *M. anisopliae*, obtained from Project Directorate of Biological Control (PDBC) now known as National Bureau of Agriculturally Important Insects were evaluated against nine important coleopteran pests *viz. Aulacophora foveicollis* Lucas, *Basilepta fulvicorne* Jacoby, *Cylas formicarius* F., *Cosmopolites sordidus* Germ., *Henosepilachna vigintioctopunctata* F., *Lanka ramakrishnai* Prathapan & Viraktamath, *Metriona circumdata* H., *Oryctes rhinoceros* Linn. and *Rhynchophorus ferrugineus* F. The quintessences of the results are presented below.

- Application of spore suspensions from fourteen day old cultures of *B. bassiana* and *M. anisopliae* in potato dextrose broth showed that both the fungi were pathogenic to all the nine insects tested. The grubs as well as adults of all these insects were susceptible to infection by these fungi. The

pathogenicity to the pupal stages of all these insects except to that of *A. foveicollis* and *L. ramakrishnai* were evaluated. The pupa of *R. ferrugineus* treated with *M. anisopliae* alone was not infected while all other pupae treated with *B. bassiana* and *M. anisopliae* were infected. The pathogenicity to eggs were evaluated for three insects *viz. H. vigintioctopunctata*, *M. circumdata* and *O. rhinoceros*. The egg stages of all these insects were also susceptible to the infection of the fungi.

- The symptoms produced in the test insects by *B. bassiana* were more or less the same but the period taken for the expression of symptoms varied. The infected adults of the test insects were active for two to four days. As pathogenesis advanced food uptake and movement were reduced considerably. Upon acquiring infection the adults were hideous. The size of the infected grubs was reduced. The cadavers, both adults and grubs initially were soft and later hardened, ultimately white fungal mycelia covered all over the body of the dead insects. Distinctive behavior of remaining in an upside down position on the surface of top most leaves by *B. bassiana* infected adults of *B. fulvicorne* and *M. circumdata* was also observed.

- Ma 4 infected insects showed sluggishness, reduced feeding, loss of colour and development of darkened spots and patches on the body. The colour of the mycelia produced in *M. anisopliae* infection was initially white, which changed to olive green and ultimately dark green.

- Assessment of the virulence of the two fungi at the comparable dose of 10^{8} spores ml^{-1} revealed that Bb 5 was superior to Ma 4 in its ability to infect the adults of all the test insects except that of *O. rhinoceros* and *B. fulvicorne*. At this concentration of 10^{8} spores ml^{-1} Ma 4 did not produce infection in *R. ferrugineus* adults. A similar trend in the infection of *M. anisopliae* was

noted against the grubs of all the test insects except to that of the grubs of *O. rhinoceros* i.e. Bb 5 was superior to Ma 4.

- Bioassay of *B. bassiana* and *M. anisopliae* was conducted against the adults and grubs of all the nine test insects using varying concentrations of the pathogens prepared from fourteen day old culture of the fungi grown in potato dextrose broth. From the probit analysis, it was seen that to achieve fifty per cent mortality within the shortest periods, for the adult *A. foveicollis* a concentration of 5. 27 × 10^8 spores ml^{-1} was essential and for the adults of *B. fulvicorne*, *C. sordidus*, *C. formicarius*, *H. vigintioctopunctata*, *L. ramakrishnai*, *M. circumdata*, *O. rhinoceros* and *R. ferrugineus* the corresponding values were 6.53 × 10^8, 4.24 ×10^9, 5.62 × 10^7, 3.99 × 10^8, 6.76 × 10^8, 7.26 × 10^9, 1.56 × 10^{15} and 3.76 × 10^{13} spores ml^{-1}, respectively.

