PATHOGENICITY OF INDIGENOUS ENTOMOPATHOGENIC FUNGI AGAINST SELECT LEPIDOPTERAN PESTS

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KERALA, INDIA

2016

DECLARATION

I, hereby declare that this thesis entitled **"PATHOGENICITY OF INDIGENOUS ENTOMOPATHOGENIC FUNGI AGAINST SELECT LEPIDOPTERAN PESTS"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVATIONS AND SYMBOLS USED

@	At the rate of
^0C	Degree Celsius
%	Per cent
µm	Micro metre
µL	Micro litre
∞	Infinite
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
bp	Base pair
C	Cultivated soil
CD	Critical difference
cfu	Colony forming unit
cm	Centimetre
CRD	Completely Randomised Design
CTAB	Cetyl Trimethyl Ammonium Bromide
DAI	Days after inoculation
DAS	Days after sowing
DAT	Days after treatment
DNA	Deoxyribonucleic acid
et al.	And others
EPF	Entomopathogenic fungi

g	Gram
g L^{-1}	Gram Per litre
h	Hour
ha	Hectare
ha^{-1}	Per hectare
HAT	Hours after treatment
i. e.	That is
ITS	Internal Transcribed Spacer
KAU	Kerala Agricultural University
Kg	Kilogram
Kg ha^{-1}	Kilogram per hectare
L	litre
L^{-1}	Per litre
LD	Lethal dose
LT	Lethal time
Ltd	Limited
M	Mortality
mg	Milligram
mL^{-1}	Per millilitre
mm	Millimetre
NBAIR	National Bureau of Agricultural Insect Resources
NS	Non significant

OA	Oatmeal Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
pH	Per Hydrogen
$Plant^{-1}$	Per Plant
Pvt	Private
RAPD	Random Amplified Polymorphic DNA
$replication^{-1}$	Replication
RFLP	Restriction Fragment Length Polymorphism
RH	Relative humidity
S	Survivability
SCAR	Sequence Characterized Amplified Regions
sp. or spp.	Species (Singular and Plural)
SSCP	Single Strand DNA conformation Polymorphisms
t	Tonnes
UC	Uncultivated soil
UV	Ultra Violet
viz.	Namely
web^{-1}	Per web

Introduction

1. INTRODUCTION

Lepidoptera is the second largest order in the class Insecta, and it is almost entirely associated with angiospermous plants (Scoble, 1992). Coevolutionary dynamics of the order Lepidoptera with the angiospermous host plants are thought to have influenced the mega diversification of this insect order (Wahlberg *et al*., 2013). Immature stages of Lepidoptera, commonly referred to as caterpillars have diverse feeding habits. They feed the plant by remaining exposed on plants, within leaf rolls or leaf mines or even within tunnels made in the plants.

The important lepidopterans with different modes of feeding that infest the widely cultivated vegetables in Kerala are snake gourd caterpillar, *Diaphania indica* Saunders; amaranthus leaf webber, *Hymenia recurvalis* Fabricius; brinjal shoot and fruit borer *Leucinodes orbonalis* Guenee and bhindi leaf roller *Sylepta derogata* Fabricius. Banana, an important fruit crop grown in Kerala is highly prone to the attack of leaf eating caterpillar *Spodoptera litura* Fabricius.

D. indica has worldwide distribution, the larvae feed on leaves of bitter gourd, cucumber and pumpkin, and it almost defoliates the plants when populations are high (George *et al*., 2002). Yield loss by *D. indica* was estimated as 10 per cent when the pest populations reached one per leaf (Schreiner, 1991). The pest is also known to feed on fruits of pointed gourd, gherkin, bitter gourd, pumpkin, sweet melon (Patel and Kulkarny, 1956; Ravi, 1998; Singh and Naik, 2006; Mohamed *et al*., 2013). The damage caused by the larvae of *D. indica* on the leaves of bittergourd was recorded by Singh and Naik (2006) as 3 to 14 per cent.

The leaf webber, *H. recurvalis* that shelters within the leaves and defoliate amaranthus plants is of common occurrence both in hills and plains of India (Ebert *et al*., 2011).

Brinjal, a globally important crop is the second highest consumed vegetable in India (Hanur *et al.*, 2014). The annual production of brinjal in India is 8-9 million

tons and it constitutes eight per cent of the total vegetable production (Hanur *et al*., 2009; Hanur, 2010). *L. orbonalis*, the shoot and fruit borer ranks top among the pests of brinjal and reduces the yield of brinjal to the tune of 70 per cent. Substantial reduction in marketable quality was reported due to the infestation of this pest.

Leaf caterpillar, *S. litura* is an important polyphagous, sporadic pest in tropical and subtropical regions. It has wide host range and known to infest 40 species of plants from India (Singh *et al*., 1998) and cause enormous economic loss to the crops such as cotton, soyabean, groundnut, tobacco and vegetables (Qin *et al*., 2004).

The caterpillars of *S. derogata*, with a different mode of feeding *i. e.,* by rolling the leaves, sheltering and feeding within the rolls is an important pest that substantially defoliate bhindi and cotton (Sohi, 1964; Mariselvi and Manimegalai, 2016).

In the stride to develop tools for managing insect pests, an array of materials and technologies have been experimented, from inorganic chemicals to organic chemicals, crude forms of botanicals to plant alkaloids and secondary metabolites, bioagents consisting of microbials to large macrobial such as birds and much more, the transgenic crops. Though the developed technologies are capable of containing the pests problems, they have also limitations which often necessitate further research for novel methods. Recently, world over, emphasis is given for development of technologies that are safe to non targets and environment and there is a revival of interest in the exploitation of microbial pathogens of insects especially entomopathogenic fungi.

Entomopathogenic fungi, numbering more than ninety genera and 750 species have been found to infect an array of insects (Hejek and St. Leger, 1994). Of these fungi, *Beauveria, Metarhizium, Lecanicillium, Nomuraea* and *Paecilomyces* are now recognized as important genera for biocontrol as they seems fit in the chapter to go

green in pest management. Recent research unveil that these genera are assemblages of many potential species and that within these species many isolates exist that vary in their infectivity to different groups of insects (Driver *et al.*, 2000). Rai *et al.* (2014) stated that the speed of kill of the host insects is dependent upon the fungal strain and species. In this context, identification of virulent species and isolates, that infect the target pest species in a shorter period, would in turn help in overcoming the limitation of slow action exhibited by the entomopathogenc fungi compared to the chemical pesticides. Moreover, investigations on indigenous fungal isolates that can adapt to specific climatic regimes are also essential.

Entomopathogenic fungi are known for their self perpetuation in nature due to its ability to switch back to hyphal mode on death of infected host and produce infective conidial saprophytic growth under humid conditions. Environmental conditions were reported to greatly influence the performance of these fungi (Benz, 1987; Inglis *et al.*, 1996; Han *et al.*, 2014). Such observations necessitate evaluation of the fungi in the field for fixing the effectiveness of fungal pathogens and also the schedule of applications, for effective crop protection. Considering the aforesaid aspects, a project entitled ″Pathogenicity of indigenous entomopathogenic fungi against select lepidopteran pests″was undertaken with the following objectives

- To isolate and identify indigenous entomopathogenic fungi,
- to assess their pathogenicity to lepidopteran pests of banana and vegetables in the laboratory and
- to evaluate the pathogenicity of the fungal isolates against the lepidopteran leaf webbers of amaranthus through pot culture experiment.

Review of Literature

2. REVIEW OF LITERATURE

Entomopathogenic fungi are gaining importance as a safe tool in pest management and hence, recent research is focused on these microbes. Literature pertaining to the isolation, identification, pathogenicity and field efficacy of the fungal pathogens is reviewed below.

2.1 ISOLATION AND IDENTIFICATION OF ENTOMOPATHOGENIC FUNGI

2.1.1 Isolation

Entomopathogenic fungi are generally isolated from insect cadavers or soil.

2.1.1.1 Isolation from Insect Cadaver

Natural infections of entomopathogenic fungi occur in over 700 species of insects. Cadavers of insects act as a reservoir of entomopathogenic fungi (Hajek and St. Leger, 1994; Bernabeu and Llorca, 2002; Meyling, 2007).

Many researchers have isolated entomopathogenic fungi from insect cadavers. Some of the fungi isolated were *Metarhizium anisopliae* (Metschnikoff) Sorokin from *Heliothis armigera* Hubner (Gopalakrishnan and Narayanan, 1988), *Fusarium pallidoroseum* (Cooke) Sacc. from *Aphis craccivora* Koch (Hareendranath, 1989), *Fusarium solani* (Mart.) Sacc. and *Aspergillus niger* Van Tieghem from *Odoiporous longicollis* Olivier (Anitha *et al.,* 1999), *Aspergillus parasiticus* Speare from *O. longicollis* (Beegum, 2005), *Mucor hiemalis* f *hiemalis* Wehmer from *Pagria signata* Motschulski (Sudharma and Rani, 2005), *Aspergillus flavus* Link, *A. niger* and *Beauveria bassiana* (Balsamo) Vuillemin from *Dolycoris baccarum* Linnaeus (Assaf *et al*., 2011), *Lecanicillium* sp. from *Coccus viridis* Green (Ramanujam *et al.,* 2011), *M. anisopliae* from *Chilo venosatus* Walker (Liu *et al.,* 2012), *Hirsutella* sp. from *Oliarus dimidiatus* Berg (Toledo *et al*., 2013), *B. bassiana* and *Metarhizium* sp. from *Basilepta fulvicorne* Jacoby (Anis, 2014), *Isaria javanica*

(Friedrichs and Bally) Samson and Hywel-Jones from *Bemisia tabaci* Gennadius (Lokesh, 2014) and *A. flavus* from *Leptocorisa acuta* (Thunb) (Nilamudeen, 2015).

Entomopathogenic fungi can be isolated directly from the insect cadaver into Potato Dextrose Agar (PDA) medium wherein the fungus has already sporulated. If external sporulation of fungus has not occurred, insect cadavers can be placed in moisture chamber to produce hyphae or conidia externally, prior to placement in PDA (Goettel and Inglis, 1997). For the isolation of entomopathogenic fungi, Mythili *et al.* (2010) surface sterilized the cadavers in two per cent sodium hypochlorite for three minutes and washed in sterile water thrice. Further, they placed the cadavers in PDA slants and after sporulation, primary cultures of these fungi were isolated by scraping the surface of cadavers with a needle and inoculating into agar slants. In the absence of sporulation, they homogenized the cadaver using micro-pestle and the homogenate was inoculated into agar medium. For obtaining pure culture of the fungus repeated hyphal tip culture was done by Aiin *et al*. (2014).

2.1.2 Isolation from Soil

Soil is the main reservoir of entomopathogenic fungi and it has an essential influence on the occurrence and expansion of insect mycoses (Ignoffo *et al.,* 1978; Mietkiewski and Tkaczuk, 1998). Keller and Zimmerman (1989) reported soil as an excellent environmental shelter for entomopathogenic fungi since it was protected from UV radiations and other adverse abiotic and biotic influences.

Different species of entomopathogenic fungi were isolated from soil (Keller and Zimmerman, 1989; Ali-Shtayeh *et al*., 2002; Klingen and Haukeland, 2006; Hu *et al*., 2011; Rani *et al*., 2015).

Several researchers studied the effect of factors such as geographical location, climatic conditions, habitat type, cropping system, soil properties as well as the

effects of biotic factors on the occurrence and distribution of entomopathogenic fungi (Chandler *et al*., 1997; Bruck, 2004; Sanchez-Pena *et al*., 2011). Soil factors (temperature, pH, organic content, moisture, minerals and biotic factors) can affect fungal persistence and activity (Charnley, 1997). Mader *et al*. (2002) reported the increased abundance and diversity of entomopathogens in organically cultivated soils in comparison to the soil where in conventional tillage was practised.

Species such as *B. bassiana*, *M. anisopliae* var. *anisopliae* and *Paecilomyces fumosoroseus* (Wize) Brown and Smith were detected in both field crop and orchard soils but their frequency in the two agroecosystems differed significantly. *B. bassiana* and *P. fumosoroseus* were more frequently detected in orchard soils, while *M. anisopliae* var. *anisopliae* was more frequently detected in field crop soils (Sun *et al*., 2008). Scheepmaker and Butt (2010) reported that entomopathogenic fungi, especially hypocrealean Ascomycetes, occur widely in the soil environment.

2.1.2.1 Methods of Isolation

Entomopathogenic fungi are isolated from soil, adopting soil dilution plate method, soil plate method, selective media method and insect bait method.

2.1.2.1.1 Soil Dilution Plate Method

Dilution plate method was followed for the isolation of fungi from soil by Waksman (1922) and other researchers (Suhail *et al*., 2006; Gaddeyya *et al.,* 2012). In this method, one gram of soil sample was mixed with sterile distilled water and a series of dilutions were made. From this, one ml of the dilution was plated onto PDA medium mixed with one per cent streptomycin solution which was then incubated for fungal development (Waksman, 1922).

Dilution plate method reduces the number of colonies per plate and thereby degree of interference between the developing colonies will be less (Garret, 1951).

2.1.2.1.2 Soil Plate Method

In Soil plate method, about 0.005 g of soil was scattered on the bottom of sterile Petri dish and molten cooled (40-45°C) agar medium containing Cetyl Trimethyl Ammonium Bromide (CTAB) and antibiotic was added, which was then rotated gently to disperse the soil particles in the medium. The Petri dishes were then incubated at 28 ± 20 ^{0}C in dark for three days for the development of fungal colonies (Warcup, 1950). Johnson and Manka (1961) reported that greater number of species of fungi can be isolated by soil plate than dilution plate method.

2.1.2.1.3 Selective Media

Selective media containing various fungicides and antibiotics were evaluated for the isolation of entomopathogenic fungi from soil (Veen and Ferron, 1966; Liu *et al*., 1993; Keller *et al*., 2003; Wraight *et al*., 2007).

Doberski and Tribe (1980) reported that the growth of *B. bassiana* was slow in Veens semiselective medium. Chase *et al.* (1986) reported that selective medium containing 0.55 g L^{-1} of dodine and 5 mg L^{-1} of chlortetracycline selectively isolated *Beauveria* sp. Decreasing the amount of dodine to 0.46 g L^{-1} and adding 0.38 benomyl allowed the growth of *B. bassiana* and *M. anisopliae*. Hu and St. Leger (2002) used Veens semiselective medium for the isolation of *Metarhizium* spp. The medium contained the antibiotic chloramphenicol as well as the fungicides dodine and cyclohexamide.

Unavailability of dodine in the market lead further research on selective media and in 2010, Fernandes *et al*. developed a novel dodine free selective medium

(designated CTC medium) which consisted of Potato Dextrose Agar and Yeast extract (PDAY) supplemented with chloramphenicol, thiabendazole and cycloheximide for the efficient isolation of *Metarhizium acridum* (Driver and Milner) J. F. Bisch. The medium was effective for selective isolation of *Metarhizium brunneum* Petch, *Metarhizium robertsii J. F. Bisch., B. bassiana* and *Beauveria brongniartii* (Sacc.) Petch from non sterile field collected soil samples.

Posadas *et al*. (2012) evaluated the quaternary ammonium compound Cetyl Trimethyl Ammonium Bromide (CTAB) as an alternative to the chemically related dodecylguanidine (dodine) for the selective isolation of entomopathogenic fungi. Oatmeal agar (OA) with chloramphenicol was used as basal medium, and three concentrations of CTAB (0.5, 0.6, 0.7 g L^{-1}) were evaluated and compared against OA + 0.46 g L^{-1} dodine. Selective isolation and growth studies were performed with the entomopathogens *B. bassiana, M. anisopliae* and *Paecilomyces lilacinus* (Thom) Samson and five common non-entomopathogenic non-target species. Results of their study suggest that OA + 0.6 g L^{-1} CTAB was a suitable, simple and inexpensive medium to replace OA + 0.46 g L^{-1} dodine for the selective isolation of these fungi.

2.1.2.1.4 Insect Bait Method

Zimmermann (1986) isolated entomopathogenic fungi from soil using *Galleria mellonella* (L.) insect bait. Though this traditionally preferred bait insect was highly susceptible, the mealworm larva, *Tenebrio molitor* L. was also used as bait insect (Sanchez-Pena *et al*., 2011; Rudeen *et al*., 2013). Keyser *et al*. (2015) reported that diversity within the entomopathogenic fungal species *Metarhizium flavoviride* W. Gams and Rozsypal was obtained through *T. molitor* baiting.

Baiting soil samples with larvae of *G. mellonella* is a widely applied tool to screen for indigenous species of entomopathogenic fungi (Vanninen, 1996; Chandler

et al., 1997; Bidochka *et al.*, 1998; Klingen *et al.*, 2002; Keller *et al.*, 2003; Meyling and Eilenberg, 2006).

2.2 IDENTIFICATION

2.2.1 Morphological Identification

Riddell (1950) prepared slide cultures of entomopathogenic fungi from 14 day old cultures for the studies on morphotaxonomic characteristics of conidia forming mycelia and conidial structures. Such characters were also examined by De Hoog, 1972; Barnett and Hunter, 1987; Samson *et al.,* 1988; Humber, 1997 and Luangsa-Ard *et al.,* 2007 for identification of fungi.

Morphological identification of the fungi can be done based on shape and size of spores in slide cultures (Domsch *et al.,* 1980; Nelson *et al.,* 1983; Samson *et al.,* 1988).

Studies on fungal morphology were undertaken macroscopically by observing colony features (colour and texture) and microscopically by staining with lactophenol cotton blue and observing under compound microscope for the conidia, conidiophores and arrangement of spores (Aneja, 2001). Taxonomic keys based on morphological characters were used for the identification of entomopathogenic fungi (Gilman, 2001; Humber, 2005; Nagamani *et al*., 2006).

Light microscopy using low (10X) and (40X) can be used to observe slides of fungal cultures. Lactophenol Cotton Blue is a reagent which stains the outer wall of fungi, aiding in the microscopic examination of the fungal mycelium and fruiting structures (Girija and Naseema, 2011).

Microscopic preparations from fungal mycelium and spores which grew on the insect's surface provided the basis for the determination of fungal species (Tkaczuk, 2014).

2.2.3 Molecular Identification

Molecular techniques were widely used in recent years for the identification and genetic variation studies of fungal isolates (Clarkson, 1992; Khachatourians, 1996).

Restriction fragment length polymorphism (RFLP) is a method used in molecular charaterisation of fungus, which generates restriction endonuclease digestion patterns of total or specific (e.g. mitochondrial) DNA. Derived patterns on analysis assist in the differentiation between species (Entz, 2005).

The examination of mitochondrial (mt) DNA showed the existence of RFLP in five species of entomopathogenic fungi which can be used for species and strain identifications (Hegedus and Khachatourians, 1993). Pipe *et al*. (1995) grouped *M. anisopliae* isolates based on geographical origin using RFLP, but they observed little difference of isolates from the same geographical region.

Several researchers adopted the Polymerase Chain Reaction (PCR) based technology involving Random amplified polymorphic DNA (RAPD) analysis to identify the genomic variability between different isolates of entomopathogenic fungi (Fegan *et al*., 1993; Strongman and MacKay, 1993; Bidochka *et al*., 1994).

The development of polymerase chain reaction (PCR)-based systems, including single-strand DNA conformation polymorphisms (SSCP) analysis allowed the identification of isolates without probe hybridisation or DNA sequencing (Hegedus and Khachatourians, 1996).

For fungal isolates that sporulated poorly, the internal transcribed spacer (ITS) region of ribosomal DNA was amplified and sequenced following the procedure of White *et al*. (1990).

Srain-specific molecular markers based on polymerase chain reaction amplification of sequence-characterized amplified regions (SCAR) were used by several researchers for the sensitive diagnosis of the fungal isolates and SCAR assays obviated the need for establishment of single spore isolates (Schilling *et al*., 1996; Abbasi *et al*., 1999; Castrillo *et al.,* 2003).

The internal transcribed spacer (*ITS*) region of the nuclear ribosomal repeat unit is the most popular locus for species identification and subgeneric phylogenetic inference in sequence-based mycological research (Horton and Bruns, 2001; Nilsson *et al*., 2008).

To obtain target DNA, Sun *et al*. (2008) followed PCR amplification using the primer pairs ITS5 (5′- GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGC TTATTGATATGC-3′) and ITS sequences were then subjected to BLAST searches against the GenBank database. Results from BLAST searches were used to guide further morphological examination and identification.

2.3 PATHOGENICITY OF FUNGI TO INSECTS

2.3.1 Importance of Select Insects in the Study

Insect pests are the major limiting factor in the vegetable production which accounts to 8.7 per cent crop loss (Sithanatham *et al*., 2003). The lepidopteran pests of vegetables *viz., Diaphania indica* Saunders*, Hymenia recurvalis* Fabricius, *Leucinodes orbonalis* Guenee*, Sylepta derogata* Fabricius and *Spodoptera litura*

Fabricius are the insects selected in the present study. Importance and nature of damage of these pests are reviewed below.

2.3.1.1 D. indica

Pumpkin caterpillar, *D. indica*, a serious pest of cucurbitaceous vegetable was first reported as pest of cucurbits in India by Lefroy (1906). Cucumber (*Cucumis sativus* L.), melon (*C. melo* L.), gherkin (*C. sativus* L.), bottle gourd (*Lagenaria siceraria* Molina), bitter gourd (*Momordica charantia* L.), snake gourd (*Trichosanthes anguina* L.), Luffa (*Luffa aegyptiaca* Mill.), little cucumber (*Melothria* spp), and cotton (*Gossypium hirsutum* L.) were reported as the important host species in which attack and considerable damage has been reported (Ayyar, 1923; Tripathi and Pandy, 1973; Clavijo *et al*., 1995; Bacci *et al*., 2006). The damage caused by larvae of *D. indica* on the leaves of bittergourd was recorded by Singh and Naik (2006) as 3 to 14 per cent. Ravi (1998) reported that apart from leaves, larvae damage the fruits. The occurrence of *D. indica* on cucumbers and melons in greenhouse crops was also reported (Shimizu, 2000; Kinjo and Arakaki, 2002).

2.3.1.2 H. recurvalis

H. recurvalis was identified as one of the important pests of leafy vegetables especially amaranthus (Ebert *et al*., 2011; Joseph *et al*., 2016). Aderolu *et al.* (2013) conducted a survey in the southern part of Nigeria and identified insect pest as major constraints in the production of amaranthus with *H. recurvalis* as one of the major pest species in the foliage.

The host plants of *H. recurvalis* include maize, beet root, eggplant, bean, cucurbit, sweet potato and Palak (Narayanan *et al*., 1957; James and Atcha-Ahowe,

2010; Manjula, 2014). The larvae cause significant yield loss in amaranthus through webbing and leaf skeletonization (Hsu and Srinivasan, 2012).

2.3.1.3 L. orbonalis

Brinjal fruit and shoot borer, *L. orbonalis* was observed as one of the major pests of brinjal causing considerable yield loss. Crop loss due to fruit and shoot borer damage in brinjal varied from 20 to 100 per cent (Lall and Ahmad, 1965; Alam, 1969; Raju *et al*., 2007: Rahman, 2007; Singh *et al*., 2008; Chakraborty and Sarkar, 2011; Salunke *et al*., 2015). Naresh *et al*. (1986) reported that pest attack caused a yield loss up to 95 per cent in India.

The first instar larvae of *L. orbonalis* bore into the tender shoot, flower and fruit of brinjal plants and the larvae also bore into the petioles and midribs of brinjal leaves which resulted in the dropping off of leaves (Butani and Jotwani, 1984).

Apart from brinjal, the pest attacks and cause yield loss in *Solanum tuberosum* Linn., *Solanum nigrum* Linn., *Solanum indicum* Linn., *Momordica charantia* Linn (Nayar *et al*., 1995).

2.3.1.4 S. litura

S. litura has a wide host range of more than 120 host plants including vegetables and ornamentals (Prasad and Bhattacharya, 1975; Ramana *et al*., 1988; Rao *et al*., 1993; Matsuura and Naito, 1997; Sharma and Bisht, 2008). Several outbreaks of this pest on cotton, tobacco and chillies have been reported in Tamil Nadu (Rao *et al*., 1983).

S. litura, the polyphagous sporadic pest (Holloway, 1989) was reported to have wide distribution throughout tropical and temperate Asia (Monobrullah and Shankar, 2008).

The loss caused by *S.litura* was reported to range between 25.8-100 per cent depending upon the crop stage and its infestation level. A single larva caused an average yield loss of 27.3 per cent in groundnut through damage to various plant parts like leaves, flowers and pods (Dhir *et al*., 1992). Larvae damaged the crops by extensive feeding and heavy feeding resulted in stunted growth (USDA, 2005). Vashisth (2009) cited by Sharma and Pathania (2015) reported that the infestation of *S. litura* in sweet pepper, tomato and cucumber in polyhouses ranged from 25 to 75 per cent.

2.3.1.5 S. derogata

S. derogata, reported as a pest of cotton from India by Sohi (1964) is a polyphagous pest that attacks *Abelmoschus esculentus* (L.) and other malvaceous plants, *Amaranthus* spp and Soyabean (Aderolu *et al*., 2013; Degri and Randy, 2014; Mariselvi and Manimegalai, 2016).

The larvae caused damage by making rolls and feeding the green tissues of leaves at early stages and defoliate the leaves at later stages (Mariselvi and Manimeghalai, 2016).

2.3.2 Pathogenicity of Fungi to *D. indica*

2.3.2.1 B. bassiana

B. bassiana was reported to infect *D. indica* and a mortality percentage of 86. 67 per cent was recorded against the larvae (Jiji *et al*., 2008).

2.3.2.2 M. anisopliae

No literature stating the pathogenicity of *M. anisopliae* to *D. indica* could be retrieved.

2.3.2.3 Other Entomopathogenic Fungi

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

2.3.3 Pathogenicity of Fungi to *H. recurvalis*

2.3.3.1 B. bassiana

A strain of *B. bassiana* Bba5648 showed 100 per cent mortality in the larvae of *H. recurvalis* five days after inoculation (James *et al*., 2007).

2.3.3.2 M. anisopliae

No literature on the infectivity of *M. anisopliae* to *H. recurvalis* is available.

2.3.3.2 Other Entomopathogenic Fungi

P. farinosus was reported to infect the larvae of *H. recurvalis* (Kuruvila and Jacob, 1980).

2.3.4 Pathogenicity of Fungi to L. orbonalis

2.3.4.1 B. bassiana

Pal and Ghosh (2014) evaluated *B. bassiana* against larva of *L. orbonalis* at the median lethal dose (LD_{50}) of 4.0×10^7 spores $mL^{-1}$and the time taken to reach 50 per cent mortality (LT_{50}) was recorded as 9.77 days.

2.3.4.2 M. anisopliae

Persual of literature showed that there was no documented information on pathogenicity of *M. anisopliae* to *L. orbonalis.*

2.3.4.3 Other Entomopathogenic Fungi to L. orbonalis

Yasodha and Natarajan (2009) reported *Aspergillus ochraceous* (Kent) as a pathogen of the larvae of *L. orbonalis*.

Pathogenicity of *N. rileyi* to *L. orbonalis* was reported by Koushik *et al*. (2013). He observed significantly higher mortality (93.06 per cent) of the first instar larvae at six days after spraying with *N. rileyi* at 3.20 x 10^9 spores mL^{-1} compared to 25.31 per cent mortality at the lower concentration of 3.20 x 10^5 spores mL^{-1}, signifying susceptibility of first instar larvae to higher concentration and the lesser effectiveness of lower concentration.

