MANAGEMENT OF BANANA PSEUDOSTEM WEEVIL *Odoiporus longicollis* Oliv. USING ENTOMOPATHOGENIC FUNGI

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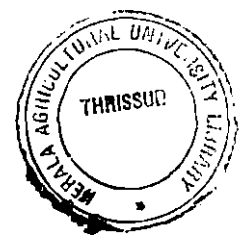
Thesis submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture



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2005

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DECLARATION

I hereby declare that this thesis entitled "**Management of banana pseudostem weevil *Odoiporus longicollis* Oliv. using entomopathogenic fungi**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award 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 "**Management of banana pseudostem weevil *Odoiporus longicollis* Oliv. using entomopathogenic fungi**" is a record of research work done independently by Ms. Sahi Beegum, M.K. (2003-11-08) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Dedicated to
My Beloved Parents

ACKNOWLEDGEMENT

In the name of God, most gracious, most merciful.

I wish to express my deep sense of indebtedness and gratitude to Dr. N. Anitha, Assistant Professor, Department of Agricultural Entomology and Chairperson of my Advisory Committee for her sincere guidance and encouragement throughout the course of this investigation and in the preparation of this thesis.

I extend my heartfelt gratitude to Dr. T. Nalinakumari, Professor and Head, Department of Agricultural Entomology for her valuable suggestions, constant inspiration and help rendered throughout the course of study.

I express my sincere thanks to Dr. K. Sudharma, Associate Professor, Department of Agricultural Entomology and member of my Advisory Committee for her valuable help rendered throughout the period of investigation.

I am deeply indebted to Dr. A. Naseema, Associate Professor, Department of Plant Pathology and member of my Advisory Committee for her constructive suggestions, timely help and encouragement during the research work and the preparation of this thesis.

I am grateful to Dr. Thomas Biju Mathew for providing me the facilities for taking photographs.

I sincerely thank Dr. Vijayaraghavakumar, Associate Professor, Department of Agricultural Statistics and Mr. C.E. Ajithkumar, Programmer for the valuable help extended in statistical analysis and interpretation of data.

It is a pleasure to keep in mind the affection, help and constant encouragement rendered by beloved teachers in the Department of Agricultural Entomology.

I am also thankful to Sheena, Jayakumar and Lekha for the help rendered in taking photographs.

I thankfully acknowledge the invaluable company given by Praveena chechi, Rani chechi and Ambily chechi for their constant inspiration and help.

I am indebted to my friends, Vidya, Shaiju, Manu, Asha, Nanditha, Abhilsh, Chitra, Anjana, Priya, Jyosna, Smitha and Thamilvel.

Words fail to express my gratitude and love to Vappa, Umma, Shami ittha, Shiras ikka, Najma mol, Vava, and Shaji itha Azim ikka and Ajmal mon, for the overwhelming love, care, encouragement and inspiration given to me throughout the course of study. Their prayers, love and expectations acted as a source of inspiration to complete this work in time.

It is my pleasure to express my thanks to Biju, P., ARDRA for prompt and skillful typing, co-operation and patience showed in the preparation of this thesis.

The supply of culture needed for the study by CPCRI, Kayamkulam and PDBC, Bangalore are greatly acknowledged.

I am grateful to Sanjay K. Singh, Scientist, Mycology and Plant Pathology, Agharkar Research Institute, Pune for identification of culture.

The awarding of PG fellowship by Kerala Agricultural University is gratefully acknowledged.

Sahi Beegum, M.K.
(2003–11–08)

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LIST OF ABBREVIATIONS

%	Per cent
°C	Degree Celsius
CD	Critical difference
cm	Centimetre(s)
DAI	Days after inoculation
EC	Emulsifiable concentrate
et al.	And others
Fig.	Figure
G	Granule
g	Gram
h	Hour
kg	Kilogram
m	Metre
mg	Milligram
ml	Millilitre
mm	Millimeter
PDA	Potato dextrose agar
ppm	Parts per million
spp.	Different species
viz.	Namely
WAI	Weeks after inoculation

Introduction

1. INTRODUCTION

Banana, 'apple of the tropics' is rich in essential nutrients and vitamins. It is cultivated in Kerala in an area of 50,871 hectares with an annual production of 3,75,903 tonnes (FIB, 2004). However, there are a number of factors, which limit the production and productivity of the crop. One of the major constraints is the high incidence of pests and diseases throughout the year. Among the pests, pseudostem weevil, *Odoiporus longicollis* Oliv. is the most serious and destructive one. Ten to ninety per cent yield loss has been reported due to the pseudostem weevil infestation (Padmanabhan *et al.*, 2001).

The adult weevil causes damage to the plant by making oviposition punctures on the outer sheath and also by feeding on the pseudostem. The first visible symptom of infestation is gummy exudation from oviposition punctures. The grubs tunnel the central core of the plant and feed on the internal tissues. As feeding of grubs progreses, feeding holes are visible on the pseudostem with gummy exudates. The pseudostems of severely infested plants break and topple.

The present strategy for controlling the pest is centered on insecticides. It is necessary to evolve effective and ecologically sound alternative management tools, which can be used either alone or in combination with less hazardous insecticides for the management of the pest. In this context, a feasible approach to combat this pest is to employ effective biocontrol agents.

Among the bioagents, entomopathogenic fungi have many advantageous over chemical insecticides. They are host specific, economical and environment friendly. The major factor limiting the use of insect pathogenic fungi as biological control agent is their requirement of high humidity and temperature for germination, growth and sporulation.

These conditions are satisfied when entomopathogenic fungi are used in the case of banana pseudostem borer as the microclimate is very conducive for the fungal development around the grubs inside the bore holes. Further, frequent rainfall and humidity prevailing in Kerala offers considerable scope for the use of entomopathogenic fungi. Combining the microbial activity of entomopathogenic fungi with other interventions and technologies promises to produce both additive and synergistic results in pest management programmes.

Considering these objectives in mind a research programme was carried out

1. to screen four entomopathogenic fungi for their pathogenicity,
2. to evaluate the biocontrol potential of these fungi against banana pseudostem weevil and
3. to determine the effective doses of the fungi and methods of application for the management of the pest.

Review of Literature

2. REVIEW OF LITERATURE

The banana pseudostem weevil (*Odoiporus longicollis* Oliv.) is one of the most injurious pests of banana and plantains. This insect was originated in South and South East Asia. It is now distributed in India, Malaysia, Indonesia and Thailand and has become a great threat to banana cultivation in these countries (Valmayor *et al.*, 1994). The borer is becoming serious to banana cultivation in Tamil Nadu also (Janakiraman and Rao, 2001).

Visalakshi *et al.* (1989) reported occurrence of banana pseudostem weevil for the first time from Kerala. According to them Nendran and Red Kappa varieties of banana were highly susceptible to the pest.

Information on the nature of damage, symptoms of attack and biology of the insect are vital in evolving strategies for the management of the pest. Hence literature related to these aspects are reviewed here.

2.1 NATURE OF DAMAGE

Jayasree (1992) observed that the emerging grubs bored into the inner leaf sheath causing extensive internal riddles and tunnels. Grubs fed on the central portion of the pseudostem by tunneling towards the base as well as towards the top portion (Anitha, 2000). Padmanabhan *et al.* (2001) reported that emerging larvae fed on tissues of succulent sheath. If larvae emerged during advanced preflowering stage of the plant, the ascending flower bud and peduncle inside the pseudostem were decayed, resulting in non-emergence of flower buds.

2.2 SYMPTOMS OF ATTACK

Anitha (2000) reported that the first visible symptom of attack was the gummy exudation from the oviposition punctures on the pseudostem. Later large quantity of gummy exudates was found oozing through the

feeding holes. The infested plant showed reduction in leaf size and yellowing of lamina. According to Padmanabhan *et al.* (2001) early symptoms of attack include the presence of small pin sized holes on the stem, fibrous extrusion from the base of leaf petiole, adult weevil and gummy exudates from the holes on the pseudostem. Fruit do not develop properly in later stages of infestation.

2.3 BIOLOGY OF *ODOIPORUS LONGICOLLIS*

The adult weevil is robust, reddish brown to black in colour measuring 1.3 to 2 cm in length (Singh, 1966). The adult females deposited eggs in chambers of the outer leaf sheath. The eggs were cream in colour and cylindrical in shape with round ends. The incubation period ranged from 3 to 8 days. The grubs were apodous soft, fleshy and covered with brownish setae of different length. Larval period lasted for 23 to 46 days with five larval instars. Pupa was exarate and pale yellow in colour. Pupal period lasted for 12 - 14 days. The total life cycle was completed in about 42 days and the adult longevity was about 90 to 120 days (Dutt and Maiti, 1972; Visalakshi *et al.*, 1989; Jayasree, 1992; Anitha, 2000).

2.4 MANAGEMENT OF PSEUDOSTEM WEEVIL

2.4.1 Cultural Control

Removal of dried leaves and leaf sheath from the plants reduced the hiding place of the adult weevil and thereby reduced the infestation (Mathew *et al.*, 1997; Anitha, 2000). Removal of affected plant parts and destroy them by burning or burying in deep pits in soil (KAU, 2002).

2.4.2 Biological Control

Janakiraman (1998) reported a reduvid bug *Sycanus* sp. feeding on *O. longicollis* grubs and adults. He also reported a mite *Centrouropoda* sp. predacious on the adult weevil.

Anitha *et al.* (1998) reported *Metarhizium anisopliae* (Met.) as a potent pathogen of pseudostem weevil. *Mucor heimalis* (Wehmer) and

Fusarium solani (Martius) Sacc. were also reported from this pest (Anitha *et al.*, 1999a, 1999b). Adult weevils of *O. longicollis* were found naturally infected with entomopathogenic fungi, such as *M. anisopliae, Beauveria bassiana* (Bals.), *Scoupulariopsis brevicollis* (Sacc.) and *Aspergillus flavus* (Link.) (Padmanabhan *et al.*, 2001, 2002). Yue *et al.* (2003) also observed *M. anisopliae* as a pathogen of the banana pseudostem weevil.

2.4.3 Chemical Control

Application of granular insecticides like phorate or carbofuran @ 25 g plant^{-1} at the basal region was found effective in controlling the pest (Visalakshi *et al.*, 1989). Carbofuran 3G at 6 g per banana pseudostem trap and benfuracarb 3G gave effective control of banana weevils (Wijesekara and Menike, 1991). Reghunath *et al.* (1992) reported that in heavily infested plants, application of aldrin (0.1 %) could bring the highest recovery of plants from infestation. The three insecticides aldrin, HCH and monocrotophos proved effective in preventing infestation in apparently healthy plant also.

Smearing the pseudostem of banana with mud slurry containing 0.25 per cent carbaryl, 0.1 per cent chlorpyriphos or 0.5 per cent neem oil was recommended for the management of the weevil (Abraham and Thomas, 1995). Mathew *et al.* (1997) reported that swabbing chlorpyriphos (0.05 per cent) was the best treatment for controlling the weevil and that carbaryl 0.5 per cent in mud slurry and 0.1 per cent carbaryl spray on the pseudostem and the leaf axils, injecting chlorpyriphos or cypermethrin through the bore holes were also effective. Monocrotophos injected into the pseudostem of infected plants during flowering caused death of grubs and adults inside the plants (Janakiraman and Rao, 2001).

2.4.4 Use of Botanicals

Padmanabhan *et al.* (2001) reported plant products *viz.*, leaf extract of *Vitex negundo* L., seed extract of *Terminalis chebula* L. and rhizome

extract of *Acorus calamus* L. against adult weevils of *O. longicollis*. These extract could be sprayed over pseudostem to prevent oviposition of the stem weevil.