- The LC_{50} value were much less for the grubs when compared to the adult coleopterans. The LC_{50} values for the grubs of the above mentioned insects were 2. 15 × 10^7, 1.87 × 10^6, 3.83 × 10^8, 2.94 × 10^7, 2.79 × 10^7, 5.72 × 10^6, 4.96 × 10^8, 6.22 × 10^{11} and 4.64 × 10^{11} spores ml^{-1}, respectively. Corresponding LC_{90} values were also worked out. The lethal time to obtain fifty per cent mortality also varied with insects.

- The lethal concentrations of *M. anisopliae* required to obtain fifty and ninety per cent mortalities in all the nine test insects were also worked out. With respect to *M. anisopliae* also the values of LC_{50}, LC_{90} and LT_{50} for the grubs of the test insects were less compared to the adults. To attain LC_{50} within the shortest periods in the adults of *A. foveicollis, B. fulvicorne, C. sordidus, C. formicarius, H. vigintioctopunctata, L. ramakrishnai, M. circumdata, O. rhinoceros* and *R. ferrugineus* the concentrations of the spores of Ma 4 required were 11.42 × 10^{10}, 3.99 × 10^8, 8.86 × 10^{10}, 6.27 × 10^9, 6.96 × 10^8,

3.66×10^{11}, 6.13×10^{10}, 2.58×10^{13} and 2.73×10^{15} spores ml^{-1}, respectively. With respect to the grubs, the values were 4.91×10^{7}, 6.09×10^{8}, 4.81×10^{8}, 5.83×10^{8}, 4.95×10^{7}, 2.42×10^{8}, 4.64×10^{9}, 3.79×10^{8} and 10.29×10^{13} spores m^{-1}, respectively. The minimum period to obtain fifty per cent mortality was observed in the case of the grubs of *C. sordidus* (8. 309 days).

- The adult of *A. foveicollis*, *B. fulvicorne*, *L. ramakrishnai*, *M. circumdata*, *H. vigintioctopunctata* and *C. formicarius* feed on the foliage and since a higher dose is required for managing the adults when compared to the grubs, the dose corresponding to LC_{90} for the adults needs to be recommended for foliar spray in the field. The spore concentrations of Bb 5 required for these insects were 10.22×10^{8}, 12.65×10^{8}, 12.65×10^{8}, 14.04×10^{9}, 7.91×10^{8} and 11.54×10^{7} spores ml^{-1}. The corresponding concentrations for Ma 4 were 20.62×10^{10}, 7.58×10^{8}, 7.69×10^{11}, 14.03×10^{10}, 12.27×10^{8} and 14.21×10^{9} spores ml^{-1}, respectively.

- The adults and grubs of *C. formicarius* are present both in the aerial parts as well as in tubers and for managing them the LC_{90} for the adults *i.e.* 11.54×10^{7} and 14.21×10^{9} spores ml^{-1} are required for Bb 5 and Ma 4, respectively.

- Experiment was conducted to identify cost effective substrates for *B. bassiana* and *M. anisopliae*. Good growth of *B. bassiana* was seen in cow dung, neem cake, rice bran and wheat bran. *M. anisopliae* grew profusely in cow dung, wheat bran and ground nut oil cake. Estimation of spore count one month after storage revealed that the spore count of *B. bassiana* was the highest in cow dung (127.69×10^{5} spores ml^{-1}) and this was significantly higher than in other substrates. The peak sporulation was observed in the samples drawn two months after storage. The determination of cfu at different intervals after storage of the fungi in different substrates

revealed that during the first month after storage, in general the viable units were also low as in the case of spore count, thereafter there was substantial increase and during the second month the colony forming units of *B. bassiana* was high, 7717. 42 × 10^5 cfus g^{-1} in cow dung and it was significantly superior than in other treatments. The count of the colony forming units in wheat bran, rice bran and neem cake were also significantly higher than in other substrates. There was a decline in the cfu during the third month in all the substrates except in saw dust which showed a slight increase. The ideal substrates that maintained the viability of *B. bassiana* were cow dung, wheat bran, rice bran and neem cake.