Aspergillus niger van Tieghem and *Penicillium expansum* (Link) Thom. showed pathogenicity to *L. orbonalis*, and the LD_{50} values were 9.06×10^7, and 1.50×10^8 spore mL^{-1}, respectively, and the time taken to reach 50 per cent mortality (LT_{50}) were 10.56 and 10.60 days, respectively (Pal and Ghosh, 2014).

2.3.5 Pathogenicity of Fungi to *S. litura*

2.3.5.1 B. bassiana

Gopalakrishnan and Narayanan (1989) reported the pathogenicity of *B. bassiana* to *S. litura*. Among the different fungal concentrations evaluated, they observed that 2.4×10^7 spores mL^{-1} was the effective concentration that caused 43.33 per cent mortality of the pest.

Baskar *et al*. (2012) evaluated the larvicidal and growth inhibitory activities of 10 different isolates of *B. bassiana* at four different concentrations against the third instar larvae of *S. litura*. After four days of treatment, isolate Bb10 was reported to cause maximum larval mortality of 68.60 per cent, which was followed by isolate Bb6 (50.27 per cent) at 10^8 spores mL^{-1}. Minimum (22.91 per cent) adult emergence and cent per cent abnormal adults were reported in Bb10 isolate at 10^8 spores mL^{-1}.

According to *Petlamul and Prasertsan (2012),* a strain of *B. bassiana* BNBCRC showed 80 per cent mortality to the third instar larvae of *S. litura*.

Asi *et al*. (2013) evaluated the efficacy of eight fungal isolates against third instar larvae of *S. litura*. The results showed that the highest mortality was caused by *B. bassiana* 25 and *I. fumosorosea* 32 and the lowest mortality was caused by *M. anisopliae* 443. Similarly, Ummidhi *et al*. (2014) observed mortality ranging from 60-99 per cent in the second instar larvae of *S. litura* treated with different isolates of *B. bassiana*.

2.3.5.2 M. anisopliae

Patait *et al*. (2009) reported the pathogenicity of the *M. anisopliae* to *S. litura* infecting cabbage during rainy season. An isolate of *M. anisopliae,* Ma2, isolated from Tamil Nadu was found more effective against *S. litura* compared to other isolates Ma1 and Ma3 (Moorthi *et al*., 2011).

Asi *et al*. (2013) reported that *M. anisopliae* was effective against eggs and different larval instars of *S. litura*. Ummidhi *et al*. (2014) observed that *M. anisopliae* M20 at a concentration of 1 x 10^8 conidia mL^{-1} was pathogenic to second instar larvae of *S. litura*. Prakash *et al*. (2015) found that the third instar larvae of *S. litura* when sprayed with infective propagules of *M. anisopliae,* the mortality ranged from 40 to 88.33 per cent.

2.3.5.3 Other Entomopathogenic Fungi

When the first instar larvae of *S. litura* were treated with *N. rileyi* at 10^8 to 10^{11} spores mL^{-1}, 100 per cent mortality of the larvae occurred in five days (Devi, 1994).

Lin *et al*. (2007) compared the pathogenicity of several fungal species against *S. litura* and he observed that *B. brongniartii* and *N. rileyi* showed 100 and 95.2 per cent mortality after treatment with 8×10^7 conidia mL^{-1} with LT_{50} of 3.0 and 4.1 days, respectively. The eggs and second instar larvae of *S. litura* were reported susceptible to the isolates of fungi belonging to five species *viz.,* *Lecanicillium muscarium* (Petch) Zare, *Cordyceps cardinalis* G.H. Sung and Spatafora*, Fusarium lateritium* Nees and *Aspergillus* sp. Two promising isolates against larvae, *viz*., *F. lateritium* and *L. muscarium* caused 89 and 77 per cent mortality, respectively at 10^8 conidia mL^{-1} (Anand and Tiwary, 2009).

The pathogenicity of different isolates of *N. rileyi* against *S. litura* was recorded by Rajan and Muthukrishnan (2009). Ramegowda *et al*. (2010) analysed the variations in severity of mycosis caused by *N. rileyi* on three lepidopteran hosts *viz., H. armigera, S. litura* and *Thysanoplusia plusia* Orichalcea (F.). Study report showed that *N. rileyi* caused higher mortality to *S. litura.*

Asi *et al*. (2013) reported that all biological stages of *S. litura* were not equally susceptible to entomopathogenic fungi. Eggs and larvae were found more susceptible, while pupae were less susceptible.

2.3.6 Pathogenicity of Fungi to *S. derogata*

2.3.6.1 B. bassiana

Ramesh *et al*. (1999) evaluated the pathogenicity of indigenous isolates of *B. bassiana* on larvae of *S. derogata* and results showed that the fungus caused a

mean mortality of 89.34 per cent. The pathogenicity of *B. bassiana* to *S. derogata* was reported by Jiji *et al.* (2006) also.

2.3.6.2 M. anisopliae

The literature pertaining to the pathogenicity of *M. anisopliae* to *S. derogata* was not seen documented.

2.3.6.3 Other Entomopathogenic Fungi

P. farinosus was reported to infect larvae of *S. derogata* (Kuruvila and Jacob, 1980).

2.3.7 Pathogenicity of *B. bassiana* to Other Lepidopteran Pests

B. bassiana was reported pathogenic to *Spodoptera exigua* Hubner (Hung and Bouicas, 1992). Diamond back moth *Plutella xylostella* (Linnaeus) when treated with *B. bassiana* caused 80 per cent mortality (Masuda, 1998). Kempraj and Gopalan (1999) observed that *B. bassiana* exhibited 98.7 per cent mortality against larvae of *Aproraema modicella* (Deventer). An isolate of *B. bassiana* was reported effective against *Chilo suppressalis* Wlk. infecting rice in Iran, (Majidi *et al.*, 2002). Kumar and Chowdhry (2004) evaluated the virulence of different isolates of *B. bassiana* to the second instar larvae of *H. armigera.* Isolates caused mortality ranging from 40 to 90 per cent with the highest percentage of larval mortality (96.6 per cent) at the highest concentration (1 x 10^9 spores mL^{-1}) and lowest (41.1 per cent) at lowest concentration ($1x10^1$ spores mL^{-1}).

Four isolates of *B. bassiana* were evaluated against second instar larvae of *Chilo partellus* (Swinhoe) by Tefera (2004). Among these isolates, *B. bassiana* (BB-01) was found highly pathogenic inducing 90 to 100 per cent mortality seven days after treatment. Among the various instars treated, second and sixth instar larvae

were reported more susceptible to these isolates than third, fourth and fifth instar larvae.

Larva of *P. xylostella*, *Pieris rapae* Linnaeus and *S. exigua* were reported susceptible to the infection by *B. bassiana* (Sabbour and Sahab, 2005).

Cent per cent mortality was recorded in the larvae of *Psara basalis* Walker when treated with an isolate of *B. bassiana* Bba5644 whereas 97 per cent mortality was recorded in another isolate of *B. bassiana* Bba5653 (James et al., 2007).

Two isolates (274 and 373) of *B. bassiana* were evaluated for the pathogenicity against eggs, larvae and pupae of rice stem borer, *Scirpophaga incertulas* (Walker). Results showed that the isolate, 274 was more pathogenic (Dhuyo and Soomro, 2008).

Lozano-gutierrez and Espana-luna (2008) evaluated the pathogenicity of *B. bassiana* against pyralid caterpillar, *Laniifera cyclades* Druce and in their study, the two strains BbZ3 and BbZ4 were observed pathogenic, that caused 100 per cent mortality to the larva.

Abood *et al*. (2010) evaluated the pathogenicity of seven isolates of *B. bassiana* against the Tiger moth, *Atteva sciodoxa* Meyrick at a concentration of $5x10^7$ conidia mL^{-1}. All the isolates were reported pathogenic and Bba-Pp was identified as the most virulent isolate causing 100 per cent mortality with a median infective time (ET_{50}) of 3.6 days.

Migiro *et al*. (2010) reported that three isolates of *B. bassiana* were pathogenic to the adult pea leaf miner, *Liriomyza huidobrensis* (Blanchard) in the laboratory.

Trizelia and Nurdin in 2010 studied the effect of *B. bassiana* isolates to *Crocidolomia pavonana* F. An isolate HhTK9 was observed as the most virulent one that caused 82.50% mortality to the larvae of *C. pavonana* at 10^8 conidia mL^{-1}.

The pathogenicity of *B. bassiana* against third instar larvae of European corn borer, *Ostrinia nubilalis* Hubner was also investigated (Safavi *et al*., 2010). Results showed that isolate BEH at 10^8 conidia mL^{-1}caused the highest mortality (57.67 per cent) in the larvae.

B. bassiana was found effective against *P. xylostella* attacking cruciferous crops (Loc and Chi, 2007). Revathi *et al*. (2011) isolated *B. bassiana* strains from the different regions in Tamil Nadu by *Galleria* bait method and evaluated the pathogenicity against *H. armigera*. More than 70 per cent mortality was reported in the larvae.

Isolates of *B. bassiana*, (strain DPK-02-d) were tested on the third and fourth instar larvae of pine defoliator, *Bupalus piniaria* (L.). The isolates were reported to cause mortality of 100 per cent to the *B. piniaria* larvae (Nedveckyte *et al.,* 2011).

Fazeli-Dinan *et al*. (2012) conducted a study on the bioefficacy of *B. bassiana* isolates against green semi-looper, *Naranga aenescens* Moore. A local isolate of *B. bassiana* DEBI003 showed complete mortality in the eggs of *N. aenescens.*

Iskender *et al*. (2012) reported that *B. bassiana* isolates (PaF04, PaF09, PaF76) were pathogenic to the larvae of arctiid, *Hyphantria cunea* (Drury). Oreste *et al*. (2012) evaluated the pathogenicity of 23 isolates of *B. bassiana* against *G. mellonella* at 2 x 10^6 conidia mL^{-1}. Results showed that the two isolates of *B. bassiana* AL1 and ALB55 caused mortality in the larvae of *G. mellonella* within the shortest time of 2.2 and 2.3 days, respectively.

Ritu *et al*. (2012) found that larvae of *H. armigera* developed infection by *B. bassiana* at 2.7 x 10^9 spores mL^{-1} in seven days.

Shalaby *et al*. (2013) studied the efficacy of *B. bassiana* for the control of tomato borer, *Tuta absoluta* (Meyrick) and the fungus was reported to give the highest effect on egg and neonate larva of *T. absoluta*, at a spore concentration of 10^7 spores mL^{-1}.

Zibaee *et al.* (2013) noted that an isolate of *B. bassiana* EUT105 caused 76 per cent mortality in the larvae of Fall Webworm, *Hyphantria cunea* Drury.

A study was conducted on the effect of *B. bassiana* on eggs and larvae of Indian meal moth, *Plodia interpunctella* Hubner by Sedehi *et al.* (2014). They found that *B. bassiana* had a strong lethal effect on the egg and larvae of the insect.

Different larval instars of *Pericallia ricini* Fab. were evaluated using different concentrations of *B. bassiana.* A concentration of 2.0x10^6 spores mL^{-1} was observed to give maximum mortality on *P. ricini* (Shophiya *et al*., 2014).

2.3.8 Pathogenicity of *M. anisopliae* to Other Lepidopteran Pests

The pathogenicity *M. anisopliae* to *H. armigera* was reported by Gopalakrishnan and Narayanan (1988). According to Kempraj and Gopalan, (1999), larvae of *Aproraema modicella* (Deventer) exhibited 85.2 per cent mortality to *M. anisopliae*. Revanna *et al.* (2003) reported *M. anisopliae* as a pathogen of *T. orichalcea* in sunflower.

Migiro *et al*. (2010) assessed the pathogenicity of seventeen isolates of *M. anisopliae* against the adult pea leaf miner, *Liriomyza huidobrensis* (Blanchard) and observed that all the isolates were pathogenic and caused mortality between 40 and 100 per cent at five days after exposure.

Loc and Chi (2007) reported that *M. anisopliae* (OM3-STO) isolated from naturally infected Diamondback moth had the highest infectivity to the pest compared to *B. bassiana* isolate (OM2-SDO).

Moorthi *et al*. (2011) evaluated the pathogenicity of *M. anisopliae* against the castor hairy caterpillar, *Euproctis fraterna* (Moore). Among the various isolates, Ma2 isolate at 10^8 spore mL^{-1} was found more effective than Ma1 than and Ma3 isolates.

Nedveckyte *et al.* (2011) found *M. anisopliae* DPK-06-d pathogenic to pine defoliator, *B. piniaria* and the pathogen was reported to cause 100 per cent mortality 18 days after application.

An isolate of *M. anisopliae* obtained by soil plating method caused greater than 70 per cent mortality on larvae of *H. armigera* (Revathi *et al*., 2011).

The second instar larvae of *H. armigera* when exposed to the various isolates of *M. anisopliae*, the isolate SBT 27 showed 98 to 100 per cent mortality in eight days and SBT 28 showed 90 to 92 per cent mortality in eight days (Vijayavani *et al.,* 2010).

M. anisopliae was found pathogenic to the eggs and neonate larvae of *T. absoluta* infecting tomato (Shalaby *et al*., 2013).

Hua *et al*. (2014) conducted a study on the pathogenicity of *M. anisopliae* against *Orthaga achatina* (Butler) larvae. Among the various strains, Mal291-2 strain was reported as the most effective one.

Third instar larvae of tiger moth, *A. sciodoxa* was seen pathogenic to an isolate of *M. anisopliae* isolated from bagworm *Pteroma pendula* Joannis (Sajap *et al*., 2014).

2.3.9 Pathogenicity of *Fusarium* spp.

More than 13 *Fusarium* species was reported pathogenic to insects and host range includes Lepidoptera, Coleoptera, Hemiptera and Diptera (Teetor-Barsch and Roberts, 1983; Humber, 1992). Some of the *Fusarium* species are weak, facultative pathogens, especially of the lepidopteran and coleopteran orders, and they colonize their dead hosts as saprophytes. Strong pathogens were reported primarily from homopterans and dipterans which was evident from field observations on natural mortalities as well as from pathogenicity tests (Teetor-Barsch and Roberts, 1983). *Fusarium semitectum* Berk. and Rav. (ARSEF 7233) isolated from diseased cadavers of aphid (*Aphis gossypii* Glover) was reported to cause mortality to sucking pests such as chilli thrips (*Scirtothrips dorsalis* Hood), broad mite (*Polyphagotarsonemus latus* Banks), sugarcane wooly aphid (*Ceratavacuna lanigera* Zehntner), spiralling whitefly (*Aleyrodicus dispersus* Russell), whitefly (*Bemisia tabaci* Gennadius) and coconut mite (*Aceria guerroronis* Keifer). The fungus did not cause mortality on larvae of lepidopteran insect pests (Mikunthan and Manjunatha, 2006).

Fusarium oxysporum Schlecht was found pathogenic to the rice greenhorned caterpillar *Melanitis leda ismene* Cramer (Nayak and Srivastava, 1978). Kuruvila and Jacob (1979) evaluated the pathogenicity of *F. oxysporum* against brown plant hopper in rice and found cent per cent mortality at a dose of 1.6×10^6 spores mL^{-1}.

Rani (2001) reported that the *Fusarium pallidoroseum* (Cooke) Sacc. caused cent per cent mortality on *A. craccivora*. 100 per cent mortality was observed on the eggs of *S. litura* treated with another species, *F. lateritium* at 10^6 conidia mL^{-1} by Anand and Tiwary (2009).

2.3.10 Pathogenicity of *Purpureocillium lilacinum* (Thorn) Samson

P. lilacinum is one of the important biocontrol agents against nematode pest (Dube and Smart, 1987; Schenck, 2004; Mendoza *et al*., 2007). The entomopathogenic potential of *P. lilacinum* was reported by (Suh *et al*., 2002; Wakil *et al*., 2012).

Kepenekci *et al*. (2015) evaluated the effects of *P. lilacinum* TR1 on the black cherry aphid adults at three conidial suspensions (10^6, $10^7$and 10^8 cfu mL^{-1}). The mortality rate reported was 73.48 and 83.64 per cent after six and eight days, respectively at 10^8cfu mL^{-1}.

2.4 EFFECTS OF FUNGAL PATHOGENS UNDER FIELD CONDITIONS

2.4.1 Field Efficacy of Fungal Pathogens on Select Test Insects

2.4.1.1 D. indica

The extent of damage by *D. indica* in bittergourd was only 8.43 per cent when treated with *B. bassiana* 2g L^{-1} as against 23.49 per cent in the untreated plot (Lenin, 2011). Amala (2010) reported that the soil drenching + spraying of *P. lilacinus* resulted a lower incidence of pumpkin caterpillar.

2.4.1.2 H. recurvalis

No documented references on the field efficacy of fungal pathogens to *H. recurvalis* were available.

2.4.1.3 L. orbonalis

Suradkar *et al*. (2008) reported that a combination of *M. anisopliae* and spinosad reduced the shoot infestation (7.47 per cent) and fruit damage (25.59 per

cent) caused by *L. orbonalis* and provided the maximum yield (81.82 q ha^{-1}). The fungus, *Lecanicillium lecanii* (Zimm.) Zare and W. Gams reduced the damage of brinjal shoot and fruit borer *L. orbonalis* in the range 58.67 to 66.79 per cent (Ghatak *et al*., 2009). Karkar *et al.* (2014) conducted a field experiment to evaluate the bioefficacy of entomopathogenic fungi *B. bassiana* (2×10^8 cfu g^{-1}), *L. lecanii* (2×10^8 cfu g^{-1}), *M. anisopliae* (2×10^8 cfu g^{-1}) and *N. rileyi* (2×10^8 cfu g^{-1}) at two different doses (30 and 40 g $10L^{-1}$ water) against *L. orbonalis*. Reduced shoot damage (9.61 to 9.80 per cent) were observed on plots sprayed with *B. bassiana* and *M. anisopliae* applied at higher dosage than lower dosage. On yield basis, higher fruit yield (16049 kg ha^{-1}) was observed in the field treated with *B. basssiana* followed by *L. lecanii*, *N. rileyi* compared to the untreated field (12500 kg ha^{-1}).

2.4.1.4 *S. litura*

Udayababu *et al*. (2012) assessed the effect of fungal strains against *S. litura* on cabbage. Results showed that *B. bassiana* (Bb-L-2) @ 1×10^{13} gave a superior yield of 14741.0 kg ha^{-1} compared to lowest 10946.3 kg ha^{-1} yield in control. He further reported that the treatment *M. anisopliae* (Ma-L-1) @ 1×10^{13} reduced the damage by *S. litura* on cabbage and increased the yield.

Patel *et al.* (2014) reported a reduction in larval population of *S. litura* at three days after application of *M. anisopliae* at 10^{13} spores ha^{-1} compared to control. Prakash *et al.* (2015) observed that field application of *M. anisopliae* at 1 x 10^{12} conidia mL^{-1} on *Vigna sinensis L. caused* 51.35 to 88.65 per cent mortality of larvae of *S. litura*.

2.4.1.5 *S. derogata*

No literature pertaining to the field efficacy of fungal pathogens to *S. derogata* was available.

2.4.2 Field Efficacy of *B. bassiana* to Other Lepidopteran Pests

Ritu *et al*. (2012) noted that when *B. bassiana* applied in experimental plots at a dosage of 2.1 x 10^4 spores mL^{-1}, it caused infection in the larvae of *H. armigera*.

Patel *et al*. (2014) conducted a field trial for the evaluation of entomopathogens against lepidopteran defoliators infesting soyabean. Results of the study showed that *B. bassiana* at 10^{13} spores ha^{-1} was the most effective treatment compared to *M. anisopliae, L. lecanii, Bacillus thuringiensis* var. *kurstaki* at 5 g L^{-1} when applied as foliar sprays in 38, 41 and 45 days old crop. Higher grain yield was also recorded in the field treated with *B. bassiana* (415.97 kg ha^{-1}) compared to lowest yield in control (215.23 kg ha^{-1}).

2.4.3 Field Efficacy of *M. anisopliae* to Other Lepidopteran Pests

Loc and Chi (2007) conducted a field experiment on cauliflower using isolates of *M. anisopliae* against Diamondback moth. Among the isolates, *M. anisopliae* (OT_3 STO) treated field obtained highest cauliflower yield of 6.98 t ha^{-1} compared to control 4.03 t ha^{-1}.

Materials and Methods

3. MATERIALS AND METHODS

Indigenous fungi were isolated from different sources and identified. Further, laboratory and pot culture experiments were carried out to assess the pathogenicity and field performance of the fungi at Biocontrol Laboratory for Crop Pest Management, Department of Agricultural Entomology and at the Instructional Farm, College of Agriculture, Vellayani, during 2014- 2016.

3.1 ISOLATION AND IDENTIFICATION OF INDIGENOUS ENTOMOPATHOGENIC FUNGI

3.1.1 Isolation of Entomopathogenic Fungi

Entomopathogenic fungi were collected from mycosed cadavers as well as from soil.

3.1.1.1 From Mycosed Cadavers

3.1.1.1.1 Collection of Mycosed Cadaver

Survey was conducted in five agroecological units in Thiruvananthapuram district at bimonthly intervals during 2015-16 for the collection of mycosed cadavers, using stratified random sampling technique. The agroecological units surveyed were southern coastal plain (Chirayinkeezhu), southern laterite (Kattakada), southern central laterite (Nedumangad), southern and central foot hills (Vellanadu) and southern high hills (Peringammala). In each unit fifteen cultivated and fifteen uncultivated fields were surveyed. Dead cadavers with or without the external symptoms of mycosis were collected from these places and brought to the laboratory.

3.1.1.1.2 Isolation of Fungi from Mycosed Cadaver

The cadavers collected from the fields were kept in Petri plates with moistened filter paper for one to two days for the development of fungal mycelia if any. The cadavers with mycelial growth were surface sterilized with two per cent sodium hypochlorite for three minutes and then washed in sterile water thrice under aseptic conditions in a laminar air flow chamber. Excess moisture from cadaver was removed by placing them in sterilised filter paper. The cadavers were then placed in Potato Dextrose Agar (PDA) slants for development of mycelia. (Quesada-Moraga *et al.,* 2008). Hyphal tip culture was repeatedly done to obtain pure culture of the fungus. The fungus thus obtained was maintained in PDA under refrigerated condition for further studies.

3.1.1.2 From Soil

3.1.1.2.1 Collection of Soil Samples

Thirty soil samples, 15 from cultivated and 15 samples from uncultivated plots were collected from each of the agroecological units (as mentioned in 3.1.1.1.1) at bimonthly intervals. Each sample consisted of 200g soil, collected from a depth of 10 -15cm using an auger. The collected soil samples were bagged separately in clean polythene bags and brought to the laboratory (Hasan *et al*., 2012) and subsequently fungi were isolated adopting different methods.

3.1.1.2.2 Isolation of Fungi by Soil Plate Method

Two gram soil was scattered on the bottom of the sterile Petri dish and sterile, molten and cooled PDA medium was poured over the soil and it was then rotated gently to disperse the soil particles in the medium. Cetyl Trimethyl Ammonium Bromide (CTAB) at 600 mg L^{-1} and antibiotic (Ampicillin 500) at 0.1 per cent were

added to the molten PDA medium before pouring into the Petri plates. The medium containing soil was allowed to settle and then wrapped the Petri plates with cling film. The Petri plates were then incubated at 30 ± 2^0C for fungal growth (Warcup, 1950).

3.1.1.2.3 Isolation of Fungi using Insect Bait Method

Larvae of wax moth, *Galleria melonella* L. was used as the bait insect to trap entomopathogenic fungi (Meyling, 2007). In addition, grubs of pseudostem weevil, *Odoiporus longicollis* Oliv. were also used to trap soil fungi.

3.1.1.2.3.1 Diet Preparation for G. melonella

The diet for the larvae of *G. melonella* was prepared as per the method of Woodring and Kaya (1988). The diet contained wheat flour (200 g), corn flour (200 g), milk powder (100 g), honey (150 mL), Yeast powder (100 g) and glycerol (150 mL). Initially, corn flour, wheat flour and milk powder were thoroughly mixed in a basin and the required amount of honey and glycerol were mixed homogenously in another container. Honey glycerol mixture was then mixed with the flour mixture and sterilized at 121^0 C and 1.06 Kg cm^{-2} for 20 minutes. Yeast powder was then added into the mixture. The diet prepared was stored in air tight containers for one month for feeding the larvae.

3.1.1.2.3.2 Stock Culture of G. melonella

Larvae of *G. melonella* were collected from honey bee combs and were reared on the artificial diets (as mentioned in 3.1.1.2.3.1) in glass jars of 15 cm x 20 cm. Mouth of the glass jars were covered with muslin cloth and secured tightly using rubber band. Cream coloured larvae after completing fifth instars in the artificial diet pupated in white tough coccons on the artificial diet or in muslin cloth used for

covering the jar. The adults emerged were transferred into another glass jar (15 x 20 cm) using a glass vial. 10 per cent of honey solution in cotton swab was given as feed for adults. Inside the glass jars folded paper strips were also provided for facilitating oviposition. Eggs collected from paper strips were transferred to Petri plate and the larvae on emergence within three to four days were reared on artificial diets in glass jars.

3.1.1.2.3.3 Stock Culture of O. longicollis

Grubs of the pseudostem weevil *O. longicollis* collected from the banana fields at the Instructional Farm, Vellayani were reared on cut pseudostem pieces (Anitha, 2000). Pseudostem was changed once in two days until pupation. The pupae in cocoons were collected and placed in another container for adult emergence and further, the adults were transferred into glass jars (15 x 20 cm size) containing pseudostem of 15 cm size for oviposition and further rearing.

3.1.1.2.3.4 Trapping Entomopathogenic Fungi

3.1.1.2.3.4.1 Using G. melonella

Soil samples collected were air dried initially for two days so as to avoid any entomopathogenic nematodes in soil. Afterwards, air dried soils were moistened with sterile water and transferred to plastic containers of 8 x 11cm size. The fourth instar larvae of *G. melonella* were used for trapping. The larvae were dipped in hot water at 40^{0} C for 10 seconds and then washed in running water for 30 seconds. Moisture was removed by wrapping the larvae with tissue paper and further kept in darkness for three hours inorder to prevent the webbing of the larvae. Thereafter, 10 larvae were released into each container with 50 g soil (Plate 1A). The containers were then covered with lids having holes and placed in a basin with water for facilitating quick

(A) *G. melonella*

(B) *O. longicollis*

Plate 1. Bait trapping of entomopathogenic fungi

infection by fungal pathogens present in the soil. The dead larvae were collected and examined for fungal infection.

3.1.1.2.3.4.2 Using O. longicollis

Soil samples were prepared as mentioned in 3.1.1.2.3.4.1 and fourth instar grub of *O. longicollis* were placed on the surface of soil samples in a plastic containers of 8 x 11cm size (Plate 1B). After 24 h of exposure, cut pseudostem of 15 cm size was provided as feed. Dead grubs were collected after four days and kept in moisture chamber for the development of fungal infection.

3.1.1.2.3.5 Isolation and Maintenance of Fungi

The cadavers of *G. melonella* and *O. longicollis* were placed in moist chambers for fungal growth. Larvae with fungal growth were surface sterilised as mentioned in 3.1.1.1.2. These were then placed in PDA plates containing streptomycin and incubated at room temperature 30 ± 2^{0}C. The fungus developed from the cadaver was isolated in PDA slants and maintained with a code number for each isolate.

