ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF BANANA RHIZOME WEEVIL (*Cosmopolites sordidus* Germer)

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF BANANA RHIZOME WEEVIL (*Cosmopolites sordidus* Germer")** a bona fide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF BANANA RHIZOME WEEVIL (*Cosmopolites sordidus* Germer)"** is a record of research work done independently by Ms. Varsha Vijayan under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

@	At the rate of
^{0}C	Degree Celsius
%	Per cent
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
bp	Base Pair
CD	Critical difference
cm	Centimetre
CRD	Completely Randomised Design
DAT	Days after transplanting
DNA	Deoxyribonucleic acid
et al.	And others
EPF	Entomopathogenic fungi
Fig.	Figure
g	Gram
g L^{-1}	Gram Per litre
h	Hour
ha	Hectare
ha^{-1}	Per hectare
HAT	Hours After Treatment
ie.	That is
ITS	Internal Transcribed Sequence

KAU	Kerala Agricultural University
kg	Kilogram
kg ha^{-1}	Kilogram per hectare
L	litre
L^{-1}	Per litre
LC	Lethal Concentration
LT	Lethal Time
mg	Milligram
mL^{-1}	Per millilitre
mm	Millimetre
NBAIR	National Bureau of Agricultural Insect Resources
NS	Non -significant
No.	Number
$Plant^{-1}$	Per Plant
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
RAPD	Random Amplified Polymorphic DNA
Sl.	Serial
sp. or spp.	Species (Singular and Plural)
viz.	Namely

Introduction

1. INTRODUCTION

Banana, the staple fruit crop of India is widely grown in tropical and subtropical regions with an area covering 0.88 million ha and productivity of 34.1 t ha^{-1} (Mamtha *et al.,* 2015). Banana rhizome weevil, *Cosmopolites sordidus* (Germer) is a destructive pest of banana all over the world. The weevils are nocturnal and are seen around or in between the cracks and crevices of rhizome. Grubs are the most destructive stage of the pest, which make longitudinal tunnels in the rhizome by feeding. They destroy the vascular system of the plant and interfere with the transport of nutrients resulting in reduction of leaf size, leaf number and bunch size which leads to weakening of the plant and toppling of the plant.

Yield loss of 40 to 100 per cent has been reported when the infestation by the pest is severe (Seshu *et al.*, 1998). Management of the pest becomes challenging because the grubs lie inside the rhizome. Almost all varieties have been identified as susceptible to rhizome weevil attack. Recommended practices for controlling the weevil population are mainly based on the cultural and chemical methods. Eventhough insecticides are effective, high cost, environmental pollution, health hazards and development of resistance warrant the development of biorational alternatives for its management. There is a high demand for organically produced food, adopting ecologically safe pest control methods. In this context, biological control of insect pest using entomopathogenic fungi has its relevance. Due to their environmental safety and self perpetuating nature, they can be successfully incorporated in the integrated pest management programmes.

Entomopathogenic fungi have the potential to grow, multiply and persist on the insects they attack. The infected insect can carry the pathogen throughout the pest's habitat leading to epizootic situation (Ferron, 1981).

More than 750 species of entomopathogenic fungi have been identified to cause mycosis in insects (Hejek and St. Leger, 1994). Soil acts as a reservoir of entomopathogenic fungi, and hence natural infection occurs on insects. Infected weevils carry the pathogens and disseminate them among healthy insects (Godonou, 1999). The gregarious life cycle of weevils cause easier transmission of diseases.

Performance of entomopathogenic fungi in laboratory conditions cannot be taken as a measure for determining its efficiency in field conditions. Sometimes control of the pest using entomopathogenic fungi in field conditions is a challenging task because of susceptibility of fungal spores to adverse climatic conditions (Inglis *et al.*, 1996). Those spores which can overcome the limitations in field conditions only can survive and infect pests.

In Kerala, prevalence of frequent rainfall and high humidity favours the growth and sporulation of fungi. Therefore, pest management using entomopathogenic fungi has a great future. Evaluation of their field efficacy is a mandate to develop suitable management practices.

The aforesaid aspects necessitated the research entitled "Entomopathogenic fungi for the management of banana rhizome weevil, *Cosmopolites sordidus* Germer" with an objective to evaluate the biocontrol potential of entomopathogenic fungi for the management of banana rhizome weevil.

Review of literature

2. REVIEW OF LITERATURE

Banana covers an area of 61936 ha with a productivity of 8806 kg ha^{-1} in Kerala (FIB, 2017). The borer pests of banana namely pseudostem weevil and the rhizome weevil have been identified as the major constraints in banana production all over the world. Among these rhizome weevil, *Cosmopolites sordidus* (Germer) has been identified to cause severe damage to the banana and has become the most destructive pest of banana. Literature related to biology of the weevil, management of the pest with special reference to entomopathogenic fungi, their pathogenicity, bioassay and field efficacy is reviewed below.

2.1 BANANA RHIZOME WEEVIL (*Cosmopolites sordidus* Germer)

Banana rhizome weevil is a nocturnal insect with 10-15 mm body length and was evolved in Southeast Asia causes economic losses in production. When the attack of the weevil continues for a long period, it can cause 60 per cent reduction in yield (Rukazambuga, 1996). Seshu *et al.* (1998) reported that the yield loss ranged from 40 to 100 per cent when there were severe infestations. The weevil has become a major constraint in the production of banana in east Africa and has emerged as a serious threat to banana cultivation causing 100 per cent yield loss (Akello *et al.,* 2008).

Adult weevils can be observed around the rhizome or in the base of leaf sheath. They are seen gregariously in moist and wet conditions and they remain sedentary for a long period of time unless disturbed. Adults lay oval shaped white eggs singly either on the surface of rhizome or in the base of leaf sheath. Ninety per cent of the eggs are laid in the pseudostem base and the remaining on the rhizome in the cavities made by the female by chewing (Zimmerman, 1968). Weevils mainly preferred flowered plants for oviposition and the egg period was found to be 6-8 days (Abera *et al.*, 1999; Gold and Messiaen, 2000). Egg to adult period under tropical climatic conditions was around 5 to 7 weeks (Shukla, 2010). The grub is having 5 instars which last for 2 to 6 week and it pupates at the end of the tunnel near the surface of the rhizome (Cheraghian, 2015).

2.1.1 Damage and Symptoms of Attack

Adult weevils are seen in the soil around the rhizomes. Grubs of the weevil are the destructive stage of the pest with their strong mandibles, they hollow out the rhizome and there by create numerous tunnels or galleries which may sometimes extend up to the pseudostem through the leaf sheath. Grubs feed inside the rhizome and causes decay of corm which later favors the attack of secondary organisms (Franzmann, 1972).

According to Shillingford (1988) root initiation of the plants also hampered due to the feeding of the grubs during early stages of plant growth. The peripheral damage caused by the grubs is mere when compared to the overall reduced plant performance. Damage inside the plant caused reduced nutrient transport and growth (Taylor, 1991). According to Gold *et al.* (2003) weevils develop inside the rhizome of plant and found destroying the vascular system which later caused death of the plant.

Above ground symptom of infestations are dull yellowish green and floppy foliage and quick withering (Zimmerman, 1968). Extensive tunneling caused reduced nutrient uptake by the plant, bunch filling may get reduced and leaf senescent prematurely (Rukazambuga *et al.*, 1996). Attack of the weevil also interfere the root growth and it also causes susceptibity to other pest and diseases (Gold and Messiaen, 2000). In newly established farms, plant stability gets weakened and crop failures occurs due to the feeding by the grubs while in established farms, reduction in bunch weight was observed (Gold *et al.*, 2001). In thoroughly riddled rhizomes, rotting occurred due to fungal attack and the entire rhizome found converted into a black mass of tissue (Cheraghian, 2015).

2.2 MICROBIAL CONTROL OF *C. sordidus*

Entomopathogenic fungi *viz, Beauveria bassiana* Vuill (Balsamo) and *Metarhizium anisopliae* (Metchnikoff) Sorokin, entomopathogenic nematodes (*Heterorhabditis* sp. and *Steinernema* sp.) and some endophytes such as

Fusarium sp. were found effective against banana rhizome weevil. Role of entomopathogenic bacteria in the management of *C. sordidus* is seldom explored by the researchers. Adult weevils can be killed by microbes such as entomopathogenic fungi and the nematodes whereas other stages can be controlled by endophytes (Gold, 2001).

2.2.1 Entomopathogenic Fungi

Management of banana rhizome weevil using entomopathogenic fungi has gained momentum since 1970s. Management of banana rhizome weevil using biological methods are becoming popularized nowadays because of the severe environmental problem of chemical management methods and high labour cost of cultural management methods (Godonou *et al.*, 2000). Fancelli *et al.* (2013) reported that since the reduced use of pesticides started protecting the environment and human health, biological control of pests such as use of entomopathogenic fungi can be considered as a convenient tool of pest management.

Mechanism of action of the fungi on insect body follows a series of processes. Ferron (1981) reported that entomopathogenic fungi can kill the insect by growing, multiplying and persisting in their body. Spores of these fungi when come in contact with the insect cuticle, start germinating after successful establishment with insect body. When the fungus enters inside the cuticle, it invades the body and the circulatory system of the insect (Samson *et al.*, 1988). Death of the host occurred due to the invasion of the pathogen, nutrient depletion and the toxin production (Navon and Ascher, 2005).

2.2.1.1. Beauveria bassiana

B. bassiana is one of the major organisms on which insect pathological research has been carried out (Steinhaus, 1963). It is also the superior fungus for managing the pests effectively than other entomopathogens (Verma *et al.*, 1988). Most of the economically important agricultural pests can be suppressed using

B. bassiana, the emerging biocontrol agent (Feng *et al.*, 1994; Coates *et al.*, 2002; Araujo *et al.*, 2009). Akello *et al.* (2009) stated that *B. bassiana* can be used as an ecofriendly measure for managing the pests since they are safe to the environment and non target organisms.

2.2.1.1.1 Pathogenicity of B. bassiana

Kaaya *et al* (1992) reported that local isolates of *B. bassiana* and one exotic isolate (268-86) were found pathogenic to the third instar grubs of banana rhizome weevil. Nankinga (1994) reported 90 per cent mortality of *C. sordidus* when treated with *B. bassiana* under laboratory conditions. Pena *et al.* (1995) reported that under natural conditions, *B. bassiana* was found to be one of the primary factor responsible for the mortality of *C. sordidus* in southern Florida.

Fancelli *et al.* (2013) reported that three isolates of *B. bassiana,* CNPMF 407, CNPMF 218 and CNPMF 416 were found to be highly effective against *C. sordidus* under laboratory conditions. Isolates of *B. bassiana* were found to be pathogenic to the adult weevils of *C. sordidus* when the weevils were dipped in spore suspensions of the fungi for 11 seconds and observed for mycosis after giving fresh feed (Omukoko *et al.*, 2014).

B. bassiana was also found pathogenic to *Oryctes rhinoceros* L. (Latch, 1976). Keller *et al.* (1986) reported that *B. brongniartii* can be used for controlling the European cockchafer, *Melolontha melolontha* L. It was found to be pathogenic to Colorado potato beetle (Anderson *et al.*, 1988) and coffee berry borer (Varela and Morales, 1996).

Zimmerman (1992) reported that white grubs were effectively controlled by applying *B. brongniartii* in Europe. According to Moina *et al.* (1998) rice weevil (*Sitophilus oryzae* L.) infestation can be effectively suppressed by application of *B. bassiana*.

Pathogenicity of *B. bassiana* was reported in banana pseudosem weevil, *Odoiporus longicollis* (Olivier) (Anitha *et al.,* 1999; Padmanabhan *et al.* (2001). One of the important coleopteran storage pests saw toothed grain beetle, *Oryzaephilus surinamensis* L. can be controlled with *B. bassiana* and it can cause 100 per cent mortality (Wakefield , 2005). Natural infestation of *B. bassiana* was reported by Agullo *et al.* (2010) on red palm weevil, *Rhynchophorus. ferrugineus* (Olivier).

2.2.1.1.2 Symptoms of Infection

Jiji *et al.* (2008) reported that spotted beetle, *Henosepilachna viginctioctopunctata* Fabricius when treated with *B. bassiana*, the grubs showed reduced feeding and the spines became brown which later changed to black. In the case of adults, brightness of the elytra was lost. Later, the body got covered by white cottony mycelia.

Grubs of *C. sordidus* when treated with *B. bassiana,* became shrunken and dull spots developed intersegmentally, dark color appeared all over the body and cottony mycelia covered the whole body after two days of incubation. Elytra of the infected adults lost their luster after death and after two days in moist chamber, the whitish mycelia started developing from the neck region (Anis, 2014).

2.2.1.1.3 Bioassay

Kaaya *et al.* (1992) reported that the three local isolates of *B. bassiana* (Isolate *I, II, III*) along with one exotic isolate (268-86) caused 98-100 per cent mortality after nine days of treatment with dry fungal spores and their LT_{50} values were 3.5, 3.3, 3.6 and 4 days respectively . In the case of adults, same isolates caused 63-97 per cent mortality in 35 days of treatment and the LT_{50} were 17.5, 12.5, 8.0, and 22 days respectively.

Nankinga (1999) reported that, in laboratory assays, *B. bassiana* isolates caused more than 90 per cent of mortality of *C. sordidus* in two weeks. *C. sordidus* when treated with *B. bassiana* recorded an LC_{50} of 4.5×10^7 spores mL^{-1}(Khan and Gangaprasad, 2001). In 14 days 50-100 per cent mortality of adults of *C. sordidus* was recorded by Magara *et al.* (2004) when treated with *B. bassiana*. Omukoko *et al.* (2014) has conducted an experiment in which they dipped 20 adult weevils of *C. sordidus* in a conidial suspension of isolates of *B. basiana viz,* ICIPE 603, ICIPE 289, ICIPE 50, ICIPE 284, ICIPE, 283, ICIPE 647, ICIPE 279 at a concentration of 1×10^8 spores mL^{-1} under laboratory conditions caused mortality of 20-51 per cent after 40 days of treatment.

Sweet potato weevil, *Cylas formicarius* F. grubs showed a mortality of 100 per cent when treated with *B. bassiana* as reported by Diaz *et al* (1986). According to Agarwal (1990) rice hispa, *Dicladispa armigera* Oliv. has got high mortality when treated with *B. bassiana* @ 10^7 to 10^8 spores mL^{-1}. At a concentration of 1.6×10^4 conidia mL^{-1}, *B. bassiana* caused a mortality of more than 80 per cent on *Cylas formicarius* as observed by Su (1991a and 1991b).

Selvasundaram and Muraleedharan (2000) reported 100 per cent mortality of tea shot hole borer when sprayed with *B. bassiana* @ 10^7 and 10^8 spores mL^{-1}. Varghese *et al.* (2003) conducted a study in mango stone weevil and reported infection of *B. bassiana* and within seven days it caused 100 per cent mortality @1.3×10^9 spores mL^{-1}. Adults of *Metamasius spinolae* (Gylh.), cactus weevil were susceptible to *B. bassiana* at a concentration of 1×10^8 conidia mL^{-1} (Tafoya *et al.*, 2004). After 20 days of treatment with *B. bassiana,* cardamom root grub *Basilepta fulvicorne* Jocobsy showed a mortality of 65-75 per cent (Varadarasan *et al.*, 2006). *B. bassiana* infection on *H. viginctioctopunctata* grubs caused 63.33 per cent mortality (Jiji *et al.*, 2008).

2.2.1.1.4 Field Efficacy

Akello *et al.* (2008) conducted a field study and reported that grub of *C. sordidus* when introduced into the roots of plants dipped in *B. bassiana*

suspension containing 1.5×10^7 spores mL^{-1} recorded 23.50-88.90 per cent mycosis and a reduction in damage of 42.00-86.70 per cent in plants due to colonization of endophytic *B. bassiana* on plant roots.

An infectivity rate of 48 per cent and 20 per cent was observed in *C. sordidus* when *B. bassiana* was applied as conidial powder applied with corn bran around the plants at one and five months after application respectively (Nankinga, 1999). Godonou (1999) reported that *B. bassiana* (IMI331094) when applied as oil palm cake-based formulation of conidia (OPKC-C) and as conidial powder (CP) applied after release of weevil in the planting holes and suckers caused 75 per cent mortality whereas the untreated control gave one per cent mortality on 60 days after treatment. Plants treated with OPKC-C were healthy without the damage, whereas 17 per cent and 19 per cent of plants treated with CP and the control plants respectively were killed. They were also reported the possibility of movement of infected weevil to transmit the pathogen to the healthy weevil up to 18 m from their point of release.

Schoeman and Botha (2003) reported that under field conditions, *B. bassiana* infected adults of *C. sordidus* could be observed from the control group which were not treated with the fungus. They illustrated that this was because of the transmission of the pathogen from the infected weevil to the healthy one due to the migration of the weevils between the plots. They also explained that the subsequent application of the fungus can build up an inoculum in soil and can spectacularly control the weevil.

A cost effective strategy for managing banana rhizome weevil was experimentally developed by integrating the pheromone trapping and *B. bassiana* application and obtained 14.20 per cent mortality of weevils but only 7.20 per cent in pheromone trap (Tinzaara *et al.*, 2004; Tinzaara, 2005). Among the biotic and abiotic factors, soil temperature was the one which had significant impact on the pathogenicity of *B. bassiana* against *C. sordidus*. The application rates and intervals were also examined for their efficacy and found that 100 g of cracked

maize formulation of *B. bassiana* applied during 90 days interval per mat was found to help in cost effective management of the pest (Nankinga and Moore, 2000).

B. bassiana was found to colonize the roots of banana plants even after 4 months of application when the treatments were applied by dipping the rhizome along with the roots in conidial suspension. The presence of *B. bassiana* was identified by reisolating the fungus from the roots and rhizomes of inoculated plants. Colonization was found to be more in the roots (91.5 per cent) compared to rhizome (75.6 per cent) and pseudostem base (58.9 per cent) (Akello *et al.*, 2008).