- With respect to *M. anisopliae* also cow dung supported maximum cfu. Significantly superior counts were noted in cow dung during the first and third months after storage compared to other substrates, the cfu in cow dung during these months were 23. 98 × 10^5 and 7.29 × 10^5 cfu g^{-1} respectively but during the second month after storage maximum cfu was noted in wheat bran.

- The bioefficacy of fungi cultured in different substrates and stored for different months was also evaluated against the grubs and adults of *C. formicarius*. With respect to bioefficacy also, the fungus cultured in cow dung ranked the highest. When sprayed with spore suspensions of *B. bassiana* stored for one month the mean mortality percentage of the grubs was 72.34 at 14 DAT. This was followed by wheat bran and differed significantly. Moderate efficacy of the fungus cultured in neem cake, groundnut oil cake, rice bran, coconut oil cake and rice husk and lowest efficacy of fungus cultured in saw dust was also evident. As in the case of *B. bassiana* the bioefficacy of *M. anisopliae* cultured in different substrates revealed that the fungus grown in cow dung was superior in their efficacy to the adults and grubs of *C. formicarius* and that the effect was maximum in the spore suspension prepared and stored for two months.

- Talc based formulations of both fungi maintained the required standards of colony forming units in the formulation up to three months after storage. Application of talc based formulation of *B. bassiana* @ 20 g l^{-1} against adults and grubs of *C. formicarius* showed 85.16 and 100 per cent mortality respectively. When sprayed with talc based formulation of *M. anisopliae* @ 20 g l^{-1} the mortality percentages were 55.46 and 68.49 respectively.

- To assess the effect of *B. bassiana* Bb 5 and *M. anisopliae* Ma 4 in the field, spore suspensions of the fungi, fungal culture in cow dung and neem cake substrates and the talc based formulations were evaluated in comparisons with insecticides and untreated control against *C. sordidus* in banana variety Nendran. Two trials, one in main crop and another in the succeeding crop were undertaken. The treatments given at three months after planting showed that the best treatment was talc based *B. bassiana* @ 30 g l^{-1} excepting the insecticide check, chlorpyriphos 0.03 per cent. In the succeeding crop talc based *B. bassiana* @ 30 g l^{-1} was even superior to the insecticide check, chlorpyriphos 0.03 per cent. The least number of galleries (0.63) and the least number of grubs in the rhizomes (0.29) besides the lowest number of adult *C. sordidus* in soil samples were seen in talc based *B. bassiana* @ 30 g l^{-1} treated banana in the succeeding crop.

- All the other treatments also effectively checked the incidence of *C. sordidus*. The number of galleries observed in the rhizome and the number of grubs in the rhizome in untreated control during the main crop were 8.33 and 12.27 and corresponding values for the succeeding crop were 10.94 and 15.65 respectively, which were significantly higher than in all other treatments. The effect of spore suspension as well as talc based formulation of *B. bassiana* were better than the talc based formulation of *M. anisopliae* as well as its spore suspension and came on par with the granular application of carbofuran 3G @ 20 g $plant^{-1}$ in the main crop. The mean number of galleries noted

were 2.32, 1.31, 0.99 and 0.91 in talc based *B. bassiana* @ 20 g l^{-1}, *B. bassiana* @ 5×10^{11} spores ml^{-1}, carbofuran 3 G @ 20 g plant $^{-1}$ and talc based *B. bassiana* @ 30 g l^{-1}, respectively. The effect of application of *B. bassiana* and *M. anisopliae* in cow dung and neem based substrates showed moderate effect with respect to the number of galleries and number of grubs.

- The B : C ratio of 1.57 calculated for the organically produced banana using *B. bassiana* talc based formulation @ 30 g plant $^{-1}$ was also higher compared to the B : C ratio of 1.24 for chlorpyriphos 0.03 per cent in the main crop. Similarly the B : C ratios for the treatments with *B. bassiana* spore suspension @ 5×10^{11} spores ml $^{-1}$ and talc based formulation of *M. anisopliae* @ 30 g l^{-1} were also higher than chlorpyriphos 0.03 per cent treatment.