3.1.2 Identification

3.1.2.1 Morphological Identification

Mycelial discs of 5 mm were taken using cork borer from fungal cultures maintained in Petri plates and placed in the center of Petri plate containing PDA for studying the morphological and cultural characteristics of the fungus. Radial growth and colour of mycelia were recorded daily. Slide cultures were prepared (Harris, 1986) from 14 day old cultures and characters of conidiophore, size and shape of conidia were studied using Motic BA 210 compound microscope under 40X magnification. Measurements of 50 conidia in µm were taken using Motic Image Plus (version 2.0ML) and spore size was determined from the mean values. The

photomicroghaphs of the slide cultures were also taken with the facilities available in the Department of Agricultural Entomology and Plant Pathology.

3.1.2.2 Molecular Identification

Molecular identification of the select fungi was done through sequencing of the Internal Transcribed Spacer (ITS) of 5.8S rDNA conserved region at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram. The ITS regions of the isolates were amplified using the ITS- 1F and ITS- 4R primers. The following protocol was adopted for the DNA barcoding using universal primers of ITS.

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

Initially, 25 mg of the tissue / mycelium was homogenized using liquid nitrogen. The powdered tissue was transferred into a microcentrifuge tube and 400 µl of buffer PL1 was added and vortexed for one minute. RNase A solution (10 µl) was then added and inverted to mix. Incubation of the homogenate was done at 65°C for 10 minutes. After transferring the lysate to a Nucleospin filter, it was centrifuged at 11000 x g for two minutes. The flow through liquid was collected and the filter was discarded. Buffer PC (450 µl) was added, mixed well and the solution was transferred to a Nucleospin Plant II column, centrifuged for one minute and the flow through liquid was discarded. Later, the buffer PW1 (400 µl) was added to the column, centrifuged at 11000 x g for one minute and flow though liquid was discarded. Subsequently, PW2 (700 µL) was added centrifuged at 11000 x g and flow through liquid was discarded. Finally PW2 (200 µL) was added and centrifuged at 11000 x g for two minutes to dry the silica membrane. Then the column was transferred to a new 1.7 ml tube. Buffer PE (50 µL) was further added and incubated at 65°C for five minutes. To elute the DNA the column was then centrifuged at 11000 x g for one minute. Storing of the eluted DNA was done at 4°C.

3.1.2.2.2 Agarose Gel Electrophoresis for DNA Quality Check

DNA isolated was checked for quality using agarose gel electrophoresis. To 5 µL of DNA, one µL of 6X gel-loading buffer (0.25 per cent bromophenol blue, 30 per cent sucrose in TE buffer pH-8.0) was added. The samples were then 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 done with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. Visualisation of the gels was done in a UV transilluminator (Genei). Using Gel documentation system (Bio-Rad), the image was captured under UV light.

3.1.2.2.3 PCR Analysis

PCR amplification reactions was done in a 20 µL reaction volume that contained 1X Phire PCR buffer (contains 1.5 mM $MgCl_2$), 0.2mM each dNTPs (dATP, dGTP, dCTP and dTTP), 1 µL DNA, 0.2 µL Phire Hotstart II DNA polymerase enzyme, 0.1 mg mL^{-1} BSA and 3 per cent DMSO, 0.5M Betaine, 5pM of forward and reverse primers. The primers used were,

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

PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) was used for PCR amplification.

PCR amplification profile

ITS

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

3.1.2.2.4 Agarose Gel Electrophoresis of PCR Products

The PCR products were checked in 1.5 per cent agarose gels prepared in 0.5X TBE buffer containing 0.5 µg mL^{-1} ethidium bromide. 6X loading dye (One µL) was mixed with five µL of PCR products and this was loaded and electrophoresis was done at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 h, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard was used on the 2-log DNA ladder (NEB). Visualisation of the gels was done in a UV transilluminator (Genei). The image was captured using Gel documentation system (Bio-Rad) under UV light.

3.1.2.2.5 ExoSAP-IT Treatment

Five µL of PCR product was mixed with two µL of ExoSAP-IT and incubated at 37°C for 15 min. The ExoSAP-IT (GE Healthcare) consisted of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications. After incubation, enzyme inactivation was done at 80°C for 15 min.

3.1.2.2.6 Sequencing using BigDye Terminator v3.1

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

The PCR mix consisted of 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) and sterile distilled water (made up to 10µL).

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

3.1.2.2.7 Post Sequencing PCR Clean up

Master mix I was prepared with 10µL milli Q and two µL 125mM EDTA. 12µL of master mix I was then added to 10µL of reaction contents and were properly mixed. Master mix II was prepared by mixing two µL of 3M sodium acetate (pH 4.6) and 50 µL of ethanol per reaction. 52 µL of master mix II was added to each reaction and inverted to mix the contents. Then the tubes were incubated at room temperature for 30 minutes. The tubes were centrifuged at 14,000 rpm for 30 minutes. Supernatant was decanted and 100 µL of 70 per cent ethanol was added and again centrifuged at 14,000 rpm for 20 minutes. Ethanol wash was repeated after decanting the supernatant. The pellet was then air dried afterwards, the cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.1.2.2.8 Sequence Analysis

The quality of the sequence was checked using Sequence Scanner Software v1 (Applied Biosystems). The sequence alignment and required editing of the obtained

sequences were carried out using Geneious Pro v5.1 (Drummond *et al*., 2010). The identity of the indigenous isolates was established based on the ITS- rDNA conserved region of the isolates. A similarity search was performed using Basic Local Alignment Search Tool (BLAST) in the National Centre for Biotechnology Information (NCBI) database and species confirmation was performed using existing available database. Based on the results, rDNA sequences were submitted in the NCBI database and accession numbers were obtained.

3.1.2.3 Sporulation

3.1.2.3.1 Maintenance of Fungi

The indigenous isolates *viz., Beauveria bassiana* (Balsamo) Vuillemin (SP2), *B. bassiana* (SP4), *Fusarium oxysporum* Schlecht. (SP1), *Fusarium solani* (Mart.) Sacc. (SP6), *Metarhizium anisopliae* Metschnikoff (Sorokin) (SP7), *M. anisopliae* (SP8), *M. anisopliae* (SP9), *M. anisopliae* (SP11), *M. anisopliae* (SP13), *Purpureocillium lilacinum* Thorn (Samson) (S10) and the cultures obtained from National Bureau of Agricultural Insect Resources (NBAIR), previously known as National Bureau of Agriculturally Important Insects (NBAII) cultures of *B. bassiana* (Bb5a), *M. anisopliae* (Ma4) were maintained in PDA. Virulence was maintained by applying spore suspension of the *B. bassiana, F. oxysporum, F. solani* and *P. lilacinum* isolates on aphid, *A. craccivora* whereas *M. anisopliae* isolates on *D. indica.*

3.1.2.4.2 Preparation of Spore Suspension

Spore suspension was prepared from 14 day old culture of the fungi grown in Petri plates. 10 mL of the sterile water was added to each Petri plate and using a sterile needle the spores were scraped into sterile water and from the spore suspension spore count was estimated.

3.1.2.4.3 Estimation of Spore Count

Spore count was taken using Neubaeur's haemocytometer. Initially, haemocytometer and cover slip were cleaned and dried using tissue paper. Ten µL of the fungal suspension was drawn using a sterile pipette and poured into each of the counting chamber. Cover slip was placed carefully over the counting chamber to avoid air bubbles. Spores in each of the four $0.1mm^3$ corner squares were counted with the help of a compound microscope under 40X magnification. Total spore count was calculated using the following equation.

Spores $mL^{-1} = (n) \times 10^4$, where n= mean spore count per corner square.

The sporulation of the different indigenous isolates and NBAIR isolates of *B. bassiana* (Bb5a) and *M. anisopliae* (Ma4) were assessed on seventh and fourteenth day.

3.2 PATHOGENICITY OF FUNGAL PATHOGENS TO INSECTS

3.2.1 Stock Culture of Test Insect

3.2.1.1 Diaphania indica Saunders

Larvae of *D. indica* collected from field were brought to the laboratory and reared on bitter gourd leaves in glass jars of 15 cm x 20 cm with its mouth tightly covered with a fine muslin cloth and tied with rubber band. Larvae on maturity pupated in tough brown coloured cocoons. These were collected and placed in another glass jar for adult emergence. Adult that emerged were fed with 10 per cent honey solution in cotton pads. The female laid cream coloured eggs on the side of the glass jars or in the muslin cloth either singly or in clusters. Larvae that emerged from eggs within three to four days were fed with fresh bitter gourd leaves daily and the culture was maintained continuously in the laboratory.

3.2.1.2 Hymenia recurvalis Fabricius

Larvae of amaranthus leaf webber collected from field were brought to the laboratory and reared on fresh amaranthus leaves in glass jars of 15 x 20 cm. Fresh leaves were provided once in two days. Larvae that underwent pupation in the amaranthus leaves were collected and kept in another glass jar for adult emergence. Adults were provided with 10 per cent honey solution in cotton swabs. Eggs laid on amaranthus leaves were collected and kept for larval emergence and culture was continuously maintained.

3.2.1.3 Leucinodes orbonalis Guenee

Field collected larvae of *L. orbonalis* were released on fresh brinjal fruits in glass jars of 15 cm x 20 cm. The Pink larvae when full grown came out of their feeding tunnels and pupated in tough silken cocoons in the bits of cloth pieces provided in the jar. Adults that emerged from the brown boat shaped pupae were collected using a vial and 10- 20 adults consisting of males and females were transferred into glass jars (15 x 20 cm) for oviposition. Cotton dipped in 10 per cent honey solution was kept as feed for adults. The female laid eggs on the muslin cloth either singly or in groups and also on the sides of the glass jar. Fresh brinjal fruits were kept as food with in the glass jar for the newly emerged larvae from the creamy white eggs. Brinjal fruits for rearing were collected from unsprayed plants maintained in pots. The newly hatched larvae after moving for a short while entered through the soft region below the calyx and developed within the fruits. In each fruit five to seven larvae developed. Thus, the stock culture for the experiment was maintained.

3.2.1.4 Spodoptera litura Fabricius

Leaf caterpillar, *S. litura* larvae were collected from banana leaves in the field and brought to the laboratory. Early instar larvae were mass reared on castor leaves

in glass jars of 15 x 20 cm. Later instar larvae were reared on castor leaves with 10 larvae in each glass jar. The jars were cleaned daily and provided with fresh castor leaves. When they reached the final instar, sterilised soil was provided in glass jars for pupation. Adults that emerged were transferred to another glass jar for egg laying and were provided with 10 per cent honey solution as feed. Folded paper strips were also kept for egg laying. The egg masses collected were kept for larval emergence and for further rearing.

3.2.1.5 Sylepta derogata Fabricius

Larvae of leaf roller, *S. derogata* were collected from the field and reared on bhindi leaves in glass jars of 15 x 20 cm. Larvae that pupated in the leaf rolls were collected and kept for adult emergence. Adults collected were kept for oviposition, along with 10 per cent honey solution as feed. Eggs laid in the bhindi leaves were collected and transferred to another container for hatching and further larval rearing.

3.2.2 Assessment of Pathogenicity

3.2.2.1 Maintenance of Fungi

The fungal isolates *viz., B. bassiana* (SP2 and SP4), *F. oxysporum* (SP1), *F. solani* (SP6), *M. anisopliae* (SP7), *M. anisopliae* (SP8), *M. anisopliae* (SP9), *M. anisopliae* (SP11), *M. anisopliae* (SP13) *P. lilacinum* (S10) and NBAIR cultures of *B. bassiana* (Bb5a), *M. anisopliae* (Ma4) were maintained in PDA (as mentioned in 3.1.2.3.1). Virulence was maintained as mentioned in 3.1.2.3.1.

3.2.2.2 Preparation of Spore Suspensions and Application

Spore suspension was prepared as mentioned in 3.1.2.4.2. This spore suspension was sprayed over the second instar larvae of the test insects using an atomiser.

Subsequently the insects were provided with their respective feed and observed daily for symptom development and mortality.

3.2.2.3 Assessment of Virulence of Fungi

Virulence of ten indigenous isolates and two NBAIR isolates were determined at three different concentrations, 10^7, 10^8 and 10^9 spores mL^{-1} from spore suspension prepared from 14 day old culture grown in PDA plates against five test insects *viz., D. indica, H. recurvalis*, *L. orbonalis, S. derogata* and *S. litura*. The experiments were done in CRD with three replications, each replication having ten insects.

3.2.3.4 Against D. indica

The virulence was tested against second instar larvae of *D. indica* taken in sterile Petri plates. The spore suspensions at the respective concentrations were sprayed over the larvae using an atomiser. Larvae treated with sterile distilled water were maintained as control. Treated larvae were then transferred to sterile Petri plate containing filter paper and provided with fresh bittergourd leaves as feed. Larval mortality was recorded daily until death or pupation. Mycosed cadavers were kept in moisture chamber for mycelial growth and further examination done for confirmation of the pathogen. The data on mortality was subjected to ANOVA after angular transformation.

3.2.3.5 Against H. recurvalis

The virulence test was conducted on the second instar larvae of *H. recurvalis* as mentioned in 3.2.3.2.

3.2.3.6 Against L. orbonalis

First instar larvae were used for assessing the virulence of fungal isolates. Spore suspensions were sprayed over the larvae of *L. orbonalis* taken in Petri plates. After thirty minutes, treated larvae were released on the surface of fresh brinjal fruits. Five larvae were released on each fruit. Ten larvae in two fruits formed one replication. Larvae sprayed with sterile water and released on the surface of brinjal fruits served as control. After five days, fruits were cut opened and the number of larvae that were present within the fruits was counted and the percentage survivability and mortality were determined from the data. The percentage mortality data was then statistically analysed after correction with Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{Mortality in treatment} - \text{Mortality in control}}{100 - \text{Mortality in control}} \times 100$$

3.2.3.7 Against S. litura

Virulence was assessed as mentioned in 3.3.3.2 using second instar larvae of *S. litura.* The treated larvae were provided with fresh castor leaves as feed. The data on mortality was statistical analysed using t test.

3.2.3.8 Against S. derogata

Second instar larvae were used for the study. The treated larvae were fed with bhindi leaves. The experiment and analysis was done as mentioned in 3.2.3.4.

3.2 POT CULTURE EXPERIMENT

The field efficacy of the select indigenous fungi and NBAIR isolates were evaluated against amaranthus leaf webbers in pot culture experiment conducted at

Instructional Farm, College of Agriculture, Vellayani during April to June 2016. Seeds were sown directly in the growbags and after two weeks, seedlings were thinned out to maintain two seedlings in each growbag (Plate 2). Crop was maintained as per the crop management practices suggested in Package of Practices Recommendations of the KAU (2011) excluding plant protection measures. Details of the experiment as follows.

Design : CRD

Replications : 4

Treatments : 10

Number of plants replication^{-1}: 5

T1 : *B. bassiana* (SP2) at 10^8 spores mL^{-1}

T2 : *B. bassiana* (SP4) at 10^8 spores mL^{-1}

T3 : *M. anisopliae* (SP7) at 10^8 spores mL^{-1}

T4 : *M. anisopliae* (SP8) at 10^8 spores mL^{-1}

T5 : *M. anisopliae* (SP9) at 10^8 spores mL^{-1}

T6 : *M. anisopliae* (SP11) at 10^8 spores mL^{-1}

T7 : *P. lilacinum* (S10) at 10^8 spores mL^{-1}

T8 : *B. bassiana* (Bb5a) at 10^8 spores mL^{-1} (NBAIR isolate)

T9 : *M. anisopliae* (Ma4) at 10^8 spores mL^{-1} (NBAIR isolate)

Plate 2. Experimental plot

T10 : Untreated

3.3.1 Preparation of Spray Solutions

3.3.1.1 Preparation of Potato Dextrose Broth (PDB)

One litre PDB was prepared using potato (200 g) and dextrose (20 g). Potato was peeled, cubed and boiled in 500 mL water. Extract was obtained by sieving through muslin cloth. Twenty gram dextrose was mixed in 500 mL water and it was added to the potato extract and made up to one litre. 100 mL of prepared media was added to each 250 mL conical flask plugged with cotton and then sterilised at 121^0 C at 1.06 kg cm^{-2} pressure for 15 minutes. Sterilised and cooled media was then inoculated with fungal cultures.

3.3.1.2 *Preparation of Broth Culture of Fungi*

Spore suspension of the fungi *B. bassiana* (SP2), *B. bassiana* (SP4), *M. anisopliae* (SP7), *M. anisopliae* (SP8), *M. anisopliae* (SP9), *M. anisopliae* (SP11), *P. lilacinum* (S10), *B. bassiana* (Bb5a), *M. anisopliae* (Ma4) were prepared from 14 day old culture of the respective fungi grown in PDB. Spore count was estimated using Neubaeur's haemocytometer and spore concentration was maintained at 10^8 spores ml^{-1}.

3.3.2 Assessment of Pest Population and Intensity of Damage

Pre treatment and post treatment population of pests and natural enemies and extent of damage by leaf webbers were recorded. Post treatment observations were taken at weekly intervals.

3.3.2.1 Extent of Damage

The extent of damage was assessed in terms of the percentage of plants infested and the number of webs plant^{-1}.

The number of plants infested by leaf webbers out of the observational plants was counted and from these values the mean percentage of infested plants was worked out. The number of webs present in each observational plant was recorded and from this, the mean values were worked out. The data was subjected to Analysis of Variance.

3.3.2.2 Population

The total number of larvae of *H. recurvalis* and *Psara basalis* Walker present in each web of the observational plants were counted and the mean population web^{-1} was calculated. The data was subjected to Analysis of Variance.

The population of other pests and natural enemies present in each observational plant was also counted.

3.3.3 Yield

Harvesting was done at 35 and 50 days after sowing. The weight of cuttings from each observational plant was recorded separately at harvest and the mean weight was recorded as g plant^{-1}. The data was statistically analysed using Analysis of Variance.

Results

4. RESULTS

4.1 ISOLATION AND IDENTIFICATION OF ENTOMOPATHOGENIC FUNGI

4.1.1 Isolation

Ten indigenous isolates of entomopathogenic fungi were collected from five agroecological zones *viz.*, southern coastal plain (Chirayinkeezhu), southern laterite (Kattakada), southern central laterite (Nedumangad), southern and central foot hills (Vellanadu) and southern high hills (Peringammala) during 2015-16. Of these, three were collected from insect cadavers and seven from soil. Among the soil collected isolates, five isolates were obtained adopting baiting with *Galleria melonella* L. and one isolate was collected adopting trapping with *Odoiporus longicollis* Oliv. One isolate was collected by adopting soil plate method, Details of the ten isolates of entomopathogenic fungi are given in Table 1.

4.1.1.1 From Mycosed Cadavers

Two isolates of *Beauveria bassiana* (Balsamo) Vuillemin (SP2 and SP4) and one isolate of *Fusarium oxysporum* Schlecht (SP1) were collected from mycosed cadavers. The isolate SP2 of *B. bassiana* was isolated from the mycosed cadaver of Tortoise beetle *Metriona circumdata* Weise present in sweet potato fields at Vellayani. The other isolate SP4 of *B. bassiana* was collected from the cadaver of an unidentified caterpillar. *F. oxysporum* (SP1) was isolated from the cadaver of white leaf hopper, *Cofana spectra* Distant present in amaranthus fields of Vellayani which were adjacent to rice fields.

Table 1. Details of fungi isolated from the different agroecological zones in Thiruvananthapuram district

Sl. No.	Isolate and Isolate code	Source	Agroecological zone and Place of collection	NCBI GenBank Accession number	Reference isolates
1.	*Beauveria bassiana* (Balsamo) Vuillemin (SP2)	Insect cadaver	Southern laterite, Vellayani	KX171222	*B. bassiana* ARSEF 751 (HQ880763.1)
2.	*B. bassiana* (SP4)	Insect cadaver	Southern laterite, Vellayani	KX171224	*B. bassiana* Bb 9016 (AY334539.1)
3.	*Fusarium oxysporum* Schlecht (SP1)	Insect cadaver	Southern laterite, Vellayani	KX171221	*F. oxysporum* CJ2 (JQ771175.1)
4.	*Fusarium solani* (Mart.) Sacc. (SP6)	Soil (UC)*	Southern laterite, Kattakada	KX171215	*F. solani* CBPPR0036 (KT211528.1)
5.	*Metarhizium anisopliae* Metschnikoff (SP7)	Soil (C)*	Southern coastal plain, Chirayinkeezhu	KX171216	*M. anisopliae* 100513 11-1 (JF495767.1)
6.	*M. anisopliae* (SP8)	Soil (UC)*	Southern and central foot hills, Vellanadu	KX171217	*M. anisopliae* M5 (LK995311.1)

C: Cultivated soil UC: Uncultivated soil

*Baiting with *G. melonella* **Baiting with *O. longicollis* *** Soil plate method

Table 1. Details of fungi isolated from the different agroecological zones in Thiruvananthapuram district (continued)

7.	*M. anisopliae* (SP9)	Soil (C)*	Southern high hills, Peringammala	KX171218	*M. anisopliae* S4ST7 (KM463106.1)
8.	*M. anisopliae* (SP11)	Soil (C)*	Southern and central foot hills, Vellanadu	KX171220	*M. anisopliae* CNGD4 (FJ545279.1)
9.	*M. anisopliae* (SP13)	Soil (UC)**	Southern central laterite, Nedumangad	KX550275	*M. anisopliae* DhMz3R (KU983783.1)
10.	*Purpureocillium lilacinum* Thorn (Samson) (S10)	Soil (C)***	Southern central laterite, Nedumangad	KX219589	*P. lilacinum* 001JFC (KR025540.1)

C: Cultivated soil UC: Uncultivated soil

*Baiting with *G. melonella* **Baiting with *O. longicollis* *** Soil plate method

4.1.1.2 From Soil

4.1.1.2.1 Using Insect Bait, G. melonella

Two isolates of *Metarhizium anisopliae* Metschnikoff (Sorokin) (SP7 and SP9) were isolated from the cultivated soils collected from southern coastal plain, (Chirayinkeezhu) and southern high hills (Peringammala), respectively and the other two isolates (SP8 and SP11) were collected from uncultivated and cultivated soils respectively of southern and central foot hills (Vellanadu). One isolate of *Fusarium solani* (Mart.) Sacc. was isolated from the uncultivated soil collected from southern laterite (Kattakada).

4.1.1.2.2 Using Insect Bait, O. longicollis

One isolate of *M. anisopliae* (SP13) was isolated from the uncultivated soil of southern central laterite, Nedumangad using *O. longicollis* as bait insect.

4.1.1.2.3 Using Soil Plate Method

Adopting soil plate method, 106 fungal isolates were preliminarily obtained from 900 soil samples, of which 70 were from cultivated fields and 36 were from uncultivated fields. Among the 106 fungal isolates, 45 were species of *Aspergillus* (13 from uncultivated soil and 32 from cultivated soil), 19 were *Fusarium* sp. (5 from uncultivated soil and 14 from cultivated soil), 14 were *Penicillium* sp. (4 from uncultivated soil and 10 from cultivated soil), and 27 were species of *Trichoderma* (10 from cultivated soil and 17 from cultivated soil). One isolate *Purpureocillium lilacinum* Thorn (Samson) was obtained from cultivated soil collected from southern central laterite, Nedumangad.

4.1.2 Identification

4.1.2.1 *Morphological Identification*

Morphological and cultural characteristics of the ten indigenous isolates collected from mycosed cadaver and soil are given in Table 2 and 3.

4.1.2.1.1 B. bassiana (SP2)

The fungus isolated from the mycosed cadaver of tortoise beetle *M. circumdata* produced flat, cottony white colonies on Potato Dextrose Agar (PDA), and the reverse was cream coloured (Plate 3 A and A1). It had a radial growth of 3.66 cm after 14 Days after inoculation (DAI) in PDA plates. The zig zag conidiogenous cells on single, lateral conidiophores had globose conidia at its tip that had a mean diameter of 1.3 ± 0.04 µm with length / width ratio of 1.00 (Plate 3 A2).

4.1.2.1.2 B. bassiana (SP4)

The fungus produced raised cottony white mycelial colonies on the upper surface and cream coloured on the lower surface of PDA plates (Plate 3 B and B1) with a radial growth of 4.66 cm after 14 days of inoculation. The isolate had globose conidia of mean diameter 1.2 ± 0.07 µm with length / width ratio of 1.00, produced in zig zag conidiogenous cells developed on single, lateral conidiophores (Plate 3 B2).

4.1.2.1.3 F. oxysporum (SP1)

The fungal isolate cultured in PDA was floccose in texture. The colony had light purple coloured center and white coloured outer margin on the upper surface. The ventral surface had faint purple coloured center with dirty white coloured outer margin (Plate 3 C and C1). The isolate produced 9cm radial growth on 14 DAI in Petri dishes. Fungal isolate produced three septate thin walled fusiform macroconidia with pointed ends (Plate 3 C2) with a mean length of 12.8 ± 2.22.