Akello *et al.* (2009) also observed that *B. bassiana* can colonize the tissues of plants when the roots were dipped in spore suspension before planting and they concluded that the increasing dose of the fungus will not cause any impact on growth of the plant. Plants treated with *B. bassiana* showed less than 50 per cent damage with a mortality of 24-89 per cent in the case of grubs of *C. sordidus*. Under field conditions, isolate of *B. bassiana* (CNPMF 218) was found to cause 20 per cent mortality of adults of *C. sordidus* and when the population size was assessed using pseudostem traps, it has been observed that within 12 months, the population size of the weevil was reduced by 40 per cent (Fancelli *et al.*, 2013). Anis (2014) reported that talc based formulation of *B. bassiana* @ 30g L^{-1} when applied in the succeeding crop as soil drenching was effective with least number of tunnels (0.63) and (0.29) grubs of *C. sordidus* which was superior compared to other treatments.

According to Karthikeyan and Jacob (2010), under field conditions, *B. bassiana* was found to be equally effective as neem oil and azadirachtin 1 % (Econeem) for the management of rice blue beetle.

2.2.1.2. Metarhizium anisopliae

The fungus is known as green muscardine fungus and was first time isolated from coackchafer beetle by Metschinikoff (1879). More than 200 species of agricultural insect pests were found as hosts for *M. anisopliae* (St. Leger, 1993). *M. anisopliae* can be used as an effective management tool against many important insect pests and can be incorporated in biological control of pests (Murad *et al.*, 2006).

2.2.1.2.1 Pathogenicity of M. anisopliae

M. anisopliae recorded 98 per cent mortality of grubs of *C. sordidus* in nine days after treatment under laboratory conditions (Lopes *et al.*, 2013). Anis (2014) conducted a study and proved the pathogenicity of *M. anisopliae* on grubs and adults of *C. sordidus*.

Zacharuk and Tinline (1968) reported that *M. anisopliae* can be used for controlling the soil inhabiting elaterids (Coleoptera). Larval stage of *Oryctes rhinoceros* F. survives in moist habitat which helps in the development and infection of *M. anisopliae* by the grubs easily. *M. anisopliae* has been widely used for the control of *O. rhinoceros* (Pillai, 1987). Butt *et al.* (1992) has tested six different isolates of *M. anisopliae* for their pathogenicity against mustard beetle, *Phaedon cochleariae* Fabricius and the cabbage stem flea beetle *Psylliodes chrysocephala* L. and they observed that, among them five isolates were pathogenic to both the pests.

According to Yip *et al.* (1992) among the 184 isolates from the soils of Tasmanian pasture, 50 per cent were found to be pathogenic to the grub of the scarab, *Adoryphorus couloni* (Burmiestor). According to Varadarasan *et al.* (1993) *B. fulvicorne* adults has got infection of *M. anisopliae* naturally and was also pathogenic to its grubs.

Ekesi *et al.* (2001) reported that five different isolates of *M. anisopliae* was found to be pathogenic to the groundnut bruchid, *Caryeidon serratus* (Olivier). *M. anisopliae* was found to be pathogenic to pseudostem weevil, *O. longicollis* (Yue *et al.*, 2003). Natural infestation of *M. anisopliae* was reported by Sujatha and Rao (2004) on *O. rhinoceros* grubs. Lesser grain borer, *Rhizopertha dominica* Fabricius infestation was found to be significantly reduced in the treatment with formulation of *M. anisoplae* in invert emulsion (0.7 per cent) and wheat flour (1.0 per cent), whereas control treatment recorded 19.0-23.3 per cent infestation in *Cicer arietinumm* L. grains (Batta, 2005).

The serious pest of coffee, *viz.* coffee berry borer *Hypothenemus hampei* Ferrari could be effectively managed by the application of *B. bassiana* (Ripoll *et al.*, 2008). Swaminathan *et al.* (2010) reported that formulation of *M. anisopliae* at a concentration of 5×10^{12} conidia effectively managed *H. viginctioctopunctata.*

2.2.1.2.2 Symptoms of Infection

M. anisopliae infected grubs of *O. rhinoceros* started developing whitish mycelia initiating from the joints and later the color changed to greenish, covered all over the body (CPCRI, 1999). *M. anisopliae* treated grubs and adults of *O. longicollis* started sluggish movement and the white mycelia later changed to greenish color (Anitha, 2000).

2.2.1.2.3 Bioassay

Dry fungal spores of *M. anisopliae* when treated on third instar grubs of *C. sordidus* caused 98-100 per cent mortality after nine days of treatment with LT_{50} of 4.2 days and failed to kill 50 per cent of the adults even after 35 days of treatment (Kaaya *et al.*, 1992).

Anitha (2000) reported that *M. anisopliae* when treated on early instar grubs of *O. longicollis* @ 10×10^5 spores mL^{-1} caused 98.67 per cent mortality and on late instar grubs recorded 97. 33 per cent mortality @ 15×10^5 spores mL^{-1}.

Leaf axil appication of *M. anisopliae* on @ 15×10^{15} conidia mL^{-1} could curatively manage the pest.

Charcoal and oven ash formulations of *M. anisopliae* when applied before and after the infestation of *Sitophilus oryzae* L. at 2.0 per cent or 2.8 mg cm^{-2} caused a mortality of 73.3–86.7 per cent after seven days (Batta, 2004). Hundred per cent mortality of larvae of red palm weevil, *R. ferrugineus* was reported in 6-7 days when treated with *M. anisopliae*. The hatchability of eggs was also found to be reduced with increased mortality (Gindin *et al.*, 2006). A mortality of 62.5-89.2 per cent was reported in sweet potato weevil, *Cylas puncticollis* (Boheman) when treated with *M. anisopliae* @ 1.7×10^{7} conidia mL^{-1} (Ondiaka *et al.*, 2008).

2.2.1.3 Purpureocillium lilacinum (Thom) Samson.

The entomopathogenic fungus, *P. lilacinum* formerly known as *Paecilomyces lilacinus* is widely used to manage the nematodes (Dube and Smart, 1987; Mendoza *et al.*, 2007; Hererro, 2016).

Isolate CG117 of *P. lilacinum* was found to be pathogenic to eggs of cucurbit beetle, *Diabrotica speciosa* Germer (Coleoptera: Curculionidae) causing 40 per cent infection on eggs (Tiganomilani *et al.*, 1995).

P. lilacinus (P251) was found to be pathogenic to the maize weevil (*Sitophilus zeamais* Motsch) which caused significant mortality compared to the control treatment (Ahmed, 2007). According to Barra *et al.* (2013), stored pests of maize such as Darkilng beetle, *Tribolium confusum* Jacquelin du Val, *R. dominica*, and *Sitophilus zeamais* Motschulsky can be controlled by using *P. lilacinum* in maize.

Two strains of *P. lilacinus viz.* JQ926223 and JQ926212 were found to be pathogenic to *Tribolium confusum* and their virulence was found to be increased when grown in media containing hydrocarbons (Barra *et al.*, 2015).

2.2.1.4 Fusarium sp

Around 13 species of *Fusarium* were observed to infect the insects especially in the order Lepidoptera, Diptera and Coleoptera (Humber, 1992).

Fusarium solani (Mart.) Sacc. can cause the mortality of whitefly, *Bemisia tabaci* (Gennadius) which is greater than that caused due to its predators or parasites (Venugopal *et al.*, 1989). Gupta *et al.* (1991) reported that the toxin Beauvericin produced by *Fusarium* sp can cause 50 per cent mortality of colorado potato beetle, *Leptinotarsa decemlineata* (Say).

F. solani treated grubs of *O. longicollis* developed dark brown coloration and white mycelia developed all over the body after death. After eight days of treatment, *F. solani* recorded 93 per cent mortality @ 2×10^{5} spores mL^{-1} with an LC_{50} of 0.505×10^{5} spores mL^{-1} (Anitha, 2000).

F. solani was found to be naturally associated with pupae of sugarbeet maggot *Tetranops myopaeformis* (Roder) as an entomopathogen in North Dakota (Majumdar *et al.*, 2007). Shukla (2010) isolated endophytic *Fusarium* strains from banana plants and were found to cause 30-48 per cent mortality of grubs of *C. sordidus.*

2.2.2 Entomopathogenic Bacteria

Pathogenicity of *Serratia marcescens* Bizio, was tested on the third instar grubs and adults of banana rhizome weevil and the LT_{50} was found to be 2.8 days but was found ineffective against the adult weevils even the concentration treated was increased 10 times that applied on grubs (Kaaya *et al.,* 1992).

Martin *et al.* (2004) reported that the *Photorhabdus luminescens* Thomas and *Chromobacterium* sp were found to be pathogenic against second instar larvae of Colorado potato beetle *Leptinotarsa decemlineata* (Say.)

2.2.3 Entomopathogenic Nematodes

Field trials conducted using *Steinernema carpocapsae* (Weiser) by releasing the nematodes through the holes made on the rhizome resulted in significant mortality of *C. sordidus* grubs (Treverrow *et al.*, 1991). Pseudostems of banana plants baited with *S. carpocapsae* by spraying a concentration of 5×10^6 nematodes m^{-2} in 0.4 L water on pseudostem caused 70 per cent mortality of adult weevils of *C. sordidus* at seven days after treatment (Schmitt *et al.,* 1992).

S. carpocapsae was found to be effective to manage the adult *C. sordidus* when 250000 infective juveniles of the nematode were released into the rhizomes of the plants which act as bait for the weevils (Treverrow and Bedding, 1993).

Remya, (2007) conducted a study in which the potential of indigenous strains of entomopathogenic nematodes were evaluated for the control of weevil pests of banana. The results revealed that the native strains of EPN were found to be more virulent than the standard strains. A minimum of 200 IJ (Infective Juveniles) per insect was required for the mortality of adults and grubs of rhizome weevil.

A study conducted in Tanzania by Mwaitulo *et al.* (2011) reported natural occurence of entomopathogenic nematodes in banana plantations which were further tested for their virulence and found that they were more effective against the larvae than the adults in which they failed to cause infection.

2.3 CULTURAL CONTROL

Gold *et al.* (1998) reported that crop sanitation and use of clean planting material, improved the plant vigor and tolerance to pest attack. Destroying the feeding and sheltering places of the weevil could control them up to an extent. The left over pieces of pseudostems of banana from which bunches were harvested could be chopped after cutting at ground level and scattered in the field which makes them dry and degrade as soon as possible (Gonodou, 1999). Gold *et*

al. (2001) reported that use of healthy planting material, use of pheromone traps and good crop husbandry helped in reducing the weevil population. According to Masanza *et al.* (2005), increased crop sanitation caused reduction of population of *C. sordidus* adults, reduced corm damage and increased yield.

2.4 MECHANICAL METHODS

Hot water treatment of the peeled rhizomes of suckers at 54°C for 10 minutes was found to be effective in managing the weevil (Stover and Simmonds, 1987). More than 99 per cent mortality of eggs and grubs were observed by Gettman *et al.* (1992) when they placed the rhizomes in a water bath of 43°C for 3 h before planting.

According to Alpizar and Falias (1997) pheromone trapping gave reasonable long lasting effective control of the weevil. Weevil control along with the nematode attack is reduced by the paring of the rhizomes and removal of outer skin which could kill the eggs and nematodes associated with the outer sheath (Gold *et al.*, 1998). Tinzaara *et al*, (2000) reported that the pitfall-cosmolure traps were found to be effective to trap 18 times more weevils than the pseudostem traps.

2.5 CHEMICAL CONTROL

Newer pesticide, bifenthrin 0.25 g a.i plant^{-1} was found to be effective by Smith (1995) but the most effective chemical against the weevil was found to be prothiophos 5 g a. i plant^{-1}. Application of carbofuran granules 20g plant^{-1} on 3rd, 5th and 7th month after the planting can be followed for the management of *C. sordidus* (Reghunadhan, 2002).

Chlorpyrifos 20 EC @ 2.5 ml L^{-1} applied around the base of plant and spraying on pseudostem after the infestation of weevil can be done. Followed by this, drenching of malathion 50 EC @ 2 ml L^{-1} was found effective in managing rhizome weevil. Before planting, dipping of the suckers in carbaryl 50 WP 0.4 per cent (50WP) for 30 minutes can control the weevils effectively (Shinde *et al.*, 2015).

2.6 OTHER METHODS

In banana plantations, beneficial pathogens, predators and parasitoids occur naturally which can control the weevil up to some extent. Myrmicine ants , *Pheidole megacephala* F. were allowed to nest in pseudostems and these pseudostems were then transferred to the banana plantations, there by the ants were propogated and disseminated in the plantation. This could control the banana weevil population in the field (Roche and Abreu, 1982). The myrmicine ants, and *Tetramorium guineese* (Bernard) was identified as the predators of *C. sordidus* in Cuba. They could potentially attack the eggs and grubs of *C. sordidus* in field conditions (Castenerias and Ponce, 1991). Weevil multiplication reduction of 20.5, 28 and 44 per cent was caused due to egg predation by *Eutochia pulla* (Erichs.) (Coleoptera: Tenebrionidae), *Euborellia annulipes* (Luc.) (Dermaptera: Carcinophoridae) and *Dactylosternam abdominale* (F.) (Coleoptera: Hydrophilidae) respectively (Kopperhofer, 1993).

A better control of *C. sordidus* was achieved when the powdered neem seed @ 60-100 g was applied around the plant bases at an interval of 4 months. Weevils laid 3-10 times less eggs in the treatment with neem-treated suckers and hatchability of eggs were only 30 per cent (Musabyimana *et al.,* 2000).

Materials and methods

3. MATERIALS AND METHODS

Experiments were conducted to study the pathogenicity of entomopathogenic fungi against banana rhizome weevil, *C. sordidus* under laboratory condition. Effective concentration of the fungi was fixed and its field performance was evaluated by conducting pot culture experiment at Instructional Farm, College of Agriculture, Vellayani during 2015 to 2017.

3.1 ISOLATION OF ENTOMOPATHOGENIC FUNGI

3.1.1 Collection of Adults and Grubs of *C. sordidus*

Adults and grubs of *C. sordidus* were collected from farmer's fields of Thiruvananthapuram district and also from College of Agriculture, Vellayani. Pseudostem traps of 10-15cm pseudostem splits were placed in different parts of banana fields, near to the rhizome region of the plant. The traps were observed daily and weevils were collected.

Adults and grubs were also collected from the decaying rhizomes of uprooted banana plants. Grubs were collected by cutting the rhizomes without damaging the grubs. Collected weevils and grubs were maintained in rhizomes inside rearing jars of 25×15 cm size. The jars were covered with muslin cloth.

3.1.2 Isolation

The collected insects were observed daily for infection and dead insects were separated. They were kept in a moist chamber made of petridish lined with moistened filter paper. Cadavers were kept for one to two days for the development of mycosis if any.

Cadavers with mycelial growth were surface sterilized using 1 per cent sodium hypochlorite solution followed by three changes of sterile distilled water. Specimens were then wiped using a tissue paper. The whole process was done inside a laminar air flow chamber. Cadavers were then placed in petriplates

containing Potato Dextrose Agar (PDA) media for further develpoment of mycelia. Pure culture was prepared after repeated hyphal tip culturing.

3.1.3 Identification

3.1.3.1 Morphological Identification

The morphological characters were studied by preparing the slide cultures of the fungi from 14 day old culture (Harris, 1986). The slides were viewed under a Motic BA 120 microscope for observing size and shape of the conidia, conidiophore and phialides. The colony characters were studied by growing them in PDA plates. The color and radial growth of the fungi was measured. For measuring the radial growth, PDA plates were inoculated with fungal discs cut out from a 14 days old culture using a cork borer and color of the mycelia was also observed daily.

3.1.3.2 Molecular Identification

Molecular identification was done through ITS (Internal Transcribed Sequencing). The primers used for amplification of the isolate were ITS-1F and ITS-4R. The protocol followed is given below.

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

About 100 mg of the tissue/mycelium was taken and using liquid nitrogen this was homogenized. The powdered tissue was transferred to a microcentrifuge tube. Buffer PL 1 of four hundred microlitres was added and this was vortexed for 1 minute. Into this ten microlitres of RNase A solution was added and inverted to mix. This homogenate was incubated at a temperature of 65°C for 10 minutes. The above lysate was transferred to a Nucleospin filter. Centrifugation was done at 11000 x g for 2 minutes. The filter was discarded after collecting the flow through liquid. Four hundred and fifty microlitres of buffer PC was added to this flow through liquid and mixed well. After transferring the solution to a Nucleospin Plant II column, solution was centrifuged for 1 minute. The flow

through liquid was discarded. Same amount of buffer PW1 was added to the column and centrifugation was done at 11000 x g for 1 minute and flow though liquid was discarded. In the next step about 700 µl of PW2 was added and centrifuged at 11000 x g and flow through liquid was discarded. At last in order to dry the silica membrane, 200 µl of PW2 was added followed by centrifugation at 11000 x g for 2 minutes. This column was transferred to a new 1.7 ml tube. Fifty µl of buffer PE was added to this and was incubated at a temperature of 65°C for 5 minutes. The column was then centrifuged at 11000 x g for 1 minute to elute the DNA. The eluted DNA was stored at 4°C.

3.1.3.2.2. Agarose Gel Electrophoresis for DNA Quality check

Agarose gel electrophoresis was done to check the quality of the DNA isolated. 1µl of 6X gel-loading buffer which was prepared with 0.25% bromophenol blue and 30 per cent sucrose in TE buffer at a pH of 8.0 was added to 5µl of DNA. 0.8 per cent agarose gel was prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg mL^{-1} ethidium bromide and the samples were loaded. At 75V the electrophoresis was performed with electrophoresis buffer 0.5X TBE. This was performed until bromophenol dye front has migrated to the bottom of the gel. UV transilluminator (Genei) was used to visualize the gels. The image was captured under UV light. Gel documentation system (Bio-Rad) was used for capturing the image (Plate 1a).