- The B : C ratios calculated for the treatments with talc based *B. bassiana* @ 30 g l^{-1} and spore suspension of *B. bassiana* 5×10^{11} spores ml^{-1} in the succeeding crop of banana were 1.78 and 1.49 which were higher than that for chlorpyriphos 0.03 per cent.

- Field experiments conducted in sweet potato to evaluate the fungal pathogens in comparison with synthetic chemicals using the variety Sree Bhadra during 2008-10 showed that talc based *B. bassiana* @ 30 gl^{-1} ranked first in reducing the extent of damage inflicted to the tubers in terms of galleries produced by the weevils excepting the treated checks. With respect to the population of grubs present in the treated tubers during the first field trial in sweet potato talc based *B. bassiana* @ 30 g l^{-1} was on par with imidacloprid 0.006 per cent. While considering the extent of damage inflicted to the tubers in terms of galleries produced by the weevils also a similar trend was seen. Neem based *M. anisopliae* proved better than that in cow dung and was on par with the talc based formulations of *B. bassiana* @ 20 g l^{-1} and *M. anisopliae* @ 30 g l^{-1} with respect to the number of galleries. The neem based *B. bassiana* @

50 g was on par with that of the talc based formulation of *B. bassiana* @ 30 g l^{-1} with respect to the number of grubs present in tubers in the second trial.

- The marketable yield was also highest in talc based *B. bassiana* @ 30 g l^{-1} excepting the yield in the insecticide checks, imidacloprid 0.006 per cent and lambda cyhalothrin 0.025 per cent in the first field trial. Though more or less a similar trend in marketable yield was noted in the second field trial also, the B : C ratio of the treatment with talc based *B. bassiana* @ 30 g l^{-1} was higher, the value being 3.00 whereas in imidacloprid 0.006 per cent it was 2.56 and in lambda cyhalothrin it was 2.25.

- From the B : C ratios calculated in the three field experiments it is inferred that drenching of talc based formulation of *B. bassiana* @ 30 g l^{-1} was better than the foliar treatment.

- The population of hymenopteran parasitoids and predatory spiders in the sweet potato field in the different treatments was not significant.

- The compatibility of *B. bassiana* and *M. anisopliae* with two fungicides *viz.* carbendazim 0.1 per cent and mancozeb 0.2 per cent and six insecticides *viz.* carbofuran 0.3 ppm, carbosulfan 0.1 ppm, imidacloprid 0.006 per cent chlorpyriphos 0.03 per cent lambda cyhalothrin 0.25 per cent and malathion 0.15 per cent were studied using poison food technique. It was seen that the sporulation of *B. bassiana* in media mixed with all the insecticides were on par and it varied from 0.04×10^8 spores ml^{-1} in mancozeb 0.2 per cent treated media to 35.45×10^8 spores ml^{-1} in imidacloprid 0.006 per cent mixed media. The sporulation of *M. anisopliae* in imidacloprid (26.74×10^8 spores ml^{-1}) was superior while that in all the other pesticides were on par and it ranged from 1.47×10^8 spores ml^{-1} in carbosulfan 0.1 ppm to 0.03×10^8 spores ml^{-1}. Good compatibility of the insecticide, imidacloprid 0.006 per cent with *B. bassiana* and *M. anisopliae* was evident from the radial growth of these

two fungi in the media poisoned with imidacloprid. The bioefficacy of both *B. bassiana* and *M. anisopliae* cultured in imidacloprid 0.006 per cent mixed media indicated higher mortality among the different pesticides.

- Attempts were made to develop pesticide tolerant strains of *B. bassiana* and *M. anisopliae.* For this they were grown continuously in media with varying doses of pesticides. A total of ten passages through poison food media was made. It was seen that both the fungi tolerated high doses of pesticides though there was inhibition in growth, sporulation and bioefficacy. *B. bassiana* and *M. anisopliae* tolerated even 32 times higher the recommended field dose of imidacloprid.