Table 2. Morphological characters of the indigenous isolates of entomopathogenic fungi

Sl. No	Isolate and Isolate code	Conidiophore arrangement	Phialide	Conidial shape	Conidial size (µm) * Mean ± S. E		Length/ width ratio
					Length	Width	
1.	*B. bassiana* (SP2)	Single and lateral	No phialide	Globose	1.3 ± 0.04	1.3 ± 0.04	1.00
2.	*B. bassiana* (SP4)	Single and lateral	No phialide	Globose	1.2 ± 0.07	1.2 ± 0.07	1.00
3.	*F. oxysporum* (SP1)	Single, lateral and branched	Mono and cylindrical	Macroconidia - Three septate thin walled fusiform with pointed ends Microconidia - Aseptate, oval	12.8 ± 2.22 3.3 ± 0.11	1.3 ± 0.05 1.1 ± 0.05	9.84 3.00
4.	*F. solani* (SP6)	Single and unbranched	Mono and subcylindrical	Macroconidia - Three septate thick walled fusiform Microconidia- Aseptate, ovoid	10.8 ± 0.39 4.5 ± 0.14	1.8 ± 0.02 1.6 ± 0.02	6.00 2.81
5.	*M. anisopliae* (SP7)	Lateral or terminal and branched	Clavate	Cylindrical with middle constricted	3.02 ± 0.02	1.01± 0.05	2.99

*Mean of 50 conidia

Table 2. Morphological characters of the indigenous isolates of entomopathogenic fungi (continued)

6.	*M. anisopliae* (SP8)	Lateral or terminal and branched	Clavate	Cylindrical	2.5 ± 0.01	0.9 ± 0.08	2.77
7.	*M. anisopliae* (SP9)	Lateral or terminal and branched	Columnar	Cylindrical	2.4 ± 0.02	0.9 ± 0.06	2.66
8.	*M. anisopliae* (SP11)	Lateral or terminal and branched	Columnar	Cylindrical with middle constricted	3.5 ± 0.07	1.0 ± 0.06	3.50
9.	*M. anisopliae* (SP13)	Lateral or terminal and branched	Columnar	Cylindrical	2.3 ± .0.01	1.0 ± 0.03	2.30
10.	*P. lilacinum* (S10)	Verticillate	Ovate to cylindrical with distinct neck	Round	0.9 ± 0.003	0.9 ± 0.003	1.00

*Mean of 50 conidia

Table 3. Cultural characteristics of the indigenous isolates of entomopathogenic fungi

Sl. No	Isolate	Colony colour		Colony texture	Radial growth at 14 DAI (cm)
		Upper	Lower		
1.	*B. bassiana* (SP2)	White	Cream	Flat and cottony	3.66
2.	*B. bassiana* (SP4)	White	Cream	Raised and cottony	4.66
3.	*F. oxysporum* (SP1)	Light purple	Faint purple	Floccose	9.00
4.	*F. solani* (SP6)	Creamy white	Cream	Floccose	9.00
5.	*M. anisopliae* (SP7)	White	Cream	Thick and raised	4.83
6.	*M. anisopliae* (SP8)	Creamy white	Light brown	Cottony and flat	6.43
7.	*M. anisopliae* (SP9)	White	Cream	Thick and cottony	6.23
8.	*M. anisopliae* (SP11)	White	Cream	Cottony	6.83
9.	*M. anisopliae* (SP13)	Creamy white	Light brown	Cottony	6.00
10.	*P. lilacinum* (S10)	Vinaceous brown	Brown	Floccose	6.33

DAI - Days after inoculation

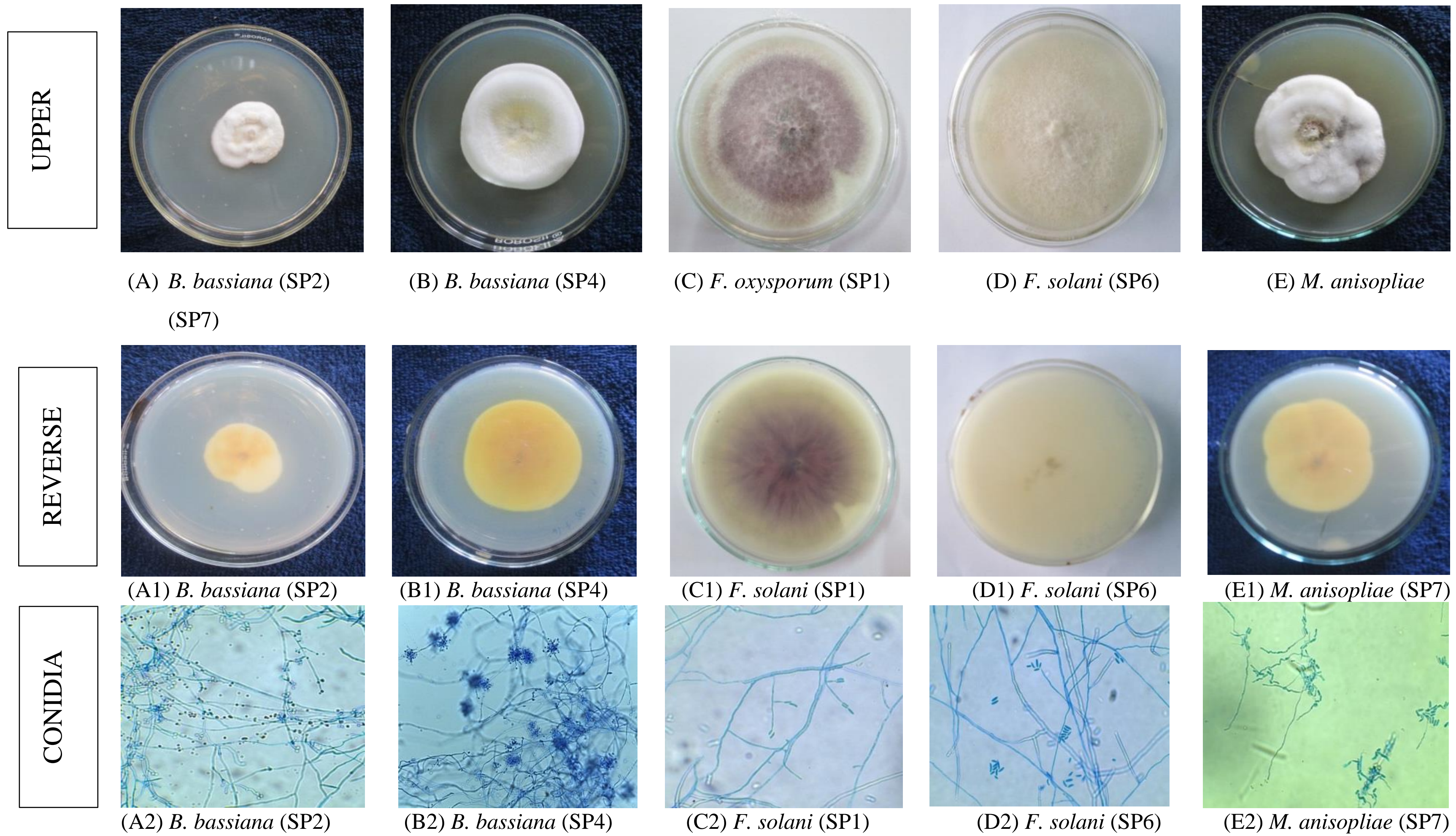


Plate 3. Growth and Photomicrographs of indigenous isolates of entomopathogenic fungi

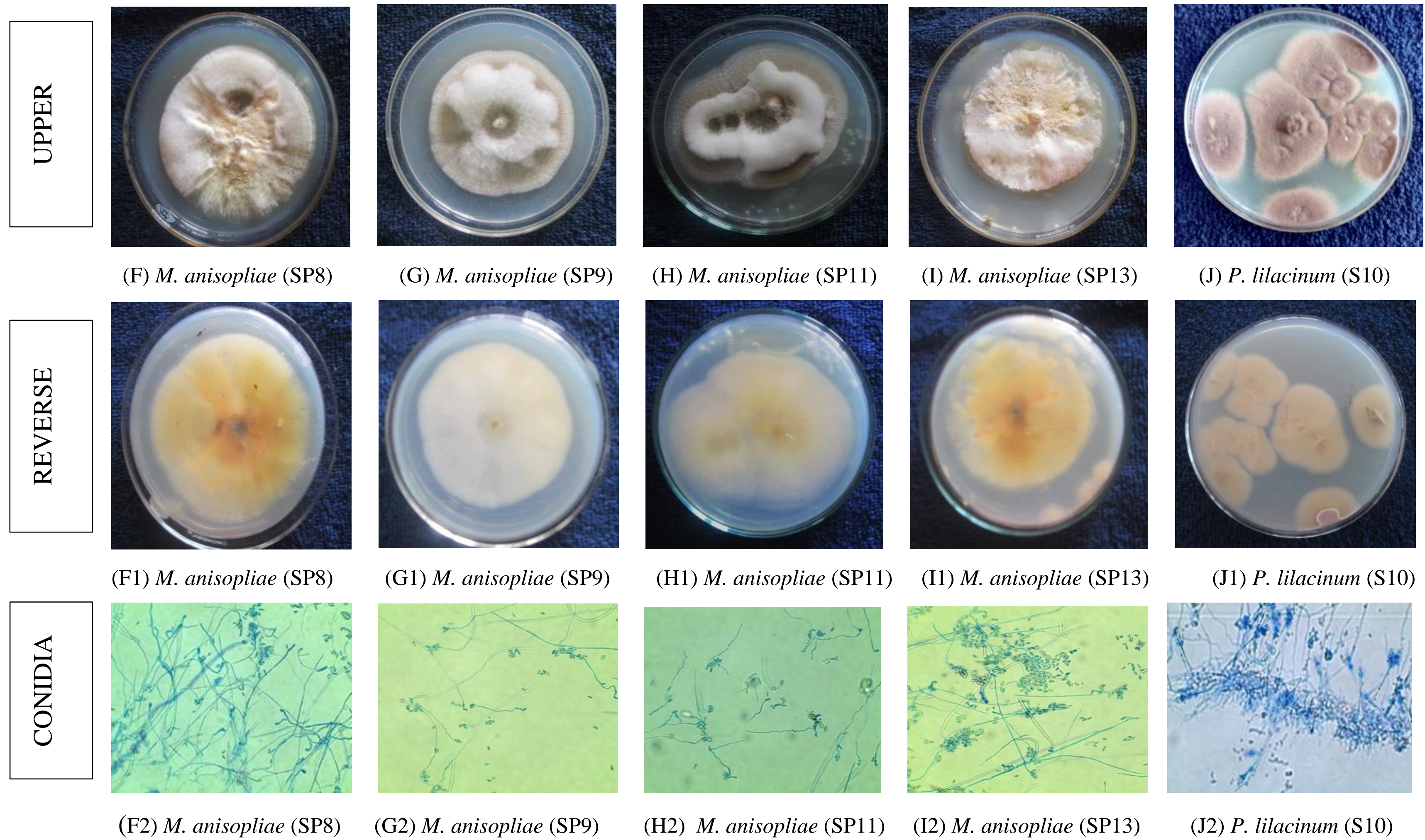


Plate 3. Growth and Photomicrographs of indigenous isolates of entomopathogenic fungi (continued)

μm and width of 1.3 ± 0.05 μm and having length / width ratio of 9.84. It also produced microconidia which was aseptate oval, with a mean length of 3.3 ± 0.11 μm and width of 1.1 ± 0.05 μm and had length / width ratio of 3.00. Spores were developed in single cylindrical phialides arise from lateral branched conidiophores.

4.1.2.1.4 F. solani (SP6)

Colonies appeared creamy white floccose on the upper surface and cream on the reverse (Plate 3 D and D1). The fungus attained a radial growth of 9 cm at 14 DAI. The isolate produced three septate thick walled fusiform macroconidia with a mean length of 10.8 ± 0.39 μm and mean width of 1.8 ± 0.02 μm having length / width ratio of 6.00. Aseptate ovoid microconidia had a mean length of 4.5 ± 0.14 μm and width of 1.6 ± 0.02 μm having length / width ratio of 2.81. Mono and subcylindrical phialides developed on single and unbranched conidiophores (Plate 3 D2).

4.1.2.1.5 M. anisopliae (SP7)

The colonies were initially white which later turned gray due to the production of gray coloured cylindrical spores with middle constriction. The phialides were clavate. The isolate produced white thick raised floccose colonies with white upper surface and cream venter (Plate 3 E and E1). The isolate had a radial growth of 4.83 cm on PDA after 14 DAI. Conidiophores developed on lateral and terminal branches of hyphae (Plate 3 E2). The mean length of conidia was 3.02 ± 0.02 μm and it had a mean width of 1.01 ± 0.05 μm. The length / width ratio was 2.99.

4.1.2.1.6 M. anisopliae (SP8)

The *M. anisopliae* (SP8) isolate was characterised by white mycelial growth initially which turned to shades of green on sporulation. The surface layer

of colonies were seen cottony and flat creamy white in colour, and reverse side was light brown (Plate 3 F and F1). The isolate produced a radial growth of 6.43 cm after 14 DAI in PDA. The conidia were cylindrical with round ends had a mean length of 2.5 ± 0.01 µm and width of 0.9 ± 0.08 µm. The length / width ratio was 2.77. These were produced on clavate phialides, on elongated branched conidiophores (Plate 3 F2).

4.1.2.1.7 M. anisopliae (SP9)

The fungus produced thick and cottony colonies of white mycelia with gray coloured spores on the upper surface and reverse cream colour (Plate 3 G and G1). Isolate had a radial growth of 6.23 cm 14 DAI. The elongated branched conidiophore produced columnar phialides which bears cylindrical conidia (Plate 3 G2) with a mean length of 2.4 ± 0.02 µm and width of 0.9 ± 0.06 µm having length / width ratio of 2.66.

4.1.2.1.8 M. anisopliae (SP11)

The isolate of *M. anisopliae* (SP11) produced cottony white mycelia initially which became gray coloured within 4-5 days on sporulation and showed zonations. The fungus produced cream colour on the lower surface of PDA plates (Plate 3 H and H1). The isolate had a radial growth of 6.83 cm after 14 days of inoculation in PDA. The columnar phialides bears cylindrical conidia with middle constriction and with a mean length of 3.5 ± 0.07 µm and width of 1.0 ± 0.06 µm with length / width ratio of 3.50 produced in elongated conidiophores (Plate 3 H2).

4.1.2.1.9 M. anisopliae (SP13)

The colony appeared similar to SP8 (Plate 3 I and I1). The isolate had a radial growth of 6.00 cm after 14 days of inoculation in PDA. The conidia were cylindrical

with a mean length of 2.3 ± 0.01µm and width of 1.0 ± 0.03 µm had length / width ratio of 2.30 (Plate 3 I2).

4.1.2.1.10 P. lilacinum (S10)

The fungus produced floccose white colonies initially that became shades of vinaceous brown with the onset of sporulation on the upper surface and cream colour on lower surface in PDA. (Plate 3 J and J1). The isolate had a radial growth of 6.33 cm after 14 days of inoculation. Verticillate conidiophores bearing ovate to cylindrical phialides which abruptly tapered into a distinct neck were observed (Plate 3 J2). The conidia were smooth walled round and were produced in chains with a mean diameter 0.9 ± 0.003 µm .

4.1.2.2 ***Molecular Identification***

The details of the molecular identification of the fungi done at Rajiv Gandhi Center for Biotechnology, Thiruvananthapuram are presented under here.

4.1.2.2.1 B. bassiana (SP2)

The Internal Transcribed Spacer (ITS) sequencing of the fungus yielded 530 base pair (bp) sequences are given below.

AGGGATCATTACCGAGTTTTCAACTCCCCAACCCTTCTGTGAACCTACCTA
TCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACTGGACCAGCGG
CCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCTTCTGAATACGCCGC
AAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCTCTTGGCTCTG
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
ATCCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTC
TGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCGACCTCCCCTTGGG
GACGTCGGCGTTGGGGACCGGCAGCACACCGCCGGCCCTGAAATGGAGT

GGCGGCCCGTCCGCGGCGACCTCTGCGTAGTAATACAGCTCGCACCGGAA
CCCCGACGCGGCCACGCCGTAAAACACCCAACTTCTGAACGTTGACCTCG
AATCAGGTAGGACTACCCGCTGAACTTAA

The Basic Local Alignment Search Tool (BLAST) analysis of above nucleotide sequence showed cent per cent similarity with *B. bassiana* ARSEF 751 having accession number HQ 880763.1. The accession number assigned by GenBank to the isolate SP2 was KX171222.

4.1.2.2.2 B. bassiana (SP4)

The Internal Transcribed Spacer (ITS) sequencing of the fungus yielded 530 base pair (bp) sequences are given below.

AGGGATCATTACCGAGTTTTCAACTCCCCAACCCTTCTGTGAACCT
ACCTATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACTGGACC
AGCGGCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCATCTTCTGAATA
CGCCGCAAGGCAAAACAAATGAATCAAAACTTTCAACAACGGATCTCTTG
GCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATCCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAG
CATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCGACCTCCCC
TTGGGGACGTCGGCGTTGGGGACCGGCAGCACACCGCCGGCCCTGAAAT
GGAGTGGCGGCCCGTCCGCGGCGACCTCTGCGTAGTAATACAGCTCGCAC
CGGAACCCCGACGCGGCCACGCCGTAAAACACCCAACTTCTGAACGTTGA
CCTCGAATCAGGTAGGACTACCCGCTGAACTTAA

The above sequence showed cent per cent similarity with *B. bassiana* Bb 9016 having accession number AY 334539.1 when subjected to nucleotide BLAST analysis. The accession number assigned by GenBank to this isolate was KX171224.

4.1.2.2.3 F. oxysporum (SP1)

The Internal Transcribed Spacer (ITS) sequencing of the fungus yielded 519 base pair (bp) sequences are given below.

AGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCACT
TGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCA
GAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAA
ATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAG
AACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC
ATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGC
CTGTTCGAGCGTCATTTCAACCCTCAAGCCCTCGGGTTTGGTGTTGGGGAT
CGGCGAGCCCTTGCGGCAAGCCGGCCCCGAAATCTAGTGGCGGTCTCGCT
GCAGCCTCCATTGCGTAGTAGTAAAACCCTCGCAACTGGAACGCGGCGCG
GCCAAGCCGTTAAACCCCCAACTTCTGAATGTTGACCTCGGATCAGGTAG
GAATACCCGCTGAACTTAA

The above sequence when subjected to nucleotide BLAST showed cent per cent similarity with *F.* oxysporum CJ2 having accession number JQ 771175.1. The accession number assigned by GenBank to the isolate SP1was KX 171221.

4.1.2.2.4 F. solani (SP6)

The ITS sequencing of the fungus yielded 529 base pair (bp) sequences are given below.

AGGGATCATTACCGAGTTATACAACTCATCAACCCTGTGAACATACCTAT
AACGTTGCCTCGGCGGGAACAGACGGCCCCGTAACACGGGCCGCCCCCG
CCAGAGGACCCCCTAACTCTGTTTCTATAATGTTTCTTCTGAGTAAACAAG
CAAATAAATTAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATG

AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCA
TGCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTGGCGTTGG
GGATCGGCGGAAGCCCCCTGCGGGCACAACGCCGTCCCCCAAATACAGT
GGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAGCTAACACCTCGCAACTG
GAGAGCGGCGCGGCCACGCCGTAAAACACCCAACTTCTGAATGTTGACCT
CGAATCAGGTAGGAATACCCGCTGAACTTAA

The Nucleotide BLAST analysis of above sequence showed cent per cent similarity with *F. solani* CBPPR0036 having accession number KT 211528.1. The accession number assigned by GenBank to this isolate was KX 171215.

4.1.2.2.5 M. anisopliae (SP7)

The ITS sequencing of the fungus yielded 520 base pair (bp) sequences are given below.

AGGGATCATTACCGAGTTATCCAACTCCCAACCCCTGTGAATTATACCTTT
AATTGTTGCTTCGGCGGGACTTCGCGCCCGCCGGGGACCCAAACCTTCTG
AATTTTTTAATAAGTATCTTCTGAGTGGTTAAAAAAAAATGAATCAAAAC
TTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAA
TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA
ACGCACATTGCGCCCGTCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTC
ATTACGCCCCTCAAGTCCCCTGTGGACTTGGTGTTGGGGATCGGCGAGGC
TGGTTTTCCAGCACAGCCGTCCCTTAAATTAATTGGCGGTCTCGCCGTGGC
CCTCCTCTGCGCAGTAGTAAAACACTCGCAACAGGAGCCCGGCGCGGTCC
ACTGCCGTAAAACCCCCCAACTTTTTATAGTTGACCTCGAATCAGGTAGG
ACTACCCGCTGAACTTAA

The above sequence when subjected to Nucleotide BLAST analysis showed cent per cent similarity with *M. anisopliae* 100513 11-1 having accession number JF 495767.1. The GenBank accession number to this isolate was KX 171216.

4.1.2.2.6 M. anisopliae (SP8)

The ITS sequencing of the fungus yielded 519 base pair (bp) sequences are given below.

AGGGATCATTACCGAGTTATCCAACTCCCAACCCCTGTGAATCATACCTTT
AATTGTTGCTTCGGCGGGACTTCGCGCCCGCCGGGGACCCAAACCTTCTG
AATTTTTTAATAAGTATCTTCTGAGTGGTTAAAAAAAATGAATCAAAACT
TTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT
GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACATTGCGCCCGTCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCA
TTACGCCCCTCAAGTCCCCTGTGGACTTGGTGTTGGGGATCGGCGAGGCT
GGTTTTCCAGCACAGCCGTCCCTTAAATTAATTGGCGGTCTCGCCGTGGCC
CTCCTCTGCGCAGTAGTAAAGCACTCGCAACAGGAGCCCGGCGCGGTCCA
CTGCCGTAAAACCCCCCAACTTTTTATAGTTGACCTCGAATCAGGTAGGA
CTACCCGCTGAACTTAA

The above sequence when subjected to Nucleotide BLAST analysis showed cent per cent similarity with *M. anisopliae* M5 having accession number LK 995311.1. The accession number assigned to this isolate by NCBI GenBank was KX 171217.

4.1.2.2.7 M. anisopliae (SP9)

The ITS sequencing of the fungus yielded 520 base pair (bp) sequences are given below.

AGGGATCATTACCGAGTTATCCAACTCCCAACCCCTGTGAATTATACCTTT
AATTGTTGCTTCGGCGGGACTTCGCGCCCGCCGGGGACCCAAACCTTCTG
AATTTTTTAATAAGTATCTTCTGAGTGGTTAAAAAAAAATGAATCAAAACT
TTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT
GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACATTGCGCCCGTCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCA
TTACGCCCCTCAAGTCCCCTGTGGGACTTGGTGTTGGGGATCGGCGAGGC
TGGTTTTCCAGCACAGCCGTCCCTTAAATTAATTGGCGGTCTCGCCGTGGC
CCTCCTCTGCGCAGTAGTAAAGCACTCGCAACAGGAGCCCGGCGCGGTCC
ACTGCCGTAAAACCCCCCAACTTTTTATAGTTGACCTCGAATCAGGTAGG
ACTACCCGCTGAACTTAA

Nucleotide BLAST analysis of above sequence showed cent per cent similarity with *M. anisopliae* S4ST7 having accession number KM 463106.1. The accession number assigned by GenBank to this isolate was KX 171218.

4.1.2.2.8 M. anisopliae (SP11)

The ITS sequencing of the fungus yielded 520 base pair (bp) sequences are given below.

AGGGATCATTACCGAGTTATCCAACTCCCAACCCCTGTGAATTATACCTTT
AATTGTTGCTTCGGCGGGACTTCGCGCCCGCCGGGGACCCAAACCTTCTG
AATTTTTTAATAAGTATCTTCTGAGTGGTTAAAAAAAAAATGAATCAAAAC
TTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAA
TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA
ACGCACATTGCGCCCGTCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTC
ATTACGCCCCTCAAGTCCCCTGTGGACTTGGTGTTGGGGATCGGCGAGGC
TGGTTTTCCAGCACAGCCGTCCCTTAAATTAATTGGCGGTCTCGCCGTGGC
CCTCCTCTGCGCAGTAGTAAAACACTCGCAACAGGAGCCCGGCGCGGTCC

ACTGCCGTAAAACCCCCCAACTTTTTATAGTTGACCTCGAATCAGGTAGG
ACTACCCGCTGAACTTAA

Nucleotide BLAST analysis of above sequence showed cent per cent similarity with *M. anisopliae* CNGD4 having accession number FJ 545279.1. The accession number assigned by GenBank to this isolate was KX 171220.

4.1.2.2.9 M. anisopliae (SP13)

The isolate SP13 obtained 508 base pair (bp) on ITS sequencing are given below.

AGGGATCATTACCGAGTTATCCAACTCCCAACCCCTGTGAATCATACCTTT
AATTGTTGCTTCGGCGGGACTTCGCGCCCGCCGGGGACCCAAACCTTCTG
AATTTTTTAATAAGTATCTTCTGAGTGGTTAAAAAAAATGAATCAAAACT
TTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT
GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACATTGCGCCCGTCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCA
TTACGCCCCTCAAGTCCCCTGTGGACTTGGTGTTGGGGATCGGCGAGGCT
GGTTTTCCAGCACAGCCGTCCCTTAAATTAATTGGCGGTCTCGCCGTGGCC
CTCCTCTGCGCAGTAGTAAAGCACTCGCAACAGGAGCCCGGCGCGGTCCA
CTGCCGTAAAACCCCCCAACTTTTTATAGTTGACCTCGAATCAGGTAGGA
CTACCC

Nucleotide BLAST analysis of above sequence showed cent per cent similarity with *M. anisopliae* isolate DhMz3R having accession number KU 983783.1. The accession number assigned by GenBank to this isolate was KX550275.

4.1.2.2.10 P. lilacinum (S10)

The ITS sequencing of the fungus yielded 557 base pair (bp) sequences are given below.

AGGGATCATTACCGAGTTATACAACTCCCAAACCCACTGTGAACCTTACC
TCAGTTGCCTCGGCGGGAACGCCCCGGCCGCCGGCCCCCGCGCCGGCGCC
GGACCCAGGCGCCCGCCGCAGGGACCCCAAACTCTCTTGCATTACGCCCA
GCGGGCGGAATTTCTTCTCTGAGTTGCACAAGCAAAAACAAATGAATCAA
AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
TGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGC
GTCATTTCAACCCTCGAGCCCCCCCGGGGGCCTCGGTGTTGGGGGACGGC
ACACCAGCCGCCCCCGAAATGCAGTGGCGACCCCGCCGCAGCCTCCCCTG
CGTAGTAGCACACACCTCGCACCGGAGCGCGGAGGCGGTCACGCCGTAA
AACGCCCAACTTTCTTAGAGTTGACCTCGGATCAGGTAGGAATACCCGCT
GAACTTAA

Nucleotide BLAST analysis of above sequence showed cent per cent similarity with *P. lilacinum* 001JFC having accession number KR 025540.1. The accession number assigned by GenBank to this isolate was KX 219589.

4.1.3 Comparison of Radial Growth of Fungi

The indigenous isolates *B. bassiana* (SP2), *B. bassiana* (SP4), *F. oxysporum* (SP1), *F. solani* (SP6), *M. anisopliae* (SP7), *M. anisopliae* (SP8), *M. anisopliae* (SP9), *M. anisopliae* (SP11), *M. anisopliae* (SP13) and *P. lilacinum* (S10), and *B. bassiana* (Bb5a), *M. anisopliae* (Ma4) obtained from National Bureau of Agricultural Insect Resources (NBAIR) were grown in PDA plates and radial growth was compared on seventh and fourteenth DAI (Table 4).

Among various isolates on the seventh DAI, *F. oxysporum* (SP1) recorded a mean radial growth of 8.40 cm which was significantly higher than that of other isolates. *F. solani* (SP6) had a mean radial growth of 7.76 cm on seventh DAI. The *M. anisopliae* isolates SP8 and Ma4 had a mean radial growth of 4.26 and 3.93 cm respectively on seventh DAI. The isolate of *M. anisopliae*, (Ma4) (3.93 cm) is on par with the isolates *M. anisopliae* (SP11) (3.66 cm) and *P. lilacinum* (S10) (3.56 cm). This was followed by the isolates *M. anisopliae* (SP9) (3.03 cm), *M. anisopliae* (SP13) (3.00 cm), *B. bassiana* (Bb5a) (2.70 cm) and *M. anisopliae* (SP7) (2.60 cm) and were on par. Mean radial growth of the isolate *B. bassiana* (Bb5a), *M. anisopliae* (SP7) and *B. bassiana* (SP4) were 2.70, 2.60 and 2.33 cm, respectively. The lowest mean radial growth of 1.96 cm was recorded in *B. bassiana* (SP2).

At 14 DAI, the isolates *F. oxysporum* (SP1) and *F. solani* (SP6) had a maximum radial growth of 9.00 cm which was statistically superior to other treatments. The next higher growth was seen in the isolate *M. anisopliae* (Ma4) with a mean radial growth of 7.83 cm. The isolates *M. anisopliae* (SP11), *M. anisopliae* (SP8), *P. lilacinum* (S10), *M. anisopliae* (SP9) and *M. anisopliae* (SP13) were on par with a mean radial growth of 6.83, 6.43, 6.33 6.23 and 6.00 cm respectively. The isolate *B. bassiana* (Bb5a), *M. anisopliae* (SP7) and *B. bassiana* (SP4) had 5.20, 4.83 and 4.66 cm mean radial growth respectively and were on par. The isolate *B. bassiana* (SP2) recorded lowest mean radial growth on 14th DAI, the value being 3.66 cm.