3.1.3.2.3 PCR Analysis

In a 20 µl reaction volume, the PCR amplification reactions were carried out. The reaction volume contained several compounds named 1X Phire PCR buffer which contains 1.5 mM $MgCl_2$, 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 1 µl of DNA, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg mL^{-1} BSA and 3 per cent DMSO, 0.5M Betaine, 5pM of forward and reverse primers.

Primers used

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

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

N PCR amplification profile

ITS & LSU

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

3.1.2.3.4. Agarose Gel electrophoresis of PCR products

Agarose gels 1.2 per cent were prepared in 0.5X TBE buffer which contains 0.5 µg mL^{-1} ethidium bromide. This gel was used for checking in the PCR products. 5 µl of PCR products were mixed with 1 µl of 6X loading dye. This was loaded to carry out the electrophoresis at 75V power supply. 0.5X TBE was used as electrophoresis buffer. Until the bromophenol blue front had migrated to almost the bottom of the gel, the process was continued for about 1-2 hours. 2-log DNA ladder (NEB) was used as the molecular standard. The

visualization of gel was done with a UV transilluminator (Genei) and the image obtained was captured using a UV light with a Gel documentation system (Bio-Rad) (Plate 1b).

3.1.2.3.5 ExoSAP-IT Treatment

For the removal of unwanted primers and dNTPs from a PCR product mixture and to avoid the interference in downstream applications, ExoSAP-IT (GE Healthcare) which consisted of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP) in a specially formulated buffer.

Two microliters of ExoSAP-IT was mixed with five micro litres of PCR product and incubated. This was incubated at 37°C for 15 minutes and enzyme inactivation was done at 80°C for 15 minutes.

3.1.2.3.6 Sequencing Using BigDye Terminator v3.1

A PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) was used for carrying out the sequencing reaction. By following the manufactures protocol, BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems , USA) was used for sequencing.

The components of a PCR mix were PCR Product (ExoSAP treated) (10-20 ng), Primer (3.2 pM) (either Forward or Reverse), Sequencing Mix (0.28 µl), 5x Reaction buffer (1.86µl), and sterile distilled water which was made up to 10µl.

Several cycles were involved in the sequencing of PCR temperature profile. The 1st cycle at 96°C for 2 minutes which was followed by 30 cycles at 96°C for 30 sec, 50°C for 40 sec and 60°C for 4 minutes for all the primers.

3.1.2.3.7 Post Sequencing PCR clean up

After sequencing, PCR Clean up was performed which has several procedures. Initially e master mix I was prepared using 10µl milli Q and 2 µl

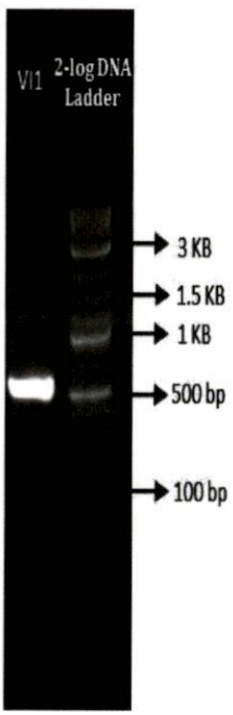


Plate 1a. DNA

Plate 1b. PCR products

Plate 1. Agarose gel electrophoresis profile

125mM EDTA per reaction. Twelve microliters of master mix I was added to each reaction containing 10µl of reaction contents. The contents were properly mixed. Master mix II was prepared with 2 µl of 3M sodium acetate at a pH of 4.6 along with 50 µl of ethanol per reaction. Master mix II (52 µl) was added to each reaction. Contents were mixed thoroughly by inverting. This was incubated at room temperature for 30 minutes and centrifuged at 14,000 rpm for 30 minutes. The supernatant was decanted and 100 µl of 70% ethanol was added followed by centrifugation at 14,000 rpm for 20 minutes. The supernatant was decanted and washed with 70% ethanol. The pellet obtained was air dried after decanting supernatant. The air dried product after cleaning was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.1.2.3.8 Sequence Analysis

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

3.1.2.3.9 Nucleotide Sequence Analysis

Nucleotide sequence was analysed using BLAST (Basic Local Alignment Searching Tool) programme. The obtained nucleotide sequence was given as query sequence. Based on percentage of similarity with the displayed sequences the organism was identified.

3.2 PATHOGENICITY OF ENTOMOPATHOGENIC FUNGI

Adults and third instar grubs of *C. sordidus* were used for the study. Pathogenicity test was conducted with fungi *viz. Fusarium solani, Metarhizium anisopliae* (Ma4), *Beauveria bassiana* (Bb5), *Beauveria bassiana* (ITCC6063), and *Purpureocillium lilacinum* (ITCC6064).

3.2.1 Rearing of *C. sordidus*

Weevils were reared in pieces of banana rhizomes. Vertically cut pieces of rhizomes were placed in rearing jars of 25 cm diameter and 15 cm height (Plate 2). The field collected adult weevils were released into the trays and the trays were covered with muslin cloth. Rhizome pieces were replaced regularly. Reared insects were used the experiment.

3.2.2 Maintenance of Fungal Pathogens

Fungal isolates of *M. anisopliae* (Ma4) and *B. bassiana* (Bb5) were obtained from National Bureau of Agricultural Insect Resources (NBAIR). *P. lilacinum* (ITCC6064) and *B. bassiana* (ITCC6063) were local isolates available in Department of Agricultural Entomology. *F. solani,* was indigenous isolate obtained from present experiment. These fungi were maintained in PDA slants at a temperature of 27 ± 5^{0}C.

3.2.3 Preparation of Spore Suspension

The fungal pathogens were inoculated in Potato Dextrose Broth (PDB) and allowed to sporulate. After 14 days of inoculation, the culture was blended in a mixer for two minutes. The suspension was filtered using a strainer. The strained spore suspension was used for the study.

3.2.4 Estimation of Spore Count

Neubaeur's heamocytometer was used to estimate the spore count. Before taking the spore count, the heamocytometer and the coverslip were cleaned with a tissue paper. Using a sterile pipette, 10 µl of spore suspension was taken and poured into the counting chambers of heamocytometer. In order to avoid air bubbles, the coverslip was placed over the counting chamber carefully. With the help of a compound microscope, the spores in 0.1 mm^3 corner squares were counted under 40X magnification. The following formula was used to calculate the total spore count.

Plate 2. Rearing of *C. sordidus*

Spores mL^{-1} = (n) $\times 10^4$, where n is the mean number of spores in each corner square

3.2.5 Pathogenicity tests

The spore suspension was uniformly sprayed over test insect using an atomiser. The treated insects were given fresh feed. Mortality of the weevils and grubs along with the disease symptoms and the behavioural changes in feeding were observed daily. Pathogenicity was proved by observing the spores under compound microscope.

3.2.6 Bioassay

The effective concentration of fungi against the adults and grubs of *C. sordidus* was determined based on mortality observed at 24 h interval. The spore suspension was prepared as described in 3.2.3. After estimating the spore count, the suspension was serially diluted to lower concentrations (Table 1). Spore suspension was sprayed uniformly over third instar grubs and adults of *C. sordidus*. Four replications were maintained for each dose with ten insects in one replication. Insects sprayed with distilled water were kept as control. Mortality of insects were recorded daily. Fresh rhizomes were provided as feed.

With the help of SPSS software version 21, the log dose probit mortality data was statistically analyzed and the LC_{50}, LC_{90}, LT_{50} values, fiducial limits and other parameters were calculated (Fang *et al.*, 2005). The effective concentration was selected for evaluation of entomopathogens in pot culture study.

Fungi	Concentration used for bioassay (spores mL^{-1})	
	Grubs	Adult
F. solani	1.5×10^{7} 1.5×10^{6} 1.5×10^{5}	2.4×10^{7} 2.4×10^{6} 2.4×10^{5}
M. anisopliae	1.3×10^{8} 1.3×10^{7} 1.3×10^{6} 1.3×10^{5}	2.1×10^{8} 2.1×10^{7} 2.1×10^{6}
B. bassiana (Bb5)	1.2×10^{8} 1.2×10^{7} 1.2×10^{6} 1.2×10^{5} 1.2×10^{4}	2.5×10^{8} 2.5×10^{7} 2.5×10^{6}
B. bassiana (ITCC6063)	1.6×10^{8} 1.6×10^{7} 1.6×10^{6}	2×10^{8} 2×10^{7} 2×10^{6}
P. lilacinum (ITCC6064)	1.9×10^{8} 1.9×10^{7} 1.9×10^{6} 1.9×10^{5} 1.9×10^{4}	2.1×10^{8} 2.1×10^{7} 2.1×10^{6}

Table 1. Details of fungi and doses selected for bioassay

3.3 EVALUATION OF ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF BANANA RHIZOME WEEVIL

Variety Nendran was selected for evaluating the effectiveness of entomopathogenic fungi against banana rhizome weevil. The effective concentrations of different entomopathogens fixed in experiment 3.2.6 was tested in field, with four replication and nine treatments. Five plants were maintained per replication. Treatments applied are given below.

T1: Talc formulation of *M. anisopliae* 10^8 spores mL^{-1}@ 30 g L^{-1}
T2: Talc formulation of *B. bassiana* 10^8 spores mL^{-1} @ 30 g L^{-1}
T3: Talc formulation of *P. lilacinum* 10^8 spores mL^{-1} @ 30 g L^{-1}
T4: Spore suspension of *M. anisopliae* @ 10^8 spores mL^{-1}
T5: Spore suspension of *B. bassiana* @ 10^8 spores mL^{-1}
T6: Spore suspension of *P. lilacinum* @ 10^8 spores mL^{-1}
T7: Chlorpyrifos 0.05%
T8: Talc solution 30 g L^{-1}
T9: Control (untreated check)

3.3.1 Preparation of Talc Formulation

Talc based formulation of the fungi were prepared from 14 day old culture grown in PDB. The culture was blended in a mixer for two minutes and the suspension was strained. Spore concentration was adjusted by enumerating the count as mentioned in 3.2.6. Talc was sterilized in a polypropylene cover and mixed with the spore suspension in 1: 3 proportion. Then allowed to dry for two days. The talc based formulation was stored inside a polythene cover and kept air tight.

3.3.2 Pot Culture Study

For confining the weevils, the growbags used for the study were lined with net inside before filling the potting mixture (Plate 3a) and the top exposed portion

of the growbag was also covered with net (Plate 3b). Suckers were planted in these growbags (Plate 4). Three sets of experiments were conducted.

1. Rhizome dip method (prophylactic)
2. Prophylactic soil drenching
3. Curative soil drenching

3.3.2.1 Rhizome Dip Method

The rhizomes were dipped in two litres of the spore suspension/ talc formulation/ chemical for 30 minutes. These suckers were planted in the growbags lined with net. After the establishment of suckers, three pairs of weevils were released near the rhizome region and were confined inside the growbag. After one month, observations were taken by destructive sampling.

3.3.2.2 Prophylactic Soil Drenching

In this experiment, treatments were applied after establishment of the planted suckers. Two litres of the spore suspensions/ formulations/chemical were drenched in each of the growbags. One day after treatment application, three pairs of weevils were released near the rhizome region. After one month, observations were taken by destructive sampling.

3.3.2.3 Curative Soil Drenching

After the establishment of suckers, three pairs of weevils were released into the growbag near the rhizome region of the suckers and confined the weevils inside the growbag. Two days after the release of weevil, treatments were applied by drenching two litres of the spore suspension/ talc formulation/ chemical around the rhizomes. One month after the treatment, observations were taken by destructive sampling.

Plate 3a. Growbag lined with net

Plate 3b. Top portion of growbag covered with net

Plate 3. Growbag for confining weevils

Plate 4. Experimental plot

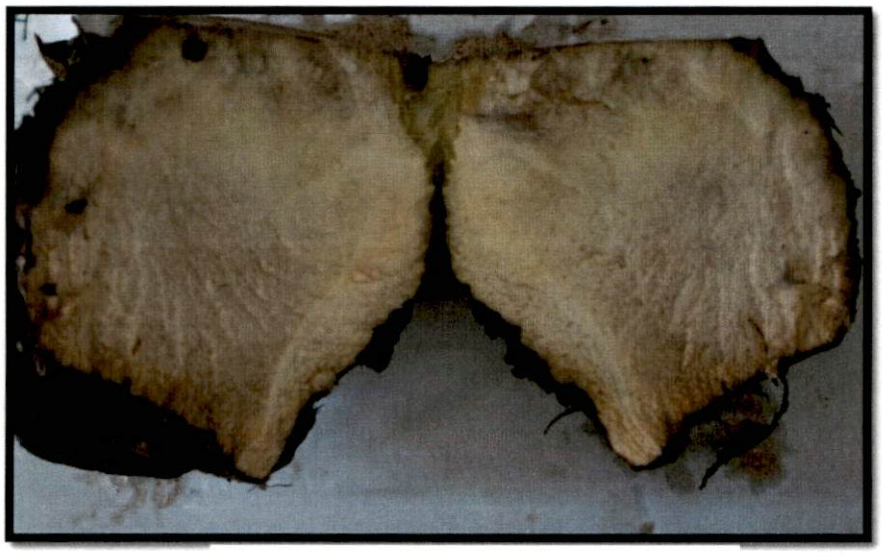
3.3.2.4 Destructive Sampling

After one month, observations were taken by destructive sampling of the suckers. Suckers were uprooted slowly. Soil filled in the growbag and soil adhering the rhizome was observed for dead weevils. Rhizomes were cut horizontally and number of tunnels was recorded. Then the rhizome was made into pieces. The rhizome damage percentage was assessed using modified method of scoring by Vilardebo (1973) (Plate 5). Number of live grubs and pupae were also recorded. Scoring scale followed is given below.

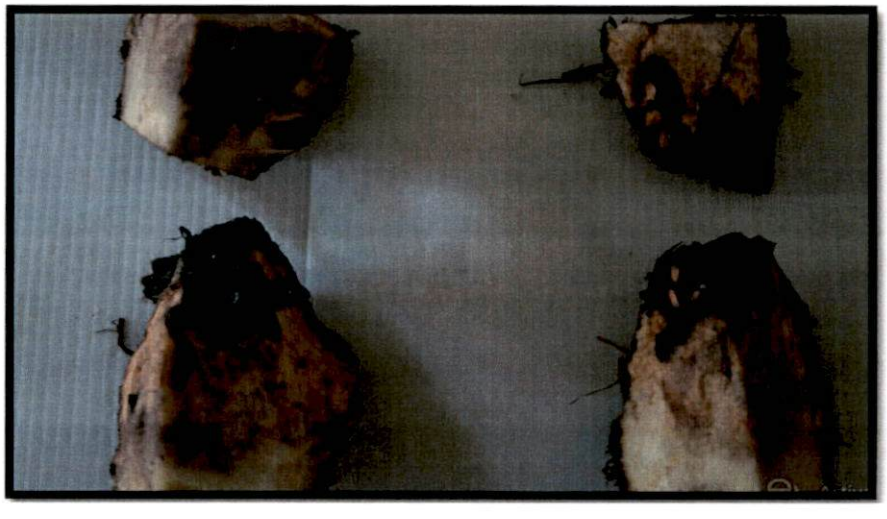
0 - No damage
10- Traces of galleries
20- Galleries on approximately 1/4th of rhizome
30- Galleries on approximately 1/3rd of rhizome
40- Galleries on approximately 1/2nd of rhizome
60- Galleries on approximately 3/4th of rhizome
100-Galleries present on totality of the rhizome

3.4 Statistical analysis

Analysis of the tabulated data was carried out using ANOVA (Analysis of Variance) (Panse and Sukhatme, 1967).



0-No damage



10-Traces of galleries

20- Galleries on approximately 1/4th of rhizome

Plate 5. Scoring scale of rhizome damage

30- Galleries on approximately 1/3rd of rhizome

40- Galleries on approximately 1/2nd of rhizome

60- Galleries on approximately 3/4th of rhizome

100- Galleries present on totality of the rhizome

Results

4. RESULTS

Entomopathogenic fungi associated with banana rhizome weevil *C. sordidus* were isolated and identified. Pathogenicity test and bioassay studies were carried out and the field efficacy was also evaluated. The results of which are detailed below.

4.1 ISOLATION OF ENTOMOPATHOGENIC FUNGI

4.1.1 Isolation

One entomopathogenic fungus, *Fusarium solani* was isolated from the adults of *C. sordidus* which was subjected to morphological and molecular identification.

4.1.2 Identification

4.1.2.1 Morphological Identification

The fungus isolated from the mycosed cadaver of the weevil produced creamy white floccose colonies on the upper surface of the PDA plate (Plate 6a) and cream color on the reverse side (Plate 6b). Within 14 Days After Inoculation (DAI) the fungus attained radial growth of 9 cm. The macroconidia was 3 to 4 septate, fusiform with blunt end having a mean length of 15.91 ± 5.20 µm and width of 4.09 ± 0.93 µm. Ovoid aseptate microconidia were also observed with a mean length of 11.10 ± 2.6µm and width of 3.7 ± 0.93 µm (Plate 7a). The conidia had a length /width ratio of 3.88. The conidiophores were unbranched bearing a monophialide (Plate 7b).