2.5 MANAGEMENT OF PESTS USING ENTOMOPATHOGENIC FUNGI

The use of entomopathogenic fungi in the management of pests is gaining momentum in many countries with a view to reduce environmental pollution and health hazards. They are important regulating factors of insect population and many of them are used as biocontrol agents of insect pest (Lancey *et al.*, 2001).

Among the microbial pathogens, fungi are the only group, which can invade the insects body actively through the cuticle. Nearly 700 species of fungi under 90 genera are known to be entomopathogens (Charnley, 1989). These fungi have a wide host range. But many workers have reported the species specificity of entomopathogenic fungi (Goettel *et al.*, 1990).

Literarure related to four fungi viz., *Metarhizium anisopliae* (Metsch.) Sorokin, *Beauveria bassiana* (Balsamo) Vuillemin, *Paecilomyces lilacinus* (Thom.) Samson, *Nomuraea rileyi* (Farlow) Samson are reviewed here.

2.5.1 *Metarhizium anisopliae* (Metsch.) Sorokin

M. anisopliae is an imperfect entomopathogenic fungi found in soil throughout the world. It was first recognized as a biocontrol agent in 1879 (Metschnikoff, 1879).

2.5.1.1 Pathogenicity

Pathogenicity of *M. anisopliae* was reported against sweet potato weevil *Cylas formicarius* (Fabr.) (Khan *et al.*, 1990) and mulberry brown chaffer, *Holotrichia paralella* (Fabr.) (Chen *et al.*, 1995). *M. anisopliae* was found to cause disease in white grubs in field condition (Singh *et al.*,

2003). Sujatha and Rao (2004) reported natural occurrence of *M. anisopliae* on the grubs of Rhinocerous beetle.

Zimmerman (1993) reported the pathogenicity of *M. anisopliae* against *Moharva posticata* Lin. and *Nilaparvata lugens* (Stal.).

Gopalakrishnan and Narayanan (1988) isolated *M. anisopliae* from mature larvae of noctuid *Heliothis armigera* (Hubner). Sujatha *et al.* (2002) reported that 19.9 per cent of mango fruit borer larvae (*Deanolis albizonalis* Hampson) were infected naturally with *M. anisopliae*. The fungus exhibited pathogenicity to larval instars of the teak defoliator *Hyblaea puera* (Cramer) (Kalia and Harsh, 2003). Occurrence of *M. anisopliae* as natural enemy of semilooper *Thysanoplusia orichalcea* (Fab.) in sunflower ecosystem was reported by Revanna *et al.* (2003).

2.5.1.2 Spore Characters

M. anisopliae produced dark green columns of cylindrical conidia from closely packed and parallelly oriented phialides (Hammill, 1981). Conidia of *M. anisopliae* were green in colour cylindrical and aseptate. The conidiophores developed as lateral and terminal branches of hyphae (Anitha, 2000).

2.5.1.3 Symptoms of Attack

M. anisopliae on grubs and adults of pseudostem weevil, caused sluggish movement and reduced feeding. Immediately after death the body of infected grub became soft and later turned hard. White mycelial mat appeared all over the body of the grubs, later colour changed to green (Anitha, 2000). Rachappa *et al.* (2001) described the pathogenincity of *M. anisopliae* to coconut black headed caterpillar. They found that four days after treatment white mycelial mat appeared all over the cadavers and on sporulation the colour turned to green.

2.5.1.4 Bioassay

M. anisopliae at a concentration of 2.5 x 10^6 spores g^{-1} under *in vitro* conditions caused 100 per cent mortality of *Holotrichia paralella* (Fabr.) (Chen *et al.*, 1995). Anitha (2000) reported *M. anisopliae* at 10 x 10^5 spores ml^{-1} caused 98.67 per cent mortality in early instars of banana pseudostem weevil. Adult weevil when treated with 15 x 10^5 spores ml^{-1}, caused 96 per cent mortality. Rajendran (2002) reported that *M. anisopliae* at 10^8 and 10^{10} conidia ml^{-1} caused 50 and 65 per cent mortality respectively in the first instar grubs of egg plant spotted beetle during the fifth day of treatment.

Forty to fifty per cent mortality of third instar larvae of cabbage root fly *Delia floralis* (Fall) was observed when exposed to 1.5 x 10^{10} spores ml^{-1} of *M. anisopliae* (Vanninen *et al.*, 1999).

The adults and nymphs of coconut coried bug when sprayed with *M. anisopliae* at 3.2 x 10^6 spores ml^{-1} caused cent per cent mortality in nymphs and 96 per cent mortality in adults (Mohan *et al.*, 2001).

Wickramatileke *et al.* (2000) reported that spore concentration of 40 x 10^8 spores ml^{-1} caused 92 per cent mortality of 3-5 day old larvae of cabbage semilooper. *M. anisopliae* at 1.2 x 10^7 spores ml^{-1} proved pathogenic to coconut black headed caterpillar and caused 80 to 90 per cent mortality (Rachappa *et al.*, 2001). At a spore concentration of 5 x 10^7 conidia ml^{-1}, it caused 55 per cent egg mortality of *Spodoptera litura* (Pandey, 2003).

2.5.1.5 Mass Production

Daoust and Roberts (1983) observed rice medium as a better and cheaper substrate for the production of conidia of *M. anisopliae*. Coconut water was identified as the best liquid medium for mass production of this fungus (Danger *et al.*, 1991). Alvarez *et al.* (1997) reported sterile humid rice as the best substrate for growing *M. anisopliae*.

2.5.1.6 Factors Affecting Storage and Pathogenicity

The spores of *M. anisopliae* remained viable for 12 months at 8°C but lost the viability after 0.5 months at 21°C (Walstad *et al.*, 1970). Different isolates of *M. anisopliae* were evaluated for their temperature and pH requirement and found that temperature of 28°C and pH level of 6.5 were most favourable for growth and sporulation of *M. anisopliae* (Sharma *et al.*, 1998, 2002).

2.5.1.7 Formulations

M. anisopliae prepared as pellets by drying the fungus cells cultured and optimized in fermenters. The dried product named BIO 1020 consists of granules with diameter 0.5 to 1 mm (Andersch, 1992). *M. anisopliae* produced on sorghum grain were formulated on attapulgite, bentomite, clay, talc, wheat flour and maize flour (Pandey and Kanoujia, 2003). The conidia in the oil formulation (7 : 3 diesel : Sunflower oil) were found to be most effective in controlling *H. armigera* (Nahar *et al.*, 2004).

2.5.1.8 Effect on Non-target Organisms

Elkadi *et al.* (1983) carried out safety test in guinea pigs and mice with *M. anisopliae,* the fungus was administered by ingestion, inhalation cutaneous and subcutaneous injection. Anatomical and histopthological examination showed that the fungus was neither toxic nor pathogenic to test organisms. When the fungus *M. anisopliae* applied by different methods to birds, fish, mite, rats, guinea pigs or rabbit, no pathological or toxicological symptoms was observed (Zimmerman, 1993). Ivashchenko and Dolgushina (2002) noticed that *M. anisopliae* did not suppress indigenous soil microflora compared to insecticides.

The fungus was also safe to predators like *Cyrtorhinus lividipennis,* spiders, coccinellid beetle and parasitoids (Rao, 1989). Jeevanand and Kannan (1995) evaluated the mammalian toxicity of this

biocontrol agent and found that the fungus did not cause any change in appearance and behaviour of albino rats even 21 days after administration.

2.5.1.9 Method of Application

Soil application of *M. anisopliae* gave 45 per cent mortality of *Agrotis segetum* (Schiff.) at 1.2 x 10^7 spores g^{-1} of soil (Patel *et al.*, 1988). Anitha (2000) described application of *M. anisopliae* spore suspension as leaf axil filling @ 1 litre $plant^{-1}$ for the management of *O. longicollis*. Saminathan *et al.* (2004) noticed that pouring spore suspension through bore holes was effective compared to soil application and swabbing of fungal spores of *M. anisopliae* on cashew for the control of stem and root borer.

2.5.1.10 Compatibility with Pesticides

Carbaryl at 0.1, 1, 10, 100 and 1000 ppm inhibited the germination of the conidia of the entomopathogenic fungus *M. anisopliae* (Aguda *et al.*, 1988). Filho and Lamas (2001) found that endosulfan, monocrotophos and deltamethrin adversely affected the growth and sporulation of *M. anisopliae* whereas thiomethoxam was compatible with the fungus. Fungicidal effect of glyphosate on *M. anisopliae* were evaluated and the result showed that the fungus was susceptible to various glyphosate formulations (Morjan *et al.*, 2002).

2.5.1.11 Field Efficacy

Application of *M.anisopliae* inoculum to *Oryctes* breeding sites caused 83 per cent infection of *Oryctes* population (Tey and Ho, 1995). The infection of *Conotrachelus humeropictus* (Fielder) by *M. anisopliae* was evaluated under field condition and a spore suspension of 9.93 x 10^{10} conidia ml^{-1} caused 42 per cent mortality (Mendes *et al.*, 2001).

2.5.2 *Beauveria bassiana* (Balsamo) Vuillemin

B. bassiana was one of the first organism linked to mycosis of the silkworm in early nineteenth century. Since that time this fungus had been recorded from approximately 175 host insects (Roberts, 1973).

2.5.2.1 Pathogenicity

B. bassiana was reported as an efficient pathogen of *Epilachna vigintioctopunctata* (Fabr) (Klochko, 1969), *Leptinotarsa decemlineata* (Say.) (Ignoffo *et al.*, 1983), *Cylas formicarius* (Boh.) and *Cosmopolites sordidus* (Diaz *et al.*, 1986). Filho *et al.* (1989) and Kaya *et al.* (1993) reported *B. bassiana* as potent biocontrol agent of banana rhizome weevil. The natural occurrence of *B. bassiana* was reported in rice hispa (Puzari and Hazarika, 1994), cowpea leaf beetle (Ekesi, 2000) and mango stone weevil (Verghese *et al.*, 2003).

The fungus was highly pathogenic to adults of *Helopeltis theobromae* (Wat.) (Lim *et al.*, 1989). Adults and nymphs of green leaf hopper were also found infected with *B. bassiana* (Narayanasamy, 1999).

The pathogenic effect of *B. bassiana* was noticed against workers of the termite, *Odontotermes obesus* (Rambur) (Gurusubramanian *et al.*, 1999) and black ant, *Componotes compresses* Fab. (Sreeramuli *et al.*, 1999).

Pathogenicity of *B. bassiana* was reported in *Chilo infuscatellus* (Snellen) (Sivasankaran *et al.*, 1990), *Heliothis armigera* Hub. (Gowda and Prasad, 1992), sugarcane root borer (Easwaramoorthy and Santhalakshmi, 1993), mango fruit borer, *Deanolis albizonalis* Hampson (Sujatha *et al.*, 2002), *Thysanoplusia orichalcea* (Revanna *et al.*, 2003), *Anomis flava* Fab. (Mohapatra and Sahu, 2004) and rice stem borer and leaf folder (Joshi *et al.*, 2004).

Shivaprakash *et al.* (2004) reported pathogenicity of *B. bassiana* against red spider mite. They recorded for the first time in India the natural incidence of this fungus against red spider mite.

2.5.2.2 Spore Character

Gopalakrishnan and Narayanan (1988) reported that *B. bassiana* spores were muscardine in colour and globose in shape, measuring 1.7 to 2.5 µm. The same characters were described by Verghese *et al.* (2003) also.