Table 4. Mean radial growth of indigenous isolates and NBAIR isolates of entomopathogenic fungi

Sl. No	Isolates	Mean radial growth (cm)*	
		7 DAI	14 DAI
1.	*B. bassiana* (SP2)	1.96[g]	3.66[f]
2.	*B. bassiana* (SP4)	2.33[fg]	4.66[e]
3.	*F. oxysporum* (SP1)	8.40[a]	9.00[a]
4.	*F. solani* (SP6)	7.76[b]	9.00[a]
5.	*M. anisopliae* (SP7)	2.60[ef]	4.83[e]
6.	*M. anisopliae* (SP8)	4.26[c]	6.43[cd]
7.	*M. anisopliae* (SP9)	3.03[e]	6.23[cd]
8.	*M. anisopliae* (SP11)	3.66[d]	6.83[c]
9.	*M. anisopliae* (SP13)	3.00[e]	6.00[d]
10.	*P. lilacinum* (S10)	3.56[d]	6.33[cd]
11.	*B. bassiana* (Bb5a)	2.70[ef]	5.20[e]
12.	*M. anisopliae* (Ma4)	3.93[cd]	7.83[b]
	C. D (0.05)	0.510	0.668

DAI – Days after inoculation *Mean of three replications

4.1.4 Spore Count

The spore count of the indigenous isolates and NBAIR isolates estimated at seventh and fourteenth DAI given in Table 5.

On the seventh DAI, NBAIR isolate *M. anisopliae* (Ma4) recorded highest spore count of 42.92 x 10^7 spores mL^{-1} which was significantly superior to other treatments. The isolate, *B. bassiana* (Bb5a) produced 33.56 x 10^7 spores mL^{-1}on seventh DAI. The indigenous isolates *M. anisopliae* (SP11) and *M. anisopliae* (SP8) recorded 12.88 x 10^7 spores $mL^{-1}$and 12.11 x 10^7 spores mL^{-1} and were on par. The isolate of *M. anisopliae* (SP7) yielded 8.22 x 10^7 spores mL^{-1}. The indigenous isolates *M. anisopliae* (SP9) and *B. bassiana* (SP4) were on par and recorded spore count of 4.99 and 5.12 x 10^7 spores mL^{-1}respectively. This was followed by the isolates *M. anisopliae* (SP13) with spore count of 2.46 x 10^7 spores mL^{-1} and *P. lilacinum* (S10) with spore count 2.44 x 10^7 spores $mL^{-1}$and were on par. The isolates *F. solani* (SP6), *F. oxysporum* (SP1) and *B. bassiana* (SP2) with low spore count of 0.80, 0.68 and 0.43 x 10^7 spores $mL^{-1}$and were on par.

A similar trend in spore production was seen in the subsequent observation taken on 14 DAI. The highest spore count was recorded in the NBAIR isolate *M. anisopliae* (Ma4), with 94.20 x 10^7 spores $mL^{-1}$and was significantly superior to other treatments. This was followed by *B. bassiana* (Bb5a) with 81.17 x 10^7 spores mL^{-1}. The indigenous isolate *M. anisopliae* (SP11) had a spore count of 28.01 x 10^7 spores mL^{-1}followed by *M. anisopliae* (SP8) (26.57 x 10^7 spores mL^{-1}) and *M. anisopliae* (SP7) (21.57 x 10^7 spores mL^{-1}) on fourteenth DAI. *M. anisopliae* (SP9) and *B. bassiana* (SP4) were on par and had spore count of 12.46 and 12.24 x 10^7 spores mL^{-1}respectively. The isolate *M. anisopliae* (SP13) recorded 9.13 x 10^7 spores mL^{-1}. The isolates *P. lilacinum* (S10) (6.32 x 10^7 spores mL^{-1}) and *F. solani* (SP6) (6.10 x 10^7 spores mL^{-1}) were on par. This was followed by the isolate *F. oxysporum*

Table 5. Mean spore count of indigenous and NBAIR isolates of entomopathogenic fungi

Sl. No	Isolate	Spore count (n x 10^7 spores mL^{-1})	
		7 DAI	14 DAI
1.	*F. oxysporum* (SP1)	0.68 (0.82)^{gh}	5.25 (2.29)^{i}
2.	*B. bassiana* (SP2)	0.43 (0.65)^{h}	4.08 (2.01)^{j}
3.	*B. bassiana* (SP4)	5.12 (2.26)^{e}	12.24 (3.49)^{f}
4.	*F. solani* (SP6)	0.80 (0.89)^{g}	6.10 (2.46)^{h}
5.	*M. anisopliae* (SP7)	8.22 (2.86)^{d}	21.57 (4.64)^{e}
6.	*M. anisopliae* (SP8)	12.11 (3.47)^{c}	26.57 (5.15)^{d}
7.	*M. anisopliae* (SP9)	4.99 (2.23)^{e}	12.46 (3.52)^{f}
8.	*M. anisopliae* (SP11)	12.88 (3.58)^{c}	28.01 (5.29)^{c}
9.	*M. anisopliae* (SP13)	2.46 (1.56)^{f}	9.13 (3.02)^{g}
10.	*P. lilacinum* (S10)	2.44 (1.56)^{f}	6.32 (2.51)^{h}
11.	*B. bassiana* (Bb5a)	33.56 (5.79)^{b}	81.17 (9.00)^{b}
12.	*M. anisopliae* (Ma4)	42.92 (6.54)^{a}	94.20 (9.70)^{a}
	CD (0.05)	(0.194)	(0.137)

DAI – Days after inoculation, Figures in parentheses are $\sqrt{x}$ transformed values

(SP1), which recorded 5.25 x 10^7 spores mL^{-1}. The lowest spore count was recorded in the isolate *B. bassiana* (SP2) (4.08 x 10^7 spores mL^{-1}).

4.2 PATHOGENICITY

The pathogenicity of indigenous isolates *viz*., *B. bassiana* (SP2), *B. bassiana* (SP4), *F. oxysporum* (SP1), *F. solani* (SP6), *M. anisopliae* (SP7), *M. anisopliae* (SP8), *M. anisopliae* (SP9), *M. anisopliae* (SP11), *M. anisopliae* (SP13), *P. lilacinum* (S10), and NBAIR isolates *B. bassiana* (Bb5a), *M. anisopliae* (Ma4) were evaluated against the larvae of test insects *viz*., *Diaphania indica* Saunders, *Hymenia recurvalis* Fabricius, *Leucinodes orbonalis* Guenee, *Sylepta derogata* Fabricius and *Spodoptera litura* Fabricius by applying spore suspensions obtained from fourteen days old culture. The results showed that *M. anisopliae* (SP7), *M. anisopliae* (SP8), *M. anisopliae* (SP9), *M. anisopliae* (SP11), *M. anisopliae* (SP13) and NBAIR isolates *B. bassiana* (Bb5a), *M. anisopliae* (Ma4) were pathogenic to the larvae of test insects. The isolates *B. bassiana* (SP2), *B. bassiana* (SP4), *F. oxysporum* (SP1), *F. solani* (SP6) and *P. lilacinum* (S10) recorded no mortality on the larvae of the test insects (Table 6).

4.2.1 Symptoms of Fungal Infection on Test Insects

4.2.1.1 D. indica

M. anisopliae (SP7, SP8, SP9, SP11, SP13 and Ma4) and *B. bassiana* (Bb5a) infected larvae showed reduced feeding and movement during the initial period of fungal infection. Death commenced on three DAT in all the isolates. The infected larvae were mummified. *M. anisopliae* (Ma4) treated larvae developed black coloured spots along the spiracular regions, later white coloured fungal growth was initiated through the intersegmental regions which completely covered the cadaver

Table 6. Pathogenicity of fungal isolates to the larvae of test insects

Sl. No	Isolates	Test insects				
		D. indica	*H. recurvalis*	*L. orbonalis*	*S. litura*	*S. derogata*
1.	*B. bassiana* (SP2)	-	-	-	-	-
2.	*B. bassiana* (SP4)	-	-	-	-	-
3.	*F. oxysporum* (SP1)	-	-	-	-	-
4.	*F. solani* (SP6)	-	-	-	-	-
5.	*M. anisopliae* (SP7)	+	+	+	-	+
6.	*M. anisopliae* (SP8)	+	+	+	-	+
7.	*M. anisopliae* (SP9)	+	+	+	-	+
8.	*M. anisopliae* (SP11)	+	+	+	+	+
9.	*M. anisopliae* (SP13)	+	+	+	-	+
10.	*P. lilacinum* (S10)	-	-	-	-	-
11.	*B. bassiana* (Bb5a)	+	+	+	+	+
12.	*M. anisopliae* (Ma4)	+	+	+	-	+

+ Pathogenic - Non pathogenic

within three days after death, which turned gray after two days in the larvae treated with isolates of *M. anisopliae* (SP7, SP9 and SP11) and green in the larvae treated with isolates of *M. anisopliae* (SP8, SP13 and Ma4). *B. bassiana* (Bb5a) infected cadaver developed a slight pinkish colour and were completely covered with white fungal growth within four days of death (Plate 4).

4.2.1.2 H. recurvalis

The larvae of *H. recurvalis* showed similar symptoms of fungal infection as mentioned in 4.2.1.1 when treated with various isolates except the development of black coloured spots in *M. anisopliae* (Ma4) and pink colour in *B. bassiana* (Bb5a) (Plate 5).

4.2.1.3 L. orbonalis

The larvae of *L. orbonalis* came out of their feeding tunnels prior to death when treated with different isolates of *M. anisopliae* (SP8, SP9, SP11 and Ma4). After death, the mycosed cadaver developed similar symptoms of fungal infection as mentioned in 4.2.1.1 except for development of pink colour in *B. bassiana* (Bb5a) and black coloured spots in *M. anisopliae* (Ma4) (Plate 6).

4.2.1.4 S. litura

No symptoms and mortality was induced by the different isolates of *M. anisopliae* in *S. litura* except SP11. The isolates *M. anisopliae* (SP11) and *B. bassiana* (Bb5a) produced similar symptoms of fungal infection as mentioned in 4.2.1.1 (Plate 7) except the development of pink colour in *B. bassiana* (Bb5a).

M. anisopliae (SP7)

M. anisopliae (SP8)

M. anisopliae (SP9)

M. anisopliae (SP11)

M. anisopliae (SP13)

M. anisopliae (Ma4)

B. bassiana (Bb5a)

Healthy larva of *D. indica*

Plate 4. Symptoms of infection of different fungal isolates on *D. indica*

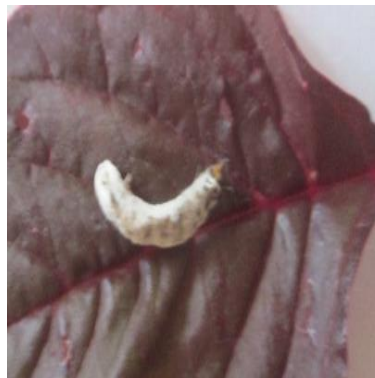
M. anisopliae (SP7)

M. anisopliae (SP8)

M. anisopliae (SP9)

M. anisopliae (SP11)

M. anisopliae (SP13)



M. anisopliae (Ma4)

B. bassiana (Bb5a)

Healthy larva of *H. recurvalis*

Plate 5. Symptoms of infection of different fungal isolates on *H. recurvalis*

M. anisopliae (SP8)

M. anisopliae (SP9)

M. anisopliae (SP11)

M. anisopliae (Ma4)

Healthy larvae of *L. orbonalis*

Plate 6. Symptoms of infection of different fungal isolates on *L. orbonalis*

B. bassiana (Bb5a)

M. anisopliae (SP11)

Healthy larvae of *S. litura*

Plate 7. Symptoms of infection of different fungal isolates on *S. litura*

4.2.1.5 S. derogata

The symptoms developed on the larvae of S. derogata by various isolates were similar as mentioned in 4.2.1.2 (Plate 8).

4.2.2 Virulence of Fungi

4.2.2.1 D. indica

The data on mean percentage mortality of indigenous isolates and NBAIR isolates on the larvae of *D. indica* at 10^7, 10^8 and 10^9 spores $mL^{-1}$are given in Table 7.

At 72 HAT, the isolate *M. anisopliae* (SP8) caused a mean mortality of 23.33 per cent at 10^7 spores mL^{-1}which was on par with that of *M. anisopliae* (Ma4) (13.33 per cent) and was significantly superior to the mortality recorded in other treatments. The isolates *M. anisopliae* (Ma4), *M. anisopliae* (SP7), *M. anisopliae* (SP11) and *M. anisopliae* (SP9) with a mean per cent mortality of 13.33, 10.00, 10.00 and 6.66 per cent respectively were statistically on par. The isolate *M. anisopliae* (SP9) caused mean mortality of 6.66 per cent was statistically on par with *M. anisopliae* (SP13) with 3.33 per cent mortality. The isolate *B. bassiana* (Bb5a) did not cause mortality at 72 HAT.

The mortality was found to increase in the subsequent observations. The *M. anisopliae* isolates (SP8) and (Ma4) caused mean mortality of 53.33 and 50.00 per cent respectively were statistically on par at 120 HAT as observed at 72 HAT. *M. anisopliae* (SP11) that caused mean mortality of 33.33 per cent and was statistically on par with *M. anisopliae* (SP9) and *M. anisopliae* (SP7) with mean mortality of 26.66 and 23.33 per cent, respectively. The isolate *M. anisopliae* (SP13) and *B. bassiana* (Bb5a) were statistically on par with mean mortality of 13.33 per cent each.

M. anisopliae (SP7)

M. anisopliae (SP8)

M. anisopliae (SP9)

M. anisopliae (SP11)

M. anisopliae (SP13)

M. anisopliae (Ma4)

B. bassiana (Bb5a)

Healthy larvae of *S. derogata*

Plate 8. Symptoms of infection of different fungal isolates on *S. derogata*

Table 7. Virulence of various isolates to *D. indica* at different concentrations

Isolate	Mortality (%)										
	n x10^7 spores mL^{-1}			n x10^8 spores mL^{-1}				n x10^9 spores mL^{-1}			
	72 HAT	120 HAT	168 HAT	48 HAT*	72 HAT	120 HAT	168 HAT	48 HAT*	72 HAT	120 HAT	168 HAT
B. bassiana (SP2)*	0	0	0	0	0	0	0	0	0	0	0
B. bassiana (SP4)*	0	0	0	0	0	0	0	0	0	0	0
F. oxysporum (SP1)*	0	0	0	0	0	0	0	0	0	0	0
F. solani (SP6)*	0	0	0	0	0	0	0	0	0	0	0
M. anisopliae (SP7)	10.00 (18.43)^{b}	23.33 (28.78)^{b}	56.66 (48.84)^{b}	0	13.33 (21.14)^{bc}	36.66 (37.22)^{b}	63.33 (52.77)^{d}	0	16.66 (23.85)^{b}	40.00 (39.23)^{c}	76.66 (61.21)^{c}
M. anisopliae (SP8)	23.33 (28.78)^{a}	53.33 (46.92)^{a}	83.33 (66.14)^{a}	13.33	36.66 (37.22)^{a}	76.66 (61.21)^{a}	100.00 (89.09)^{a}	23.33	43.33 (41.15)^{a}	80.00 (63.43)^{a}	100.00 (89.09)^{a}
M. anisopliae (SP9)	6.66 (12.59)^{bc}	26.66 (30.99)^{b}	63.33 (52.77)^{b}	0	10.00 (15.30)^{c}	33.33 (35.21)^{b}	70.00 (56.99)^{cd}	0	20.00 (26.56)^{b}	43.33 (41.15)^{bc}	80.00 (63.43)^{c}
M. anisopliae	10.00	33.33	63.33 (52.77)	0	16.66 (23.85)	40.00	76.66	0	23.33 (28.28)	53.33	86.66

Mean of three replications , 10 insects $replication^{-1}$, Figures in parentheses are angular transformed values

* Excluded for statistical analysis HAT- Hours after treatment

Table 7. Virulence of various isolates to *D. indica* at different concentrations (continued)

(SP11)	(18.43)[b]	(35.21)[b]	b		bc	(39.23)[b]	(61.21)[c]		b	(46.92)[b]	(68.85)[b]
M. anisopliae (SP13)	3.33 (6.74)[cd]	13.33 (21.14)[c]	36.66 (37.22)[c]	0	6.66 (12.59) d	23.33 (28.78)[c]	46.66 (43.07)[e]	0	16.66 (23.85) b	33.33 (35.00)[cd]	56.66 (48.84)[d]
P. lilacinum (S10)*	0	0	0	0	0	0	0	0	0	0	0
B. bassiana (Bb5a)	0 (0.90)[d]	13.33 (21.14)[c]	33.33 (35.21)[c]	0	0 (0.90)[cd]	16.66 (23.85)[c]	46.66 (68.85)[e]	0	3.33 (6.74)[c]	23.33 (28.78)[d]	53.33 (46.92)[d]
M. anisopliae (Ma4)	13.33 (21.14)[ab]	50.00 (28.78)[a]	76.66 (61.21)[a]	10.0 0	26.66 (30.99)[a] b	73.33 (59.00)[a]	86.66 (52.77)[b]	16.66	30.00 (33.00)[a] b	76.66 (61.21)[a]	100.00 (89.09)[a]
Untreated *	0	0	0	0	0	0	0	0	0	0	0
CD (0.05)	(10.281)	(6.509)	(6.498)		(12.298)	(6.296)	(6.973)		(10.881)	(6.785)	(5.089)

Mean of three replications, 10 insects replication^{-1}, Figures in parentheses are angular transformed values

* Excluded for statistical analysis HAT- Hours after treatment

A similar trend in virulence was observed at the 168 HAT. 83.33 per cent mean mortality was caused by the isolate *M. anisopliae* (SP8) which was statistically on par with *M. anisopliae* (Ma4) with mean mortality of 76.66 per cent. The isolates *M. anisopliae* (SP11), *M. anisopliae* (SP9) and *M. anisopliae* (SP7) were statistically on par with mean mortality ranging from 63.33 to 56.66 per cent. The isolate *M. anisopliae* (SP13) that caused mean mortality of 36.66 per cent was statistically on par with that of *B. bassiana* (Bb5a) (33.33 per cent).

At 10^8 spores mL^{-1}, mortality was initiated at a shorter interval of 48 HAT in the isolates *M. anisopliae* (Ma8) (13.33 per cent) and *M. anisopliae* (Ma4) (10.00 per cent). In other isolates, no mortality was recorded at 48 HAT.

The mean mortality of 36.66 per cent recorded in the treatment *M. anisopliae* (SP8) was statistically on par with that in the isolate *M. anisopliae* (Ma4) (26.66 per cent) at 72 HAT. The isolate *M. anisopliae* (SP11) caused mean mortality of 16.66 per cent and was statistically on par with *M. anisopliae* (SP7), *M. anisopliae* (SP9), *M. anisopliae* (SP13) in which the mortality ranged from 13.33 to 6.66 per cent. The isolate *B. bassiana* (Bb5a) caused no mortality on the larvae of *D. indica* at 72 HAT.

The superiority of the isolates SP8, Ma4 and SP11 was evident at 120 HAT and 168 HAT also. At the 120 HAT, the isolate *M. anisopliae* (SP8) (76.66 per cent) was statistically on par with the isolate *M. anisopliae* (Ma4) (73.33 per cent). *M. anisopliae* (SP11) recorded 40.00 per cent mean mortality and it was on par with *M. anisopliae* (SP7), *M. anisopliae* (SP9) that caused mean mortality of 36.66 and 33.33 per cent, respectively. The isolate *M. anisopliae* (SP13) was statistically on par with *B. bassiana* (Bb5a) showed 23.33 and 16.66 per cent mortality, respectively.

Cent per cent mortality was recorded in the isolate *M. anisopliae* (SP8) at 168 HAT which was significantly superior to the other treatments. The isolate

M. anisopliae (Ma4) caused 86.66 per cent mortality. *M. anisopliae* (SP11) and (SP9) were statistically on par and indicated mean mortality of 76.66 and 70.00 per cent respectively. The mean mortality of 63.33 per cent was recorded in the treatment *M. anisopliae* (SP7). The isolate *M. anisopliae* (SP13) and *B. bassiana* (Bb5a) were on par with mean mortality of 46.66 per cent each.

At 10^9 spores mL^{-1}also, only the isolates of *M .anisopliae* (SP8) and (Ma4) recorded mortality of *D. indica* and it was 23.33 and 16.66 per cent respectively at 48 HAT.

At the 72 HAT, the mortality percentage increased in the isolate *M. anisopliae* (SP8) and (Ma4) and they were more virulent than other treatments. The isolate *M. anisopliae* (SP8) caused a mean mortality of 43.33 per cent which was statistically on par with isolate *M. anisopliae* (Ma4) (30.00 per cent). The isolates *M. anisopliae* (Ma4) (30.00 per cent), *M. anisopliae* (SP11) (23.33 per cent), *M. anisopliae* (SP9) (20.00 per cent), *M. anisopliae* (SP7) (16.66 per cent) and *M. anisopliae* (SP13) (16.66 per cent) were statistically on par. The isolate *B. bassiana* (Bb5a) was statistically inferior with mean mortality of 3.33 per cent.

In the subsequent observations also the superiority of *M. anisopliae* (SP8) and (Ma4) was evident. The isolates *M. anisopliae* (SP8) and *M. anisopliae* (Ma4) at 120 HAT were statistically on par with 80.00 and 76.66 per cent mortality respectively. The isolate *M. anisopliae* (SP11) recorded mean mortality of 53.33 per cent. *M. anisopliae* (SP9) caused 43.33 per cent mortality and it was on par with the isolate *M. anisopliae* (SP7) (40.00 per cent). The isolate *M. anisopliae* (SP7) (40.00 per cent) was statistically on par with *M. anisopliae* (SP13) (33.33 per cent) and *B. bassiana* (Bb5a) (23.33 per cent).

Cent per cent mortality of *D. indica* was seen in *M. anisopliae* (SP8) and *M. anisopliae* (Ma4) at 168 HAT and it was significantly superior to other treatments.

This was followed by isolate *M. anisopliae* (SP11) that caused 86.66 per cent mean mortality. The isolates *M. anisopliae* (SP9) and *M. anisopliae* (SP7) were statistically on par with 80.00 and 76.66 per cent mortality. The isolates *M. anisopliae* (SP13) and *B. bassiana* (Bb5a) caused mean mortality of 56.66 and 53.33 per cent, respectively and were on par.

No mortality was recorded in the isolates *B. bassiana* (SP2), *B. bassiana* (SP4), *F. oxysporum* (SP1), *F. solani* (SP6) and *P. lilacinum* (S10) at 10^7, 10^8 and 10^9 spores mL^{-1}on the larvae of *D. indica.*

4.2.2.2 H. recurvalis

The data on mean mortality of indigenous isolates and NBAIR isolates at 10^7, 10^8 and 10^9 spores $mL^{-1}$are given in Table 8.

At 10^7 spores mL^{-1}, mortality initiated in the larvae of *H. recurvalis* at 72 HAT when treated with all isolates. The NBAIR isolate *M. anisopliae* (Ma4) caused a mean mortality of 26.66 per cent at 72 HAT which was on par with that of *M. anisopliae* (SP11), *M. anisopliae* (SP9) and *M. anisopliae* (SP8) with mean mortality of 23.33, 16.66 and 13.33 per cent respectively. The isolate *M. anisopliae* (SP8) recorded a mean mortality of 13.33 per cent, which was on par with that of *M. anisopliae* (SP7) (6.66 per cent). *M. anisopliae* (SP7) and *M. anisopliae* (SP13) were on par with a mean mortality of 6.66 and 3.33 per cent respectively. The isolate *B. bassiana* (Bb5a) did not cause mortality on the larvae of *H. recurvalis* at 10^7 spores mL^{-1}.

An increase in mortality of *H. recurvalis* was noted over the period. At 120 HAT, the highest percentage mortality recorded was 53.33 in the isolate *M. anisopliae* (SP11) and it was statistically on par with that of *M. anisopliae* (Ma4)

Table 8. Virulence of various isolates to *H. recurvalis* at different concentrations

Isolate	Mortality (%)										
	10^7 spores mL^{-1}			10^8 spores mL^{-1}				10^9 spores mL^{-1}			
	72 HAT	120 HAT	168 HAT	48 HAT*	72 HAT	120 HAT	168 HAT	48 HAT*	72 HAT	120 HAT	168 HAT
B. bassiana (SP2)*	0	0	0	0	0	0	0	0	0	0	0
B. bassiana (SP4)*	0	0	0	0	0	0	0	0	0	0	0
F. oxysporum (SP1)*	0	0	0	0	0	0	0	0	0	0	0
F. solani (SP6)*	0	0	0	0	0	0	0	0	0	0	0
M. anisopliae (SP7)	6.66 (12.59)^{bc}	16.66 (23.85) $_{c}$	33.33 (35.21) $_{d}$	0	13.33 (21.14)^{bc}	23.33 (28.78) $_{c}$	56.66 (48.84) $_{c}$	0	16.66 (23.85) $_{bc}$	36.66 (37.22) $_{d}$	66.66 (54.78)^{c}
M. anisopliae (SP8)	13.33 (21.14)^{ab}	43.33 (41.15) $_{ab}$	56.66 (48.84) $_{bc}$	0	23.33 (28.78)^{ab}	56.66 (48.84) $_{a}$	83.33 (66.14) $_{b}$	16.66	30.00 (33.21) $_{a}$	63.33 (52.77) $_{b}$	86.66 (68.85)^{b}
M. anisopliae (SP9)	16.66 (23.85)^{a}	33.33 (35.21) $_{b}$	46.66 (43.07) $_{c}$	0	20.00 (26.07)^{ab}	36.66 (37.22) $_{b}$	66.66 (54.78) $_{c}$	0	23.33 (28.78) $_{ab}$	53.33 (46.92) $_{c}$	73.33 (59.00)^{c}

Mean of three replications, 10 insects $replication^{-1}$, Figures in parentheses are angular transformed values,

* Excluded for statistical analysis HAT- Hours after treatment

(46.66 per cent) and *M. anisopliae* (SP8) (43.33 per cent). The isolate *M. anisopliae* (SP8) and *M. anisopliae* (SP9) were statistically on par which caused mean mortality ranging from 43.33 to 33.33 per cent. *M. anisopliae* (SP7) recorded 16.66 per cent mortality which was statistically on par with the isolate *M. anisopliae* (SP13) (13.33 per cent). The isolate *B. bassiana* (Bb5a) recorded 10.00 per cent mortality.

The isolate *M. anisopliae* (SP11) recorded 73.33 per cent mortality on the larvae of *H. recurvalis* at 168 HAT which was significantly superior to other treatments and statistically on par with that of *M. anisopliae* (Ma4) (63.33 per cent). The mean mortality caused by *M. anisopliae* (SP8) was 56.66 per cent and this was followed by *M. anisopliae* (SP9), which recorded mean mortality of 46.66 per cent. The isolate *M. anisopliae* (SP7) caused mean mortality of 33.33 per cent. The isolate *B. bassiana* (Bb5a) (26.66 per cent) was statistically on par with that of *M. anisopliae* (SP13) (23.33 per cent).

At the higher concentration of 10^8 spores mL^{-1}, the mortality initiated at 48 HAT in the isolates *M. anisopliae* (Ma4) which recorded 13.33 per cent mortality and *M. anisopliae* (SP11) (6.66 per cent). Mortality of *H. recurvalis* was not observed in all other isolates at 48 HAT.