4.1.2.2 Molecular Identification

The details of the 532 base pair (bp) sequences are given below

AGGGATCATTACCGAGTCTAAACAACTCATCAACCCTGTGAACATACC
TAAAACGTTGCTTCGGCGGGAACAGACGGCCCCGTAACACGGGCCGC

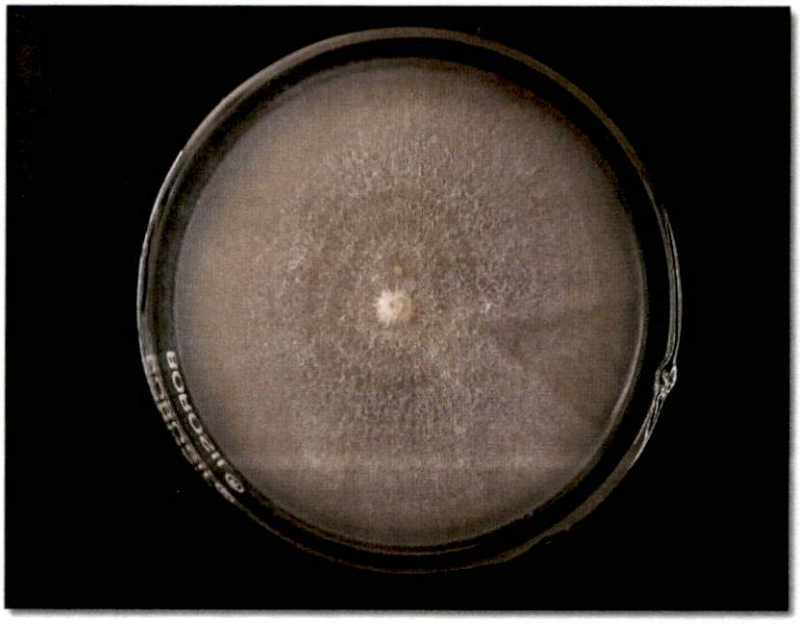


Plate 6a. Upperside of PDA plate

Plate 6b. Reverse side of PDA plate

Plate 6. Growth of *F. solani* in PDA plate

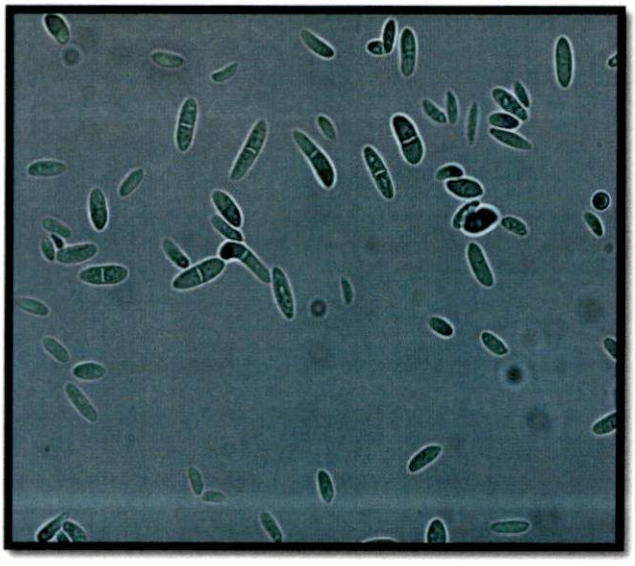


Plate7a. Macroconidia and microconidia of *F. solani*

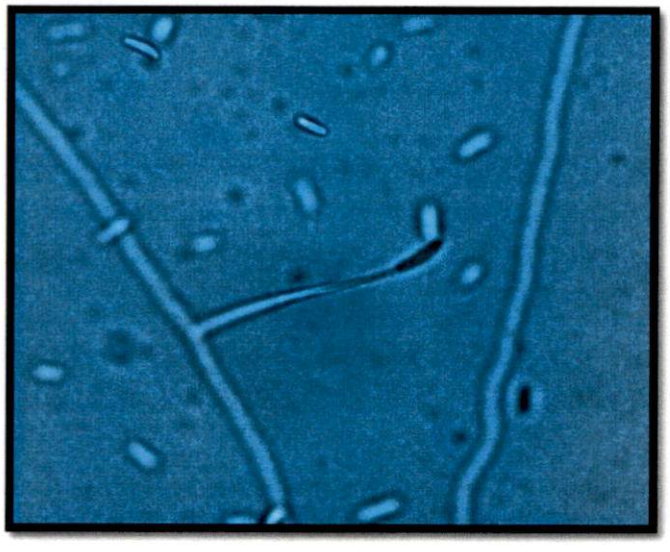


Plate 7b. Monophialide produced from unbranched conidiophore

Plate 7. Morphological characters of *F. solani*

CCCCGCCAGAGGACCCCTAACTCTGTTTATATTATGTTTTTTCTGAGTA
AACAAGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGGCTCTGG
CATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG
AATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTA
TTCTGGCGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCC
GGGCCTGGCGTTGGGGATCGGCGGAAAGCCCCCTGTGGGCATACGCC
GTCCCCTAAATACAGTGGCGGTCCCGCCGCAGCTTCCATTGCGTAGTA
GCTAACACCTCGCAACTGGAGAGCGGCGCGGCCAAGCCGTAAAACCC
CCAACTTCTGAATGTTGACCTCGAATCAGGTAGGAATACCCGCTGAAC
TTAA

The above nucleotide sequence was analysed using NCBI BLAST (Basic Local Alignment Searching Tool) which showed cent per cent similarity with *F. solani.*

4.2 PATHOGENICITY TO BANANA WEEVILS

All the isolates tested *viz.* the indigenous isolate *F. solani,* NBAIR isolates *M. anisopliae* (Ma4) and *B. bassiana* (Bb5), along with isolates maintained in Department of Agricultural Entomology, *B. bassiana* (ITCC6063) and *P. lilacinum* (ITCC6064) were found to be pathogenic to both the adults and grubs of *C. sordidus* when treated with spore suspension of 14 day old culture.

4.2.1 Symptoms of Infection

4.2.1.1 F. solani

Grubs were observed with reduced feeding and movement two days of treatment. Adults did not show any changes in feeding behavior. Mortality was observed two days after treatment. A brownish discoloration developed at the ventral thoracic region and the body was shrunken (Plate 8a). Initially white creamy mycelial growth appeared in the ventral thoracic region and later the

entire body was covered with mycelial growth (Plate 8b). Grubs became hard and mummified (Plate 8c).

Adult weevils were infected after 5 days of treatment and the creamy white growth initiated from the intersegmental regions (Plate 9).

4.2.1.2 B. bassiana (Bb5)

Feeding was found to be reduced after one day after treatment and movement was also reduced. Adult weevils showed no changes in feeding. Inside the tunnel, the third instar grubs of the weevil showed shrinkage of integument and development of pink color after three days of infection (Plate 10a). On fourth day, the pink color changed and whitish mycelia started developing from the posterior part of abdomen intersegmentally (Plate 10b). Slowly the whole body was covered with the white cottony mycelia (Plate 10c).

Infected adult weevils showed white mycelial growth from the intersegmental region after two days of death (Plate 11a). On the ventral part also the mycelia started initiating intersegmentally (Plate 11b). The growth became so extensive that after four days, the mycelia covered all the body regions except the dorsal part of elytra (Plate 11c).

4.2.1.3 B. bassiana (ITCC6063)

Feeding and movement was found to be reduced one day after treatment. There were no marked changes in feeding behavior of adults. Initially pink color was observed after three days of treatment. White cottony mycelia started initiating 4 DAT, which later covered the entire body of the grub (Plate 12a). In the case of adults mortality occurred 3 DAT and the white mycelia started initiating 5 DAT (Plate 12b).

Plate 8a. Grub with brownish discoloration

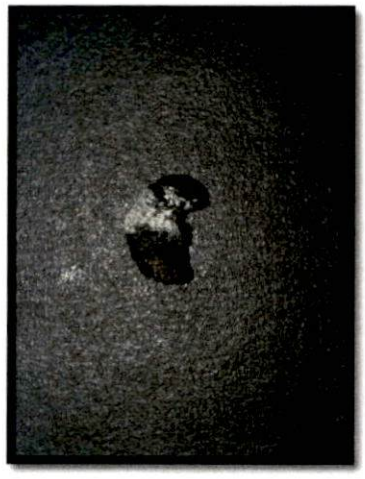


Plate 8b. Grub with creamy white growth

Plate 8c. Grub with hard and mummified body

Plate 8. Symptoms of infection by *F. solani* on grub

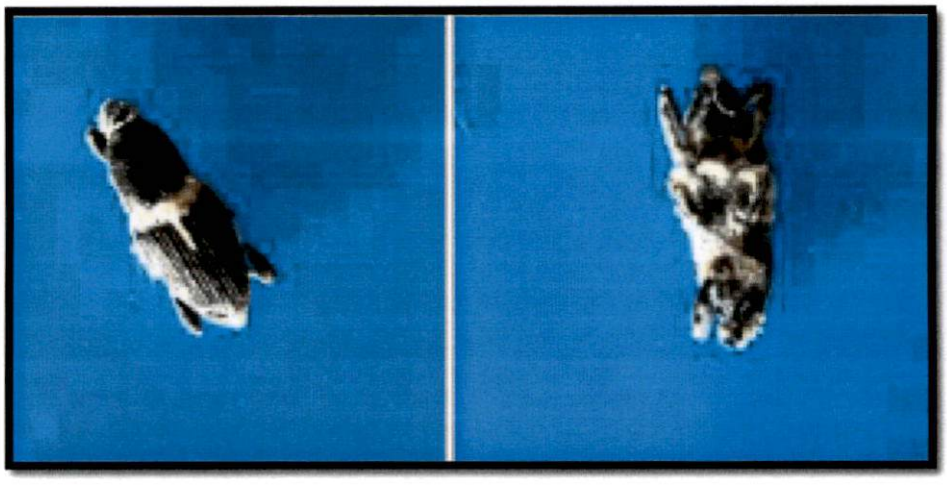


Plate 9. Symptoms of infection by *F. solani* on adults

Plate 10a. Grub with Pink coloration

Plate 10b. Grub with white mycelia initiated from the posterior abdomen

Plate 10c. Grub with white cottony mycelia

Plate 10. Symptoms of infection by *B. bassiana* on grub

Plate 11a. Adult with white mycelia originating intersegmentally

Plate 11b. Ventral part of adult body with mycelial growth

Plate 11c. Adult with extensive mycelia

Plate 12a. Grub with white mycelial growth

Plate 12b. Adult with white mycelial growth

Plate 12. Symptoms of infection by *B. bassiana* (ITCC6063) on grub and adult

4.2.1.4 M. anisopliae

The infected grubs showed reduced feeding and movement. After two days of treatment, grubs remained static within the tunnels inside the rhizome. Adult weevils expressed no changes in feeding behavior. The infected grubs were mummified and started developing whitish mycelial growth two days after treatment. The whitish mycelia covered all over the body except the sclerotised head region (Plate 13a). After two days, greenish growth appeared all over the body and the larvae degraded into greenish powder. (Plate 13b).

The adult weevils infected with the fungus developed white mycelial growth after four days of death. The mycelia developed from the intersegmental regions of the weevil especially from the ventral region of thorax (Plate 14a). The dorsal elytra, legs and the spiracular area were also covered with white mycelia which later changed into green color (Plate 14b). Mycelia were also developed even at the tip of the antennae.

4.2.1.5 P. lilacinum (ITCC6064)

Reduced feeding was observed one day after treatment in the case of grubs. Adults showed no special behavioural changes in feeding. The grubs developed a pink color after one day of infection (Plate 15a) and the next day onwards the whitish mycelia developed from the abdominal region. Later the mycelial growth covered all over the body and was not as much thick as *B. bassiana* (Plate 15b).

Adult weevils produced white mycelial growth in two days. The growth was not thick but extensive on the ventral and dorsal sides of the body (Plate 16a, 16b)

4.2.2 Bioassay

Bioassay was carried out on both adults and grubs of weevil using the different fungi selected for the study.

Plate 13a. Grub with white mycelial growth

Plate 13b. Grubs with greenish growth

Plate 13. Symptoms of infection by *M. anisopliae* on grub

Plate 14a. Adult ventral part with white mycelial growth

Plate 14b. Legs and spiracular area of adult covered with greenish mycelia

Plate 14. Symptoms of infection by *M. anisopliae* on adult

Plate 15a. Grub with pink coloration

Plate 15b. Grub with white mycelial growth

Plate 15. Symptoms of infection by *P. lilacinum* on grub

Plate 16a. Adult with white mycelial growth on dorsal side

Plate 16b. Ventral part of adult with mycelial growth on ventral side

Plate 16. Symptoms of infection by *P. lilacinum* on adult

4.2.2.1 F. solani

4.2.2.1.1 Mortality of C. sordidus Grubs

F. solani, when applied on third instar grubs at different concentrations 1.5×10^7,10^6 and 10^5 spores mL^{-1} recorded no mortality at 24 HAT (Table 2). At 48 HAT, 20 per cent mortality was observed at highest concentration (10^7 spores mL^{-1}) followed by 13.33 per cent at 10^6 spores mL^{-1}. The highest mortality observed during 72 HAT was 26.66 per cent at 10^7 spores mL^{-1}followed by 33.33 per cent at 10^6 spores mL^{-1}. The concentration, 10^5 spores mL^{-1} initiated mortality at 96 HAT and was found to be 20 per cent and constant even at 120 HAT. Grubs treated with 10^6 spores mL^{-1} caused mortality of 40 per cent and was statistically on par with the highest concentration (46.66 per cent). The lowest concentration at 120 HAT caused a mortality of 20 per cent.

4.2.2.1.2 Mortality of C. sordidus Adults

A maximum of 20 per cent mortality was observed when adults of *C. sordidus* was treated with *F. solani* even at highest dose of 10^7 spores mL^{-1} at 360 HAT.

4.2.2.2 B. bassiana (Bb5)

4.2.2.2.1 Mortality of C. sordidus Grubs

The mean percentage mortality of *B. bassiana* at 10^8,10^7,10^6, 10^5, and 10^4 spores mL^{-1} are given in Table 3.

At 10^8 spores mL^{-1}, mortality initiated at 24 HAT in the third instar grubs. It progressively increased to 30 per cent whereas the lower concentrations recorded no mortality till 72 HAT. At 96 HAT, the highest mortality (45 per cent) was observed in grubs treated with spore concentration of 10^8 spores mL^{-1}, while the lower concentrations 10^7 spores mL^{-1} recorded a mean per cent mortality of 40 which were statistically on par.

Table 2. Mortality of *C. sordidus* grubs treated with *F. solani*

	Mortality at 24 h interval (%)				
Concentration (Spores mL^{-1})	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT
1.5×10^{7}	0	20.00	26.66	46.66 $(43.07)^{a}$	46.66 $(43.07)^{a}$
1.5×10^{6}	0	13.33	33.33	40.00 $(38.85)^{ab}$	40.00 $(38.85)^{ab}$
1.5×10^{5}	0	0	0	20.00 $(26.56)^{b}$	20.00 $(26.56)^{b}$
Water spray	0	0	0	0 $(1.28)^{c}$	0 $(1.28)^{c}$
CD(0.05)	-	-	-	13.001	13.001

Figures in parentheses are angular transformed values
HAT- Hours after treatment

Table 3. Mortality of *C. sordidus* grubs treated with *B. bassiana* (Bb5)

	Mortality at 24 h interval (%)								
Concentration (Spores mL^{-1})	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	144 HAT	168 HAT	192 HAT	216HAT
1.2×10^{8}	10.00	25.00	30.00	45.00 (42.11)^{a}	55.00 (47.88)^{a}	75.00 (57.10)^{a}	90.00 (76.07)^{a}	90.00 (76.07)^{a}	95.00 (82.39)^{a}
1.2×10^{7}	0	0	0	40.00 (39.23)^{a}	45.00 (41.88)^{a}	55.00 (47.88)^{b}	70.00 (57.10)^{b}	75.00 (60.26)^{b}	85.00 (69.76)^{b}
1.2×10^{6}	0	0	0	10.00 (13.92)^{b}	20.00 (23.41)^{b}	30.00 (32.89)^{c}	40.00 (39.23)^{c}	45.00 (42.11)^{c}	45.00 (42.11)^{c}
1.2×10^{5}	0	0	0	0 (1.28)^{c}	0 (1.28)^{c}	0 (1.28)^{d}	35.00 (36.06)^{c}	45.00 (42.11)^{c}	50.00 (45.00)^{c}
1.2×10^{4}	0	0	0	0 (1.28)^{c}	0 (1.28)^{c}	0 (1.28)^{d}	0 (1.28)^{d}	20.00 (23.41)^{d}	35.00 (26.5)^{d}
Water spray	0	0	0	0 (1.28)^{c}	0 (1.28)^{c}	0 (1.28)^{d}	0 (1.28)^{d}	0 (1.28)^{d}	0 (1.28)^{e}
CD (0.05)	-	-	-	9.517	12.422	7.183	10.623	10.842	12.087

Figures in parentheses are angular transformed values, HAT-Hours After Treatment

Even after 120 hours of treatment, no mortality was observed for the grubs treated with 10^5 and 10^4 spores mL^{-1}. The spore concentration of 10^8 spores mL^{-1} was superior to other treatments which recorded 55 per cent mortality, while the other treatments caused less than 50 per cent mortality at the same time. The second highest mortality recorded during 120 HAT was 45 per cent which was obtained with spore concentration of 10^7 spores mL^{-1} which was followed by 10^6 spores mL^{-1} (20 per cent).

A mean mortality of 75 per cent was recorded at 144 HAT. Spore concentration of 10^7 spores mL^{-1} showed 55 per cent mortality at 144 HAT, at the same time 10^6 spores mL^{-1} showed 30 per cent mortality. A similar trend was observed with spore concentration of 10^8 spores mL^{-1} also. At 168 HAT, the mean per cent mortality was highest in treatment, 10^8 spores mL^{-1} (90) which was statistically superior compared to other treatments. Second highest mortality was observed in 10^7 spores mL^{-1} which was 70 per cent. The mean per cent mortality obtained at 10^6 spores mL^{-1} (45) and 10^5 spores mL^{-1} (35) were statistically on par.