2.5.2.3 Symptoms of Attack

The sugarcane root borer larvae infected by *B. bassiana* became hard and brittle. The body showed pinkish discolouration in initial stages of infection and was covered with fluffy fungal mat in the advanced stages (Easwaramoorthy and Santhalakshmi, 1993). Infection of *B. bassiana* on termite caused body surface shrinkage, colour change, hardened and brittle appendages. Later stage mycelial growth was recorded on joints of legs, abdomen, thorax and head region (Gurusubramanian *et al.*, 1999). Simon (2002) reported that *B. bassiana* infected *Spodoptera litura* (Fabr.) larvae became restless, unstable and showed ceased feeding. At death the body became tough and mummified, white mycelial growth was visible all over the cadaver. Same fungus was found associated with cocoon of red palm weevil also. The infected cocoon had a layer of fungal growth and the malformed adults which emerged did not survive (Simon *et al.*, 2003).

2.5.2.4 Bioassay

Leaf surface treatment for determining the activity of *B. bassiana* against *L. decemlineata* revealed that 200, 16.25 and 0.75 kg conidia ha^{-1} caused 99, 90 and 50 per cent mortality of larvae respectively (Ignoffo *et al.*, 1983). Schoeman and Schoeman (1999) observed 100 per cent mortality of banana rhizome weevil treated with *B. bassiana* within a period of six weeks in the laboratory. Khan and Gangaprasad (2001) reported LC_{50}

value of 4.5 x 10^7 spores ml^{-1} against *C. sordidus*. The fungus at 1.3 x 10^9 spores ml^{-1} caused cent per cent mortality of mango stone weevil in 2-7 days of incubation period (Verghese *et al.*, 2003). In white grub *Cyclocephala signaticolis* Burm, *B. bassiana* caused 70 per cent mortality at 1 x 10^8 spores ml^{-1} (Beron *et al.*, 2005).

Fabio *et al.* (2003) reported that *B. bassiana* at 1 x 10^7 spores ml^{-1} spore load caused 77.5 per cent mortality of fifth instar nymph of *Rhodnius pallenscens*. Fagade *et al.* (2004) reported LC_{50} of 10^7 spores ml^{-1} against grasshopper.

Seventy six per cent mortality of larvae of *C. infuscatellus* was obtained when *B. bassiana* at 10^7 spores ml^{-1} was sprayed on them (Sivasankaran *et al.*, 1990). Hafez *et al.* (1994) conducted bioassay using *B. bassiana* against *Phthorimaea operculella* Seller. and they obtained LC_{50} value of 4.7 x 10^8 conidia ml^{-1}. The percentage larval mortality was higher in *H. armigera* larvae at 10^9 spores ml^{-1} (Manjula and Padmavathamma, 1999). It caused more than 95 per cent mortality of *Plutella xylostella* Linn. at 10^7 conidia ml^{-1} (Masuda, 2000). Simon (2002) reported LC_{50} of 7.87 x 10^8 and 5.75 x 10^8 spores ml^{-1} against fourth instar larvae of *Sylepta derogata* (Fabr.) on eight and nine days after inoculation respectively. She also reported that the spore load of 5.56 x 10^5 spores ml^{-1} caused 50 per cent mortality on third instar larvae of *Pericallia ricini* F. The fungus at 10^7 spores ml^{-1} caused 86 per cent mortality of the larvae of *Heortia vitessoides* Moore (Barman and Nath, 2002) and 49 to 61 per cent egg mortality of *S. litura* Fabr. at 5 x 10^7 conidia ml^{-1} (Pandey, 2003).

2.5.2.5 Mass Production

Filho *et al.* (1989) observed better conidial production of *B. bassiana* in bran broth compared to rice and potato broth. Growth and sporulation of *B. bassiana* was maximum on rice among the different solid media tested (Ibrahim and Low, 1993). They identified coconut water as the best liquid medium for mass production of *B. bassiana*. A mixture of rice hull, saw

dust and rice bran in the ratio 75 : 25 : 100 to be an ideal substrate for mass production of *B. bassiana* (Puzari *et al.*, 1997). Molasses and yeast broth produced 1 x 10^9 spores ml^{-1} (Sharma *et al.*, 2002). Chaudhuri *et al.* (2001) tested various substrate including grains and agricultural byproducts for mass multiplication of *B. bassiana* and based on biomass production and viability of conidia, sorghum grain was found to be the best substrate to support growth and sporulation of the fungus. *B. bassiana* grew well on rice bran (fine and coarse) and wheat bran (Haraprasad *et al.*, 2001) and Sabouraud's dextrose agar medium (Verghese *et al.*, 2003) yielding maximum sporulation.

2.5.2.6 Factors Affecting Storage and Pathogenicity

The spores of *B. bassiana* stored at -13°C maintained their viability for 10 months, while the spores stored at 5°C showed a fall in viability (Filho and Cardelli, 1988). Pathogenicity test with *B. bassiana* proved that they were highly virulent and causing 100 per cent mortality of their host at 25 to 27°C and relative humidity 80 to 90 per cent (Gopalakrishnan and Narayanan, 1988). Lim *et al.* (1989) noticed that *B. bassiana* grew and sporulated well at 25°C but the growth was inhibited at temperature higher than 35°C. *B. bassiana* could grow at a temperature range of 20 to 28°C, but 25°C was best for conidial production and a pH level of 6.5 was found favourable for the growth (Sharma *et al.*, 1998, 2002). Shipp *et al.* (2003) reported that at 97.5 per cent relative humidity, *B. bassiana* caused 88 per cent infection in greenhouse pest, while 75 per cent relative humidity caused only 43 per cent infection.

2.5.2.7 Formulations

Zhang *et al.* (1992) developed a wettable powder formulation of *B. bassiana* against larvae of *Ostrinia furnacalis* Guence. This formulation when sprayed at 50 x 10^9 spores g^{-1} resulted in 95 per cent death of the target insect. Wettable powder formulation retained more than 85 per cent spore germination even after eight months under refrigerated condition. Lima

(1992) reported that the conidia of *B. bassiana* formulated as bran bait gave good control of *Schistocera gregaria* Forsk. Dry rice grain based inoculum was formulated by Maniana (1993) for controlling the maize stem borer. Chiue (1993) prepared granules of *B. bassiana* using sand and wine derivatives for controlling *O. furnacalis.* The powder formulation of *B. bassiana* was effectively used against the fire ant *Solenopsis invicta* Buren. (Oi *et al.*, 1994). For the control of pear psylla, the conidial suspension of *B. bassiana* in water was found to be very effective (Putreka *et al.*, 1994). Manjun *et al.* (2000) reported that the conidia of *B. bassiana* in oil formulation exhibited higher virulence to *P. xylostella* than conidia in water formulation. Maize based formulations of *B. bassiana* reduced the banana rhizome weevil population (Nankinga and Moore, 2000).

2.5.2.8 Effect on Non-target Organisms

Ramesh and Selvasundaram (2001) observed that *B. bassiana* spore suspension had no adverse effect on any of the predator species of tea pests.

2.5.2.9 Compatibility with Pesticides

The wettable and flowable formulations of pesticides such as Carbaryl 50 WP, Diflubenzuron 25 WP, Endosulfan 50 WP and Carbofuran caused no inhibition and often increased the colony counts whereas emulsifiable concentrate of pesticides *viz.*, Endosulfan 3 EC, Fenvalerate 2 EC, Permethrin 2 EC, frequently inhibited *B. bassiana* germination (Anderson and Roberts, 1983). Carbaryl at 0.1, 1, 10, 100 and 1000 ppm inhibited the germination of *B. bassiana* (Aguda *et al.*, 1988). Abamectin, triflumuron, thuringiensin and carbaryl showed no significant inhibition of colony growth of the fungus (Anderson *et al.*, 1989). *B. bassiana* showed better growth on medium containing 25 per cent diflubenzuron at the two lower doses while at ten times the field dose, growth was inhibited (Sapieha and Mietkiewski, 1992). Simon (2002) reported complete inhibition of growth of *B. bassiana* by dimethoate 0.05 per cent. Chlorpyriphos 0.05 per cent,

malathion 0.1 per cent and quinalphos 0.05 per cent considerably reduced the sporulation of the fungus.

Todorova *et al.* (1998) studied the effect of six fungicides (Chlorothalonil, maneb, thiophonate, mancozeb, metalaxyl + mancozeb and zineb) on *B. bassiana in vitro*. All the six fungicides inhibited the mycelial growth and sporulation. However application of fungal isolation of *B. bassiana* 2 to 4 days before applying fungicides (metalaxyl, mancozeb, copper oxcychloride) synergised the effect, while application of fungicides 2 to 4 days before applying the fungus antogonised the effect (Kouassi and Coderre, 2003). The use of α, cypermethrin and thiomethoxam were found to be compatible with entomopathogenic fungus *B. bassiana* (Oliveira *et al.*, 2003).

Devaprasad *et al.* (1989) observed that, neem oil and neem seed kernel extract were most deleterious to the germination of conidia of *B. bassiana* whereas *Ocimum sanctum* L. and *Acorus calamus* L. were not as deleterious as the neem products. Gupta *et al.* (1999) reported that the commercial neem product like neemgold, neemmark and neemcake extract had inhibitory effect on mycelial growth of *B. bassiana*.

2.5.2.10 Field Efficacy

Badilla and Alves (1991) reported that the spore suspension of *B. bassiana* at a concentration of 4.5×10^{11} conidia per ml resulted 92.3 per cent mortality of sugarcane weevil under field condition. *B. bassiana* at 10^7 spores ml^{-1} caused considerable reduction in the population of *Maruca testulatis* on redgram at dry land farm condition (Manjula and Padmavathamma, 1996). A spore load of 1.18×10^{10} caused 2 to 72 per cent mortality of all stages of *H. armigera* in groundnut under field condition (Rathod, 2002). Oil based formulation of indigenous fungal isolates of *B. bassiana* showed 51.25 per cent efficacy against *H. armigera* infestation on pigeon pea under field condition (Nahar *et al.*, 2004).

Purwar and Yadav (2002) reported Dispel, a formulation of *B. bassiana* at 1 ml ha^{-1} gave 100 per cent mortality of leaf folder on soyabean one day after treatment under field condition. *B. bassiana* at 6 g / tree recorded control of coffee berry borer (Saravanan and Chozhan, 2003).

2.5.3 *Paecilomyces lilacinus* (Thom.) Samson

P. lilacinus is a soil inhabiting fungus that is capable of parasitizing nematode eggs, juveniles and females and reducing soil populations of parasitic nematodes. It has been found infecting a wide range of hosts (Samson, 1974). It was first discovered in soil and observed to control root-knot nematodes on potato in Peru (Jatala *et al.*, 1979). The fungus was commercially developed for the use against whitefly *Bemisia tabaci* Gennadius (Osborne and Landa, 1992).

2.5.4 *Nomuraea rileyi* (Farlow) Samson

N. rileyi is an important entomopathogenic fungus recorded from several lepidopteran pests (Ignoffo, 1976).

2.5.4.1 Pathogenicity

Natural epizootics caused by the mitosporic fungus *N. rileyi* frequently arise in field population of lepidopteran pest (Thorvilson and Pedigo, 1984). *Acontia groellsii* Westw. was found naturally infected with *N. rileyi* (Gopalakrishnan and Narayanan, 1988).

Natural incidence of *N. rileyi* was reported on *H. armigera* (Men *et al.*, 1990; Manjula *et al.*, 2004), *S. litura* (Patil *et al.*, 2003) and cotton semilooper *Anomis flava* (Fab.) (Mohapatra and Sahu, 2004). It was recorded as potential natural control agent of *Nephopetryx eugraphella* Rag. infesting Chiku (Ingle *et al.*, 2004).