At 72 HAT, the highest mean mortality of 30.00 per cent was recorded in the treatment *M. anisopliae* (Ma4), which was statistically on par with that of *M. anisopliae* (SP11), *M. anisopliae* (SP8), *M. anisopliae* (SP9) and, recorded a mean mortality of 26.66, 23.33 and 20.00 per cent respectively. The isolate *M. anisopliae* (SP9) (23.33 per cent) was statistically on par with the isolates *M. anisopliae* (SP9) (20.00 per cent) and *M. anisopliae* (SP7) (13.33) per cent. The isolates *M. anisopliae* (SP7) and *M. anisopliae* (SP13) were on par and recorded 13.33 and 6.66 per cent mortality respectively. *M. anisopliae* (SP13) (6.66 per cent) and *B. bassiana* (Bb5a) (3.33 per cent) were on par.

The mortality percentage progressively increased at 120 HAT, the isolate *M. anisopliae* (Ma4) recorded the highest mortality of 63.33 per cent and on par with that of the isolates *M. anisopliae* (SP11) and *M. anisopliae* (SP8) that caused 60.00 and 56.66 per cent respectively. This was followed by the isolate *M. anisopliae* (SP9) which recorded 36.66 per cent mortality. The isolate *M. anisopliae* (SP7) caused mortality of 23.33 per cent, which was on par with that of mortality caused by isolate *B. bassiana* (Bb5a) (16.66 per cent). The isolates *B. bassiana* (Bb5a) and *M. anisopliae* (SP13) were statistically on par with a mortality of 16.66 and 13.33 per cent respectively.

A similar trend in the virulence of the isolates was observed in the subsequent observations also. At 168 HAT, the isolate *M. anisopliae* (Ma4) and *M. anisopliae* (SP11) recorded higher mortality of 96.66 and 93.33 per cent respectively. The isolate *M. anisopliae* (SP8) recorded 83.33 per cent mortality. The mean mortality caused by *M. anisopliae* (SP9) and *M. anisopliae* (SP7) was 66.66 and 56.66 per cent respectively and are on par. The isolate *B. bassiana* (Bb5a) and *M. anisopliae* (SP13) recorded lower mortality of 36.66 and 33.33 per cent.

Mortality of *H. recurvalis* initiated earlier (48 HAT) at the higher concentration of 10^9 spores mL^{-1}in the isolate *M. anisopliae* (Ma4) (20.00 per cent), *M. anisopliae* (SP8) (16.66 per cent) and *M. anisopliae* (SP11) (13.33 per cent).

At the 72 HAT, *M. anisopliae* (Ma4) still higher mortality of 33.33 per cent was recorded which was on par with that of the isolates *M. anisopliae* (SP8) (30.00 per cent), *M. anisopliae* (SP11) (26.66 per cent) and *M. anisopliae* (SP9) (23.33 per cent). The isolate *M. anisopliae* (SP7) caused 16.66 per cent mean mortality and are on par with *M. anisopliae* (SP13) (10.00 per cent). The isolates *M. anisopliae* (SP13) and *B. bassiana* (Bb5a) recorded 13.33 and 6.66 per cent mortality respectively were on par.

The isolate *M. anisopliae* (Ma4) caused mean mortality to the tune of 73.33 per cent and statistically on par with that of *M. anisopliae* (SP11) (70.00 per cent) at 120 HAT. The mean mortality caused by the isolate *M. anisopliae* (SP8) was 63.33 per cent. The isolate *M. anisopliae* (SP7) recorded 36.66 per cent mortality. This was followed by the isolate *B. bassiana* (Bb5a) (26.66 per cent) and *M. anisopliae* (SP13) (23.33 per cent) and were on par.

Over the period of 168 HAT, *M. anisopliae* (Ma4) caused 100 per cent mortality and it was statistically on par with that of *M. anisopliae* (SP11) recorded 96.66 per cent mortality. The isolate *M. anisopliae* (SP8) recorded 86.66 per cent mortality. This was followed by the isolates *M. anisopliae* (SP9) (73.33 per cent) and *M. anisopliae* (SP7) (66.66 per cent). The isolates *B. bassiana* (Bb5a) and *M. anisopliae* (SP13) were significantly inferior with 46.66 and 43.33 per cent mortality respectively and were on par.

The isolates *B. bassiana* (SP2), *B. bassiana* (SP4), *F. oxysporum* (SP1), *F. solani* (SP6) and *P. lilacinum* (S10) recorded no mortality in the larvae of *H. recurvalis* at 10^7, 10^8 and 10^9 spores mL^{-1}.

4.2.2.3 L. orbonalis

The mean mortality caused by various indigenous isolates and NBAIR isolates in the first instar larvae of *L. orbonalis* five DAT at 10^7, 10^8 and 10^9 spores mL^{-1}on the basis of the percentage mortality determined from the survived larvae within the fruits provided as food are given in Table 9.

The isolate *M. anisopliae* (SP8) recorded the highest mortality of 64.44 per cent at 10^7 spores mL^{-1} but it was on par with that of *M. anisopliae* (Ma4) (60.74 per cent), *M. anisopliae* (SP11) (57.04 per cent) and *M. anisopliae* (SP9) (53.33 per cent). The NBAIR isolate *B. bassiana* (Bb5a) recorded 39.25 per cent mortality. This was followed by the isolate *M. anisopliae* (SP7) which recorded 21.11 per cent mortality. Only 10.37 per cent mortality was recorded by the isolate *M. anisopliae* (SP13).

Table 9. Virulence of various isolates to *L. orbonalis* at different concentrations

	Mortality / Survivability (%) at 5 DAT					
	n x 10^7 spores mL^{-1}		n x10^8 spores mL^{-1}		n x 10^9 spores mL^{-1}	
Isolate	M	S*	M	S*	M	S*
B. bassiana (SP2)*	0	100	0	100	0	100
B. bassiana (SP4)*	0	100	0	100	0	100
F. oxysporum (SP1)*	0	100	0	100	0	100
F. solani (SP6)*	0	100	0	100	0	100
M. anisopliae (SP7)	21.11 (26.93)^{cd}	73.33	34.44 (35.90)^{d}	63.34	51.85 (46.06)^{c}	43.34
M. anisopliae (SP8)	64.44 (53.41)^{a}	33.34	75.55 (60.53)^{a}	23.34	95.83 (82.49)^{a}	3.34
M. anisopliae (SP9)	53.33 (46.92)^{ab}	40.00	58.52 (49.90)^{bc}	33.34	81.29 (64.65)^{b}	10.00
M. anisopliae (SP11)	57.04 (49.05)^{ab}	43.34	65.18 (53.92)^{ab}	40	88.33 (73.55)^{ab}	16.67
M. anisopliae (SP13)	10.37 (15.64)^{de}	83.34	20.74 (27.08)^{e}	76.67	36.11 (36.75)^{c}	56.67
P. lilacinum (S10)*	0	100	0	100	0	100
B. bassiana (Bb5a)	39.25 (38.76)^{bc}	56.67	44.81 (42.01)^{cd}	53.34	51.48 (45.85)^{c}	43.34
M. anisopliae (Ma4)	60.74 (51.23)^{a}	36.67	72.22 (45.32)^{ab}	26.67	95.83 (82.49)^{a}	3.34
Untreated	6.66 (12.59)^{e}	93.34	3.33 (6.74)^{f}	96.66	10.00 (15.30)^{d}	96.66
CD (0.05)	(11.875)		(8.510)		(16.854)	

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

* Excluded for statistical analysis   M- Mortality, S- Survivability

A similar effect was noted at the higher concentrations of 10^8 and 10^9 spores ml^{-1}. At 10^8 spores mL^{-1} 75.55 per cent mortality was recorded in the isolate *M. anisopliae* (SP8) which was on par with that of *M. anisopliae* (Ma4) (72.22 per cent). The isolates Ma4 and SP11 of *M. anisopliae* were on par however, the isolate of *M. anisopliae* (SP11) (65.18 per cent) was statistically on par with that of *M. anisopliae* (SP9) which recorded 58.52 per cent mortality. Only 44.81 per cent was recorded in the isolate *B. bassiana* (Bb5a). This was followed by the isolate *M. anisopliae* (SP7) (34.44 per cent) and *M. anisopliae* (SP13) (20.74 per cent). At 10^9 spores $mL^{-1}$the isolates *M. anisopliae* (SP8) and (Ma4) that caused 95.83 per cent mortality were on par with *M. anisopliae* (SP11) (88.33 per cent). The isolates of *M. anisopliae* (SP11) and *M. anisopliae* (SP9) were statistically on par with mean mortality of 88.33 and 81.29 per cent respectively. The pathogenicity recorded in the isolates *M. anisopliae* (SP7) (51.85 per cent), *B. bassiana* (Bb5a) (51.48 per cent) and *M. anisopliae* (SP13) (36.11 per cent) were on par.

4.2.2.4 S. litura

The mean mortality caused by indigenous isolates and NBAIR isolates on the larvae of *S. litura* at 10^7, 10^8 and 10^9 spores mL^{-1} was given in Table 10.

Among the two isolates pathogenic to *S. litura,* only the isolate *M. anisopliae* (SP11) caused 6.66 per cent mortality at 72 HAT at 10^7 spores mL^{-1}. Subsequently at 120 HAT, mortality was observed in both the isolates, the mean per cent mortality caused by *M. anisopliae* (SP11) isolate was 15.00 per cent, which was statistically superior to the mortality caused by *B. bassiana* (Bb5a) (8.33 per cent). An increase in mortality was observed in both the isolates as time advanced, but the

Table 10. Virulence of various isolates to *S. litura* at different concentrations

Isolate	Mortality (%)								
	n x10^7 spores mL^{-1}			n x 10^8 spores mL^{-1}			n x 10^9 spores mL^{-1}		
	72 HAT	120 HAT	168 HAT	72 HAT	120 HAT	168 HAT	72 HAT	120 HAT	168 HAT
B. bassiana (SP2)	0	0	0	0	0	0	0	0	0
B. bassiana (SP4)	0	0	0	0	0	0	0	0	0
F. oxysporum (SP1)	0	0	0	0	0	0	0	0	0
F. solani (SP6)	0	0	0	0	0	0	0	0	0
M. anisopliae (SP7)	0	0	0	0	0	0	0	0	0
M. anisopliae (SP8)	0	0	0	0	0	0	0	0	0
M. anisopliae (SP9)	0	0	0	0	0	0	0	0	0
M. anisopliae (SP11)*	6.66	15.00	28.33	13.33	23.33	41.66	20.00	33.33	53.33
M. anisopliae (SP13)	0	0	0	0	0	0	0	0	0
P. lilacinum (S10)	0	0	0	0	0	0	0	0	0
B. bassiana (Bb5a)*	0	8.33	16.66	3.33	15.00	25.00	8.33	23.33	31.66
M. anisopliae (Ma4)	0	0	0	0	0	0	0	0	0
Untreated	0	0	0	0	0	0	0	0	0
t (0.05)*	3.162**	2.394 **	2.572*8	3.351**	2.716*8	4.382**	2.905**	3.351**	5.817**

Mean of three replications, 10 insects $replication^{-1}$ HAT- Hours after treatment

* Statistically analysed using t test ** Treatments are significantly different at 5% level

mortality observed was only 28.33 per cent in *M. anisopliae* (SP11) and 16.66 per cent in *B. bassiana* (Bb5a) at 168 HAT.

At 10^8 spores mL^{-1} at 72 HAT, the isolate *M. anisopliae* (SP11) recorded 13.33 per cent mortality which was significantly superior to *B. bassiana* (Bb5a), which caused mean mortality of 3.33 per cent. Afterwards at 120 HAT, the mortality increased to 23.33 in *M. anisopliae* (SP11) per cent and 15.00 per cent in *B. bassiana* (Bb5a). Subsequently at 168 HAT, 41.66 per cent and 25.00 per cent mortality in *M. anisopliae* (SP11) and *B. bassiana* (Bb5a) respectively. At 10^9 spores mL^{-1}an increase in mortality was observed 20.00 per cent and 8.33 per cent was recorded in *M. anisopliae* (SP11) and *B. bassiana* (Bb5a) respectively. Thereafter at 120 HAT and 168 HAT, the mortality increased to 33.33 and 53.33 per cent in *M. anisopliae* (SP11) and 23.33 and 31.66 per cent in *B. bassiana* (Bb5a).

4.2.2.5 S. derogata

The pathogenicity of indigenous isolates and NBAIR isolates 10^7, 10^8 and 10^9 spores mL^{-1}on the larvae of *S. derogata* is given in Table 11.

At 10^7 spores mL^{-1} the mortality initiated at 72 HAT and the mean per cent mortality recorded was 26.66 per cent in the isolate *M. anisopliae* (SP11), it was statistically on par with that of *M. anisopliae* (SP8) (23.33 per cent) and these treatments were significantly superior. The isolates *M. anisopliae* (Ma4), *M. anisopliae* (SP7) and *M. anisopliae* (SP9) were on par with 16.66, 13.33 and 13.33 per cent mortality respectively. The isolates *M. anisopliae* (SP13) and *B. bassiana* (Bb5a) did not recorded mortality at the 72 HAT.

At 120 HAT, the percentage mortality recorded was 70.00 in the isolate *M. anisopliae* (SP8) which was statistically on par with that of *M. anisopliae* (SP11)

Table 11. Virulence of various isolates to *S. derogata* at different concentrations

Isolate	Mortality (%)										
	n x 10^7 spores mL^{-1}			n x 10^8 spores mL^{-1}				n x 10^9 spores mL^{-1}			
	72 HAT	120 HAT	168 HAT	48 HAT	72 HAT	120 HAT	168 HAT	48 HAT	72 HAT	120 HAT	168 HAT
B. bassiana (SP2)*	0	0	0	0	0	0	0	0	0	0	0
B. bassiana (SP4)*	0	0	0	0	0	0	0	0	0	0	0
F. oxysporum (SP1)*	0	0	0	0	0	0	0	0	0	0	0
F. solani (SP6)*	0	0	0	0	0	0	0	0	0	0	0
M. anisopliae (SP7)	13.33 (21.14)^{c}	33.33 (35.21)^{b}	53.33 (46.92)^{c}	0	16.66 (23.85)^{b}	36.66 (37.22)^{c}	63.33 (52.77)^{b}	0	30.00 (33.21)^{ab}	46.66 (43.07)^{b}	73.33 (59.00)^{bc}
M. anisopliae (SP8)	23.33 (28.78)^{ab}	70.00 (56.78)^{a}	86.66 (68.85)^{a}	6.66	30.00 (33.21)^{ab}	80.00 (63.43)^{a}	93.33 (77.40)^{a}	10.00	36.66 (37.22)^{a}	83.33 (66.14)^{a}	96.66 (83.25)^{a}
M. anisopliae (SP9)	13.33 (21.14)^{c}	36.66 (37.22)^{b}	56.66 (48.84)^{c}	0	23.33 (28.78)^{ab}	50.00 (45.00)^{b}	76.66 (61.21)^{b}	0	30.00 (33.21)^{ab}	56.66 (48.84)^{b}	80.00 (63.92)^{bc}
M. anisopliae (SP11)	26.66 (30.99)^{a}	56.66 (48.84)^{a}	83.33 (66.14)^{a}	16.66	36.66 (37.22)^{a}	76.66 (61.21)^{a}	96.66 (83.25)^{a}	23.33	40.00 (39.23)^{a}	83.33 (66.14)^{a}	100.00 (89.09)^{a}
M. anisopliae (SP13)	0 (0.90)^{d}	10.00 (15.30)^{c}	26.66 (30.99)^{d}	0	3.33 (6.749)^{c}	16.66 (23.85)^{d}	33.33 (35.21)^{c}	0	13.33 (21.14)^{c}	26.66 (30.78)^{c}	36.66 (37.22)^{d}
P. lilacinum (S10)*	0	0	0	0	0	0	0	0	0	0	0
B. bassiana (Bb5a)	0 (0.90)^{d}	13.33 (21.14)^{c}	23.33 (28.78)^{d}	0	6.66 (12.59)^{c}	23.33 (28.78)^{d}	36.66 (37.22)^{c}	0	20.00 (26.07)^{bc}	46.66 (43.07)^{b}	66.66 (54.78)^{c}
M. anisopliae (Ma4)	16.66 (23.85)^{bc}	36.66 (37.22)^{b}	70.00 (56.78)^{b}	0	26.66 (30.99)^{ab}	46.66 (43.07)^{b}	76.66 (61.21)^{b}	0	33.33 (35.21)^{a}	53.33 (46.92)^{b}	83.33 (66.14)^{b}
Untreated *	0	0	0	0	0	0	0	0	0	0	0
CD (0.05)	(6.474)	(10.284)	(6.475)		(10.849)	(5.714)	(10.889)		(7.863)	(7.887)	(9.771)

Mean of three replications, 10 insects replication^{-1}, Figures in parentheses are angular transformed values,

* Excluded for statistical analysis HAT- Hours after treatment

(56.66 per cent). The isolates *M. anisopliae* (Ma4), *M. anisopliae* (SP9) and *M. anisopliae* (SP7) were statistically on par which caused mean mortality ranging from 36.66 to 33.33 to per cent. The mortality caused by the isolate *B. bassiana* (Bb5a) was significantly lower (13.33 per cent) and on par with that of *M. anisopliae* (SP13) (10.00 per cent). The mean mortality caused by the isolates *M. anisopliae* (SP8) and *M. anisopliae* (SP11) were 86.66 and 83.33 per cent respectively and were statistically on par at 168 HAT. This was followed by the isolate *M. anisopliae* (Ma4) with 70.00 per cent mortality. The isolates *M. anisopliae* (SP9) and *M. anisopliae* (SP7) recorded 56.66 and 53.33 per cent mortality respectively and are statistically on par. The isolates *M. anisopliae* (SP13) and *B. bassiana* (Bb5a) were less pathogenic and were on par with mean mortality of 26.66 and 23.33 per cent respectively.

At the higher concentration of 10^8 spores mL^{-1} the mortality was initiated within 48 HAT in the larvae of *S. derogata* treated with the isolates *M. anisopliae* (SP11) (16.66 per cent) and *M. anisopliae* (SP8) (6.66 per cent).

At 72 HAT, no significant difference was recorded in the effect of *M. anisopliae* (SP11), *M. anisopliae* (SP8), *M. anisopliae* (Ma4) and *M. anisopliae* (SP9), the mortality recorded was 36.66, 30.00, 26.66 and 23.33 per cent respectively. The isolate *M. anisopliae* (SP7) caused 16.66 per cent mean mortality. *B. bassiana* (Bb5a) recorded 6.66 per cent mortality and *M. anisopliae* (SP13) recorded 3.33 per cent mortality and were statistically inferior.

At 120 HAT, the isolate *M. anisopliae,* (SP8) (80.00 per cent) and *M. anisopliae* (SP11) (76.66 per cent) were on par. The mean mortality caused by the isolate *M. anisopliae* (SP9) (50.00 per cent) and *M. anisopliae* (Ma4) (46.66 per cent) were statistically on par. This was followed by the isolate *M. anisopliae* (SP7) with mean mortality of 36.66 per cent. The isolate *B. bassiana* (Bb5a) recorded 23.33 per

cent mortality which was on par with the isolate *M. anisopliae* (SP13) (16.66 per cent).

At 168 HAT, *M. anisopliae* (SP11) caused 96.66 per cent mortality, which was statistically on par with that of *M. anisopliae* (SP8) that recorded 93.33 per cent mortality and were significantly superior to other treatments. The isolates *M. anisopliae* (Ma4) (76.66 per cent), *M. anisopliae* (SP9) (76.66 per cent) and *M. anisopliae* (SP7) (63.33 per cent) were statistically on par. The effect of *B. bassiana* (Bb5a) and *M. anisopliae* (SP13) was the least, 36.66 and 33.33 per cent respectively.

A similar trend in the performance of the isolates was seen at the higher dose of 10^9 spores mL^{-1}also. The isolates of *M. anisopliae* (SP11 and SP8) recorded 23.33 and 10.00 per cent mortality respectively at 48 HAT. At 72 HAT, the isolate *M. anisopliae* (SP11) was significantly superior with mean mortality of 40.00 per cent and statistically on par with that of *M. anisopliae* isolates (SP8) (36.66 per cent), (Ma4) (33.33 per cent), (SP7) (30.00 per cent) and (SP9) (30.00 per cent). The isolate *B. bassiana* (Bb5a) caused 20.00 per cent mortality and was on par with *M. anisopliae* (SP13) which caused 13.33 per cent mortality.

At 120 HAT also a similar trend with higher per cent mortality was recorded. The isolates *M. anisopliae* (SP8) (83.33 per cent) and *M. anisopliae* (SP11) (83.33 per cent) were significantly superior. This was followed by *M. anisopliae* isolates (SP9) (56.66 per cent), (Ma4) (53.33 per cent), (SP7) (46.66 per cent) and *B. bassiana* (Bb5a) (46.66 per cent) were statistically on par. The lowest mortality was recorded in *M. anisopliae* (SP13) 26.66 per cent and significantly inferior.

At 168 HAT, the isolate *M. anisopliae* (SP11) recorded cent per cent mortality of larvae of *S. derogata* and which was on par with *M. anisopliae* (SP8) with mean mortality of 96.66 per cent. The isolate *M. anisopliae* (Ma4) caused 83.33 per cent

mortality, which was on par with that of *M. anisopliae* (SP9) and *M. anisopliae* (SP7) with mean per cent mortality of 80.00 and 73.33 per cent. The isolate *B. bassiana* (Bb5a) recorded 66.66 per cent mortality which was followed by *M. anisopliae* (SP13) with mean mortality of 36.66 per cent.

The isolates *B. bassiana* (SP2), *B. bassiana* (SP4), *F. oxysporum* (SP1), *F. solani* (SP6) and *P. lilacinum* (S10) recorded no mortality of *S. derogata* larvae at the three different concentrations evaluated.

4.3 POT CULTURE EXPERIMENT

The effect of various isolates of entomopathogenic fungi on the management of leaf webbers is presented in Tables 12 to 15.

4. 3. 1 Population

4.3.1.1 Leaf Webber

The data on the population of the larvae of *H. recurvalis* and *Psara basalis* Walker assessed on the basis of mean number of larvae are present in each web in five observational plants in each replication are presented in Table 12.

There was no significant difference in the pretreatment count of the larval population of leaf webbers. On the seventh DAT, the treatments *M. anisopliae* (Ma4), *M. anisopliae* (SP8) and *M. anisopliae* (SP11) were statistically on par with 0.97, 1.00 and 1.01 larvae web^{-1} respectively and significant reduction in the mean number of larvae web^{-1} was observed in these treatments. The treatments *B. bassiana* (Bb5a) (1.05), *M. anisopliae* (SP7) (1.07) and *M. anisopliae* (SP9) (1.07), were statistically on par. The treatments *P. lilacinum* (S10), *B. bassiana* (SP2), and *B. bassiana* (SP4) and recorded 1.27, 1.28 and 1.39 mean larvae web^{-1} and were statistically on par with untreated.

Table 12. Mean number of larvae web^{-1} in various treatments with entomopathogenic fungi

	Mean number of larvae web^{-1}		
Treatments (n x 10^8 spores mL^{-1})	Precount	7 DAT	14 DAT
B. bassiana (SP2)	1.11	1.28^{abc}	1.28^{ab}
B. bassiana (SP4)	1.14	1.39^{ab}	1.44^{a}
M. anisopliae (SP7)	1.18	1.07^{bc}	0.95^{bc}
M. anisopliae (SP8)	1.45	1.00^{c}	0.70^{cde}
M. anisopliae (SP9)	1.07	1.07^{bc}	0.85^{cd}
M. anisopliae (SP11)	1.26	1.01^{c}	0.41^{e}
P. lilacinum (S10)	1.18	1.27^{abc}	1.41^{a}
B. bassiana (Bb5a)	1.19	1.05^{bc}	0.96^{bc}
M. anisopliae (Ma4)	1.17	0.97^{c}	0.53^{de}
Untreated	1.22	1.52^{a}	1.67^{a}
CD (0.05)	NS	0.355	0.396

DAT- Days after treatment

NS – Non significant

At 14 DAT, *M. anisopliae* (SP11) treated plants recorded 0.41 mean larvae web^{-1} and it was significantly lower than that in the other treatments. and *M. anisopliae* (Ma4) (0.53), *M. anisopliae* (SP8) (0.70) and *M. anisopliae* (SP9) (0.85) were statistically on par. The mean number of larvae web^{-1} was 0.95 in the treatment *M. anisopliae* (SP7) and it was statistically on par with *B. bassiana* (Bb5a) (0.96). The treatments *B. bassiana* (SP2) (1.28), *P. lilacinum* (S10) (1.41) and *B. bassiana* (SP4) (1.44) were on par with the untreated plants (1.67).

4.3.1.2 Population of Spiders

The population of spiders assessed in terms of mean number of spiders in each observational plants (Table 13) showed that the fungi evaluated had no effects on the population of spiders.

4. 3. 2 Extent of Damage

The data on the extent of damage assessed in terms of the percentage of plants infested by leaf webbers and number of webs plant^{-1} are given in Table 14 and Table 15 respectively.

The precount data in the various treatments of entomopathogenic fungi and untreated was statistically similar. On the seventh DAT, the treatment *M. anisopliae* (SP11) showed significant reduction in the percentage of plants infested by leaf webbers (65.00 per cent) (Table 14). The treatment *M. anisopliae* (Ma4) recorded 75.00 per cent infestation of leaf webber in treated plants whereas *M. anisopliae* (SP9) and (SP8) recorded 80.00 per cent infestation. The treatments *M. anisopliae* (SP7), *B. bassiana* (Bb5a), *P. lilacinum* (S10), *B. bassiana* (SP2) and *B. bassiana* (SP4) with mean percentage infestation of 85.00, 90.00, 90.00, 90.00 and 100.00 per cent, respectively and were statistically on par with untreated (95.00).

Table 13. Population of spiders in various treatments with entomopathogenic fungi

	Mean number of spider		
Treatments(n x 10^8 spores mL^{-1})	Precount	7 DAT	14 DAT
B. bassiana (SP2)	0.25	0.15	0.2
B. bassiana (SP4)	0.05	0.20	0.15
M. anisopliae (SP7)	0.15	0.20	0.3
M. anisopliae (SP8)	0.10	0.20	0.35
M. anisopliae (SP9)	0	0.10	0.05
M. anisopliae (SP11)	0.20	0.35	0.45
P. lilacinum (S10)	0.05	0.35	0.30
B. bassiana (Bb5a)	0.10	0.15	0.20
M. anisopliae (Ma4)	0	0.15	0.20
Untreated	0.15	0.10	0.20

DAT- Days after treatment

Table. 14 Percentage of plants infested by leaf webbers

Treatments (n x 10^8 spores mL^{-1})	Mean percentage of plants infested		
	Precount	7 DAT	14 DAT
B. bassiana (SP2)	100.00	90.00^{abc}	95.00^{a}
B. bassiana (SP4)	100.00	100.00^{a}	95.00^{a}
M. anisopliae (SP7)	95.00	85.00^{abcd}	60.00^{cd}
M. anisopliae (SP8)	95.00	80.00^{abcd}	55.00^{de}
M. anisopliae (SP9)	95.00	80.00^{cd}	65.00^{cd}
M. anisopliae (SP11)	95.00	65.00^{d}	35.00^{f}
P. lilacinum (S10)	95.00	90.00^{abc}	90.00^{ab}
B. bassiana (Bb5a)	100.00	90.00^{abc}	75.00^{bc}
M. anisopliae (Ma4)	95.00	75.00^{bcd}	40.00^{ef}
Untreated	100.00	95.00^{ab}	95.00^{a}
CD (0.05)	NS	21.737	19.909

DAT- Days after treatment

NS – Non significant

On the fourteenth DAT, the least number of plants infested by leaf webbers was observed in the treatment *M. anisopliae* (SP11), (35.00 per cent) and it was significantly lower than in other treatments. This was followed by the treatment *M. anisopliae* (Ma4) which recorded an infestation of 40.00 per cent and it was followed by *M. anisopliae* (SP8) that recorded 55.00 per cent infestation. The isolates *M. anisopliae* (SP7) and *M. anisopliae* (SP9) were statistically on par with mean per cent infestation of 60.00 and 65.00 per cent respectively. Significantly higher (75.00 per cent) infestation was observed in the treatment with NBAIR isolate *B. bassiana* (Bb5a). The isolates *P. lilacinum* (S10), *B. bassiana* (SP2) and *B. bassiana* (SP4) were ineffective treatments with mean per cent infestation of 90.00, 95.00 and 95.00 and, respectively and were on par with the untreated plants (95.00 per cent).