At 192 HAT, mortality was constant with 10^8 spores mL^{-1} while the mortality increased to 75 per cent at 10^7 spores mL^{-1}. Mortality recorded with 10^6 spores mL^{-1} and 10^5 spores mL^{-1} was 45 per cent each. The highest mortality observed was 95 per cent at 216 HAT with 10^8 spores mL^{-1} followed by 85 per cent mortality caused by 10^7 spores mL^{-1}. Fifty per cent mortality was obtained in grubs treated with 10^5 spores mL^{-1} which was statistically on par with the mortality obtained with 10^6 spores mL^{-1}(45 per cent). At same time interval, the lowest concentration of 10^4 spores mL^{-1} recorded least mortality of 35 per cent.

4.2.2.2.2 Mortality of C. sordidus Adults

B. bassiana when treated on adults at 2.5×10^8,10^7, and 10^6 spores mL^{-1} recorded a mean per cent mortality of 20 in 10^8 spores mL^{-1} 120 HAT (Table 4). At 240 HAT, 10^8 spores mL^{-1} and 10^7 spores mL^{-1}recorded mortality of 25 and 15 per cent respectively. Whereas, 10^6 spores mL^{-1} was not able to cause mortality at

Table 4. Mortality of *C. sordidus* adults treated with *B. bassiana* (Bb5)

	Mortality at 24 h interval (%)			
Concentration (Spores mL^{-1})	120 HAT	240 HAT	360 HAT	480 HAT
2.5×10^{8}	20.00	25.00 $(29.73)^{a}$	35 $(36.06)^{a}$	40 $(38.94)^{a}$
2.5×10^{7}	0	15.00 $(20.24)^{a}$	30.00 $(32.89)^{a}$	30.00 $(29.73)^{b}$
2.5×10^{6}	0	0 $(1.28)^{b}$	0 $(1.28)^{b}$	0 $(1.28)^{b}$
Water spray	0	0 $(1.28)^{b}$	0 $(1.28)^{b}$	0 $(1.28)^{b}$
CD (0.05%)	-	10.890	7.459	9.041

Figures in parentheses are angular transformed values
HAT- Hours After Treatment

240 and 480 HAT. A mean per cent mortality of 35 was observed in the highest concentration (10^8 spores mL^{-1}) and was statistically on par with 10^7 spores mL^{-1}(30) at 360 HAT. At 480 HAT, the maximum mortality recorded by 10^8 spores mL^{-1} was 40 per cent.

4.2.2.3 B. bassiana (ITCC6063)

4.2.2.3.1 Mortality of C. sordidus Grubs

The *B. bassiana* strain ITCC6063 was treated on third instar grubs of weevil at 1.6×10^8,10^7 and 10^6 spores mL^{-1} and the results are presented in Table 5.

A spore concentration of 10^8 spores mL^{-1} caused a mean mortality of 26.66 per cent at 48 HAT and remained constant up to 96 HAT. In the lower concentrations, mortality was not observed even at 48 HAT. The highest mortality observed at 10^8 spores mL^{-1} was 46.66 per cent at 120 HAT which was statistically on par with 10^7 spores mL^{-1} (33.33 per cent).

4.2.2.3.2 Mortality of C. sordidus Adults

Adults of *C. sordidus* when treated with *B. bassiana* (ITCC6063) recorded only 20 per cent mortality at 480 HAT.

4.2.2.4 M. anisopliae (Ma4)

4.2.2.4.1 Mortality of C. sordidus Grubs

Table 6 explains the mean percentage mortality of third instar grubs of *C. sordidus* treated with *M. anisopliae* at 10^8, 10^7, 10^6 and 10^5 spores mL^{-1}

At 24 HAT, a mean mortality of 6.66 per cent was observed at 10^8 spores mL^{-1} and it was 53.33 at 96 HAT, while the concentrations 10^7, 10^6, 10^5 spores mL^{-1} recorded no mortality. For the spore concentration of 10^7 spores mL^{-1}, the mortality observed was 33.33 per cent at 120 HAT. At 120 HAT, a mean mortality of 80 per cent was recorded at 10^8 spores mL^{-1} which was significantly

Table 5. Mortality of *C. sordidus* grubs treated with *B. bassiana* (ITCC6063)

	Mortality at 24 h interval (%)				
Concentration (Spores mL^{-1})	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT
1.6×10^{8}	0	26.66	26.66 (30.78)[a]	26.66 (30.78)[a]	46.66 (43.07)[a]
1.6×10^{7}	0	0	20.00 (26.56)[a]	33.33 (35.00)[a]	33.33 (35.00)[a]
1.6×10^{6}	0	0	0 (1.28)[b]	0 (1.28)[b]	0 (1.28)[b]
Water spray	0	0	0 (1.28)[b]	0 (1.28)[b]	0 (1.28)[b]
CD (0.05)	-	-	9.732	9.732	9.313

Figures in parentheses are angular transformed values
HAT- Hours After Treatment

Table 6. Mortality of *C. sordidus* grubs treated with *M. anisopliae* (Ma4)

	Mortality at 24 h interval (%)										
Concentration (Spores mL^{-1})	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	144 HAT	168 HAT	192 HAT	216 HAT	240 HAT	264 HAT
1.29×10^{8}	6.66	26.66	40.00	53.33	80.00	86.66 (71.87)^{a}	100.00 (88.71)^{a}	100.00 (88.71)^{a}	100.00 (88.71)^{a}	100.00 (88.71)^{a}	100.00 (88.71)^{a}
1.29×10^{7}	0	0	0	0	33.33	33.33 (35.00)^{b}	46.66 (43.07)^{b}	53.33 (47.29)^{b}	80.00 (67.65)^{b}	86.66 (71.87)^{b}	93.33 (80.29)^{a}
1.29×10^{6}	0	0	0	0	0	20.00 (25.66)^{b}	26.66 (30.78)^{bc}	33.33 (35.00)^{bc}	40.00 (39.25)^{c}	40.00 (39.23)^{c}	53.33 (46.93)^{b}
1.29×10^{5}	0	0	0	0	0	0 (1.28)^{c}	13.33 (18.13)^{c}	20.00 (26.56)^{c}	26.66 (30.78)^{c}	26.66 (30.78)^{c}	26.66 (30.78)^{c}
Water spray	0	0	0	0	0	0 (1.28)^{c}	0 (1.28)^{d}	0 (1.28)^{d}	0 (1.28)^{d}	0 (1.28)^{d}	0 (1.28)^{d}
CD (0.05)	-	-	-	-	-	13.290	14.346	12.831	16.802	13.288	14.346

Figures in parentheses are angular transformed values, HAT-Hours After Treatment

superior to the other treatments. In other treatments, no mortality was observed till 120 HAT. In the grubs treated with spore concentration of 10^6 spores mL^{-1} there was no mortality till 144 HAT. A mean per cent mortality of 20 was observed at 10^6 spores mL^{-1} which was statistically on par with 10^7 spores mL^{-1} (33.33 per cent).

Percentage mortality increased progressively at 168 HAT. Concentration of 10^8 spores mL^{-1} caused hundred per cent mortality, while other treatments showed significantly lower mortality. Mortality started at 168 HAT with 10^5 spores mL^{-1} (13.33 per cent) which was statistically on par with 10^6 spores mL^{-1} (26.66 per cent).

A mean mortality of 53.33 per cent was observed at 192 HAT for the grubs treated with 10^7 spores mL^{-1}. The mortality caused by 10^6 spores mL^{-1} (33.33 per cent) was statistically on par with the mortality caused by 10^7 spores mL^{-1} (53.33 per cent) and 10^5 spores mL^{-1} (20 per cent).

The mortality increased to 80 per cent at 216 HAT, in the case of 10^7spores mL^{-1} which was followed by 10^6 spores mL^{-1} (40 per cent). At 216 HAT, least mortality was observed with 10^5 spores mL^{-1} which was constant even at 264 HAT.

At 264 HAT, mean mortality of 93.33 per cent was observed with spore concentration of 10^7 spores mL^{-1} which was statistically superior to the 10^6 spores mL^{-1} and 10^5spores mL^{-1} but statistically on par with the mortality caused by 10^8 spores mL^{-1}. Spore concentrations 10^6 spores mL^{-1} and 10^5 spores mL^{-1} recorded a mean mortality of 53.33 per cent and 26.66 per cent respectively at 264 HAT.

4.2.2.4.2 Mortality of C. sordidus Adults

When the adults were treated with *M. anisopliae*, at 2.1×10^8 spores mL^{-1}, mortality observed was 25 per cent at 120 HAT (Table 7). The other lower

Table 7. Mortality of *C. sordidus* adults treated with *M. anisopliae* (Ma4)

	Mortality at 24 h interval (%)			
Concentration (Spores mL^{-1})	120 HAT	240 HAT	360 HAT	480 HAT
2.1×10^{8}	25.00	30.00	35.00 (36.06)[a]	35.00 (36.06)[a]
2.1×10^{7}	0	25.00	30.00 (32.89)[a]	30.00 (32.89)[a]
2.1×10^{6}	0	0	15.00 (20.24)[b]	20.00 (23.42)[a]
Water spray	0	0	0 (1.28)[b]	0 (1.28)[b]
CD (0.05)	-	-	7.459	14.279

Figures in parentheses are angular transformed values
HAT- Hours After Treatment

concentrations did not cause mortality even at the end of 120 HAT. At 240 HAT, a mean per cent mortality of 30 was recorded by 10^8 spores mL^{-1} followed by 10^7 spores mL^{-1}(25 per cent). The highest mortality of 35 per cent was recorded at 360 HAT (10^8 spores mL^{-1}) and the lowest mortality was 15 per cent caused by 10^6 spores mL^{-1}. The mortality caused by 10^8 spores mL^{-1}(35 per cent) and 10^7 spores mL^{-1} (30 per cent) were statistically on par.

4.2.2.5 P. lilacinum (ITCC6064)

4.2.2.5.1 Mortality of C. sordidus Grubs

The mean mortality caused by different concentrations, 10^8, 10^7, 10^6, 10^5 and 10^4 in the third instar grubs of *C. sordidus* are given in Table 8.

Mortality initiated at 48 HAT, in the grubs treated with 10^8 spores mL^{-1} (15 per cent) and 10^7 spores mL^{-1} (20 per cent). At 72 HAT, the mortality in both the concentrations increased to 30 per cent. The lower concentrations were not effective to cause mortality even at 96 HAT. A similar trend in increase of mortality was observed with both 10^8 spores mL^{-1} and 10^7 spores mL^{-1} at 96 HAT.

At 120 HAT, a mean mortality of 45 per cent was recorded at 10^7 spores mL^{-1} followed by 10^8 spores mL^{-1} (40). The spore concentration of 10^6 spores mL^{-1} recorded an initial mortality of 30 per cent at 144 HAT. Mean per cent mortality of both the higher concentrations were statistically on par which were 70 per cent at 10^8 spores mL^{-1} and 65 per cent at 10^7 spores mL^{-1}.

AT 168 HAT, the highest mortality observed was 85 per cent caused by 10^8 spores mL^{-1} followed by 10^7spores mL^{-1},10^6 spores mL^{-1}, and 10^5 spores mL^{-1} each of 65 per cent, 40 per cent , and 20 per cent respectively.

At 192 HAT, 10^8 spores mL^{-1} caused highest mortality of 85 per cent. Mortality of 70 per cent was recorded with 10^7 spores mL^{-1}, followed by 10^6 spores mL^{-1} (40 per cent) and were statistically on par with

Table 8. Mortality of *C. sordidus* grubs treated with *P. lilacinum*

	Mortality at 24 h interval (%)							
Concentration (Spores mL^{-1})	24 HAT	48 HAT	72 HAT	96 HAT	120 HAT	144 HAT	168 HAT	192 HAT
1.9×10^{8}	0	15.00	30.00	40.00	40.00	70.00 (57.10)[a]	85.00 (72.91)[a]	85.00 (72.91)[a]
1.9×10^{7}	0	20.00	30.00	40.00	45.00	65.00 (53.93)[a]	65.00 (53.93)[b]	70.00 (57.10)[b]
1.9×10^{6}	0	0	0	0	0	30.00 (32.89)[b]	40.00 (39.23)[c]	40.00 (39.23)[c]
1.9×10^{5}	0	0	0	0	0	0 (1.28)[c]	20.00 (25.56)[d]	35.00 (36.06)[c]
1.9×10^{4}	0	0	0	0	0	0 (1.28)[c]	0 (1.28)[e]	0 (1.28)[d]
Water spray	0	0	0	0	0	0 (1.28)[c]	0 (1.28)[e]	0 (1.28)[d]
CD (0.05)	-	-	-	-	-	7.352	12.130	12.914

Figures in parentheses are angular transformed values
HAT- Hours After Treatment

10^5 spores mL^{-1} (35 per cent). The lowest concentration 10^4 spores mL^{-1} was not able to cause mortality even at 192 HAT.

4.2.2.5.2 Mortality of Adults of C. sordidus

The adults when treated with *P. lilacinum* at 120 HAT recorded only 5 per cent mortality at 2.1×10^8 spores mL^{-1} and no other treatments caused mortality at this point of time (Table 9). At 240 HAT, 10^7 spores mL^{-1} recorded a mean mortality of 25 per cent and was statistically on par with 10^8 spores mL^{-1}(20 per cent). The highest mortality observed at 10^8 spores mL^{-1} was 30 per cent and was constant even at 480 HAT. Adults treated with 10^7 spores mL^{-1} recorded a mean per cent mortality of 25 per cent at 480 HAT but no mortality was caused by 10^6 spores mL^{-1}.

4.2.3 Lethal Time of EPF

Table 10 describes the lethal time required to kill the population of grubs treated with different fungi such as *M. anisopliae*, *B. bassiana*, and *P. lilacinum*.

LT_{50} of *M. anisopliae* at 1.3×10^8 spores mL^{-1} was calculated as 2.73 days. The lethal time required for killing half the population of test insect with 1.3×10^7spores mL^{-1} was 7.17 days. LT_{50} of *M. anisopliae* at 1.3×10^6 spores mL^{-1} was 10.94 days. The lowest concentration (1.3×10^5 spores mL^{-1}) of the fungus produced mortality of fifty per cent at 11.95 days.

LT_{50} of *B. bassiana* at 1.2×10^8 spores mL^{-1} was 4.01 days which was more compared to *M. anisopliae* at 1.3×10^8 spores mL^{-1} (2.73 days). Whereas 10^7 spores mL^{-1} of *B. bassiana* recorded LT_{50} of 5.61 days and was less compared to *M. anisopliae* (7.17 days). The lower concentrations of *B. bassiana* such as 10^6, 10^5 and 10^4 spores mL^{-1} recorded LT_{50} of 9.59, 9.58, and 9.19 days respectively. The LT_{50} of *M. anisopliae* at 10^6 and 10^5 spores mL^{-1} were more compared to that of *B. bassiana*.

Table 9. Mortality of *C. sordidus* adults treated with *P. lilacinum*

	Mortality at 24 h interval (%)			
Concentration (Spores mL^{-1})	120 HAT	240 HAT	360 HAT	480 HAT
2.1×10^{8}	5.00	20.00 (26.56)[a]	30.00 (32.89)[a]	30.00 (32.89)[a]
2.1×10^{7}	0	25.00 (29.73)[a]	25.00 (29.73)[a]	25.00 (29.73)[a]
2.1×10^{6}	0	0 (1.28)[b]	0 (1.28)[b]	0 (1.28)[b]
Water spray	0	0 (1.28)[b]	0 (1.28)[b]	0 (1.28)[b]
CD (0.05)	-	4.870	7.459	7.459

Figures in parentheses are angular transformed values
HAT- Hours After Treatment

LT_{50} of *P. lilacinum* at 1.9×10^8 spores mL^{-1} was 5.24 days. At the lowest dose evaluated *i.e,* @ 10^5 spores mL^{-1} LT_{50} was 8.37 days. The doses below 10^8 spores mL^{-1}were observed with lower LT_{50} values compared to the lower doses of *M. anisopliae* and *B. bassiana*.

4.2.4 Lethal Concentration of EPF

LC_{50} of *M. anisopliae* was 5.5×10^6 spores mL^{-1} and was high compared to *B. bassiana* (2×10^6 spores mL^{-1}) and *P. lilacinus* (2.7×10^6 spores mL^{-1}) at 7DAT (Table 11). The LC_{90} value was found to be 5.5×10^7 spores $mL^{-1}$for *M. anisopliae* which was less compared to other two fungi. LC_{90} of *B. bassiana* was 9.8×10^7 spores mL^{-1} followed by *P. lilacinum* (1.6×10^8 spores mL^{-1}).

4.3 FIELD EVALUATION OF ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF BANANA RHIZOME WEEVIL

4.3.1 Rhizome Dip Method

Effect of different entomopathogenic fungi on population and damage caused by *C. sordidus* was assessed based on the rhizome damage percentage, number of tunnels and number of immature stages and the results are presented in Table 12.

4.3.1.1 Rhizome Damage

Rhizomes dipped in spore suspension of *M. anisopliae* @ 10^8 spores mL^{-1} was found to be effective with least damage of 10.83 per cent. Rhizomes dipped in talc formulation of *M. anisopliae* @ 30 g L^{-1} exhibited 19.99 per cent damage and was statistically on par with that of *B. bassiana* which recorded a damage of 24.99 per cent. A mean damage of 49.99 per cent was observed in rhizomes dipped in spore suspension of *P. lilacinum* @ 10^8 spores mL^{-1}. Talc formulation of *B. bassiana* recorded a mean damage of 29.99 per cent which was statistically on par with talc formulation of *P. lilacinum* which recorded 30.83 per cent damage and

spore suspension of *B. bassiana* which recorded 24.99 per cent damage. There was no damage in the rhizomes dipped in Chlorpyrifos 0.05 % (Chemical check) and the highest damage was recorded in control plants (79.99 per cent). Rhizomes dipped in talc solution @ 30 g L^{-1} showed 69.99 damage in rhizome.

4.3.1.2 Number of Tunnels

Rhizomes dipped in spore suspension of *M. anisopliae* showed a mean number of 0.49 tunnels, whereas those dipped in spore suspension of *B. bassiana* @ 10^8 spores mL^{-1} showed a mean number of 1.58 tunnels and was statistically on par with that observed in talc formulation of *M. anisopliae* (1.99).