- Spore production was found to decrease with subculturing in both the fungi. The rate of reduction noted for *M. anisopliae* was lower when compared to *B. bassiana.* The fungus cultured in imidacloprid, carbosulfan and carbofuran were on par in their bioefficacy towards adult *C. formicarius*, but these were significantly less than that in control. Moderate mortality was seen with respect to the fungus cultured in chlorpyriphos, carbendazim and lambda cyhalothrin, the mortality percentages were 24.07, 19.62 and 17.63, respectively.

- To analyse the variations induced in *B. bassiana* and *M. anisopliae* after ten passages through poisoned media RAPD was followed and similarity coefficients were worked out. The experiments were conducted twice. In both reactions, consistent results were seen in the polymorphism developed in *B. bassiana* and *M. anisopliae* subjected to artificial selection. Among the two fungi, polymorphism was higher for *B. bassiana.* 83.19 per cent polymorphism was evident in *B. bassiana,* whereas 38.46 per cent only was seen for *M. anisopliae.* Among the ten universal fungal primers, RFu 1-10 tested, RFu 10 was found to give maximum polymorphism.

- The similarity coefficients indicates that the genetic makeup of *B. bassiana* can be altered highly even with ten passages of the fungus through carbendazim and that pesticides vary in their ability to induce such changes. A comparison of the changes induced in the two fungi revealed that changes are higher for *B. bassiana* when compared to *M. anisopliae.*

- New fungal pathogens were collected from the fields in Thiruvananthapuram and Idukki districts. These were identified through ITS sequencing. The new isolates were *Beauveria* sp. from *C. formicarius, B. bassiana* from *B. fulvicorne, B. brongniartii* from *M. circumdata, Metarhizium* sp. from *B. fulvicorne; Metarhizium* sp., *Metarhizium album* and *Metarhizium anisopliae* var *majus* from *O. rhinoceros, Fusarium moniliformae* from *H. vigintioctopunctata* and *Paecilomyces* sp. from *C. sordidus* and these isolates can be exploited in insect specific biocontrol programmes.

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EVALUATION OF ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF COLEOPTERAN PESTS AND CHARACTERISATION OF PESTICIDE TOLERANT STRAINS

by

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ABSTRACT

Development of safe and sustainable technologies for pest management is the need of the hour to counter the adversities created by the synthetic pesticides. Considering this, the research on "Evaluation of entomopathogenic fungi for the management of major coleopteran pests and characterisation of pesticide tolerant strains" was carried out in the Department of Agricultural Entomology, College of Agriculture during 2007-2014. The main objectives of the study were to assess the pathogenicity of entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillimen and *Metarhizium anisopliae* (Metschnikoff) Sorokin against coleopteran pests, determination of LC_{50}, LC_{90} and LT_{50}, development of formulations, assessment of field efficacy, compatibility with pesticides and development of pesticide tolerant strains and molecular characterization of the fungi.

The isolates PDBC Bb 5 and PDBC Ma 4 were evaluated against nine coleopteran pests *viz. Aulacophora foveicollis* Lucas, *Basilepta fulvicorne* Jacoby, *Cylas formicarius* F., *Cosmopolites sordidus* Germ., *Henosepilachna vigintioctopunctata* F., *Lanka ramakrishnai* Prathapan & Viraktamath, *Metriona circumdata* H., *Oryctes rhinoceros* Linn. and *Rhynchophorus ferrugineus* F. Both the fungi were pathogenic to the grubs as well as adults of all the nine insects tested. Though the symptoms produced in the test insects by both the fungi were more or less the same, differences in the behavior of the infected insects were seen. The period taken for the expression of symptoms also varied. The virulence of these two fungi was determined against these nine coleopterans. Ma 4 was found inferior to Bb 5 in its ability to infect the adults of all the test insects except to that of *O. rhinoceros* at the comparable dose of 10^8 spores ml^{-1}.