The pretreatment count of number of webs produced by leaf webbers, were statistically similar. On the seventh DAT, among various treatments, *M. anisopliae* (SP11), *M. anisopliae* (Ma4), *M. anisopliae* (SP8), *M. anisopliae* (SP7), *M. anisopliae* (SP9) and *B. bassiana* (Bb5a) showed significant reduction in the number of webs compared to untreated (5.25) (Table 15). The lowest number of web was observed in plants treated with *M. anisopliae* (SP11) (1.77) and *M. anisopliae* Ma4 (1.85) and were statistically on par. The treatments *M. anisopliae* (SP8), *M. anisopliae* (SP7), *B. bassiana* (Bb5a) and *M. anisopliae* (SP9) recorded 2.15, 2.30, 2.72 and 2.75 mean number of webs respectively and were on par. There was an increase in the number of webs in the treatments *P. lilacinum* (S10) (3.27), *B. bassiana* (SP4) (4.95) and *B. bassiana* (SP2) (5.40) also in the untreated plants (5.25).

On the 14 DAT, the number of webs produced was significantly lower in the treatments *M. anisopliae* (SP11) (0.70) and *M. anisopliae* (Ma4) (0.85) which were statistically on par. This was followed by the treatments *M. anisopliae* (SP8),

Table 15. Mean number of webs plant^{-1} in various treatments with entomopathogenic fungi

	Mean number of webs plant^{-1}		
Treatments (n x 10^8 spores mL^{-1})	Precount	7 DAT	14 DAT
B. bassiana (SP2)	5.00	5.40^{a}	4.85^{a}
B. bassiana (SP4)	4.25	4.95^{a}	5.30^{a}
M. anisopliae (SP7)	4.85	2.30^{bc}	1.87^{cd}
M. anisopliae (SP8)	3.85	2.15^{bc}	1.35^{cd}
M. anisopliae (SP9)	5.15	2.75^{bc}	1.50^{cd}
M. anisopliae (SP11)	4.55	1.77^{c}	0.70^{d}
P. lilacinum (S10)	3.10	3.27^{b}	3.50^{b}
B. bassiana (Bb5a)	4.40	2.72^{bc}	2.50^{bc}
M. anisopliae (Ma4)	4.40	1.85^{c}	0.85^{d}
Untreated	4.85	5.25^{a}	5.65^{a}
CD (0.05)	NS	1.349	1.131

DAT- Days after treatment

NS – Non significant

M. anisopliae (SP9) and *M. anisopliae* (SP7) which recorded 1.35, 1.50, and 1.87 webs plant^{-1} and are statistically on par. The treatment *P. lilacinum* (S10) recorded 3.50 mean number of webs plant^{-1}. *B. bassiana* (SP2) and *B. bassiana* (SP4) recorded higher number of webs 4.85, and 5.30 respectively and were on par with that of untreated plants (5.65).

4. 3. 3 Yield

The data on yield recorded in terms of the mean weight of cuttings from each observational plants are given in Table 16.

Among the various fungal pathogens, *M. anisopliae* (SP11) recorded the highest yield of 73.75 g plant^{-1} and it was significantly superior to other treatments. The isolate, *M. anisopliae* (Ma4) recorded 65.25 g plant^{-1} and was significantly superior to other treatments. A mean yield of 50.50 g plant^{-1} was recorded in the treatment *M. anisopliae* (SP8) and was statistically on par with that of *M. anisopliae* (SP9) (44.75 g plant^{-1}). The isolate *M. anisopliae* (SP7) yielded 40.00 g plant^{-1} and was on par with that of *B. bassiana* (Bb5a) which yielded 37.87 g plant^{-1}. The treatments *B. bassiana* (SP4), *B. bassiana* (SP2), *P. lilacinum* (S10) were ineffective, which recorded a lower yield of 25.50, 24.62 and 25.25 g plant^{-1} and were on par with that of the untreated plant (22.75 g plant^{-1}).

Table. 16 Yield in various treatments with entomopathogenic fungi

	Yield (g plant^{-1})	
Treatments (n x 10^8 spores mL^{-1})	Harvest at 35 DAS	Harvest at 50 DAS
B. bassiana (SP2)	24.62^{f}	32.25^{e}
B. bassiana (SP4)	25.50^{f}	37.00^{d}
M. anisopliae (SP7)	40.00^{de}	43.00^{bc}
M. anisopliae (SP8)	50.50^{c}	41.25^{cd}
M. anisopliae (SP9)	44.75^{cd}	45.25^{bc}
M. anisopliae (SP11)	73.75^{a}	50.75^{a}
P. lilacinum (S10)	25.25^{f}	37.00^{d}
B. bassiana (Bb5a)	37.87^{e}	38.00^{d}
M. anisopliae (Ma4)	65.25^{b}	46.75^{ab}
Untreated	22.75^{f}	31.25^{e}
CD (0.05)	6.266	4.461

DAS- Days after sowing

Discussion

5. DISCUSSION

Entomopathogenic fungi are important regulators of insect populations and are valued as the most versatile group of microbes for pest management in view of their wide host range and safety to non targets. Globally, there is a revival of interest in entomopathogenic fungi and research focused on these microbes unveiled the potential of the fungi in the genera *Beauveria*, *Metarhizium* and *Lecanicillium* as important bioagents (Kirk, 2003; Rai *et al*., 2014). The existence of many different species within these genera and many isolates within a species, that vary in their virulence have been documented (Soetopo, 2004; Pires *et al*., 2010; Safavi *et al.,* 2010). In this context, identification and selection of virulent isolates against target pest species is indispensible.

It is presumed that better pest management could be made feasible with indigenous isolates that can adapt better to the local environmental conditions. Moraga *et al*. (2007) was of the opinion that isolation of indigenous entomopathogenic fungi is a requisite for providing an insight into naturally occurring fungal diversity and for providing a repository of bioagents, to be conserved or used in pest management. An understanding of the factors that influence the distribution of fungi such as geographical location, habitat type as to whether natural or cultivated, soil properties etc. was considered important and investigations in these lines have been taken abroad by Rath *et al*., 1992; Vanninen, 1996; Chandler *et al*., 1997; Tarasco *et al.,* 1997; Bidochka *et al.,* 1998; Asensio *et al*., 2003; Keller *et al*., 2003; Bruck, 2004; Klingen and Haukeland, 2006; and Meyling and Eilenberg, 2006.

The immature stages of a large number of insects in the order Lepidoptera are crop pests and they have diverse feeding habits. Some remain exposed on leaf surface and feed the green matter whereas some others roll or web the leaves, remain sheltered within leaf rolls or webs and feed the leaf tissues, yet some other larvae bore into shoots and fruits and feed from within. Vegetable production in Kerala is

seriously hampered by the lepidopteran insect pests with such varied feeding habits. The pumpkin caterpillar, *Diaphania indica* Saunders is a major pest of the important vegetable, bittergourd cultivated in an area of 2302 ha in the State (GOK, 2015). The feeding of the larvae was reported to cause 60 per cent damage on the fruits of bitter gourd (Jhala *et al*., 2004). Brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee; Bhindi leaf roller, *Sylepta derogata* Fabricius; Leaf caterpillar, *Spodoptera litura* Fabricius and Amaranthus leaf webber, *Hymenia recurvalis* Fabricius are lepidopterans which unless checked, cause substantial yield reduction (Naresh *et al*., 1986; Dhir *et al*., 1992; Vashisth, 2009; Hsu and Srinivasan, 2012; Mariselvi and Manimeghalai, 2016). Currently, the demand for pesticide free vegetables and actions for their production are sky high. Against this backdrop the present project entitled "Pathogenicity of indigenous entomopathogenic fungi against select lepidopteran pests" was undertaken with the objectives of isolation and identification of indigenous isolates of entomopathogenic fungi, assessment of their pathogenicity to select lepidopteran pests and suitability of its use against lepidopteran pests of amaranthus through pot culture experiment.

Soil is an important reservoir of many different fungi including the entomopathogenic species which have an influence on the expansion of insect mycoses (Ignoffo *et al*., 1978). In 2007, Moraga *et al*. also stated that many species of hypocrealean fungi inhabit the soil for a major part of their life cycle when they are outside their host insect. *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin grow naturally in an array of environment (Rai *et al*., 2014; Wang and Leger, 2014). These fungi and other entomopathogenic fungi can be isolated from insect cadavers (Assaf *et al*., 2011; Ramanujam *et al.,* 2011; Liu *et al.,* 2012; Anis, 2014) or from soil using media (Beilharz *et al.*, 1982) or baiting soil with insects (Zimmerman, 1986; Rishi *et al.,* 2013). All these methods were followed for the isolation of indigenous fungi in the present study. Soil samples were collected from five agroecological zones *viz.*, southern coastal plain

(Chirayinkeezhu), southern laterite (Kattakada), southern central laterite (Nedumangad), southern and central foot hills (Vellanadu) and southern high hills (Peringammala) in Thiruvananthapuram district during 2015-16.

Ten isolates of entomopathogenic fungi were selected from the 115 fungal isolates which included three isolates from insect cadavers and seven isolates from soil. Two isolates of *B. bassiana* and one isolate of *Fusarium oxysporum* Schlecht were isolated from insect cadavers while five isolates of *M. anisopliae* and one isolate of *Fusarium solani* (Mart.) Sacc. were obtained from soil through baiting with the wax moth and the banana pseudostem weevil and one isolate of *Purpureocillium lilacinum* Thorn (Samson) was obtained through soil plate method (selective media). Eventhough no isolate of *B. bassiana* could be obtained using *Galleria mellonella* (L.) in the present study, Moraga *et al*. (2007) found *G. mellonella* as a suitable bait for the isolation of *B. bassiana* and a better percentage of *B. bassiana* (42.6 per cent) compared to *M. anisopliae* (7.3 per cent) was isolated using this insect.

G. mellonella is the traditionally used insect for trapping entomopathogenic fungi (Zimmeman, 1986; Meyling, 2007; Sun and Liu, 2008) though the larvae of *Tenebrio molitor* (L.), which is suitable for trapping different isolates of *B. bassiana* and *M. anisopliae* were also employed (Sanchez-Pena *et al*., 2011). *G. mellonella* is good bait for entomopathogens but its diet consisting of honey, corn flour and glycerol is costly which prompted for further identification of an alternate and cheaper bait insect during the study. The pseudostem weevil, *Odoiporous longicollis* Olivier is an important pest infesting banana in Kerala which is readily available in the field and can be easily reared on banana pseudostems (Anitha, 2000). *O. longicollis* was also tested as bait and using this insect one isolate of *M. anisopliae* was obtained. This is the first report on the use of *O. longicollis* as a bait for entomopathogenic fungi.

From among the nine hundred soil samples collected from 450 cultivated and 450 uncultivated fields in the different agroclimatic zones, adopting soil plate method with selective media, initially hundred and six fungal isolates were obtained. On analysis of the community structure of fungi, it was seen that the fungi were species of *Aspergillus, Fusarium, Penicillium, Purpureocillium and Trichoderma* (Figure 1). No isolate of *B. bassiana* and *M. anisopliae* could be isolated adopting soil plate method. According to Humber (1998) species of *Aspergillus* and *Penicillium* occur as primary pathogens or facultative pathogens of insects or as contaminant saprobes. Hasan *et al*. (2012) investigated the soil samples from insect hibernation sites following dilution plate method and observed the presence of the opportunistic fungi, *viz., Aspergillus flavus* Link, *Aspergillus niger* Tiegh., *Penicillium glabrum* (Wehmer) Westling, *Penicillium digitatum* (Pers.) Sacc. along with *B. bassiana.* Further they reported that the most frequently encountered species was *P. glabrum* followed by *A. niger*. A similar widespread distribution of *Aspergillus* sp. (42.45 per cent) and *Penicillium* sp. (13 per cent) was also seen in the present investigations. Species of *Aspergillus* (Sun and Liu 2008; Abdulla and Mohammed, 2009 and Assaf *et al*., 2011) and *Penicillium* (Abdulla *et al.,* 2002; Alishtayeh *et al*., 2002, Abdulla and Mohamed, 2009) have been earlier isolated and recorded as insect pathogens. An analysis of the fungal diversity of the rhizosphere soils in different agricultural fields done by Chandrasekhar *et al.* (2014) indicated that the dominant genera in all the agricultural soils were *Aspergillus* and *Penicillium,* which is supportive to the information garnered in the present study. Among the different isolation techniques of entomopathogenic fungi, bait trapping was adjudged as the best method considering the extent of isolation and easiness in obtaining isolates belonging to the genera *Beauveria* and *Metarhizium.*

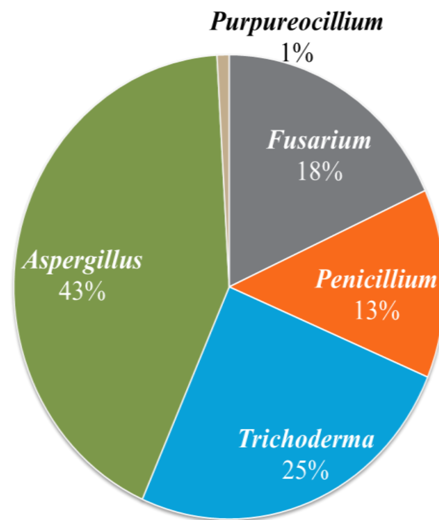


Figure 1. Fungal diversity in the isolates obtained through soil plate method

While considering the distribution of fungi in the different agroecological zones, it was seen that 30.43 per cent of the fungal isolates were present in southern laterite and 22.60 per cent were present in southern and central foot hills. The isolates of *B. bassiana* were collected only from the southern laterite whereas

M. anisopliae isolates were distributed in all agroecological zones. In other agroecological zones, the occurrence was 20.00 per cent (southern high hills), 13.91 per cent (southern central laterite) and 13.04 per cent (southern coastal plain) (Figure 2). Vanninen (1996) observed geographical location as an important factor determining the occurrence of entomopathogenic fungi. Charnley, in 1997 opined that soil factors such as temperature, pH, organic content and biotic factors affect fungal population and activity.

All these fungi were present in both cultivated and uncultivated soils but the percentage of occurrence (Figure 3) was more in cultivated soil (69 per cent) compared to uncultivated (31 per cent). Out of the ten select fungi, *B. bassiana* (SP2), *F. oxysporum* (SP1), *M. anisopliae* (SP7, SP9, SP11), and *P. lilacinum* (S10) were observed in cultivated soils and *B. bassiana* (SP4), *F. solani* (SP6) and *M. anisopliae* (SP8, SP13) were from uncultivated soil. Widespread occurrence of *Metarhizium* spp. in soils and their abundance in cultivated fields of wheat, oilseed, rapeseed and neighbouring uncultivated pastures was reported by Keyser *et al*. (2015). According to Goble *et al*. (2010) significantly higher population of entomopathogenic fungi occur in refugia compared to cultivated orchards. Medo and Cagan (2011) were of the opinion that *B. bassiana* though occur in different habits, were more abundant in natural habitats. They have also stated that *M. anisopliae* occurred primarily in field and meadow samples and that their occurrence was not negatively affected by intense cultivation.

Morphological characters formed the basis for the taxonomic studies of fungi (Riddell 1950; Domsch *et al.,* 1980; Humber, 1998). The taxonomy of

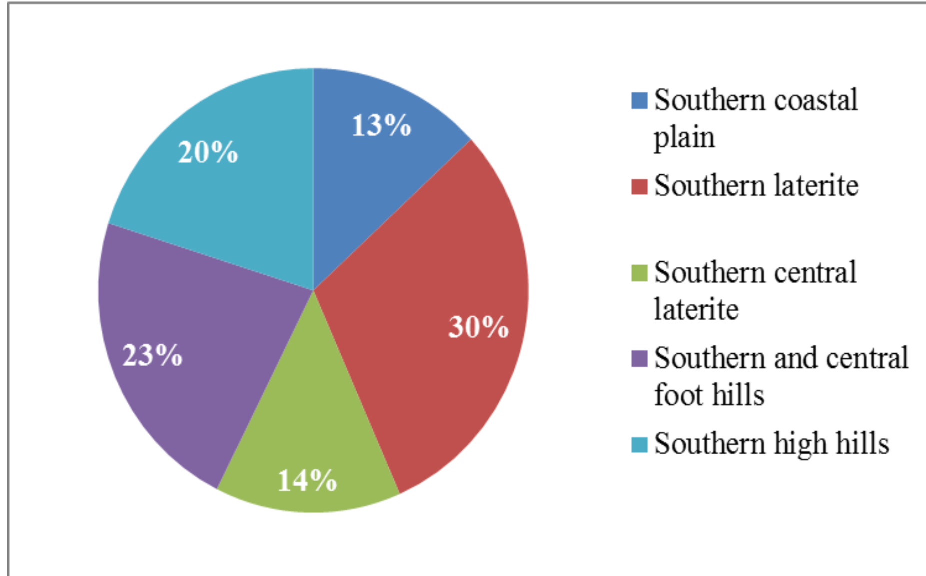


Figure 2. Fungal diversity obtained from different agroecological zones

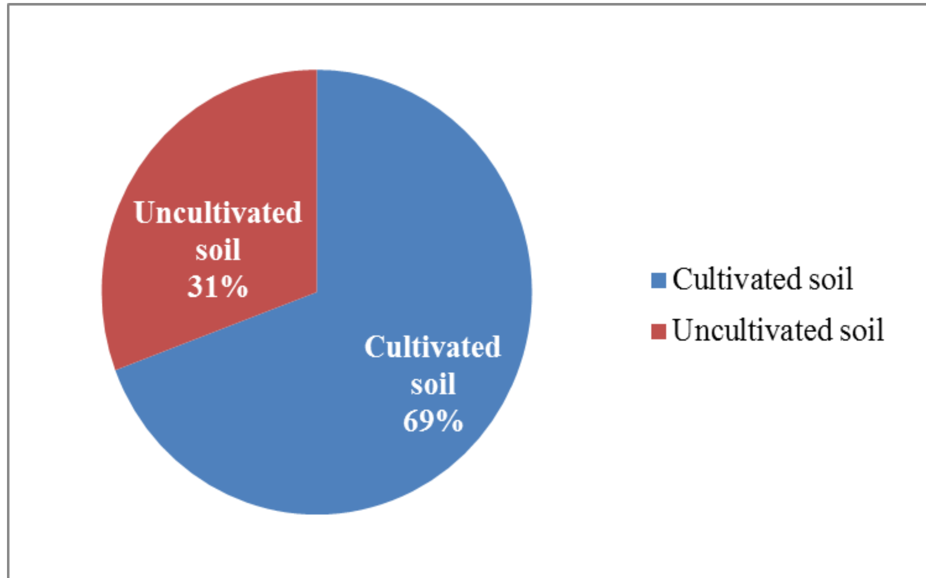


Figure 3. Fungal diversity in cultivated and uncultivated soil

entomopathogenic fungi is complex. Classification based on conidial size has been attempted by researchers (Samson *et al.,* 1988; Assaf *et al.,* 2011). As early in 1976, Tulloch recognized two species of *Metarhizium viz*., *M. flavoviridae* and *M. anisopliae*, using morphological characters of conidia and conidiogenesis cells. Further, *M. anisopliae* was subdivided into two variants *viz., M. anisopliae* var. *anisopliae* (conidia 3.5 -9 µm) and the long spored *M. anisopliae* var. *major* (conidia 9-18 µm) (Humber, 2005). In the present studies also observations on conidial size have been made. The size of conidia of the five isolates of *M. anisopliae* varied and it was in the range 2.3 x 0.9 to 3.5 x 1.01 µm which could be grouped into the category of medium and high ratio group according to length / width ratio, following the categorization of Tangthirasunun (2010) who classified the isolates of *M. anisopliae* based on length / width ratio of conidia into low, medium and high ratio groups. The present measurements noted on the conidial size of *M. anisopliae* is in contrast to that of Humber (2005) but, it agrees with that of (Bai *et al*., 2015; Ghayedi and Abdollahi, 2013), which further necessitates detailed investigations and conclusions on the conidial characters of the variants of *M. anisopliae*.

The conidial size of all other fungi, *B. bassiana*, *F. oxysporum*, *F. solani* and *P. lilacinum* noted in the study was in accordance with that mentioned for these fungi by other researchers (Draganova *et al*., 2010; Gupta *et al*., 2010; Luangsa-ard *et al*., 2011; Lokesh, 2014).

Cultural characters formed yet another basis for the classification of the fungi (Gupta *et al*., 2010; Raj and Tabassum, 2014). With respect to the cultural characters, no significant differences was observed in colony texture between the two isolates of *B. bassiana*, isolate SP2 produced flat cottony white colonies on the upper, whereas SP4 produced white, raised and cottony colonies, the reverse of which were cream. The cultural characters documented by Draganova *et al*. (2010); Mythili *et al*. (2010) and Kulu *et al.* (2015) for *B. bassiana* matches with the present

observations, however, the differences in the cultural characters of the different isolates of *M. anisopliae* was notable, the initial colour of *M. anisopliae* isolates, SP7, SP9 and SP11 were initially white which later turned gray and the reverse side of which were cream to brown, while that of the isolates SP8 and SP13 produced white growth that later turned green, the reverse of which were light brown. Striking differences in growth and sporulation of the *M. anisopliae* isolates was also evident. The spore count of the *M. anisopliae* isolates varied and highest spore count of 26.57x 10^7 spores mL^{-1} was recorded in the isolate *M. anisopliae* (SP8). The lowest spore production was noted in the isolate *B. bassiana* (SP2) (4.08 x 10^7 spores mL^{-1}). The radial growth of the isolates was in the range of 4.83 to 6.83 cm at fourteenth days after inoculation. Such variations in cultural characters were reported earlier by Tangthirasunun *et al*. (2010); Fernandes *et al.* (2010) and Ghayedi and Abdollahi, 2013). Variations in radial growth of the different isolates of was also noted by Hoe *et al.* (2009); Mishra *et al*. (2015) and Sepulveda *et al.* (2016).

Quicker and accurate identification of a broader range of fungal pathogens are feasible through molecular techniques (Bruns *et al*., 1991). Molecular characterization of different isolates of entomopathogenic fungi has been achieved through Internal Transcribed Spacer (ITS) sequencing of rDNA (Inglis and Tigano, 2006; Nilsson *et al*., 2008). Since ITS region of rDNA showed high degree of variation between closely related species this tool is widely used in fungal taxonomy (Neuveglise *et al*., 1994; Chen *et al*., 2000; Oliveira *et al*., 2012). Other molecular tools such as Random amplified polymorphic DNA (RAPD), Restriction fragment length polymorphism (RFLP), Sequence-characterized amplified regions (SCAR) were also adopted (Fegan *et al*. 1993; Hegedus and Khachatourians, 1993; Schilling *et al*., 1996). Considering the relevance of molecular identification of fungi in fungal taxonomy today, and the precision that ITS sequencing method offers, in the present studies for molecular characterisation of the newly isolated indigenous isolates ITS

sequencing of the fungal rDNA was done, with the expertise available at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

The Internal Transcribed Spacer sequencing of the fungi at 5.8S rDNA conserved region of the fungal DNA yielded 519 base pair sequences for SP1 and Basic Local Alignment Search (BLAST) in National Center for Biotechnology Information (NCBI) revealed the identity as *F. oxysporum*, Similarly for SP6, 529 base pair sequences obtained showed that the fungus was *F. solani.* 530 base pair sequences of SP2 and SP4 showed that these isolates were *B. bassiana.* The DNA size of about 320 - 2300 base pair was recorded for the *B. bassiana* isolates in earlier studies through molecular identification (Viaud *et al.* 1998; Kaur and Padmaja, 2008). Carneiro *et al*. (2008) sequenced 570 base pair for the identification of *Beauveria* isolates. From the 520 base pair sequences of SP7, SP9, SP11and the isolates were identified as *M. anisopliae*. SP8 that was also identified as *M. anisopliae* isolate yielded 519 base pair sequences whereas SP13 yielded 508 base pairs. The analysis of ITS1 -5.8S- ITS2 rDNA regions of *M. anisopliae* isolates by Destefano *et al*. (2004) yielded 540 base pairs. The isolate, SP12 yielded 557 base pair sequences and was identified as *P. lilacinum*.

Further, the pathogenicity of the newly isolated indigenous isolates and the isolates obtained from National Bureau of Agricultural Insect Resources (NBAIR) was assessed using spore suspensions from fourteen day culture of the fungus grown in Potato Dextrose Agar (PDA), against five important caterpillar pests of vegetables *viz., D. indica*, the pumpkin caterpillar; *H. recurvalis*, amaranthus leaf webber; *L. orbonalis*, Brinjal shoot and fruit borer; *S. litura*, the polyphagous leaf caterpillar and *S. derogata,* bhindi leaf roller. The indigenous isolates *M. anisopliae* (SP7, SP8, SP9, SP11 and SP13) and NBAIR isolates *B. bassiana* (Bb5a) and *M. anisopliae* (Ma4) were found pathogenic to all the test insects except *S. litura* towards which the isolate SP11 and Bb5a were only infective. The host range of entomopathogenic fungi and

their isolates were found to vary substantially, some with a very narrow host range while some were broad spectrum (MacLeod, 1963; Li, 1988; Askary *et al*. 1998; Devi *et al*., 2008). The present findings gain foothold through the statements of Butt *et al*., 2001 also that physiological factors of the insect such as defense mechanisms as well as that of the pathogen such as enzymes and toxins produced influence the pathogenicity of a fungal pathogens.

During fungal infections, though at early stages, the insects do not exhibit signs and symptoms of infection, at later stages the insects become less active with reduced appetite (McCoy *et al*., 1988; Nilamudeen, 2015) and immediately before or at death the infected insects develop characteristic colours. The typical signs and symptoms of fungal infection *i.e.,* immobility, reduced feeding and mummification in the infected insects was evident in this study too. Besides these symptoms, the infected second instar larvae of *L. orbonalis* came out of their feeding tunnels prior to death.