Number of tunnels observed in rhizomes treated with talc formulation of *M. anisopliae* (1.99) was statistically on par with talc formulation of *B. bassiana* (2.74) and spore suspension of *P. lilacinum* (2.75). Treatment with talc solution recorded 3.08 tunnels which was found to be statistically on par with the spore suspension of *P. lilacinum* (2.75) and talc formulation of *B. bassiana* (2.74).

4.3.1.3 Number of Immature Stages

No immature stages were observed in rhizome treated with spore suspension of *M. anisopliae* @ 10^8 spores mL^{-1}. The number of immature stages (Grubs and pupae) noted was minimum in spore suspension of *B. bassiana* which was statistically on par with talc formulation of *M. anisopliae,* the average number being 1.75 and 1.83 respectively. A mean number of 2.67 immature stages were observed in rhizomes treated with talc formulation of *B. bassiana* and found to be statistically on par with the spore suspension of *P. lilacinum* (3.16) and talc solution (3.24). More number of larvae and pupae were obtained from the untreated rhizomes (3.41) and was statistically on par with the talc treated rhizomes (3.24) and talc based formulation of *P. lilacinum* (3.16). No immature stages were observed in rhizomes treated with chlorpyrifos (0.05%).

Table10. LT_{50} of fungi at different concentrations on *C. sordidus* grubs

Fungi	Concentration (spores mL^{-1})	LT_{50} (Days)
M. anisopliae	1.3×10^{8}	2.73
	1.3×10^{7}	7.17
	1.3×10^{6}	10.94
	1.3×10^{5}	11.95
B. bassiana	1.2×10^{8}	4.01
	1.2×10^{7}	5.61
	1.2×10^{6}	9.59
	1.2×10^{5}	9.58
	1.2×10^{4}	9.19
P. lilacinum	1.9×10^{8}	5.24
	1.9×10^{7}	5.10
	1.9×10^{6}	8.03
	1.9×10^{5}	8.37

Table 11. LC_{50} and LC_{90} of fungi at 7 DAT

Fungi	LC_{50} (Spores mL^{-1})	Fiducial limit for LC_{50} (Spores mL^{-1})		LC_{90} (Spores mL^{-1})	Fiducial limit for LC_{90} (Spores mL^{-1})		χ^2
		Lower	Upper		Lower	Upper	
M. anisopliae	5.5×10^{6}	2.4×10^{6}	1.3×10^{7}	5.5×10^{7}	2×10^{7}	4.3×10^{8}	3.74
B. bassiana	2×10^{6}	8.3×10^{5}	5.5×10^{6}	9.8×10^{7}	2.7×10^{7}	1×10^{8}	2.36
P. lilacinum	2.7×10^{6}	1×10^{6}	7.9×10^{6}	1.6×10^{8}	4×10^{7}	2.2×10^{8}	1.35

Table 12. Effect of rhizome dip treatment with entomopathogenic fungi on

Treatments	*Rhizome damage (percentage)	**Number of tunnels rhizome^{-1}	**Number of immature stages rhizome^{-1}
Talc formulation of *M. anisopliae* @ 30 g L^{-1}	19.99 (26.52)^{e}	1.99 (1.57)^{cd}	1.83 (1.52)^{d}
Talc formulation of *B. bassiana* @ 30 g L^{-1}	29.99 (33.08)^{d}	2.74 (1.79)^{bc}	2.67 (1.77)^{bc}
Talc formulation of *P. lilacinum* @ 30 g L^{-1}	30.83 (33.72)^{d}	3.16 (1.70)^{b}	3.16 (1.90)^{abc}
Spore suspension of *M. anisopliae* @ 10^{8} spores mL^{-1}	10.83 (19.17)^{f}	0.49 (0.98)^{e}	0 (0.70)^{e}
Spore suspension of *B. bassiana* @ 10^{8} spores mL^{-1}	24.99 (29.86)^{de}	1.58 (1.43)^{d}	1.75 (1.49)^{d}
Spore suspension of *P. lilacinum* @ 10^{8} spores mL^{-1}	49.99 (44.99)^{c}	2.75 (1.78)^{bc}	2.49 (1.72)^{c}
Chlorpyrifos @ 0.05%	0 (1.65)^{g}	0 (0.70)^{e}	0 (0.70)^{e}
Talc solution 30 g L^{-1}	69.99 (56.87)^{b}	3.08 (1.88)^{b}	3.24 (1.94)^{ab}
Control	79.99 (63.74)^{a}	4.91 (2.32)^{a}	3.41 (1.81)^{a}
CD (0.05)	4.708	0.253	0.199

*Figures in parentheses are angular transformed values.
**Figures in parentheses are square root transformed values.
Mean of four replications

4.3.2 Prophylactic Soil Drenching

Table 13 details the effect of entomopathogenic fungi on *C. sordidus* when the treatments were applied as prophylactic soil drenching.

4.3.2.1 Rhizome Damage

Talc formulation of *M. anisopliae* and its spore suspension recorded least damage (14.16 per cent) followed by the talc formulation of *B. bassiana* (31.66 per cent). Spore suspension of *B. bassiana* and *P. lilacinum* recorded rhizome damage of 39.16 per cent which was statistically on par with the talc formulation of *P. lilacinum* (41.66). The untreated rhizomes recorded highest damage in rhizome (64.99 per cent). No damage was observed in rhizomes treated with chlorpyriphos 0.05% (Chemical check).

4.3.2.2 Number of Immature stages

Number of live grubs were less in those rhizomes treated with talc formulation of *M. anisopliae* (0.66). Spore suspension of *M. anisopliae* @ 10^8 spores mL^{-1} recorded 1.08 live grubs. The live grubs recorded from the rhizomes treated with talc formulation and spore suspension of *B. bassiana*, and spore suspension of *P. lilacinum* was 1.49, 1.49 and 1.83 respectively and was statistically on par.

4.3.2.3 Number of tunnels

Least number of tunnels were observed in the treatment with talc formulation of *M. anisopliae* and *B. bassiana* which was 1.16. Spore suspension of *M. anisopliae* recorded 1.25 tunnels in rhizome followed by 2.91 from the rhizomes treated with spore suspension of *B. bassiana*. *P. lilacinum* when applied as spore suspension and talc formulation was less effective with 3.41 and 3.91 tunnels in the rhizomes respectively. Maximum number of tunnels (5.24) was observed in control treatment followed by the talc treated rhizomes (5.16).

Table 13. Effect of prophylactic soil drenching with entomopathogenic fungi on population and damage caused by *C. sordidus*

Treatments	*Rhizome damage (Percentage)	**Number of tunnels rhizome^{-1}	**Number of immature stages rhizome^{-1}
Talc formulation of *M. anisopliae* @ 30g L^{-1}	14.16 $(21.88)^{c}$	1.16 $(1.28)^{d}$	0.66 $(1.07)^{e}$
Talc formulation of *B. bassiana* @ 30g L^{-1}	31.66 $(34.16)^{b}$	1.16 $(1.28)^{d}$	1.49 $(1.41)^{bc}$
Talc formulation of *P. lilacinum* @ 30g L^{-1}	41.66 $(40.15)^{b}$	3.91 $(2.09)^{b}$	1.41 $(1.38)^{cd}$
Spore suspension of *M. anisopliae* @ 10^{8} spores mL^{-1}	14.16 $(22.08)^{c}$	1.25 $(1.32)^{d}$	1.08 $(1.25)^{d}$
Spore suspension of *B. bassiana* @ 10^{8} spores mL^{-1}	39.16 $(38.71)^{b}$	2.91 $(1.84)^{c}$	1.49 $(1.40)^{bc}$
Spore suspension of *P. lilacinum* @ 10^{8} spores mL^{-1}	39.16 $(38.71)^{b}$	3.41 $(1.97)^{bc}$	1.83 $(1.52)^{bc}$
Chlorpyrifos @ 0.05%	0 $(1.65)^{d}$	0 $(0.70)^{e}$	0 $(0.70)^{f}$
Talc solution 30-g L^{-1}	54.16 $(47.39)^{a}$	5.16 $(2.37)^{a}$	1.49 $(1.40)^{bc}$
Control	64.99 $(54.36)^{a}$	5.24 $(2.39)^{a}$	2.66 $(1.77)^{a}$
CD (0.05)	7.103	0.132	0.153

*Figures in parentheses are angular transformed values.
**Figures in parentheses are square root transformed values.
Mean of four replications

4.3.3 Curative Soil Drenching

Table 14 describes the effect of entomopathogenic fungi on *C. sordidus* when applied as curative soil drenching.

4.3.3.1 Rhizome Damage

Plants treated with spore suspension of *M. anisopliae* @ 10^8 spores mL^{-1} recorded least damage in rhizomes (10 per cent) followed by talc formulation of *M. anisopliae* (12.49 per cent) and *B. bassiana* (12.49 per cent) @ 30 g $L^{-1}$and was statistically on par. Spore suspension of *B. bassiana* recorded a mean rhizome damage of 27.49 which was statistically on par with the damage recorded in rhizomes treated with spores suspension of *P. lilacinum* (34.16 per cent). Highest rhizome damage was observed in the control treatment (83.32 per cent) followed by the talc formulation of *P. lilacinum* (53.33 per cent) and found to be statistically on par.

4.3.3.2 Number of Immature Stages

No live grubs were observed from the rhizomes treated with chlorpyriphos which was followed by the talc formulation (0.57) and spore suspension of *M. anisopliae* (0.74) and were statistically on par. Spore suspension of *B. bassiana* recorded a mean number of 1.24 grubs in the treated plants. Number of live grubs recorded from the plants treated with talc formulation of *P. lilacinum* (1.91) and and its spore suspension (2.16) were statistically on par.

4.4.3.3 Number of Tunnels

Plants treated with spore suspension of *M. anisopliae* recorded least number of tunnels (1.08) followed by its talc formulation (1.16). Talc formulation of *B. bassiana* recorded a mean number of 1.82 tunnels which was statistically on par with its spore suspension of *B. bassiana* (1.99). No tunnels were observed in the chemical treated rhizomes. *P. lilacinum* when applied as talc formulation and

Table 14. Effect of curative soil drenching with entomopathogenic fungi on population and damage caused by *C. sordidus*

Treatments	*Rhizome damage (Percentage)	**Number of tunnels rhizome^{-1}	**Number of immature stages rhizome^{-1}
Talc formulation of *M. anisopliae* @ 30 g L^{-1}	12.49 (20.66)^{d}	1.16 (1.28)^{d}	0.57 (1.03)^{e}
Talc formulation of *B. bassiana* @ 30 g L^{-1}	12.49 (20.66)^{d}	1.82 (1.52)^{c}	1.74 (1.49)^{c}
Talc formulation of *P. lilacinum* @ 30 g L^{-1}	53.33 (46.90)^{b}	3.16 (1.91)^{b}	1.91 (1.55)^{bc}
Spore suspension of *M. anisopliae* @ 10^{8} spores mL^{-1}	10 (18.54)^{d}	1.08 (1.26)^{d}	0.74 (1.11)^{e}
Spore suspension of *B. bassiana* @ 10^{8} spores mL^{-1}	27.49 (31.59)^{c}	1.99 (1.58)^{c}	1.24 (1.32)^{d}
Spore suspension of *P. lilacinum* @ 10^{8} spores mL^{-1}	34.16 (35.57)^{c}	2.91 (1.84)^{b}	2.16 (1.62)^{bc}
Chlorpyrifos @ 0.05%	0 (1.65)^{e}	0 (0.70)^{e}	0 (0.70)^{f}
Talc solution 30 g L^{-1}	51.66 (45.95)^{b}	2.99 (1.86)^{b}	2.16 (1.64)^{b}
Control	83.32 (66.15)^{a}	4.66 (2.27)^{a}	2.74 (1.80)^{a}
CD (0.05)	4.122	0.134	0.135

*Figures in parentheses are angular transformed values.

** Figures in parentheses are square root transformed values.

Mean of four replications

spore suspension recorded 3.16 and 2.91 tunnels respectively and was found to be statistically on par. The control treatment recorded 4.66 tunnels in rhizomes.

Discussion

5. DISCUSSION

C. sordidus, the weevil pest of banana is a threat to banana production all over the world. Galleries made by the grubs inside the rhizome reduces the nutrient uptake and functioning of vascular system and there by death of the plant. Due to the rhizome damage, secondary infection by other fungal pathogens may occur leading to rotting of the rhizome. Most of the times external visible symptoms of infestation is of no use to identify the incidence of the pest because by the time the rhizome might have extensively be tunneled by the grub.

Clean cultivation, paring and application of chemical insecticides are the control measures followed by the farmers for managing the pest. Maintaining proper sanitation in the banana plantation is a labour intensive process and indiscriminate use of insecticides causes severe environmental problems. In this context, management of the pest using biological methods has its relevance. Among the biological control methods, entomopathogens can act as an efficient tool for controlling the population of the weevil. Entomopathogenic fungi *viz. M. anisopliae* and *B. bassiana* are identified to control the coleopteran pests and are potential pathogens of almost all insect pests (Hejek and St. Leger, 1994; Bello *et al.*, 2000).

Indigenous entomopathogens present in soil can be isolated and exploited for the successful control of agricultural pests in a biological way. Infestation of these fungi are very less in *C. sordidus* under field conditions which may be due to the reduced movement of the weevil in soil or due to intensive sclerotisation of the insect which limits the entry of the fungal spores into the body.

In the present study, natural infection of entomopathogenic fungus *F. solani* was observed on the adult weevils of *C. sordidus* collected from the banana fields of College of Agriculture, Vellayani. Natural infection of *F. solani* was observed by Anitha (2000) in pseudostem weevil and Majumdar *et al.* (2007) on the pupae of root maggot of sugarbeet, *T. myopaeformis*. *Fusarium* spp are

mainly endophytes which are associated with the roots of many plants and causing infection on the subterranean insect pests (Majumdar *et al.,* 2007).

5.1 PATHOGENICITY OF ENTOMOPATHOGENIC FUNGI

Pathogenicity studies of the entomopathogenic fungi were carried out on both the adults and grubs of banana rhizome weevil. The isolate obtained from present study, *F. solani* and the other indigenous isolates from Vellayani, *viz. B. bassiana* (ITCC6063) and *P. lilacinum* (ITCC6064) along with NBAIR isolates *viz. M. anisopliae* (Ma4) and *B. bassiana* (Bb5) were tested for their pathogenicity against grubs and adults of *C. sordidus.* All the fungi tested were found to be pathogenic to the grubs and adults of *C. sordidus.*

No specific changes in feeding behavior and movement were observed in the adults treated with these fungi. Whereas grubs showed reduced feeding and movement when treated with these fungi. *M. anisopliae* and *P. lilacinum* treated grubs became inactive one day after treatment (DAT), while those treated with *B. bassiana* (Bb5) and *B. bassiana* (ITCC6063) showed inactivity three DAT. Reduced feeding and appetite were observed by McCoy *et al.* (1988) during the later stages of infection by this fungi.

The symptom development occurred immediately after the death of the insect. Infected insects developed a distinct brownish discoloration and shrinkage of body after death. Later, the mycelia invaded the body making the grubs hard and stiff. The fungus penetrates the insect cuticle by the production of enzymes along with mechanical pressure resulting the invasion of fungus into the insect body.

Pathogenicity *of M. anisopliae* has been proved by Lopes *et al.* (2013) and Anis (2014) on adults and grubs of *C. sordidus. M. anisopliae* treated grubs after death, became mummified and developed whitish mycelia intersegmentally which later changed to greenish color due to sporulation. The intersegmental mycelial growth started from ventral thoracic region was prominent in adults, because this

region has less sclerotisation, which helps in easy emergence of the germinated condia. The growth extensively covered the spiracular area, legs and the elytra.

B. bassiana caused mycosis on *C. sordidus* (Kaaya *et al.*, 1993; Nankinja, 1999; Fancelli *et al.*, 2013; Omukoko *et al.*, 2014) and the symptoms developed were identical in the case of *B. bassiana* (Bb5) and *B. bassiana* (ITCC6063).

Not much studies were undertaken regarding the pathogenicity of *P. lilacinum* to *C. sordidus.* It was found to be pathogenic to coleopteran storage pests (Barra *et al.*, 2013; Wakil *et al.*, 2014). In present study, after one day of treatment pink coloration was observed when the grubs were treated with *P. lilacinum* and mycelial growth was initiated two DAT.

Adults of *C. sordidus* after treatment with the fungi took much more time for mycelial growth in the case of all the fungi compared to grubs. This may be due to the presence of hard elytra of adult.

Virulence of the entomopathogenic fungi is the primary factor which determines the ability to act as potential entomopathogen. Bioassay studies conducted in laboratory on adults and grubs of *C. sordidus* helped in comparison of virulence of different fungi.

Mortality occurred 24 HAT in the case of grubs of *C. sordidus* when treated with *B. bassiana* (Bb5) and *M. anisopliae* whereas *B. bassiana* (ITCC6064), *F. solani,* and *P. lilacinum* caused mortality 48 HAT.

Mortality has been progressively increased with the time irrespective of the fungi. Highest mortality of 100 per cent was observed on grubs treated with *M. anisopliae*. *M. anisopliae* at its highest dose of 10^8 spores mL^{-1} caused hundred per cent mortality of the grubs at 168 HAT, while *B. bassiana* (Bb5) and *P. lilacinum* caused only 90 per cent and 85 per cent mortality respectively. At 10^7 spores mL^{-1} also the highest mortality was observed in grubs treated with *M. anisopliae*. Contradictory results were reported by Kaaya *et al.* (1992) who

observed that *M. anisopliae* was least pathogenic to the grubs of *C. sordidus* compared to *B. bassiana*. In present study, Eventhough hundred per cent mortality was not obtained by the treatment with *B. bassiana* (Bb5) it was statistically on par with *M. anisopliae*. *M. anisopliae* was able to cause highest mortality of grubs during short period of time compared to other fungi. Virulence of *M. anisopliae* was found to be more than *B. bassiana* and this may be due to the the production of toxins/enzymes (Kershaw *et al.*, 1999).