Further, bioassay was conducted against the adults and grubs of all the nine test insects and the LC_{50}, LC_{90} and LT_{50} were worked out. From the probit analysis, it was seen that to achieve fifty per cent mortality of the adult coleopterans

with *B. bassiana* within the shortest periods, spore concentrations of 5. 27 × 10^8, 6.53 × 10^8, 4.24 ×10^9, 5.62 × 10^7, 3.99 × 10^8, 6.76 × 10^8, 7.26 × 10^9, 1.56 × 10^{15} and 3.76 × 10^{13} spores ml^{-1} was essential for *A. foveicollis, B. fulvicorne, C. sordidus, C. formicarius, H. vigintioctopunctata, L. ramakrishnai, M. circumdata, O. rhinoceros* and *R. ferrugineus* respectively. The LC_{50} values were much less for the grubs when compared to the adult coleopterans and the values were 2. 15 × 10^7, 1.87 × 10^6, 3.83 × 10^8, 2.94 × 10^7, 2.79 × 10^7, 5.72 × 10^6, 4.96 × 10^8, 6.22 × 10^{11} and 4.64 × 10^{11} spores ml^{-1} respectively for the grubs of the above mentioned insects. Corresponding LC_{90} values were also worked out. The lethal time to obtain fifty percent mortality also varied with insects. With respect to *M. anisopliae* 11.42 × 10^{10}, 3.99 × 10^8, 8.86 × 10^{10}, 6.27 × 10^9, 6.96 × 10^8, 3.66 × 10^{11}, 6.13 × 10^{10}, 2.58 × 10^{13} and 2.73 × 10^{15} spores ml^{-1} respectively was required for the adult coleopterans and 4.91 × 10^7, 6.09 × 10^8, 4.81 × 10^8, 5.83 × 10^8, 4.95 × 10^7, 2.42 × 10^8, 4.64 × 10^9, 3.79 × 10^8 and 10.29 ×10^{13} spores ml^{-1} respectively for the grubs. From the LC_{50} and LC_{90} values, information on the effective field doses of these two fungi against the nine coleopterans could be garnered.

Inorder to identify cost effective materials for the multiplication of the fungus, nine substrates were evaluated. The ideal substrates that maintained the viability of *B. bassiana* were cow dung, wheat bran, rice bran and neem cake. Cow dung and wheat bran supported maximum cfu of *M. anisopliae*. The peak sporulation of the fungi was observed in the samples drawn two months after storage. Talc based formulations of both fungi maintained the required standards of colony forming units in the formulation upto three months after storage. The cfu at third month after storage for *B. bassiana* and *M. anisopliae* were 0.2 × 10^9 and 0. 24 × 10^9 cfu g^{-1} respectively. The bioefficacy of fungi cultured in different substrates and stored for different months was also evaluated against the grubs and adults of *C. formicarius*. With respect to bioefficacy also, the fungi cultured in cow dung, wheat bran and neem cake proved better.

The results of the field experiment in banana, variety Nendran to assess the effect of spore suspensions of *B. bassiana* and *M. anisopliae,* fungi in cow dung and neem cake substrates and talc based formulations of the fungi in comparison with insecticide revealed that the best treatment was talc based *B. bassiana* @ 30 g l^{-1} excepting the insecticide check, chlorpyrifos 0.03 per cent for the management of *C. sordidus.* In the succeeding crop of banana talc based *B. bassiana* @ 30 g l^{-1} was even superior to the insecticide check, chlorpyriphos 0.03 per cent. The least number of galleries (0.63) and the least number of grubs in the rhizomes (0.29) besides the lowest number of adult *C. sordidus* in soil samples were seen in talc based *B. bassiana* @ 30 g l^{-1}. The B : C ratios calculated for the treatments with talc based *B. bassiana* @ 30 g l^{-1} in the main crop was 1.57 compared to 1.24 for chlorpyriphos 0.03 per cent. The B : C ratios for the treatments with talc based *B. bassiana* @ 30 g l^{-1} and spore suspension of *B. bassiana* 5×10^{11} spores ml^{-1} in the succeeding crop of banana were 1.78 and 1.49 which were higher than that for chlorpyriphos 0.03 per cent. The results from the succeeding crop indicate the ability of these fungi to self perpetuate and bring about long lasting effect in the treated area.