The symptoms such as colour slightly varied between isolates of a species and more pronouncedly between species. The symptoms produced by all the isolates, on application of spore suspensions from fourteen day old culture of *M. anisopliae,* SP7, SP8, SP9, SP11, SP13 and Ma4 on the larvae of *D. indica, H. recurvalis* and *S. derogata* were similar except in *D. indica* treated with *M. anisopliae* (Ma4), where the infected larvae initially developed black spots along the spiracular region. Nedveckyte *et al*. (2011) while evaluating local strains of *B. bassiana* and *M. anisopliae* against *Bupalus piniaria* L. also noted the occurrence of black drops in mycosed cadavers and this was attributed to the melanisation of internal cavity induced by the activity of polyphenoloxidase. White coloured mycelia initially developed in all the insects treated with *M. anisopliae* which changed into grayish green in *M. anisopliae* SP7, SP9, and SP11, and green in the case of *M. anisopliae* SP8, SP13 and Ma4. Han *et al*. (2014) also observed similar symptoms of fungal

infection on the larvae of *Spodoptera exigua* Hubner treated with the isolate *M. anisopliae* FT83.

On the larvae of test insects treated with NBAIR isolate *B. bassiana* (Bb5a), white coloured fungal growth was observed. Gabarty *et al*. (2014) and Nilamudeen (2015) also observed the emergence of white mycelia on the cadavers of lepidopteran pests *viz., Agrotis ipsilon* (Hufnagel) and *Cnaphalocrocis medinalis* Guen infected with *B. bassiana.* The indigenous isolates of *B. bassiana* (SP2 and SP4) obtained in the present study were not pathogenic and did not produce any symptoms of fungal infection in any of the test insects.

An assessment of the virulence of the ten different isolates at three different concentrations against the five lepidopteran test insects revealed that the indigenous isolates of *M. anisopliae* SP8, SP11 and the NBAIR isolate Ma4 are highly virulent isolates and the mortality initiated at a shorter period (48h) after treatment in these isolates compared to the initiation of mortality in other isolates. Susceptibility of the insects to the different isolates varied. Though mortality initiated 48 h after treatment when treated with SP8, SP11 and Ma4 at concentrations of 10^8 and 10^9 spores mL^{-1}, it was not uniformly expressed in all the test insects, however three DAT, mortality initiated in all the test insects except *S. litura* at 10^9 spores mL^{-1}. In all other isolates, mortality initiated at a longer period of 72 h after treatment at 10^9 spores mL^{-1}. The order of virulence observed was *M. anisopliae* SP8, SP11, Ma4, SP9, SP7, SP13 and Bb5a.

The genus *Metarhizium* is pathogenic to a spectrum of insect pests. From the performance of the five indigenous *Metarhizium* isolates and NBAIR isolate (Ma4) against the select lepidopteran pests, it is concluded that SP8 and SP11 of *M. anisopliae* are aggressive isolates, against *D. indica*, *H. recurvalis, L. orbonalis* and *S. derogata* belonging the family Pyralidae which are fragile caterpillars, whereas

the isolate SP8 was ineffective against *S. litura* and SP11 showing only 53.33 per cent against this relatively stout caterpillar of the family Noctuidae. Ravi (2013) also made a similar observation that *B. bassiana* (Bb5a) and *M. anisopliae* (Ma4) were not pathogenic to third instar larvae of *S. litura* at 10^9 and 10^{10} spores mL^{-1}. None the less, Kumar and Chowdry (2004) reported *M. anisopliae* as a good pathogen of another noctuid caterpillar pest, *Helicoverpa armigera* Hubner. Several factors influence the pathogenicity of fungal pathogens, which include nutritional deficiency, invasion by the conidia and destruction of tissues, production of toxins and defense chemicals (Roberts, 1981; Charnley, 2003; Goettel *et al*., 2010; Butt *et al*., 2001). According to Hoe *et al.* (2009) one of the deciding factors of host specificity of the fungal pathogen is the properties of the insect integument. Pucheta *et al*. (2006) and Ortiz-Urquiza and Keyhani (2013) opined that the cuticle acts as the point of contact and barrier between insect and the fungi and that heterogenous structure of the cuticle vary with insects and their life stages. Such factors might have contributed to the variable infectivity of the different isolates noted against the test insects in the present study.

The genus *Beauveria* consists of approximately 22 entomopathogenic species (Kirk, 2003). Among the different species, *B. bassiana* acts as a pathogen of various insects (Jain *et al*., 2008; Sandhu *et al.*, 2012) however, the two indigenous isolates of *B. bassiana*, SP2 and SP4 evaluated in the study were found ineffective against all the lepidopteran insects tested though they were isolated from the cadaver. Rai *et al*. (2014) stated that the important feature of *Beauveria* sp. is the high host specificity of the isolates. Differential mortality produced by isolates of *B. bassiana* have been reported by Kumar and Chowdhry (2004). In their studies, mortality per cent ranging from 40-90 per cent have been observed in *H. armigera* treated with eighteen isolates of *B. bassiana*. He further stated that isolates from a specific host are more virulent for that host than those isolated from other hosts. According to Ignoffo and Garcia (1985) microbes undergo selection, recombination and mutation depending upon

ecological conditions. These changes ultimately result in variations in their genetic makeup and with subsequent changes in factors that affect their virulence. This may be the reason for the differential pathogenicity observed for the different isolates even within a species. This result and the related references on host specificity and variability in the virulence of different isolates of entomopathogenic fungi reiterates the need for selection of the apt isolate against the pest species for obtaining the desired results in pest management.

The genus *Fusarium* contains 13 insect pathogenic species (TeetorBarsch and Roberts 1983; Humber 1992) however, *F. oxysporum* (SP1) and *F. solani* (SP6) were found non pathogenic to the lepidopteran test insects in the present study. Based on the field observations and pathogenicity tests conducted, Teetor-Barsch and Roberts (1983), observed that *Fusarium* sp. act as a weak pathogen against lepidopteran and coleopteran pests but as a virulent against homopterans and dipterans (Hajek *et al*., 1990). Contrastingly, Varma and Tandan (1996) found *F. oxysporum* as an effective pathogen of the lepidopteran pests of sugarcane *viz*., *Chilo auricilius* Dudgeon, *Chilo infuscatellus* Snellen and *S. inferens* Walker.

Purpureocillium lilacinum (Thorn) Samson is a known pathogen of insects. The isolate *P. lilacinum* TR1 was reported to cause 83.64 per cent mortality on the adults of cherry aphid after eight days, at 10^8 cfu mL^{-1} (Kepenekci *et al*., 2015). No mortality in all the lepidopteran test insects was observed due to *P. lilacinum* (S10) in the present study.

The effect of varying spore concentrations on the extent of mortality of the test insects was analysed subsequently. At the lowest dose of 10^7 spores mL^{-1} the mortality of larvae of *D. indica* observed at 168 HAT was 83.33 percent in the isolate *M. anisopliae* (SP8), which recorded the highest mortality whereas it was cent per cent at the higher spore concentration of 10^9 spores mL^{-1}. The mortality noted in

L. orbonalis also followed a similar trend at these two concentrations of SP8, while a mortality of 64.44 per cent was recorded at 10^7 spores mL^{-1} a higher mortality of 95.83 per cent was recorded at 10^9 spores mL^{-1}. It was seen that with increase in spore concentration the mortality percentage also increased irrespective of the isolates evaluated. With respect to the virulence against *H. recurvalis*, *S. litura* and *S. derogata* also a dose dependent effect was seen. *M. anisopliae* SP11 recorded maximum mortality against these insects and the percentage mortality recorded in this treatment was 73.33, 28.33 and 83.33 per cent for the three insects, respectively at 10^7 spores mL^{-1} whereas the corresponding values were 96.66, 53.33 and 100.00 per cent at 10^9 spores mL^{-1}. This dose dependent effect of entomopathogenic fungi was evident in the earlier studies also. Virulence of fungal isolates of *M. anisopliae* was reported to vary significantly with conidial concentrations in *Coptotermes curvignathus* Holmgren (Hoe *et al.,* 2009). The highest mortality of the larvae of *Tuta absoluta* Meyrick was recorded at 10^{10} spores mL^{-1} among the various concentrations of 10^7 to 10^{10} spores mL^{-1} of isolates of *M. anisopliae* evaluated (Shalaby *et al.,* 2013). Dose dependent mortality was recorded by Dhuyo and Soomro (2008) when larvae of *Scirpophaga incertulas* (Walker) were treated with different concentrations of *B. bassiana.* The superiority of the isolates *M. anisopliae* SP8 and SP11 was also evident at the two higher concentrations of 10^8 and 10^9 spores mL^{-1} evaluated in this study.

Environmental conditions have a great influence on the infectivity of the entomopathogens against insects. Ability of the fungal propagule to persist in the environment and incite infection through contact of adequate number of infective propogules are decisive factors in the efficacious control of insect pests in the field (Butt *et al.*, 2001). This calls for the evaluation of these pathogens in natural situations prior to selection as candidates in biocontrol programmes. With this purpose, a pot culture experiment was conducted, during April to June 2016 in the Instructional Farm, Vellayani with the objective of evaluating seven indigenous

isolates of fungi and two NBAIR isolates against leaf webbers infesting Amaranthus, variety Arun. It was evident from the observations that the fungal isolate, *M. anisopliae* (SP11) was the most effective treatment in which the percentage reduction of infested plants over control was 31.57 and 63.15 per cent on the seventh and fourteenth day after treatment. In the next effective treatment *M. anisopliae* (Ma4), the corresponding reduction was 21.05 to 57.89 per cent over control (Figure 4). Similarly, with respect to percentage reduction in the number of webs present (66.28 and 87.61 per cent) on seventh and fourteenth day after treatment was also less in *M. anisopliae* (SP11). This was followed by the isolate *M .anisopliae* (Ma4) that recorded 64.76 and 84.95 per cent reduction in number of webs respectively on seventh and fourteenth day after treatment (Figure 5). The isolate *M. anisopliae* (SP11) recorded 33.55 and 75.44 per cent reduction in the number of larvae web^{-1} on seventh and fourteenth day after treatment respectively over control (Figure 6). The yield recorded was also significantly higher (73.75 g plant^{-1}) in plants treated with the isolate *M. anisopliae* (SP11) compared to control.

It was seen from the present results that eventhough the larvae remained in concealed situations in webs, effective management of leaf webbers in Amaranthus was achieved with the isolates SP11 and SP8 of *M. anisopliae*, the isolates that recorded highest pathogenicity against *H. recurvalis* in the laboratory tests also. According to Inglis *et al.*, 1993 the microhabitat where the fungi are deployed for pest management has an influence on its persistence and efficiency. It is inferred from this statement that the microhabitat within the leaf webs where the larvae were present was conducive for the infection by these entomopathogenic fungi and that entomopathogens seems ideal for management of pests living in concealed situations also.

Inspite of the rain that prevailed (Appendix I) during the pot culture trial effective management with fungal isolates could be obtained. Anecdotal evidences

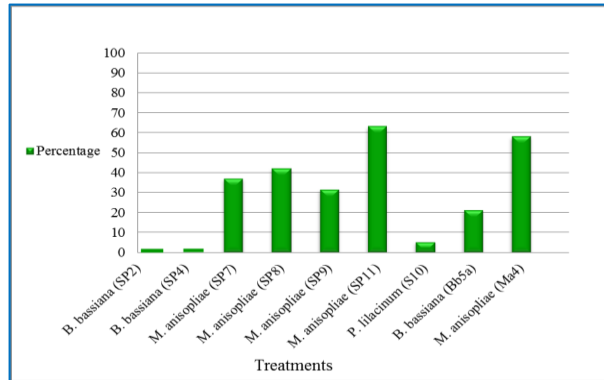


Figure 4. Mean percentage reduction of leaf webbers in different treatments over untreated

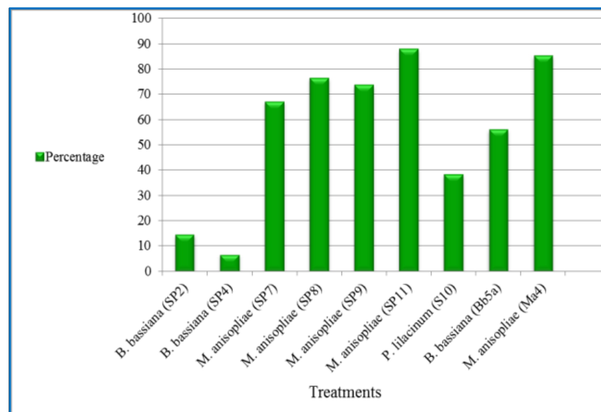


Figure 5. Mean percentage reduction in webbings per plant over untreated

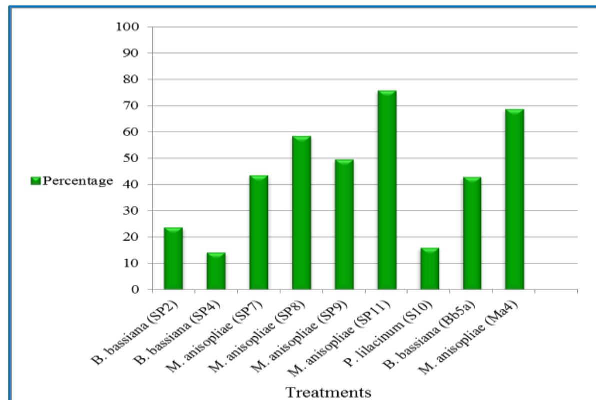


Figure 6. Mean percentage reduction in larvae per web over untreated

on the low recovery of conidia from treated insects washed vigourously with water led to the inference that rain does not remove significant number of conidia from insect integument. (Burges, 1998), Nonetheless contradictory evidences put forth by Inglis *et al.*, (1995, 2000) showed that rain removed significantly *B. bassiana* and *M. anisopliae* conidia from foliage of monocotyledonous and dicotyledonous plants and from the integument of insects. Butt *et al.* (2001) emphasized the need for the elucidation of the influence of confounding variables such as canopy density and architecture, and insect behavior and conidial persistence on the infectivity of entomopathogenic fungi. Effective management of lepidopteran and coleopteran pests under natural situations, in pot culture or in field experiments with entomopathogenic fungi were earlier reported by Sahayaraj and Namachivayam, 2011; Anis, 2014; Lokesh, 2014; Magda and Said, 2014; Nilamudeen, 2015. The present studies explicitly indicate the suitability of the fungal pathogens even on rainy days for the management of leaf webbers in Amaranthus that remain concealed within the webs.

Summary

6. SUMMARY

Entomopathogenic fungi have emerged as an important tool in pest management in lieu of the synthetic chemical insecticides that cause an array of problems to humans and environment. Safety to non targets and host specificity are some of the attributes that brought them to limelight in the recent years. Now, the existence of many species and isolates within each genera of these fungi that vary in their virulence is also evident. This demands appropriate selection of the fungal isolate for effective pest management. Isolation, identification and evaluation of virulent indigenous isolates of these fungi is the need of the hour for the better exploitation of entomopathogenic fungi in pest management. The pumpkin caterpillar, *Diaphania indica* Saunders; Brinjal fruit and shoot borer, *Leucinodes orbonalis* Guenee; Amaranthus leaf webber, *Hymenia recurvalis* Fabricius; and Bhindi leaf roller, *Sylepta derogata* Fabricius are important lepidopteran pests that substantially reduce yield in vegetables. In banana, the leaf feeding polyphagous *Spodoptera litura* Fabricius is an important pest. Considering the importance of these lepidopteran pests and the importance of entomopathogenic fungi, a study entitled "Pathogenicity of indigenous entomopathogenic fungi against select lepidopteran pests" was carried out during 2014-2016 in the Department of Agricultural Entomology, College of Agriculture, Vellayani with the objectives to isolate and identify indigenous entomopathogenic fungi, evaluate their pathogenicity to lepidopteran pests of banana and vegetables in the laboratory and to assess the pathogenicity of the fungal isolates against the leaf webbers of amaranthus through pot culture experiment.

Survey was conducted in five agroecological zones *viz.,* southern coastal plain (Chirayinkeezhu), southern laterite (Kattakada), southern central laterite (Nedumangad), southern and central foot hills (Vellanadu) and southern high hills (Peringammala) at bimonthly intervals during 2015-16, for the collection of mycosed

cadaver and soil samples. From each agroecological unit 30 samples, 15 from cultivated and 15 from uncultivated fields were collected, and a total of 900 soil samples were collected for the isolation of fungi. A total of 115 fungal isolates were obtained of which 106 isolates were obtained through soil plate method (selective media) and six isolates were obtained through baiting with *Galleria melonella* L. / *Odoiporus longicollis* Oliver. Three isolates were obtained from mycosed cadavers. Of all the isolates, 30.43 per cent were collected from southern laterite and 22.60 per cent were from southern and central foot hills. The extent of isolation from southern high hills, southern central laterite and southern coastal plain were 20.00, 13.91 and 13.04 per cent respectively. Sixty nine per cent of the fungal isolates were obtained from cultivated and 31 per cent were obtained from uncultivated soils. The fungi isolated through soil plate method consisted *of Aspergillus* spp. (42.45 per cent), *Trichoderma* spp. (25.47 per cent), *Fusarium* spp. (17.92 per cent), *Penicillium* spp. (13.20 per cent) and *Purpureocillium* spp. (1.00 per cent).

The ten isolates selected from the 115 isolates for further studies included two isolates of *Beauveria bassiana* (Balsamo) Vuillemin (SP2 and SP4), one isolate of *Fusarium oxysporum* Schlecht (SP1), one isolate of *Fusarium solani* (Mart.) Sacc. (SP6), five isolates of *Metarhizium anisopliae* Metschnikoff (Sorokin) (SP7, SP8, SP9, SP11, SP13), and one isolate of *Purpureocillium lilacinum* Thorn (Samson) (S10). The isolates of *B. bassiana* were obtained from mycosed cadavers and *M. anisopliae* isolates were obtained through bait trapping.

The studies on the morphological and cultural characters of the fungal isolates revealed that the size and shape of the conidia varied between species and isolates. The cultural characters such as colony colour, texture and radial growth of the isolates were varied.

The spore count of indigenous isolates and isolates from National Bureau of Agricultural Insect Resources (NBAIR) were estimated. The NBAIR isolate, *M. anisopliae* (Ma4) recorded the highest spore count of 42.92 x 10^7spores mL^{-1} and 94.20 x 10^7spores mL^{-1}at 7 Days after inoculation (DAI) and 14 DAI respectively and was significantly superior to spore count in other treatments. Among the various indigenous isolates, *M. anisopliae* (SP11) that recorded 12.88 x 10^7spores mL^{-1}at 7 DAI and 28.01x 10^7spores mL^{-1} at 14 DAI was significantly superior to other indigenous isolates.

The pathogenicity studies of the different fungal isolates against the five lepidopteran pests showed that the *M. anisopliae* (SP7, SP8, SP9, SP11 and SP13) and NBAIR isolate *B. bassiana* (Bb5a) and *M. anisopliae* (Ma4) were pathogenic to *D. Indica, H. recurvalis, L. orbonalis* and *S.derogata.* Only two isolates, *M. anisopliae* (SP11) and *B. bassiana* (Bb5a) were pathogenic to *S. litura.*

The infected insects showed reduced feeding and movement initially and on death, the insect became mummified. The *M. anisopliae* isolates produced white mycelia two days after death and the cadavers turned green / grayish green depending on the isolates. *B. bassiana* isolate infected insects were covered with white cottony mycelia four days after death.

Studies on the virulence of the isolates at three different concentration *viz.*, 10^7, 10^8 and 10^9 spores mL^{-1}showed that *M. anisopliae* isolate (SP8) was the most virulent isolate against *D. indica* and *L. orbonalis* at 10^7 to 10^9 spores mL^{-1}. The mortality initiated 48 Hours after treatment (HAT) (13.33 per cent) at 10^8 spores mL^{-1} in the larvae of *D. indica* and it increased over the period. The mortality ranged from 83.33 to 100 per cent in *D. indica* and 64.44 to 95.83 per cent in *L. orbonalis* at 10^7 to 10^9 spores mL^{-1} at 168 HAT. The virulence exhibited against *D. indica* by other isolates was in the order *M. anisopliae* (Ma4) > *M. anisopliae* (SP11) > *M. anisopliae*

(SP9) > *M. anisopliae* (SP7) > *M. anisopliae* (SP13) > *B. bassiana* (Bb5a). With respect to *L. orbonalis* the virulence was in the order *M. anisopliae* (Ma4) > *M. anisopliae* (SP9) > *M. anisopliae* (SP11) > *M. anisopliae* (SP7) > *B. bassiana* (Bb5a) > *M. anisopliae* (SP13).

Against the larvae of *H. recurvalis* the NBAIR isolate *M. aniopliae* (Ma4) that caused 63.33 to 100 per cent mortality at 168 HAT was the best treatment but it was on par with *M. anisopliae* (SP11) that recorded 73.33 to 96.66 per cent mortality. The superiority of the *M. anisopliae* (SP11) was evident from the virulence shown against *S. derogata* and *S. litura*, in which the mortality of 83.33 to 100 per cent and 28.33 to 53.33 was recorded respectively at 168 HAT. The effect of *M. anisopliae* (SP8) against *S. derogata* was on par with *M. anisopliae* (SP11). It was evident from the studies that *M. anisopliae* (SP11), *M. anisopliae* (SP8) and *M. anisopliae* (Ma4) were virulent isolates. Dose dependent mortality was recorded in all the isolates evaluated.

The efficacy of select indigenous fungal isolates and NBAIR isolates against amaranthus leaf webbers were evaluated through pot culture experiment conducted in the Instructional Farm, Vellayani during April to June 2016. The isolate *M. anisopliae* (SP11) @ 10^8 spores mL^{-1} that showed 63.15, 87.65 and 75.44 percentage reduction in the infestation of plants by leaf webbers, webbings $plant^{-1}$ and mean larvae web^{-1} at 14 DAT was the best treatment. It was significantly superior to all other treatments but was on par with that of *M. anisopliae* (Ma4). The yield from plants treated with *M. anisopliae* (SP11) was significantly superior (73.75 g $plant^{-1}$) and it was followed by the plants treated with *M. anisopliae* (Ma4) (63.25 g $plant^{-1}$) and *M. anisopliae* (SP8) (50.50 g $plant^{-1}$).

To sum up, the newly isolated indigenous isolates of entomopathogenic fungus, *M. anisopliae* (SP11), *M. anisopliae* (SP8) and the NBAIR isolate *M. anisopliae*

(Ma4) were found to be highly virulent isolates that can be exploited for the biological control of vegetable pests *viz., D. indica, H. recurvalis, L. orbonalis* and *S. derogata*. As these isolates varied in their virulence to the test insects and as spore concentration dependent mortality was evident, selection of the appropriate isolate and its application at the required concentration is highly essential for effective management. Further, investigations on the indigenous fungal resources, their evaluation and further, their development as biopesticides need to be hastened.

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PATHOGENICITY OF INDIGENOUS ENTOMOPATHOGENIC FUNGI AGAINST SELECT LEPIDOPTERAN PESTS

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ABSTRACT

The present study entitled "Pathogenicity of indigenous entomopathogenic fungi against select lepidopteran pests" was carried out in the Department of Agricultural Entomology, College of Agriculture, Vellayani during 2014-2016 with

the objective to identify indigenous entomopathogenic fungi and evaluate their pathogenicity to lepidopteran pests of banana and vegetables.

Survey was conducted in five agroecological zones of Thiruvananthapuram district at bimonthly intervals during 2015-16, for the isolation of fungi. Fungi from mycosed cadavers and 900 soil samples, collected from cultivated and uncultivated fields were isolated. Of the ten isolates of fungi selected from the 115 fungal isolates obtained, three were from mycosed cadavers which consisted of two isolates of *Beauveria bassiana* (Balsamo) Vuillemin (SP2 and SP4) and one isolate of *Fusarium oxysporum* Schlecht (SP1). Of the seven isolates from soil, one isolate was *Fusarium solani* (Mart.) Sacc. (SP6), five were isolates of *Metarhizium anisopliae* Metschnikoff (Sorokin) and one isolate was *Purpureocillium lilacinum* Thorn (Samson). Four isolates of *M. anisopliae* were trapped using larvae of *Galleria melonella* L. and one was trapped using grubs of *Odoiporous longicollis* Olivier. The isolate, S10 was obtained through soil plate method, with selective media.

Symptoms of fungal infection varied, which was mainly reflected in the mycelial colour and growth of the isolates. Morphological and cultural characteristics also varied among the fungal isolates. Further, molecular characterization of the fungi was done through ITS sequencing. GenBank accession numbers for all the ten isolates were obtained on submission of nucleotide sequence in National Center for Biotechnology Information (NCBI).

Among the various indigenous isolates, highest spore count was recorded in the *M. anisopliae* isolate, SP11 (28.01×10^7 spores mL^{-1}) at 14 days after inoculation.

The pathogenicity of the ten indigenous isolates and two isolates from National Bureau of Agricultural Insect Resources (NBAIR) were evaluated against five lepidopteran insects infesting banana and vegetables at different concentrations. The

isolate *M. anisopliae* (SP8) recorded the highest mortality of 83.33 to 100 per cent and 64.44 to 95.83 per cent against the second instar larvae of *Diaphania indica* Saunders and first instar larvae of *Leucinodes orbonalis* Guenee at 10^7 to 10^9 spores mL^{-1} at seven days and five days after treatment respectively. The isolates SP11 and Ma4 of *M. anisopliae* that caused mortality of 83.33 to 100 per cent and 63.33 to 100 per cent were the most effective isolates against second instar larvae of *Sylepta derogata* Fabricius and *Hymenia recurvalis* Fabricius respectively. All the isolates except *M. anisopliae* (SP11) and *B. bassiana* (Bb5a) were non pathogenic to the larvae of *Spodoptera litura* Fabricius. The colour of the mycelial growth varied with isolates.

A pot culture experiment was conducted in the Instructional Farm, Vellayani during April to June 2016, for the evaluation of seven indigenous isolates and two NBAIR isolates against leaf webbers in amaranthus, variety Arun. The lowest number of plants infested by webbers, webbings $plant^{-1}$ and larvae web^{-1} at 14 days after treatment and the highest yield was recorded in the isolate *M. anisopliae* (SP11) @ 10^8 spores mL^{-1} and it was followed by *M. anisopliae* Ma4 and SP8 .

To conclude, ten indigenous isolates of entomopathogenic fungi were collected from mycosed cadavers and soil and were identified as *B. bassiana* (SP2, SP4), *F. oxysporum* (SP1), *F. solani* (SP6), *M. anisopliae* (SP7, SP8, SP9, SP11 and SP13) and *P. lilacinum* (S10) through morphological, cultural characters and molecular characterization. Pathogenicity test to five lepidopteran pests showed that *M. anisopliae* (SP7, SP8, SP9, SP11, SP13) and NBAIR isolates of *B. bassiana* (Bb5a) and *M. anisopliae* (Ma4) were pathogenic to *D. indica, H. recurvalis, L. orbonalis* and *S. derogata.* Results of pot culture experiment showed that the indigenous isolates *M. anisopliae* (SP11) and (SP8) and NBAIR isolate *M. anisopliae* (Ma4) can be exploited for the management of leaf webbers in amaranthus.

Appendices

Appendix I

Weather parameters during April to June 2016

Period	Temperature (^{0}C)		Relative Humidity (%)	Rainfall (mm)
	Max.	Min.		
April - 3rd week	35.5	26.7	92.7	5.7
April - 4th week	35.2	27	88.6	0.0
May - 1st week	35.7	26.3	90.0	21.3
May - 2nd week	34.8	25.8	92.3	35.9
May - 3rd week	32.5	24.3	95.7	55.5
May - 4th week	33.1	25.2	88.6	24.3
June - 1st week	32.7	25.2	89.0	0.0
June - 2nd week	35.1	25.1	95.1	48.0