5.2.4.2 Spore Character

The spore of *N. rileyi* were pale green in colour and ellipsoidal in shape measuring 3 to 4.5 µm (Gopalakrishnan and Narayanan, 1988). Conidia is malachite green in colour and conidiophore bearing dense branches. Conidia broadly ellipsoidal to cylindrical (Mohapatara and Sahu, 2004).

2.5.4.3 Symptoms of Attack

The larvae of cotton semilooper infected with *N. rileyi* was covered with fluffy greenish powdery growth and the cadaver became hard and brittle (Mohapatra and Sahu, 2004). Infected larvae of *N. eugraphella* showed the symptom as loss of sensitivity, mummification and toughness with white mycelial growth from the posterior end which subsequently covering the entire body which later turned greenish due to sporulation (Ingle *et al.*, 2004).

2.5.4.4 Bioassay

N.rileyi at 5 x 10^4 spores ml^{-1} of *N. rileyi* caused 100 per cent mortality of *S. exigua* Hub. (Lopes and Boucias, 1994) and *S. frugiperda* Hub. (Lerana *et al.*, 1994). Spore suspension of 10^7 ml^{-1} caused 86 per cent mortality in *N eugraphella* (Ingle *et al.*, 2004). Manjula *et al.* (2004) reported 80 per cent mortality of larvae of *S. litura* with highest concentration of *N. rileyi* (10^9 spores ml^{-1}).

2.5.4.5 Mass Production

N. rileyi grew well and sporulated profusely on Sabouraud's Maltose Agar enriched with one per cent yeast (Gopalakrishnan and Narayanan, 1988). Julio *et al.* (2004) reported that medium with potato and yeast extract induced highest growth rate for *N. rileyi.*

2.5.4.6 Effect on Non-target Organisms

Parasites attacking *H. armigera* larvae and a spider was found susceptible to entomogenous fungus *N. rileyi* (Powers *et al.*, 1986).

2.5.4.7 Compatibility with Pesticides

Ignoffo *et al.* (1975) found that pesticide recommended for use in soybean, suppressed the growth of *N. rileyi* at one tenth of the recommended concentration. *In vitro* compatibility of *N. rileyi* with cypermethrin, dimethoate, monocrotophos and endosulfan was tested at recommended rate and also ten times recommended rate. Sporulation on surface was more than 75 to100 per cent area in all the treatments (Devi *et al.*, 2002).

Carbendazim exhibited 75.85 per cent inhibition of *N. rileyi* (Kulkarni and Lingappa, 2001) and various glyphosate formulations were also inhibited the fungal growth (Morjan *et al.*, 2002).

Neem seed kernel extract and *Vitex nigundo* enhanced bioefficacy of *N. rileyi* against *S. litura* (Patil *et al.*, 2002).

2.5.4.8 Field Efficacy

The field performance of *N. rileyi* at 2 x 10^8 conidia l^{-1} against soybean pests, *S. litura* and *H. armigera* was conducted. The larval population was reduced to 28 to 62 per cent within 10 days (Lingappa *et al.*, 2002). The fungus effectively controlled *H. armigera* in pigeon pea field and caused 60.8 per cent mortality (Nahar *et al.*, 2004).

Materials and Methods

3. MATERIALS AND METHODS

The experiment on the "Management of banana pseudostem weevil, *Odoiporus longicollis* Oliv. using entomopathogenic fungi" was carried out in the Department of Agricultural Entomology, College of Agriculture, Vellayani during 2004-2005.

3.1 SCREENING OF ENTOMOPATHOGENIC FUNGI

3.1.1 Maintenance of stock culture of *O. longicollis*

Grubs of the pseudostem weevil *O. longicollis* collected from the field were introduced into banana pseudostem pieces of 10 cm length placed inside glass troughs. The pseudostem pieces were replaced with new ones, once in three days. After pupation cocoons were collected and kept in glass troughs covered with wet muslin cloth for adult emergence. Newly emerged adults were collected and kept on pseudostem pieces for egg laying. The eggs laid in the air chambers of the sheath were carefully collected and the grubs emerged were reared separately and kept as stock culture. Grubs and adults were collected from this stock culture for further studies.

A few of the grubs and adults collected from the field were found infected with a fungal pathogen, which was isolated and maintained in Potato Dextrose Agar slants. The fungus was sent for identification to the Agharkar Research Institute, Pune.

3.1.2 Pathogenicity Test

Cultures of *Beauveria bassiana* , *Paecilomyces lilacinus* and *Nomuraea rileyi* were obtained from PDBC, Bangalore and *Metarhizium anisopliae* from CPCRI , Kayamkulam.

Pathogenicity test were conducted under laboratory conditions against grubs and adults of banana pseudostem weevil using spore suspension of the following fungi.

1. *Metarhizium anisopliae*
2. *Beauveria bassiana*
3. *Paecilomyces lilacinus*
4. *Nomuraea rileyi*

Each fungi was grown on Potato Dextrose Agar (PDA) slants seperately. Sterile distilled water (5 ml) was added to the sporulated medium. The spore suspension was sprayed on the second instar grubs and adults collected from the stock culture using an atomizer. The experiment was replicated four times for each fungi, and ten test insects were used in each replication. Second instar grubs and adults treated with sterile water served as control. After 20 minutes, treated insects were transferred to fresh pseudostem pieces placed in glass troughs covered with wet muslin cloth. The treated insects were examined daily for their feeding behaviour, morphological changes and mortality. Dead insects were transferred to a petriplates containing moist filter paper and observed for the presence of mycelial growth on the cadavers. Pathogenicity was further confirmed by proving Koch's postulate (Aneja, 1996).

3.1.3 Maintenance of Fungal Culture

The cultures of the two fungi selected from the pathogenicity test ie. *M. anisopliae, B. bassiana* were maintained on PDA slants for further studies. The virulence of the fungi was maintained by inoculating them periodically through pseudostem weevil grubs and reisolating them afresh.

3.2 AGE SUSCEPTIBILITY OF GRUBS

Experiments were conducted with first, second, third, fourth and fifth instar larvae of *O. longicollis* in completely randomized design with four replications. The test insects were sprayed with spore suspension of

M.anisopliae and *B.bassiana.* Ten grubs of each instar were taken for each replications. Mortality counts were taken at an interval of 24 hours. The data were analysed statistically (Panse and Sukhatme, 1978).

3.3 DETERMINATION OF EFFECTIVE DOSES

Seven day old fungal cultures of *M.anisopliae* and *B.bassiana* maintained in PDA were used for the experiment. For preparing different concentrations of spore suspension of the test fungi, stock suspension was prepared initially. For this, 5 mm diameter discs were placed in 10 ml of water and the suspension was shaken well for two minutes and filtered through muslin cloth. From the filtered spore suspension one ml was taken out and spore count was estimated using a haemocytometer. Required concentrations were prepared either by adding fungal discs or by adding sterile water.

Table 1.Spore concentrations of *M. anisopliae* and *B. bassiana* used for standardization of effective doses

Treatments	Fungi tested	
	M. anisopliae	*B. bassiana*
T_1	1.6×10^7 spores ml^{-1}	1.8×10^7 spores ml^{-1}
T_2	1.6×10^6 spores ml^{-1}	1.8×10^6 spores ml^{-1}
T_3	1.6×10^5 spores ml^{-1}	1.8×10^5 spores ml^{-1}
T_4	1.6×10^4 spores ml^{-1}	1.8×10^4 spores ml^{-1}
T_5	1.6×10^3 spores ml^{-1}	1.8×10^3 spores ml^{-1}
T_6	Water	Water

Ten second instar grubs were sprayed with each concentration of the fungus using an atomizer. The experiment was conducted in a completely randomized design with four replication. Grubs sprayed with

sterile water served as control. Mortality of grubs were recorded daily. The dead insects were placed in moist chamber for the development of mycelial growth. Larvae which showed mycelial growth were considered as dead due to fungal infection. Observations were recorded till pupation. The dosage mortality data were subjected to Probit analysis (Finney, 1952).

3.4 MASS PRODUCTION AND SHELF LIFE

3.4.1 Solid Media

In order to find out suitable solid media for mass production and storage of *M. anisopliae* and *B. bassiana,* experiments were conducted in CRD with six naturally available solid substrates. The media tested were rice bran, wheat bran, gingelly oil cake, neem cake, coir pith and dried guinea grass.

Sixty gram each of the substrates were powdered, moistened with water (50 ml) and taken in 250 ml conical flasks. These substrates after sterilization (121 °C for 20 minutes) were inoculated with 5 mm discs from seven day old cultures of the test fungi grown in PDA and incubated at room temperature (28 ± 2 °C). Four replications were maintained for each treatment. The flasks were shaken daily for uniform growth of the fungi. Observation on mycelial growth and sporulation were recorded.

3.4.1.1 Mycelial Growth

The nature and extent of growth of the fungi in different substrates were estimated seven days after inoculation by visual comparison based on a score chart.

+++ Profuse growth

++ Moderate growth

\+ Slight growth

0 No growth

3.4.1.2 Sporulation

Spore counts were estimated in each medium, seven days after inoculation. One gm material was diluted in 50 ml sterile water and shaken well for two minutes. The suspension obtained was filtered through muslin cloth. From this one drop of the suspension was used for estimating the spore count using haemocytometer. The spore counts were taken at weekly intervals starting from seven days after inoculation for a period of two months. The data were subjected to statistical analysis.

3.4.2 Liquid Media

The liquid substrates tested were coconut water, rice bran extract, starch solution, raw rice water, coconut cake extract and neem cake extract. The experiments were designed in CRD with four replications. The extracts were prepared by boiling and filtering 50 g of the solid material with 400 ml water for 10 minutes. 100 ml media were taken in 250 ml conical flasks, sterilized (121 °C for 20 minutes) and inoculated with 5 mm discs of seven day old cultures of fungi grown in PDA and incubated at room temperature (28 ± 2 °C). Observations on mycelial growth and sporulation were recorded.

3.4.2.1 Mycelial Growth

The growth of the fungi in liquid media were recorded by visual comparison based on score chart as in 3.4.1.1

3.4.2.2 Sporulation

The spore counts were taken at weekly intervals starting from seven days after inoculation for a period of five weeks. The inoculated liquid medium was shaken well for one minute before taking the sample. From this one ml of the medium was pipetted out into 50 ml sterile water in 250 ml conical flasks. This suspension was used for the estimation of spore count using a haemocytometer.

3.5 VIRULENCE OF THE FUNGI IN SELECTED MEDIA

To study the effect of media on the virulence of the fungi, intensity of infection was tested at weekly intervals for one month.

3.5.1 Virulence of *M. anisopliae*

Dose 1.6×10^6 spores ml^{-1}

Design CRD

Replication 5

Two solid and two liquid media that gave maximum spore count in the previous experiments were selected for this test.

Treatments

1. Rice bran
2. Wheat bran
3. Rice bran extract
4. Coconut water

Test insect 2^{nd} instar grubs of *O. longicollis*

Inoculation of the fungus was done as described in 3.4.1 and 3.4.2.

3.5.2 Virulence of *B. bassiana*

Dose 1.8×10^7 spores ml^{-1}

Design CRD

Replication 5

Two solid and two liquid media that gave maximum spore count in the previous experiments were selected for this test.

Treatments

1. Rice bran
2. Wheat bran
3. Rice bran extract
4. Coconut water

Test insect 2nd instar grubs of *O. longicollis*

Inoculation of the fungus was done as described in 3.4.1 and 3.4.2.

Spore suspension prepared was sprayed on test insect and the symptoms of infection were recorded. Mortality counts were taken at 24 hour intervals. Treatment with sterile water served as the control. The cumulative percent mortality was analysed statistically.