F. solani and *B. bassiana* (ITCC6063) at their highest spore load of 10^7 spores $mL^{-1}$and 10^8 spores mL^{-1} recorded less than fifty per cent mortality of the grubs even at 120 HAT. *B. bassiana* (Bb5) was found to produce significantly higher mortality than *B. bassiana* (ITCC6063), this may be due to variation in strains.

P. lilacinum was the least effective fungus under laboratory conditions when treated on grubs. The bioassay studies of *P. lilacinum* has not been carried out by any researchers on *C. sordidus*, but the study conducted on black cherry aphid, *Myzus cerasi* F. observed 83.64 per cent mortality at 10^8 spores mL^{-1} (Kepenekci *et al.*, 2015).

Dose-mortality relationship of different fungi on adults of *C. sordidus* showed that *B. bassiana* cause highest mortality compared to *M. anisopliae* but a significant difference in mortality was not observed. *M. anisopliae* and *B. bassiana* could not kill 50 per cent of the adults even after 20 days of treatment. Reduced mortality of adults (less than 50 per cent) was observed by Omukoko *et al.* (2014) when they dipped the adults in conidial suspension of *B. bassiana* at a concentration of 1×10^8 spores mL^{-1}. *B. bassiana* (ITCC6063) and *F. solani* showed very less mortality of adults even at their highest spore concentration.

In the case of adults, time taken for getting mortality was more when compared to grubs. This may be because of the hard elytra of the adults which act as barrier for the fungi to penetrate the cuticle and thereby cause infection.

In this study, a proportionate increase in mortality was observed with spore dose. Such positive correlation between the number of viable spores and the mortality of the host was proposed by Navon and Ascher (2005).

The lethal time period and lethal concentration required to cause 50 per cent mortality of grubs revealed that, *M. anisopliae* at its highest concentration of 1.3×10^8 spores mL^{-1}, was found with an LT_{50} of 2.73 days. Whereas *B. bassiana* and *P. lilacinum* at highest concentration of 10^8 spores mL^{-1} has taken 4.01 and 5.24 days for killing 50 per cent of the grubs. *M. anisopliae* could cause mortality of 50 per cent of grubs within a short period of time compared to the other two fungi indicating its potential as efficient biocontrol agent. Virulence of *M. anisopliae* can be explained by the action of toxin, dextruxins which causes the death of infected insect even before the establishment of fungus on its body (Kershaw *et al.,* 1999).

LC_{50} and LC_{90} of different fungi after 7 days of treatment along with their fiducial limits were computed by probit analysis. LC_{50} value of *M. anisopliae* was found to be 5.5×10^6 spores mL^{-1} which was comparatively higher than that of *B. bassiana* with an LC_{50} of 2×10^6 spores mL^{-1}. Among the three fungi, *B. bassiana* was found with lowest LC_{50} value followed by *P. lilacinum* (2.7×10^6 spores mL^{-1}). LC_{90} of *M. anisopliae* was 5.5×10^7 spores mL^{-1} which was the lowest concentration compared to *B. bassiana* (9.8×10^7 spores mL^{-1}) and *P. lilacinum* (1.6×10^8 spores mL^{-1}). Eventhough the LC_{50} value was the lowest in case of *P. lilacinum,* its LC_{90} value was higher than other fungi. Comparing the LC_{50} and LT_{50} of different fungi, *M. anisopliae* was superior with lowest LT_{50}. Hence the most effective fungus against *C. sordidus* was observed as *M. anisopliae,* followed by *B. bassiana* and *P. lilacinum*.

5.3 FIELD EFFICACY

The fungi which has been identified to cause more than 50 per cent mortality of grubs *viz., B. bassiana* (Bb5), *M. anisopliae* (Ma4) and *P. lilacinum* were tested for their field efficacy by conducting pot culture experiment.

In rhizome dip method, spore suspension of *M. anisopliae* 30 g L^{-1} @ 10^8 spores mL^{-1} was more effective than its talc formulation with a rhizome damage of 10.83 per cent. Similarly spore suspension of *B. bassiana* 30 g L^{-1} @ 10^8 spores mL^{-1} recorded a damage of 24.99 per cent which was comparatively less than its talc formulation (29.99 per cent). The number of tunnels in the rhizome was also less in the plants treated with spore suspension of *M. anisopliae* (0.49) compared to its talc formulation (1.99). Talc formulation of *B. bassiana* recorded more number of tunnels (2.74) in the rhizomes, than its spore suspension (1.58). The number of immature stages collected from the rhizomes also followed a similar trend in which the spore suspensions of the fungi recorded less number of immature stages than their talc formulation in rhizome dip method. Among the treatments, spore suspension of *M. anisopliae* was the best treatment than its talc formulation followed by the spore suspension of *B. bassiana* when applied as rhizome dip method. When the efficacy of the spore suspension and talc formulation is compared, spore suspension was highly effective than talc formulation in the case of *M. anisopliae* and *B. bassiana.* The effect of spore suspension may be due to the immediate direct contact of the spores to the insects.

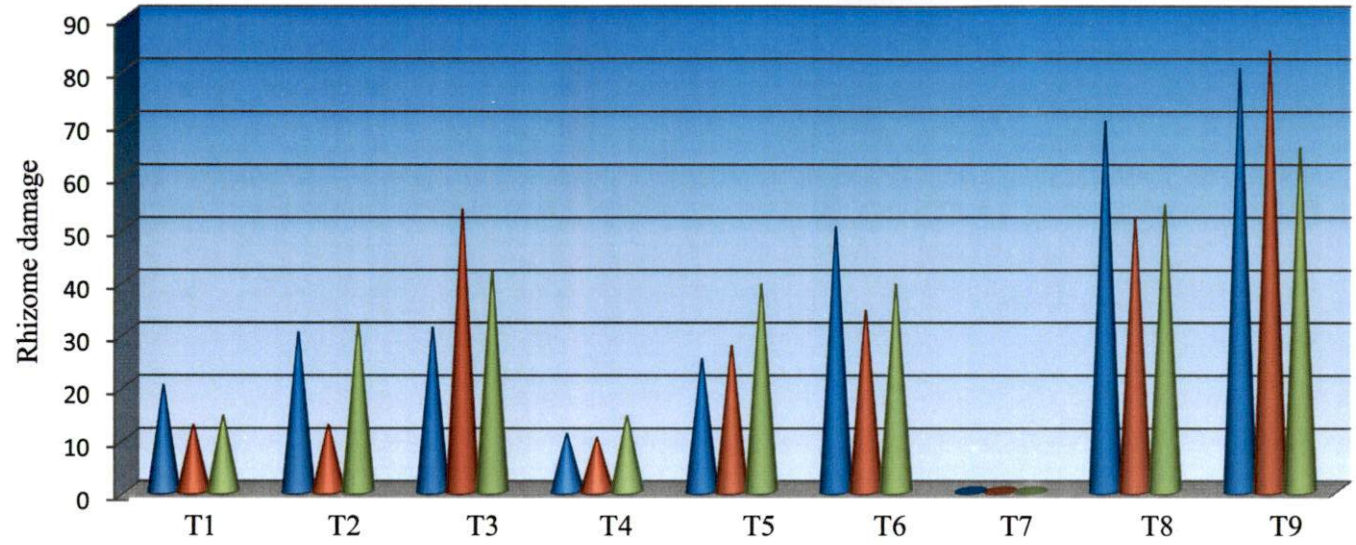
Application of the treatments as prophylactic soil drenching was carried out to compare their efficacy. Spore suspension and talc formulation of *M. anisopliae* recorded least damage in rhizome (14.16) which were statistically on par. Spore suspension of *B. bassiana* recorded 39.16 per cent damage in rhizome which was more compared to its talc formulation (31.66 per cent). Similarly the talc formulation of *P. lilacinum* was less effective compared to its spore suspension. But the damage observed in talc formulation and spore suspension treated rhizomes were statistically on par in the case of *B. bassiana* and *P. lilacinum.*

Number of immature stages recorded were least in the rhizomes drenched with talc formulation of *M. anisopliae* (0.66) which was followed by its spore suspension (1.08). *B. bassiana* when applied as talc formulation and spore suspension recorded equal number of immature stages in rhizome (1.49). Number

of tunnels was observed to be less in the rhizomes (1.16) treated with talc formulation of *M. anisopliae* and was statistically on par with that of its spore suspension (1.25). Similarly the number of tunnels was less in the treatment with talc formulation of *B. bassiana* than its spore suspension (2.91). In prophylactic soil drenching, talc formulation of *M. anisopliae* and *B. bassiana* were found to be effective than their spore suspension, when the rhizome damage, number of tunnels and immature stages were taken into account. *M. anisopliae* when applied as both talc formulation and spore suspension were equally effective and was the best treatment in prophylactic soil drenching. *B. bassiana* was found to be the next best fungi in its efficacy, when applied as talc formulation and spore suspension. Talc formulation and spore suspension of *P. lilacinum* was least effective compared to that of *M. anisopliae* and *B. bassiana* when applied as prophylactic soil drenching. The superiority of the talc formulation may be due to the presence of the talc in soil around the rhizome which may act as a physical barrier.

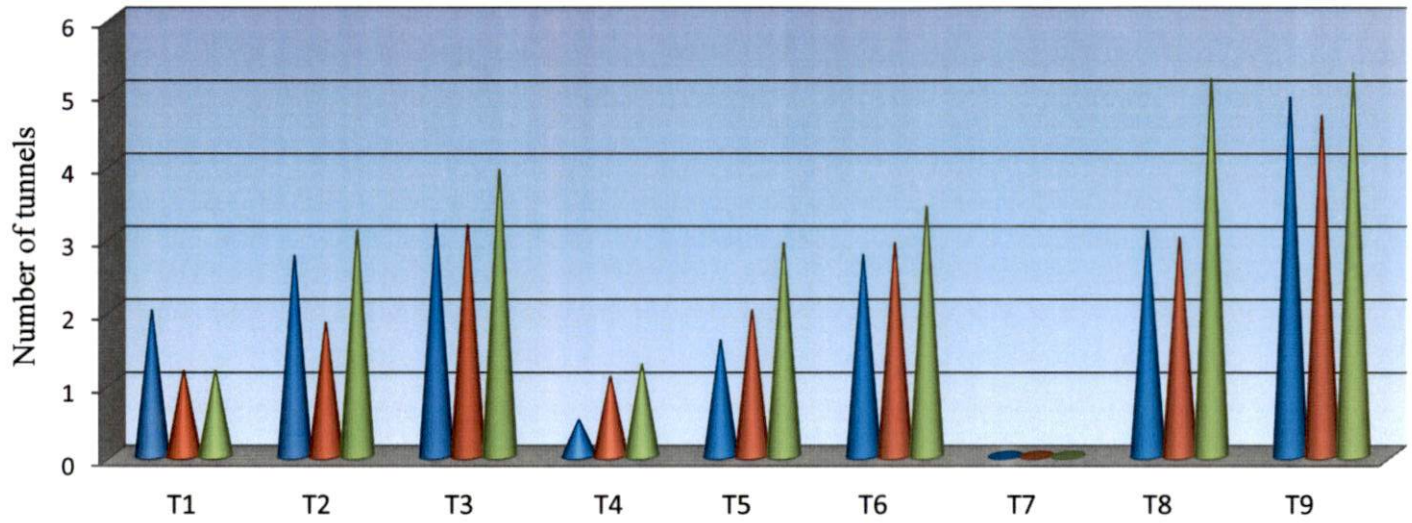
The third experiment was the application of treatments as curative soil drenching. *M. anisopliae* applied as its spore suspension recorded least rhizome damage (10 per cent) compared to its talc formulation (12.49). Number of immature stages and tunnels observed in the treatment with talc formulation and spore suspension of *M. anisopliae* were statistically on par. Talc formulation of *M. anisopliae* and *B. bassiana* recorded similar rhizome damage and were statistically on par. *B. bassiana* applied as talc formulation was effective than its spore suspension when the rhizome damage and number of tunnels were taken into consideration.

Effect of the treatments on rhizome damage and number of tunnels in the three methods such as rhizome dip, prophylactic soil drenching and curative soil drenching is represented in Figure 1 and 2 respectively. Among the three methods, curative soil drenching of talc formulation of *M. anisopliae* and *B. bassiana* recorded least rhizome damage and tunnels, and was effective than other methods of application. Number of immature stages observed in all the



T1- Talc formulation of Ma4 @ 30 g L^{-1}
T2- Talc formulation of Bb5 @ 30 g L^{-1}
T3- Talc formulation of *P. lilacinum* @ 30 g L^{-1}
T4- Spore suspension of Ma4 @ 10^8 spores mL^{-1}
T5- Spore suspension of Bb5 @ 10^8 spores mL^{-1}
T6- Spore suspension of *P. lilacinum* @ 10^8 spores mL^{-1}
T7- Chlorpyrifos @ 0.05%
T8- Talc solution 30 g L^{-1}
T9- Control

Figure 1. Effect of entomopathogenic fungi on rhizome damage



T1- Talc formulation of Ma4 @ 30 g L^{-1}
T2- Talc formulation of Bb5 @ 30 g L^{-1}
T3- Talc formulation of *P. lilacinum* @ 30 g L^{-1}
T4- Spore suspension of Ma4 @ 10^8 spores mL^{-1}
T5- Spore suspension of Bb5 @ 10^8 spores mL^{-1}
T6- Spore suspension of *P. lilacinum* @ 10^8 spores mL^{-1}
T7- Chlorpyrifos @ 0.05%
T8- Talc solution 30 g L^{-1}
T9- Control

Figure 2. Effect of entomopathogenic fungi on number of tunnels

methods is well explained in Figure 3. Since they are present in concealed condition inside the rhizome, the chance of infection may be less (Akello *et al.*, 2007).

When the number of dead adults collected from the study was observed, more number of dead insects were obtained from the rhizomes on which *M. anisopliae* was applied curatively, than other methods (Figure 4). In the case of *B. bassiana* also, curative application recorded more number of dead adults. Whereas, *P. lilacinum* caused mycosis of released adults only when applied as rhizome dip method.

A pathogen prefers nutrient rich media for its survival and growth. In curative method of application, already the nutrient rich media *ie,* the insect is present for establishing well than the soil which is not a specific media for growth of the fungi (Navon and Ascher, 2005). This may be the primary reason for the success of curative treatments.

In addition to that, the fungi such as *M. anisopliae* and *B. bassiana* can colonize on the tissues of the rhizome (Akello *et al.*, 2009) and there by establish a continuous contact with the insect. This possibility of endophytic colonization has been reported on several crop plants (Cherry *et al.*, 2004; Posada and Vega, 2005). Akello *et al.* (2009) reported successful colonization of *B. bassiana* on the roots and rhizomes of banana one month after inoculation. This type of association may also contribute a major role in the success of entomopathogenic fungi.

The failure of prophylactic soil drenching might be because of the loss of viability and survival of spores in soil due to the adverse climatic conditions especially temperature (Nankinga *et al.*, 2005) which may reduced the epizootics caused by the fungi (Charnley, 1992). Under field conditions, the performance of fungi gets reduced due the influence of temperature, humidity and the carrier substrate.