Field experiments were also conducted in sweet potato, variety Sree Bhadra to assess the effect of two fungi. Talc based *B. bassiana* @ 30 gl^{-1} was superior treatment excepting imidacloprid 0.006 per cent in reducing the galleries produced by the weevils. A similar trend was evident in the second trial also. The effect of application of *B. bassiana* and *M. anisopliae* in cow dung and neem based substrates showed moderate effect with respect to the number of galleries and number of grubs. Drenching of talc based formulation of *B. bassiana* @ 30 g l^{-1} was better than its foliar treatment.

The compatibility of *B. bassiana* and *M. anisopliae* with two fungicides and six insecticides was also evaluated inorder to evaluate the suitability of integrating the fungi with pesticides in pest management programmes. Good compatibility of the insecticide, imidacloprid 0.006 per cent with *B. bassiana* and *M. anisopliae* was seen. Bioefficacy of the fungi cultured in this insecticide was also higher. Attempts were

also made to develop pesticide tolerant strains of *B. bassiana* and *M. anisopliae*. For this they were grown continuously in media with varying doses of pesticides. A total of ten passages through poison food media was made. It was seen that both the fungi tolerated high doses of pesticides though there was inhibition in growth, sporulation and bioefficacy. *B. bassiana* and *M. anisopliae* tolerated even 32 times higher the recommended field dose of imidacloprid. Subculturing reduced the spore production of both the fungi and the rate of reduction was higher for *B. bassiana*. Variations induced in *B. bassiana* and *M. anisopliae* after ten passages through poisoned media were analysed. Pesticides varied in their ability to induce such changes in the fungi. Polymorphism was higher for *B. bassiana* cultured in carbendazim. The polymorphism exhibited in *B. bassiana* was higher (83.19 per cent) compared to *M. anisopliae* (38.46 per cent). Among the ten universal fungal primers evaluated, RFu – 10 was found to give maximum polymorphism.

To conclude, *B.bassiana* and *M.anisopliae* are pathogenic to the grubs and adults of all the nine test insects. Of the two fungi tested Bb 5 was more virulent than Ma 4 except to *O. rhinoceros*. Talc based formulation of both the fungi maintained the required standards of cfu in the formulations upto three months after storage. Cow dung, wheat bran and neem cake are ideal substrates for the multiplication of the fungi. Soil drenching of talc based formulation of *B. bassiana* @ 30 g l^{-1} three months after planting was the best treatment for the management of *C. sordidus*. For the management of *C. formicarius* in sweet potato also soil drenching of talc based formulation of *B. bassiana* @ 30 g l^{-1} was superior. Compatibility of *B. bassiana* and *M. anisopliae* with insecticides and fungicides varied, the most compatible one was imidacloprid. Maximum tolerance to this insecticide, upto 32 times higher than the field dose was shown by *B. bassiana* and *M. anisopliae*. Pesticides induced changes in DNA, the polymorphism exhibited was higher for *B. bassiana* compared to *M. anisopliae*. Nine isolates of the fungi were also identified through ITS sequencing. The new isolates were *Beauveria* sp. from *C. formicarius*, *B. bassiana*

from *B. fulvicorne, B. brongniartii* from *M. circumdata, Metarhizium* sp. from *B. fulvicorne; Metarhizium* sp., *Metarhizium album* and *Metarhizium anisopliae* var *majus* from *O. rhinoceros, Fusarium moniliformae* from *H. vigintioctopunctata* and *Paecilomyces* sp. from *C. sordidus* and these isolates can be exploited in insect specific biocontrol programmes.