3.6 EFFECT OF PESTICIDES ON GROWTH AND SPORULATION OF THE FUNGI

The experiment was aimed at testing the compatibility of *M. anisopliae* and *B. bassiana* with insecticides and fungicides used for pest management in banana.

Two insecticides and two fungicides commonly used on banana crop were evaluated for their compatibility with *M. anisopliae* and *B. bassiana* by adopting Poison food technique (Zentmeyer, 1955). The treatments used in the experiment were

T_1 – NeemAzal T/S 0.4 per cent

T_2 – Chlorpyriphos 0.03 per cent

T_3 – Mancozeb 0.3 per cent

T_4 – Copper oxychloride 0.4 per cent

T_5 – Control

Design – CRD

Replication – 4

Required concentrations of pesticide were mixed with 100 ml of sterilized PDA in 250 ml conical flask. Flasks were shaken thoroughly and the media were poured into sterile petridishes and allowed to

solidify. Circular discs (5 mm) were cut from the seven day old culture by means of sterile cork borer and placed in the centre of each petridish. Medium without pesticide inoculated with fungi served as control. Diameter of the fungal colony was measured, when maximum growth was observed in control plate. The percent inhibition over control was calculated by the formula:

$$\text{Per cent inhibition, } I = \frac{C - T}{C} \times 100$$

C – Growth in control

T – Growth in treatment

The data were analysed statistically.

3.7 FIELD EVALUATION

Effective doses of the pathogens obtained from the laboratory studies were tested under field conditions.

3.7.1 Raising of Crop

Suckers of banana variety Nendran were obtained from the Instructional Farm, College of Agriculture, Vellayani. The plants were raised in 2 m spacing, following the recommendations of the package of practices of Kerala Agricultural University (KAU, 2002) without any pesticide application, for field evaluation of the fungi.

3.7.2 Prophylactic Method

Design - RBD

Replication - 3

Treatments - 12

The experiment was conducted in Instructional Farm, College of Agriculture, Vellayani. Five month old plants were used for the study.

Five, second instar grubs of pseudostem weevil were introduced into the pseudostem three days after treatment. Mortality of grubs were recorded twenty days after treatment by destructive sampling.

Table 2. Details of prophylactic treatments with various fungi and insecticides

Treatments	Dose	Methods of application	Quantity/ plant
B. bassiana spore suspension	1.8×10^7 spores ml^{-1}	Leaf axil filling	500 ml
B. bassiana in rice bran	1.8×10^7 spores ml^{-1}	Leaf axil filling	300 g
B. bassiana spore suspension	1.8×10^7 spores ml^{-1}	Stem injection	10 ml
B. bassiana spore suspension	1.8×10^7 spores ml^{-1}	Leaf axil filling two times	500 ml
M. anisopliae spore suspension	1.6×10^6 spores ml^{-1}	Leaf axil filling	500 ml
M. anisopliae in rice bran	1.6×10^6 spores ml^{-1}	Leaf axil filling	300 g
M. anisopliae spore suspension	1.6×10^6 spores ml^{-1}	Stem injection	10 ml
M. anisopliae spore suspension	1.6×10^6 spores ml^{-1}	Leaf axil filling two times	500 ml
NeemAzal T/S	0.4 %	Leaf axil filling	500 ml
Chlorpyriphos	0.03 %	Leaf axil filling	500 ml
NeemAzal F	0.4 %	Leaf axil filling	500 ml
Water	-	Leaf axil filling	500 ml

Five month old plants were used for the study. Five, second instar grubs of the weevil were introduced into the pseudostem three days after treatment. Mortality of grubs were recorded twenty days after treatment by destructive sampling.

3.7.3. Curative method of Management

Plants were artificially infested with second instar grubs of *O. longicollis* five months after planting. Five second instar grubs were introduced in to the plants by making small holes on pseudostem. After three days the plants were given the following treatments.

Table 3. Details of curative treatments with various fungi and insecticides

Treatments	Dose	Methods of application	Quantity/ plant
B. bassiana spore suspension	1.8×10^7 spores ml^{-1}	Leaf axil filling	500 ml
B. bassiana in rice bran	1.8×10^7 spores ml^{-1}	Leaf axil filling	300 g
B. bassiana spore suspension	1.8×10^7 spores ml^{-1}	Stem injection	10 ml
M. anisopliae spore suspension	1.6×10^6 spores ml^{-1}	Leaf axil filling	500 ml
M. anisopliae in rice bran	1.6×10^6 spores ml^{-1}	Leaf axil filling	300 g
M. anisopliae spore suspension	1.6×10^6 spores ml^{-1}	Stem injection	10 ml
NeemAzal T/S	0.4 %	Leaf axil filling	500 ml
Chlorpyriphos	0.03 %	Leaf axil filling	500 ml
Water	-	Leaf axil filling	500 ml

The experiment was conducted in RBD with three replications. Destructive sampling was carried out twenty days after treatment and the mortality of the grubs were recorded and analysed statistically.

Results

4. RESULTS

Results obtained from the laboratory and field experiments for the management of *O. longicollis* using entomopathogenic fungi are presented in this chapter.

4.1 PATHOGENICITY TEST

Pathogenicity of the fungi, *M. anisopliae, B. bassiana, P. lilacinus* and *N. rileyi* to grubs and adults of *O. longicollis* were tested and the results are presented in Table 4. Grubs and adults, sprayed with the fungi were kept under observation for development of disease symptoms. Out of the four fungal pathogens tested only *M. anisopliae* and *B. bassiana* showed positive results and were pathogenic to the grubs of *O. longicollis* and none of the fungi was pathogenic to adult weevil. These two fungi were used for further studies.

4.1.1 Mycoses of *O. longicollis* Grubs Infected with *M. anisopliae*

The grubs infected with *M. anisopliae* showed reduced feeding and sluggish movement on third day of inoculation and died on six days after inoculation. The cadaver became hard and pale in colour. When dead larvae were kept in moist chamber, white mycelial growth appeared on the body two days after death which later turned green (Plate 1) .

4.1.2 Mycoses of *O. longicollis* Grubs Infected with *B. bassiana*

The grubs did not show visible symptoms upto four days after inoculation. However, the food uptake was reduced considerably on second day after inoculation. The grubs were sluggish in their movements. The infected grubs died from fourth day onwards. The cadaver appeared stiff and mummified. The body of dead larvae changed to pinkish in colour. Twenty four hours after death white mycelial growth of the fungus appeared

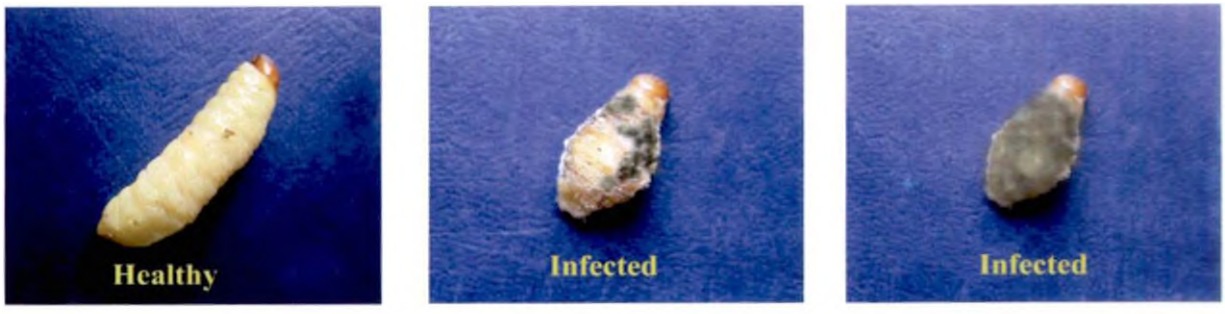


Plate 1. Mycoses caused by *Metarhizium anisopliae* on *O. longicollis* grubs

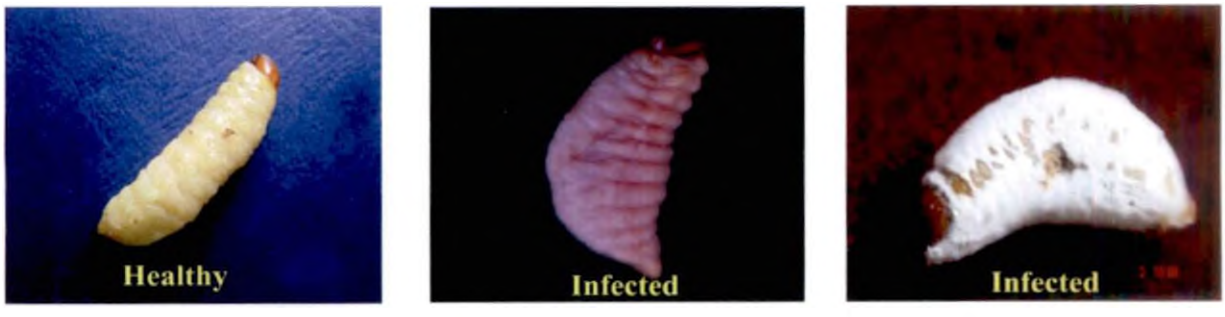


Plate 2. Mycoses caused by *Beauveria bassiana* on *O. longicollis* grubs

Plate 3. Grubs and adults infected with *Aspergillus parasiticus*

all over the cadaver. Within one week, the whole body surface was covered with white coloured powdery spores of the fungus (Plate 2).

4.1.3 New Report of a Fungus

A fungal pathogen was isolated from diseased grubs and adults of *O. longicollis* collected from the field. The dead grubs were covered with green spores of the fungus. The greenish sporulation of the fungus was observed on the appendages and body segments of the dead adults (Plate 3). The pathogenicity was confirmed by koch's postulates. The fungus was identified as *Aspergillus parasiticus* Speare by Agharkar Research Institute, Pune.

4.2 AGE SUSCEPTIBILITY OF GRUBS

4.2.1 Age susceptibility of Grubs to *M. anisopliae*

First, second, third, fourth and fifth instar grubs were used for testing the susceptibility (Table 5). Death of the grubs started on sixth day of inoculation. The mortality was 5.71, 10 and 2.57 per cent for first, second and third instar grubs respectively and they were found to be on par with each other. First instar grubs and second instar grubs showed significantly higher mortality (14.64 per cent) compared to third instar grubs (2.57 per cent) on seventh day of inoculation. Fourth and fifth instar grubs showed mortality only from eighth day onwards (2.57 per cent) which differed significantly from the mortality per cent recorded for first and second instar grubs (19.48). For third instar grubs mortality was increased to 10 per cent and was significantly higher than that of fourth and fifth instar grubs. On ninth day after inoculation first instar grubs and second instar grubs recorded 55.03 and 50.00 per cent mortality respectively and were significantly higher than third instar grubs (17.24 per cent). The fourth and fifth instar grubs recorded significantly lower mortality (2.57per cent) and it remain same on tenth day of inoculation. On tenth day after inoculation the mortality per cent did not

Table 4. Pathogenicity of entomopathogenic fungi on *O. longicollis*

Fungus	*O. longicollis*	
	Grubs	Adults
M. anisopliae	Positive	Negative
B. bassiana	Positive	Negative
P. lilacinus	Negative	Negative
N. rileyi	Negative	Negative

Table 5. Age susceptibility of grubs of *O. longicollis* to *M. anisopliae*

Stage of grubs	Percentage mortality				
	6 DAI	7 DAI	8 DAI	9 DAI	10 DAI
1st instar	5.71 (13.82)	14. 64 (22.49)	19.48 (26.18)	55.03 (47.86)	57.52 (49.31)
2nd instar	10.00 (18.43)	14.64 (22.49)	19.48 (26.18)	50.00 (44.98)	60.48 (51.03)
3rd instar	2.57 (9.21)	2.57 (9.21)	10.00 (18.43)	17.24 (24.52)	17.24 (24.52)
4th instar	0.00 (0.00)	0.00 (0.00)	2.57 (9.21)	2.57 (9.21)	2.57 (9.21)
5th instar	0.00 (0.00)	0.00 (0.00)	2.57 (9.21)	2.57 (9.21)	2.57 (9.21)
CD (0.05)	(9.484)	(8.449)	(11.661)	(11.142)	(12.232)

DAI – Days after inoculation
Figures in parentheses are values after angular transformation.

show any significant difference between first instar (57.52 per cent) and second instar grubs (60.48 per cent), whereas they showed significantly higher mortality compared to third instar (17.24 per cent), fourth instar and fifth instar grubs (2.57 per cent). Though the per cent mortality was significantly low, the mortality percentage increased from 2.57 (6 DAI) to 17.24 (10 DAI) in third instar grubs.