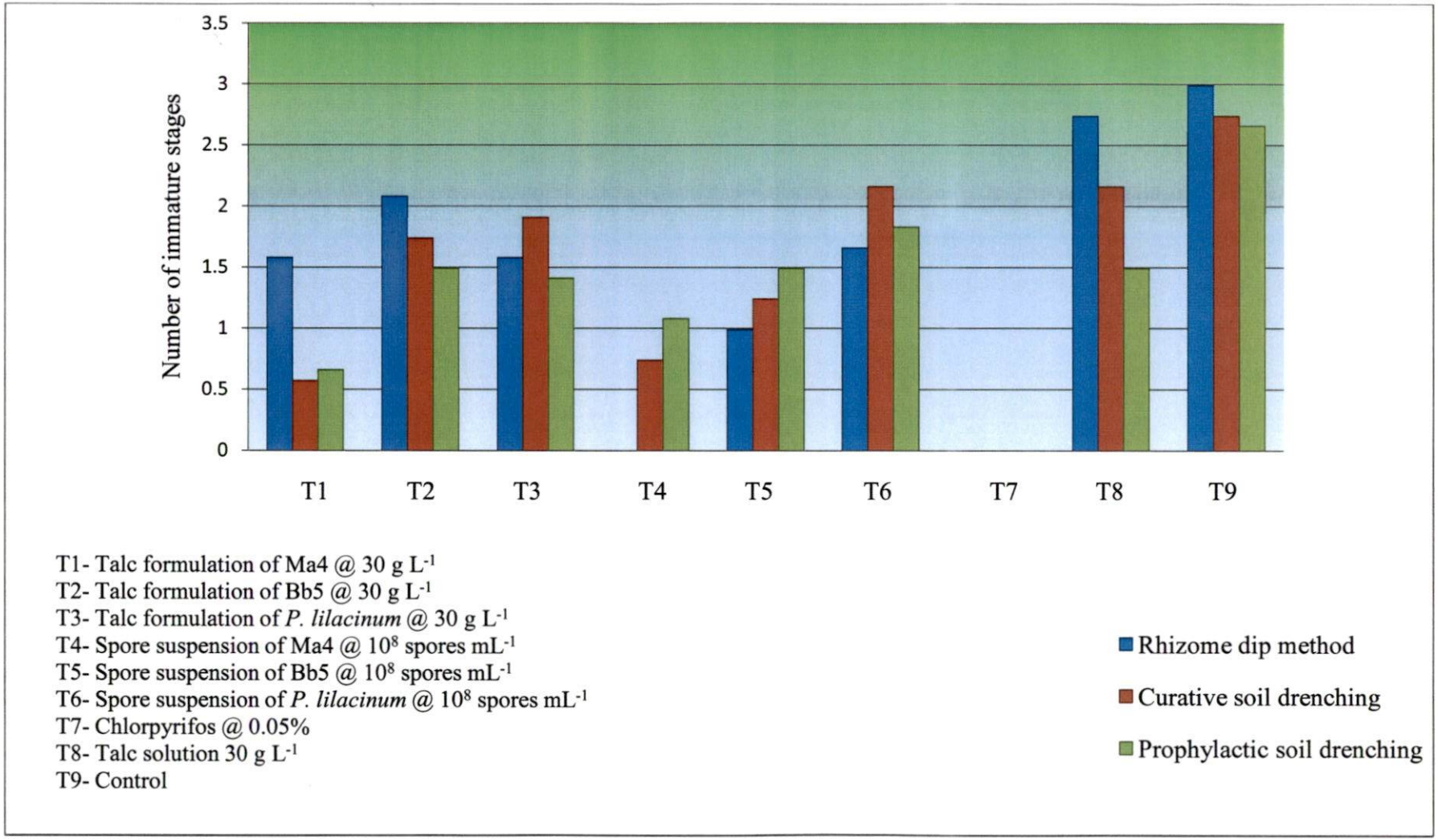


Figure 3. Effect of entomopathogenic fungi on number of immature stages

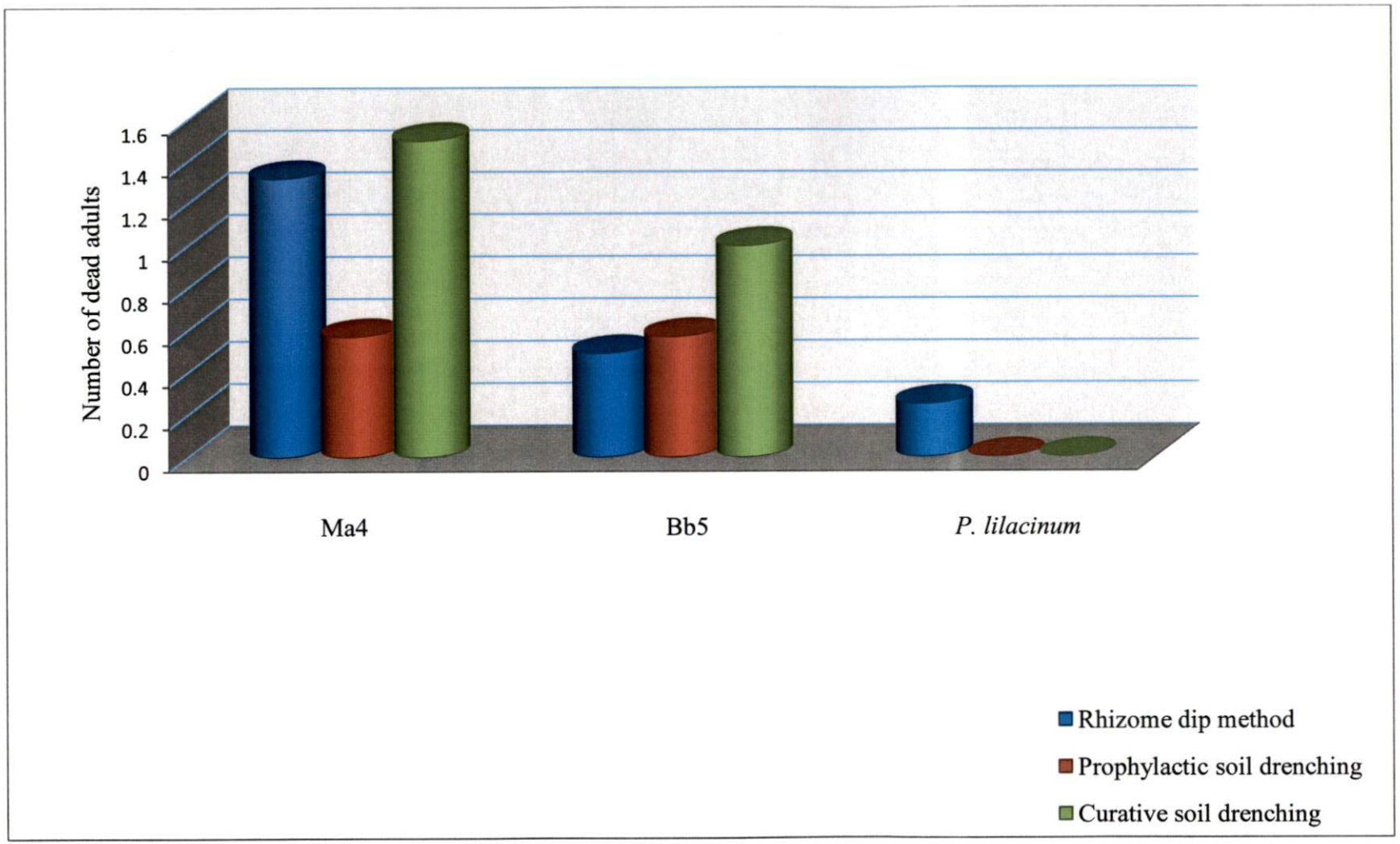


Figure 4. Effect of entomopathogenic fungi on released adults

The fungus *M. anisopliae* was found to be superior in the laboratory conditions with significantly higher mortality than other fungi and its talc formulation applied as curative method was effective in field conditions to control the population of *C. sordidus*. *B. bassiana* caused less mortality in laboratory conditions compared to *M. anisopliae* but its talc formulation was equally effective as that of *M. anisopliae* when applied curatively.

As the rhizome weevils are found in clusters in cavities and depressions of outer layer of the rhizome, horizontal transmission also play a role in transmission of fungal spores in its habitat when the infected weevil moves and carry these spores to healthy weevils. This creates an epizootic condition in field Omukoko *et al.* (2014).

Eventhough endophytic association and horizontal transmission effects are there, a significant effect on the population of the weevil is not possible in a single application. The buildup of the inoculum in soil is essential which is possible by subsequent application of the the fungus (Schoeman and Botha, 2003). Since the application of the fungi is external, it can only control the exposed stages of *C. sordidus*. In order to get an effective control of the pest, special delivery mechanisms can be developed which can allow the fungi to effectively come in contact with these hidden stages like grubs and pupae (Akello *et al.*, 2007).

Summary

6. SUMMARY

Banana rhizome weevil, *C. sordidus* have been identified as a major constraint to the production of banana all over the world. No variety of banana has been identified to be resistant to rhizome weevil. Damage caused by the grubs by making extensive tunneling of rhizome using their strong mandibles causes weakening of the plant and also the secondary infection by other pathogens. Being the major pest of banana, rhizome weevil needs special attention in its management level. Cultural and chemical methods have been employed by the farmers for the management of weevil. Considering the deleterious effects caused by the chemical pesticides towards the environment and laborious practices that followed in cultural methods necessitates an alternate method for management of the weevil.

Safety to the non target organisms and the specificity of entomopathogenic fungi were the prime reasons which helped them to emerge as an efficient tool in pest management. Natural infestation of entomopathogenic fungi can be observed on insects. So the cadavers of insects are sources for isolation of entomopathogenic fungi. Adults of rhizome weevil can be observed on cracks and crevices of rhizomes in contact with the soil, which enhances the chances of getting infection by the entomopathogenic fungi from soil. Indigenous entomopathogenic fungi in soil were identified to have more virulence and potential than the exotic fungi. Climatic conditions like frequent rainfall and humidity favours the expression of full potential of entomopathogenic fungi in its sporulation and growth under Kerala conditions.

Considering the importance of damage caused by the rhizome weevil and the efficiency of entomopathogenic fungi, a study was conducted entitled "Entomopathgenic fungi for the management of banana rhizome weevil, *Cosmopolites sordidus*, Germer" in College of Agriculture, Vellayani with an objective to evaluate the biocontrol potential of entomopathogenic fungi for the management of banana rhizome weevil.

Natural infestation of *Fusarium solani* was observed on the adult weevil collected from banana fields of College of Agriculture, Vellayani. The fungus was isolated and morphological and cultural characters were studied. Fungus produced fusiform septate microconidia and ovoid aseptate macroconidia. Isolate of *F. solani* along with the NBAIR isolates *viz, M. anisopliae* and *B. bassiana* and local isolates available in Department of Agricultural Entomology *viz, B. bassiana* (ITCC6063) and *P. lilacinum* (ITCC6064) were tested for their pathogenicity and found to be pathogenic to grubs and adults of *C. sordidus.* Symptoms developed due to the infection of fungi were different in their characters of mycelial growth on adults and grubs. Time taken for infection, color of mycelia and time taken for mycelial growth were also vary in different fungi.

Virulence of the fungi was assessed by conducting bioassay studies on grubs and adults of *C. sordidus* by spraying different concentration of fungal spore suspension on the insect and observed their mortality. Mortality was found to be increased with time irrespective of the fungi. *F. solani* and *B. bassiana* (ITCC6063) recorded less than 50 per cent mortality of grubs at their highest spore load of 10^7 and 10^8 spores mL^{-1} respectively. While on adults they caused significantly less mortality. *M. anisopliae* @ 10^8 spores mL^{-1} caused highest mortality of 100 per cent in grubs after 168 hours of treatment. *B. bassiana* caused 90 per cent mortality at 168 hours of treatment. Whereas *P. lilacinum* was least effective fungus against grubs of *C. sordidus* under laboratory conditions with maximum of 85 per cent mortality during same time period. *M. anisopliae* was found to be virulent pathogen against grubs of *C. sordidus* than other fungi under laboratory conditions.

M. anisopliae and *B. bassiana* @ 10^8 spores mL^{-1} were not effective to cause 50 per cent mortality of adult weevils even 20 days after treatment. Significantly less mortality was observed when *F. solani* and *B. bassiana* (ITCC6063) were treated on adult weevils. Time period for initiating mortality was found to be more in the case of adults compared to grubs. Mortality was

found to be increased with increasing spore concentration in the case of all the tested fungi.

LT_{50} value of *M. anisopliae* when treated on grubs of *C. sordidus* was found to be 2.73 days at 10^8 spores mL^{-1} while *B. bassiana* and *P. lilacinum* showed an LT_{50} of 4.01 and 5.24 days respectively at same concentration.

Lethal concentration to kill 50 per cent and 90 per cent grubs were calculated by probit analysis. LC_{50} value of *B. bassiana* was found to be lowest among the three fungi *ie*, 2×10^6 spores mL^{-1} at seven days after treatment. LC_{50} of *M. anisopliae* was found to be 5.5×10^6 spores mL^{-1}. LC_{90} was found to be lowest for *M. anisopliae* (5.5×10^7 spores mL^{-1}). Comaparing the performance of all the fungi in laboratory conditions, *M. anisopliae* was found to be highly virulent with lowest LT_{50} and LC_{90} value.

From the results obtained from the pot culture studies for evaluating the potential of these fungi when applied as rhizome dip method, rhizome damage observed in the treatment with spore suspension of *M. anisopliae* 30 g L^{-1} @ 10^8 spores mL^{-1} was least (10.83 per cent) and was the best treatment. Spore suspension of *B. bassiana* recorded 24.99 per cent damage and that of *P. lilacinum* caused 49.99 per cent damage. Number of tunnels observed in the treatment with spore suspension of *M. anisopliae* was 0.49 and was the least compared to all the treatments. When taking into consideration, the number of immature stages were found to be least in treatment with spore suspension of *M. anisopliae* @10^8 spores mL^{-1}.

In prophylactic soil drenching, spore suspension and talc formulation of *M. anisopliae* @ 10^8 spores mL^{-1} was found with least rhizome damage of 14.16 per cent and were equally effective. The number of tunnels and immature stages were also found to be statistically on par in these treatments. Rhizome damage observed in treatment with talc formulation and spores suspension of *B. bassiana* and *P. liacinum* was statistically on par.

In the third method of application *ie,* curative soil drenching, *M. anisopliae* applied as spore suspension recorded 10 per cent damage in rhizome while its talc formulation caused 12.49 per cent damage. Number of immature stages and tunnels observed in the treatment with talc formulation and spore suspension of *M. anisopliae* were statistically on par. *M. anisopliae* and *B. bassiana* applied as talc formulation recorded similar rhizome damage and were statistically on par.

Among the three methods, curative method of application of talc formulation of *M. anisopliae* and *B. bassiana* @ 30 g L^{-1} was found to be most effective with least rhizome damage, number of tunnels and immature stages. Number of dead adults obtained from the treatment with *M. anisopliae* as curative soil drenching was more than that obtained from other methods.

In field conditions, the performance of entomopathogenic fungi to control *C. sordidus* has a great future because of the possibility of horizontal transmission of fungal spores between the adults due to its gregarious life cycle. Moreover, there is endophytic association of these fungi with rhizome and roots of banana. Special delivery mechanisms of *M. anisopliae* and *B. bassiana* should be developed for the management of the grubs and pupae since it is present inside the rhizomes.

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ENTOMOPATHOGENIC FUNGI FOR THE MANAGEMENT OF BANANA RHIZOME WEEVIL (*Cosmopolites sordidus* Germer)

by

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ABSTRACT

The study entitled "entomopathogenic fungi for the management of banana rhizome weevil, *Cosmopolites sordidus* Germer" was conducted at College of Agriculture, Vellayani during 2015-17. Objective of the study was to evaluate the biocontrol potential of entomopathogenic fungi for the management of banana rhizome weevil. Adults and grubs of the rhizome weevil were collected from Thiruvananthapuram district and were observed for infestation by entomopathogenic fungi. From the infected adult weevil, *Fusarium solani* (Mart.) Sacc. was isolated and its pathogenicity was proved on adults and grubs of *C. sordidus.*

Pathogenicity studies with entomopathogenic fungi *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ma4), *Beauveria bassiana* (Balsamo) Vuillemin (Bb5), *Beauveria bassiana* (ITCC6063), *Purpureocillium lilacinum* (Thorn) Samson (ITCC6064) and *F. solani* were carried out on adults and grubs of *C. sordidus*. Grubs of *C. sordidus* treated with *M. anisoliae* (Ma4) caused mortality at 24 Hours after treatment (HAT) and white mycelial growth initiated two days after death which later changed to green color. In case of adults, mortality occurred 72 HAT. *B. bassiana* treated on grubs of *C. sordidus* caused mortality at 24 HAT and developed pink color after three days and later white cottony mycelial growth was observed on body. On adults, death occurred 72 HAT and extensive mycelial growth covered the body except the dorsal elytra after four days of treatment. *P. lilacinum* when treated on grubs caused mortality at 48 HAT and produced pink color one day after death and white mycelia covered the body. In adults, mortality initiated four days after treatment and extensive mycelial growth was observed on dorsal and ventral side. At 48 HAT, *F. solani* caused mortality of grubs of *C. sordidus* and produced brown color on shrunken grubs which later changed to creamy white stiff grubs. All the tested fungi were found to be pathogenic to both adults and grubs of *C. sordidus.*

Under laboratory conditions the effective spore concentrations of the fungi were found out by testing different concentrations on adults and grubs. *M. anisopliae* (Ma4) @ 1.29×10^8 spores mL^{-1}recorded 100 per cent mortality of grubs at 168 HAT. On adults *M. anisopliae* @ 2.1×10^8 spores mL^{-1} recorded 35 per cent mortality. *B. bassiana* (Bb5) @ 1.2×10^8 spores mL^{-1} when treated on grubs caused 95 per cent mortality at 216 HAT. Whereas in adults, *B. bassiana* caused 40 per cent mortality @ 2.5×10^8 spores mL^{-1}. *P. lilacinum* @ 1.9×10^8 spores mL^{-1} recorded 85 per cent mortality at 192 HAT when treated on grubs. At a spore concentration of 2.1×10^8 spores mL^{-1} *P. lilacinum* caused 30 per cent mortality. *B. bassiana* (ITCC6063) and *F. solani* caused less than 50 per cent mortality of both grubs and adults of *C. sordidus.*

Evaluation of entomopathogenic fungi for the management of *C. sordidus* under field conditions was done by conducting three pot culture experiments; *viz.* rhizome dip method, prophylactic soil drenching and curative soil drenching. The study revealed that spore suspension of *M. anisopliae* @ 10^8 spores mL^{-1} when applied as rhizome dip method was more effective with least damage in rhizomes (10.83 per cent), lowest number of tunnels (0.49) and the least number of live immature stages (0). In the prophylactic soil drenching, spore suspension of *M. anisopliae* @ 10^8 spores mL^{-1} was found to be effective with least rhizome damage (14.16 per cent), number of tunnels (1.08) and number of live immature stages (1.25) followed by its talc formulation with a rhizome damage, number of tunnels, and immature stages of 14.16 per cent, 1.16 and 0.66 respectively. In curative soil drenching, spore suspension of *M. anisopliae* recorded least rhizome damage (10 per cent) and was statistically on par with talc formulation of *M. anisopliae* and *B. bassiana* @30 g L^{-1} (12.49 per cent). Soil drenching given as curative treatment was more effective than the prophylactic method. Talc formulation of Ma4 and Bb5 was equally effective when applied as curative soil drenching.

To conclude, isolate *F. solani* was isolated from the cadaver of *C. sordidus*. *F. solani*, NBAIR isolates *viz*. *M. anisopliae* (Ma4) and *Beauveria bassiana* (Bb5) and local isolates available in department of Agricultural Entomology *B. bassiana* (ITCC6064), *P. lilacinum* (ITCC6064), were identified to be pathogenic to grubs and adults of *C. sordidus*. In laboratory conditions, *M. anisopliae* @ 10^8 spores mL^{-1} was found to cause 100 per cent mortality of grubs. Curative application by soil drenching of talc formulation of *M. anisopliae* and *B. bassiana* 10^8 spores mL^{-1} @ 30g L^{-1} was found to be effective for the manangement of *C. sordidus*.