4.2.2 Age susceptibility of Grubs to *B. bassiana*

The mortality of grubs was observed on fourth day of inoculation and the mortality per cent recorded from fourth day to eighth day after inoculation was presented in Table 6. Significantly higher mortality was observed in first and second instar grubs (29.58 per cent) compared to third instar grubs (12.19 per cent). Fifth day after inoculation the mortality per cent of first and second instar grubs were 49.75 and 52.16 respectively. Fourth instar grubs showed mortality (3.66 per cent) on fifth day of inoculation, which was significantly lower than mortality per cent of third instar grubs (19.36). The mortality per cent of first instar grubs (72.26), second instar grubs (72.11) and third instar grubs (42.39) were significantly higher than fourth instar grubs (22.32) and fifth instar grubs (14.59) on sixth day after inoculation. Mortality of grubs increased to 99.99 per cent and 82.45 per cent for first and second instar grubs respectively on seventh day of inoculation which differ significantly from fourth instar grubs (52.16 per cent) and fifth instar (42.08 per cent). On the eighth day after inoculation the mortality per cent of first and second instar grubs was 99.99, which showed significantly higher mortality compared to third instar grubs (72.44 per cent). Only 62.05 per cent mortality was recorded for fourth instar grubs, which was on par with mortality per cent of fifth instar grubs (52.16). Eventhough the percentage mortality of fourth and fifth instar grubs were not significant, mortality increased from 3.66 to 62.05 per cent for fourth instar and 14.59 to 52.16 for fifth instar grubs.

Table 6. Age susceptibility of grubs of *O. longicollis* to *B. bassiana*

Stage of grubs	Percentage mortality				
	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI
1st instar	29.58 (5.53)	49.75 (7.12)	72.26 (8.56)	99.99 (10.05)	99.99 (10.05)
2nd instar	29.58 (5.53)	52.16 (7.29)	72.11 (8.55)	82.45 (9.13)	99.99 (10.05)
3rd instar	12.19 (3.63)	19.36 (4.51)	42.39 (6.59)	67.43 (8.27)	72.44 (8.57)
4th instar	0.00 (0.00)	3.66 (2.16)	22.32 (4.83)	52.16 (7.29)	62.05 (7.94)
5th instar	0.00 (0.00)	0.00 (0.00)	14.59 (3.95)	42.08 (6.56)	52.16 (7.29)
CD (0.05)	(0.827)	(1.246)	(0.893)	(0.732)	(0.716)

DAI – Days after inoculation
Figures in parentheses are values after $\sqrt{x+1}$ transformation.

4.3 DETERMINATION OF EFFECTIVE DOSES

4.3.1 *M. anisopliae*

Effective doses of the fungus were tested and the result is presented in Table7. Five spore concentrations of the fungus were used in the bioassay against second instar grubs of *O. longicollis*. The fungus caused mortality of larvae on the sixth day of inoculation at higher spore concentration. Significantly highest mortality was recorded at 1.6×10^6 spores ml^{-1} (10.36 per cent) and 1.6×10^7 spores ml^{-1} (14.60 per cent). At lower concentration (1.6×10^4 spores ml^{-1}) mortality recorded was 1.49 per cent on seventh day of inoculation. Maximum mortality of 24.05 per cent was recorded at a spore concentration of 1.6×10^6 spores ml^{-1} followed by 1.6×10^7 spores ml^{-1} (22.32 per cent). At 1.6×10^3 spores ml^{-1}, mortality was recorded only on eighth day of inoculation (3.66 per cent) and on the same day highest mortality was recorded at 1.6×10^7 spores ml^{-1} (34.83 per cent). On ninth day after inoculation mortality increased to 52.42 and 52.16 per cent at 1.6×10^6 and 1.6×10^7 spores ml^{-1} respectively. However, there was no significant difference in mortality per cent in these two concentrations. Maximum mortality was recorded at 1.6×10^6 spores ml^{-1} (54.89 per cent) whereas lowest mortality was observed at 1.6×10^3 spores ml^{-1} (3.66 per cent) on tenth day after inoculation. On all recorded days, water spray did not cause mortality on grubs. Results of probit analysis of dose mortality of the larvae on tenth day after inoculation showed the LC_{50} value as 3.9×10^6 spores ml^{-1}.

4.3.2 *B. bassiana*

Results presented in Table 8 shows that the death of the larvae occurred on fourth day onwards. Mortality percentage were 3.66, 10 and 27.32 at spore load of 1.8×10^5, 1.8×10^6 and 1.8×10^7 spores ml^{-1} respectively. On the fifth day, significantly higher mortality was recorded at 1.8×10^7 spores ml^{-1} (39.69 per cent), followed by 1.8×10^6 spores ml^{-1} (24.76 per cent) and least per cent mortality was recorded at 1.8×10^5 spores ml^{-1} (14.59 per cent).

No mortality was recorded at lower concentration (1.8×10^3 and 1.8×10^4 spores ml^{-1}). Only on sixth day, larvae showed mortality at 1.8×10^4 spores ml^{-1} (1.49 per cent). On the same day, maximum mortality was recorded at 1.8×10^7 spores ml^{-1} (67.26 per cent). On seventh day it still increased to 92.45 per cent and was found to be on par with 1.8×10^6 spores ml^{-1} (77.44 per cent). On eighth day the mortality per cent increased to 99.99 at 1.8×10^7 spores ml^{-1} which was followed by 77.44 at 1.8×10^6 spores ml^{-1}. At 1.8×10^5 and 1.8×10^4 spores ml^{-1} mortality per cent were 54.89 and 12.2 respectively. All the treatments differed significantly from each other. The LC_{50} value on eight day was 1.6×10^5 spores ml^{-1}.

4.4 MASS PRODUCTION AND SHELF LIFE

4.4.1 Solid Media

4.4.1.1 M. anisopliae

Mycelial growth and the sporulation of the fungus in various solid media are given in the Table 9 and 10. The fungus exhibited profuse mycelial growth on rice bran and wheat bran (Plate 4). Growth on guinea grass, gingelly oil cake and neem cake was slight. The fungal growth was absolutely nil in coir pith.

Among the different solid media, rice bran (9.26×10^6 spores ml^{-1}) and wheat bran (8.71×10^6 spores ml^{-1}) recorded the maximum spore count one week after inoculation and they did not differ significantly. Guinea grass (0.83×10^6 spores ml^{-1}) gingelly oil cake (0.05×10^6 spores ml^{-1}) and neem cake (0.17×10^6 spores ml^{-1}) recorded the least number of spores. Production of fungal spores was maximum in all solid substrates except neem cake, coir pith and guinea grass on second week after inoculation. There was significant difference observed between the spore count in rice bran (230.16×10^6 spores ml^{-1}) and wheat bran (42.85×10^6 spores ml^{-1}). Production of fungal spores in guinea grass (0.23×10^6 spores ml^{-1}),

Table 7. Mortality of second instar grubs of *O. longicollis* treated with *M. anisopliae*

Dose spores ml^{-1}	Percentage mortality				
	6 DAI	7 DAI	8 DAI	9 DAI	10 DAI
1.6×10^3	0.00 (0.00)	0.00 (0.00)	3.66 (2.16)	3.66 (2.16)	3.66 (2.16)
1.6×10^4	0.00 (0.00)	1.49 (1.58)	6.49 (2.74)	3.66 (2.16)	8.33 (3.05)
1.6×10^5	0.00 (0.00)	6.49 (2.74)	6.49 (2.74)	17.19 (4.27)	19.36 (4.50)
1.6×10^6	10.36 (3.37)	24.05 (5.00)	31.94 (5.74)	52.42 (7.31)	54.89 (7.48)
1.6×10^7	14.60 (3.95)	22.32 (4.83)	34.83 (5.99)	52.16 (7.29)	54.63 (7.46)
Water	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD (0.05)	(1.116)	(1.242)	(1.416)	(1.006)	(1.424)

DAI – Days after inoculation
Figures in parentheses are values after $\sqrt{x+1}$ transformation.

Probit analysis of dose mortality response of *O. longicollis* grubs to varying doses of *M. anisopliae* on tenth day after inoculation

Slope (b)	0.497
Intercept (a)	3.209
χ^2	4.025
DF	3
LC_{50}	3.9×10^6 spores ml^{-1}

Table 8. Mortality of second instar grubs of *O. longicollis* treated with *B. bassiana*

Dose spores ml^{-1}	Percentage mortality				
	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI
1.8×10^3	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.66 (2.16)
1.8×10^4	0.00 (0.00)	0.00 (0.00)	1.49 (1.58)	6.49 (2.74)	12.20 (3.63)
1.8×10^5	3.66 (2.16)	14.59 (3.95)	24.76 (5.08)	47.4 (6.96)	54.89 (7.48)
1.8×10^6	10.00 (3.32)	24.76 (5.08)	62.22 (7.95)	77.44 (8.86)	77.44 (8.86)
1.8×10^7	27.32 (5.32)	39.69 (6.38)	67.26 (8.26)	92.45 (9.67)	99.99 (10.05)
Water	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD (0.05)	(0.864)	(0.684)	(0.933)	(0.773)	(0.944)

DAI – Days after inoculation
Figures in parentheses are values after $\sqrt{x+1}$ transformation.

Probit analysis of dose mortality response of *O. longicollis* grubs to varying doses of *B. bassiana* on eighth day after inoculation

Slope (b)	1.057
Intercept (a)	0.486
χ^2	1.204
DF	4
LC_{50}	1.6×10^5 spores ml^{-1}

gingelly oil cake (0.12 x 10^6 spores ml^{-1}) and neem cake (0.09 x 10^6 spores ml^{-1}) were found to be on par with each other. The spore count was found decreasing in all the substrates. Rice bran gave maximum sporulation (72.77 x 10^6 spores ml^{-1}) on third week after inoculation. A drastic reduction in spore count was recorded on fourth week after inoculation in rice bran (3.43 x 10^6 spores ml^{-1}) and wheat bran (2.46 x 10^6 spores ml^{-1}) and they differ significantly from each other. Only slight decrease in spore count was recorded in guinea grass (0.17 x 10^6 spores ml^{-1}) and gingelly oil cake (0.05 x 10^6 spores ml^{-1}). A steady decrease in spore count was recorded from fifth week after inoculation. Rice bran recorded 2.75 x 10^6 spores ml^{-1} followed by wheat bran (1.79 x 10^6 spores ml^{-1}). Six week after inoculation it was still decreased to 1.96 x 10^6 spores ml^{-1} and 1.03 x 10^6 spores ml^{-1} respectively for rice bran and wheat bran. Minimum spore count were recorded in gingelly oil cake and neem cake (0.09 x 10^6 spores ml^{-1}) which was on par with guinea grass (0.17 x 10^6 spores ml^{-1}). Seventh week after inoculation maximum spore count was recorded in rice bran (1.03 x 10^6 spores ml^{-1}) which was decreased to 0.27 x 10^6 spores ml^{-1} on the eighth week after inoculation. Wheat bran (0.19 x 10^6 spores ml^{-1}) and guinea grass (0.07 x 10^6 spores ml^{-1}) recorded less spore count. The fungus failed to grow in coir pith throughout the period.

4.4.1.2 B. bassiana

The mycelial growth of fungus was profuse in rice bran and wheat bran and the growth was slight in guinea grass, gingelly oil cake, coir pith and neem cake (Table 11, Plate 5).

The results presented in Table 12 shows that the sporulation was high in rice bran (503.42 x 10^6 spores ml^{-1}) followed by wheat bran (320.32 x 10^6 spores ml^{-1}) during the first week after inoculation. The sporulation was least in gingelly oil cake (0.56 x 10^6 spores ml^{-1}) coir pith (0.37 x 10^6 spores ml^{-1}) guinea grass (0.13 x 10^6 spores ml^{-1}) and neem

Table 9. Mycelial growth of *M. anisopliae* on solid media

Sl. No.	Media	Mycelial growth rate
1	Rice bran	+++
2	Wheat bran	+++
3	Guinea grass	+
4	Gingelly oil cake	+
5	Coir pith	0
6	Neem cake	+

+++ Profuse growth
++ Moderate growth
+ Slight growth
0 No growth

Table 10. Sporulation of *M. anisopliae* on solid media

Solid media	Spore count / ml x 10^6							
	1(WAI)	2(WAI)	3(WAI)	4(WAI)	5(WAI)	6(WAI)	7(WAI)	8(WAI)
Rice bran	9.26 (3.20)	230.16 (15.20)	72.77 (8.68)	3.43 (2.10)	2.75 (1.93)	1.96 (1.72)	1.03 (1.42)	0.27 (1.13)
Wheat bran	8.71 (3.11)	42.85 (6.62)	11.19 (3.49)	2.46 (1.86)	1.79 (1.67)	1.03 (4.2)	0.37 (1.17)	0.19 (1.09)
Guinea grass	0.83 (1.35)	0.23 (1.11)	0.22 (1.10)	0.17 (1.08)	0.12 (1.06)	0.17 (1.08)	0.12 (1.06)	0.07 (1.03)
Gingelly oil cake	0.05 (1.02)	0.12 (1.06)	0.07 (1.03)	0.05 (1.02)	0.05 (1.02)	0.09 (1.05)	0.07 (1.03)	0.09 (1.05)
Coir pith	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Neem cake	0.17 (1.08)	0.09 (1.05)	0.09 (1.05)	0.00 (0.00)	0.05 (1.02)	0.09 (1.05)	0.09 (1.05)	0.09 (1.05)
CD (0.05)	(0.388)	(2.767)	(3.621)	(0.164)	(0.155)	(0.168)	(0.315)	(0.065)

Figures in parentheses are values after $\sqrt{x + 1}$ transformation.
WAI – Week after inoculation

Table 11. Mycelial growth of *B. bassiana* on solid media

Sl. No.	Media	Mycelial growth rate
1	Rice bran	+++
2	Wheat bran	+++
3	Guinea grass	+
4	Gingelly oil cake	+
5	Coir pith	+
6	Neem cake	+

+++ Profuse growth
++ Moderate growth
+ Slight growth
0 No growth

Table 12. Sporulation of *B. bassiana* on solid media

Solid media	Spore count / ml x 10^6							
	1(WAI)	2(WAI)	3(WAI)	4(WAI)	5(WAI)	6(WAI)	7(WAI)	8(WAI)
Rice bran	503.42 (22.45)	73.36 (8.62)	61.60 (7.91)	0.53 (1.23)	0.22 (1.10)	0.22 (1.10)	0.15 (1.07)	0.10 (1.05)
Wheat bran	320.32 (17.93)	56.96 (7.61)	49.32 (7.09)	2.93 (1.98)	0.21 (1.10)	0.21 (1.10)	0.03 (1.02)	0.01 (1.01)
Guinea grass	0.13 (1.07)	0.12 (1.06)	0.03 (1.01)	0.02 (1.01)	0.05 (1.02)	0.05 (1.02)	0.01 (1.01)	0.01 (1.01)
Gingelly oil cake	0.56 (1.25)	0.24 (1.11)	0.23 (1.11)	0.17 (1.08)	0.14 (1.07)	0.14 (1.07)	0.00 (1.00)	0.00 (1.00)
Coir pith	0.37 (1.17)	0.03 (1.01)	0.02 (1.01)	0.03 (1.01)	0.02 (1.01)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Neem cake	0.12 (1.06)	0.01 (1.01)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
CD (0.05)	(7.004)	(0.678)	(0.881)	(0.165)	(0.165)	(0.037)	(0.017)	(0.016)

Figures in parentheses are values after $\sqrt{x+1}$ transformation.
WAI – Week after inoculation

cake (0.12 x 10^6 spores ml^{-1}) and all these treatments were found to be on par with each other. Second week onwards there was a drastic reduction in sporulation of the fungus. Spore count was reduced to 73.36 x 10^6 ml^{-1} in rice bran and 56.96 x 10^6 ml^{-1} in wheat bran. The spore count was much decreased in all other substrates. The fungus did not grow in neem cake from third week onwards. There was no significant difference between the spore count in rice bran (61.6 x 10^6 spores ml^{-1}) and wheat bran (49.32 x 10^6 spores ml^{-1}) on the third week. The sporulation reduced to 0.03 x 10^6 spores ml^{-1} in guinea grass and 0.02 x 10^6 spores ml^{-1} in coir pith. On fourth week the fungus recorded a steep decrease in sporulation in rice bran (0.53 x 10^6 spores ml^{-1}) and wheat bran (2.93 x 10^6 spores ml^{-1}). Production of fungal spores in guinea grass (0.05 x 10^6 spores ml^{-1}) and coir pith (0.02 x 10^6 spores ml^{-1}) were on par to each other, on fifth week after inoculation. No spores were produced on coir pith on sixth week onwards. On seventh week after inoculation the spore count was considerably reduced to 0.15 x 10^6 spores ml^{-1} in rice bran and it reached 0.1 x 10^6 spores ml^{-1} on eighth week.

4.4.2 Liquid Media

4.4.2.1 M. anisopliae

The resuls presented in Tables 13 and 14 shows that the mycelial growth and sporulation of fungus differ significantly in different liquid media. Among the different liquid media tried, the growth of fungus was profuse in coconut water (Plate 6). Moderate growth was observed in rice bran extract. In raw rice water, starch solution and coconut cake extract fungus showed slight growth. The fungus did not grow in neem cake extract and water.

The spore count in coconut water (7.77 x 10^7 spores ml^{-1}) was significantly higher than that recorded from other five liquid media during first week after inoculation. The spore count in rice bran extract (0.49 x 10^7 spores ml^{-1}) was found to be on par with starch solution (0.45 x 10^7

spores ml^{-1}). The fungus failed to grow in neem cake extract throughout the period. On the second week of inoculation, all the substrates showed a decrease in sporulation and the highest was recorded in coconut water (0.95×10^7 spores ml^{-1}) which was on par with rice bran extract (0.74×10^7 spores ml^{-1}). In raw rice water and starch solution the sporulation was meagre (0.02×10^7 spores ml^{-1}). In coconut cake extract the spore count was 0.01×10^7 spores ml^{-1} and the fungus failed to sporulates in these substrates from third week onwards. In starch solution and raw rice water the spore count remain same for third week also (0.02×10^7 spores ml^{-1}). On the same day spore count recorded in coconut water and rice bran extract were 0.80×10^7 spores ml^{-1} and 0.54×10^7 spores ml^{-1} respectively. Spore count in coconut water and rice bran extract were 0.29×10^7 spores ml^{-1} and 0.38×10^7 spores ml^{-1} respectively on fourth week of inoculation. On fifth week, the sporulation of fungus reached minimum with a spore count of 0.19×10^7 spores ml^{-1} in coconut water and 0.17×10^7 spores ml^{-1} in rice bran extract. Except in raw rice water (0.01×10^7 spores ml^{-1}) and starch solution (0.03×10^7 spores ml^{-1}) all other liquid media failed to support the growth of the fungus and they were found to be on par with control.

4.4.2.2 B. bassiana

The results are presented in Table 15. In coconut water and rice bran extract the fungus showed maximum mycelial growth and it was profuse (Plate 7). Moderate growth of fungus was observed in raw rice water and starch solution, while it exhibited slight growth in coconut cake extract and neem cake extract and it did not grow in water.

Coconut water (440.03×10^6 spores ml^{-1}) and rice bran extract (232.26×10^6 spores ml^{-1}) recorded maximum sporulation and they did not differ significantly (Table 16) for the first week. Production of fungal spores in raw rice water (36.37×10^6 spores ml^{-1}) and starch solution (3.33×10^6 spores ml^{-1}) were on par with each other. The spore count was much

Table 13. Mycelial growth of *M. anisopliae* on liquid media

Sl. No.	Media	Mycelial growth rate
1	Coconut water	+++
2	Rice bran extract	++
3	Raw rice water	+
4	Starch solution	+
5	Coconut cake extract	+
6	Neem cake extract	0
7	Water	0

+++ Profuse growth
++ Moderate growth
+ Slight growth
0 No growth

Table 14. Sporulation of *M. anisopliae* on liquid media

Liquid media	Spore count / ml x 10^7				
	1(WAI)	2(WAI)	3(WAI)	4(WAI)	5(WAI)
Coconut water	7.77 (2.96)	0.95 (1.39)	0.80 (1.34)	0.29 (1.13)	0.19 (1.09)
Rice bran extract	0.49 (1.22)	0.74 (1.32)	0.54 (1.24)	0.38 (1.18)	0.17 (1.08)
Raw rice water	0.04 (1.02)	0.02 (1.01)	0.02 (1.01)	0.03. (1.01)	0.01 (1.01)
Starch solution	0.45 (1.02)	0.02 (1.01)	0.02 (1.01)	0.02 (1.01)	0.03 (1.01)
Coconut cake extract	0.04 (1.02)	0.01 (1.01)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Neem cake extract	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Water	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
CD (0.05)	(0.128)	(0.256)	(0.273)	(0.083)	(0.045)

Figures in parentheses are values after $\sqrt{x + 1}$ transformation.
WAI – Week after inoculation

Table 15. Mycelial growth of *B. bassiana* on liquid media

Sl. No.	Media	Mycelial growth rate
1	Coconut water	+++
2	Rice bran extract	+++
3	Raw rice water	++
4	Starch solution	++
5	Coconut cake extract	+
6	Neem cake extract	+
7	Water	0

+++ Profuse growth
++ Moderate growth
+ Slight growth
0 No growth

Table 16. Sporulation of *B. bassiana* on liquid media

Liquid substrate	Spore count / ml x 10^6				
	1(WAI)	2(WAI)	3(WAI)	4(WAI)	5(WAI)
Coconut water	440.03 (21.00)	0.42 (1.19)	0.63 (1.28)	0.17 (1.08)	0.07 (1.03)
Rice bran extract	232.26 (15.27)	1.76 (1.66)	0.37 (1.17)	0.12 (1.06)	0.01 (1.01)
Raw rice water	36.37 (6.11)	0.19 (1.08)	0.21 (1.10)	0.12 (1.06)	0.03 (1.01)
Starch solution	3.33 (2.08)	0.20 (1.09)	0.13 (1.04)	0.08 (1.04)	0.02 (1.01)
Coconut cake extract	0.64 (1.28)	0.06 (1.03)	0.04 (1.02)	0.04 (1.02)	0.00 (1.00)
Neem cake extract	0.08 (1.04)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Water	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
CD (0.05)	(6.221)	(0.247)	(0.120)	(0.067)	(0.035)

Figures in parentheses are values after $\sqrt{x + 1}$ transformation.
WAI – Week after inoculation

reduced in coconut cake extract (0.64 x 10^6 spores ml^{-1}) and neem cake extract (0.08 x 10^6 spores ml^{-1}). Second week onwards, a drastic reduction in sporulation were recorded in all the substrates. In coconut water the spore count reached 0.42 x 10^6 spores ml^{-1} and was found to be on par with all other substrates except rice bran extract (1.76 x 10^6 spores ml^{-1}). In neem cake the fungus failed to sporulate. Coconut water recorded maximum spore count (0.63 x 10^6 spores ml^{-1}) followed by rice bran extract (0.37 x 10^6 spores ml^{-1}) during third week after inoculation. Raw rice water (0.21 x 10^6 spores ml^{-1}) and coconut cake extract (0.04 x 10^6 spores ml^{-1}) recorded least number of spores. However there was no significant difference observed between the spore count in coconut water (0.17 x 10^6 spores ml^{-1}), rice bran extract (0.12 x 10^6 spores ml^{-1}) and raw rice water (0.12 x 10^6 spores ml^{-1}) on the fourth week. Sporulation of the fungus recorded were negligible on the fifth week after inoculation. The spore count recorded in coconut water was 0.07 x 10^6 spores ml^{-1}. Rice bran extract, raw rice water and starch solution recorded spore count of 0.01 x 10^6 spores ml^{-1}, 0.03 x 10^6 spores ml^{-1} and 0.02 x 10^6 spores ml^{-1} respectively and spore count in all the growth media were statistically on par.

4.5 VIRULENCE

4.5.1 *M. anisopliae*

The results presented in Table 17 shows that the fungus grown in coconut water gave the maximum mortality of 39.91 per cent, followed by that grown in rice bran extract (33.3 per cent) during the first week of storage. Mortality was reduced to 31.74 and 27.7 per cent respectively during the second week and the virulence of the fungus did not show significant difference between first and second week after storage. The mortality caused by the fungus grown on coconut water decreased to 15.66 and 3.68 per cent on third week and fourth week respectively and they differed significantly. The fungus grown in rice bran extract, could effect

15.66 per cent and 1.65 per cent mortality on third and fourth weeks after storage. The fungus grown in rice bran and wheat bran caused (50 per cent and 47.99 per cent) mortality respectively on second week after storage. The mortality percent was only 45.98 and 39.91 respectively on first week after storage, which was found to be on par with the mortality per cent on second week after storage. The virulence of fungus in rice bran and wheat bran further reduced to 15.66 per cent on third week after storage. Mortality reduced to 15.29 per cent and 11.77 per cent respectively on fourth week after storage in rice bran and wheat bran.

4.5.2 *B. bassiana*

The results of virulence of *B. bassiana* on selected media are presented in Table 18. *B. bassiana* grown on coconut water gave maximum mortality of 88.73 per cent on first week of storage, which was reduced to 76.16 per cent on second week after storage. The mortality was reduced to 54.02 per cent and 45.97 per cent on third week and fourth week respectively and they did not differ significantly. While fungus grown in rice bran extract could effect 82.62 per cent mortality on first week of storage and it reduced to 39.83 at the end of fourth week of storage and they differed significantly in their virulence. Maximum mortality was observed in rice bran grown culture (97.56 per cent) on the first week of storage and was found to be on par with mortality on second week of storage (93.12 per cent). The virulence of fungus was reduced in third week (66.08 per cent mortality) and the fourth week (60.09 per cent mortality) of storage. Fungus grown in wheat bran caused maximum mortality on second week after storage (92.47 per cent) which was found to be on par with mortality recorded on first week of storage (90.46 per cent) whereas they differ significantly with reduced mortality on third week (64.46 per cent) and fourth week (52.01 per cent) of storage.

Table 17. Mortality of second instar grubs of *O. longicollis* treated with *M. anisopliae* grown on different media stored for different periods in room temperature

Week after storage	Percentage mortality			
	Coconut water	Rice bran extract	Rice bran	Wheat bran
1	39.91 (39.17)	33.34 (35.25)	45.98 (42.68)	39.91 (39.17)
2	31.74 (34.28)	27.70 (31.74)	50.00 (44.98)	47.99 (43.83)
3	15.66 (23.30)	15.66 (23.30)	15.66 (23.30)	15.66 (23.30)
4	3.68 (11.06)	1.65 (7.37)	15.29 (23.01)	11.77 (20.05)
CD (0.05)	(8.656)	(10.307)	(6.404)	(5.754)

Figures in parentheses are values after angular transformation.

Table 18. Mortality of Second instar grubs of *O. longicollis* treated with *B. bassiana* grown on different media and stored for different periods in room temperature

Weeks after storage	Percentage mortality			
	Coconut water	Rice bran extract	Rice bran	Wheat bran
1	88.73 (70.35)	82.62 (65.33)	97.56 (80.99)	90.46 (71.98)
2	76.16 (60.75)	66.37 (54.53)	93.12 (74.76)	92.47 (74.05)
3	54.02 (47.29)	45.98 (42.68)	66.08 (54.36)	64.46 (53.38)
4	45.97 (42.68)	39.83 (39.11)	60.09 (50.80)	52.01 (46.14)
CD (0.05)	(9.467)	(7.723)	(14.287)	(13.731)

Figures in parentheses are values after angular transformation.

4.6 EFFECT OF PESTICIDES ON GROWTH OF FUNGI

Results presented in Table 19 shows that all the pesticides inhibited the growth of both the fungi. *M. anisopliae* showed 7.29 cm of colony diametre eight days after inoculation in the control and was significantly higher compared to other treatments. The growth was only 0.57 cm and 0.55 cm in media poisoned with mancozeb 0.3 per cent and copper oxychloride 0.4 per cent respectively and were on par to each other. The fungus covered 0.7 cm and 2.99 cm in media poisoned with chlorpyriphos 0.03 per cent and NeemAzal 0.4 per cent and they differed significantly. Significantly higher inhibition of mycelial growth was noticed in the case of mancozeb 0.3 per cent, copper oxychloride 0.4 per cent and chlorpyriphos 0.03 per cent. It gave 92.49, 92.19 and 90.41 per cent inhibition respectively. The effect of NeemAzal 0.4 per cent in inhibiting the growth of the fungus was significantly low (58.89 per cent inhibition).

B. bassiana recorded 2.84 cm of colony diameter with NeemAzal (0.4 per cent) and it differed significantly from other treatments. The fungus could grow 0.77, 0.55 and 0.57 cm in media poisoned with chlorpyriphos (0.03 per cent), copper oxychloride 0.4 per cent and mancozeb 0.3 per cent respectively. No significant difference was observed among these treatments. The fungus showed 8.09 cm of colony growth in control. NeemAzal 0.4 per cent was found to inhibit the growth of fungus by 64.83 per cent and was significantly low. The per cent inhibition by chlorpyriphos 0.03 per cent was 90.45. Copper oxychloride 0.4 per cent and mancozeb 0.3 per cent gave 92.93 and 93.22 per cent inhibition and they were on par to each other.

Table 19. Effect of pesticides on the mycelial growth of *M. anisopliae* and *B. bassiana*

Pesticides	*M. anisopliae*		*B. bassiana*	
	*Colony diameter (cm)	**Per cent inhibition of mycelial growth	*Colony diameter (cm)	**Per cent inhibition of mycelial growth
NeemAzal T/S 0.4 %	2.99 (1.99)	58.89 (50.11)	2.84 (196)	64.83 (53.61)
Chlorpyriphos (0.03 %	0.70 (1.30)	90.41 (71.93)	0.77 (1.33)	90.45 (71.97)
Copper oxy chloride 0.4 %	0.55 (1.25)	92.19 (73.74)	0.55 (1.25)	92.93 (74.55)
Mancozeb 0.3 %	0.57 (1.25)	92.49 (74.06)	0.57 (1.26)	93.22 (74.87)
Control	7.29 (2.88)	-	8.09 (3.02)	-
CD (0.05)	(0.052)	(2.199)	(0.087)	(3.217)

DAI – Days after inoculation

*Figures in parentheses are values after $\sqrt{x+1}$ transformation.

** Figures in parentheses denote angular transformation

4.7 FIELD EVALUATION

4.7.1 Management of Grubs with Prophylactic Treatment

The data presented in Table 20 shows that the plants treated with leaf axil application of chlorpyriphos 0.03 per cent recorded significantly higher mortality of grubs (69.82 per cent) followed by NeemAzal T/S 0.4 per cent (54.69 per cent) and *B. bassiana* spore suspension applied two times (52.42 per cent). These treatments were statistically on par with single application of *B bassiana* spore suspension and NeemAzal F 0.4 per cent which caused 52.16 per cent and 44.3 per cent mortality of grubs respectively. Mortality per cent decreased to 37.06 when *B. bassania* grown in rice bran was applied. Only 34.83 per cent and 24.76 per cent mortality were recorded, when the plant was treated with *M. anisopliae* spore suspension two times application and single application respectively. The effect was least in stem injection of *M. anisopliae* (3.66 per cent), *B. bassiana* (12.19 per cent) and leaf axil application of *M. anisopliae* grown in rice bran (14.59 per cent).

4.7.2 Management of Grubs with Curative Treatment

The percentage mortality observed in curative treatments (Table 21) was highest for leaf axil application of chlorpyriphos 0.03 per cent (72.5 per cent). Application of NeemAzal T/S 0.4 per cent, spore suspension of *B. bassiana* (1.8×10^7 spores ml^{-1}) and *B. bassiana* grown in rice bran were found to be effective with 60, 46.66 and 35 per cent mortality respectively and these treatment were found to be on par with the chlorpyriphos 0.03 per cent. The mortality recorded in stem injection of *B. bassiana*, *M. anisopliae* and leaf axil application of spore suspension and rice bran culture of *M. anisopliae* were 15, 10, 20 and 15 per cent respectively.

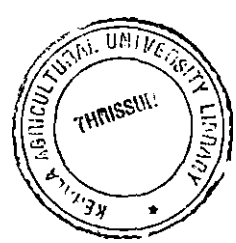


Table 20. Mortality of grubs of *O. longicollis* by prophylactic treatments in banana plants

Sl. No.	Treatments	Quantity/ plant	Percentage mortality
1	LAF of spore suspension of *B. bassiana*	500 ml	52.16 (7.29)
2	LAF of *B. bassiana* in rice bran	300 g	37.06 (6.17)
3	Stem injection of *B. bassiana*	10 ml	12.19 (3.63)
4	LAF of *B. bassiana* at two times	500 ml	52.42 (7.31)
5	LAF of spore suspension of *M. anisopliae*	500 ml	24.76 (5.08)
6	LAF of *M. anisopliae* in rice bran culture	300 g	14.59 (3.95)
7	Stem injection of *M. anisopliae*	10 ml	3.66 (2.16)
8	LAF of *M. anisopliae* at two times	500 ml	34.83 (5.99)
9	LAF NeemAzal T/S 0.4 %	500 ml	54.69 (7.46)
10	LAF of NeemAzal F 0.4%	500 ml	44.30 (6.73)
11	LAF Chlorpyriphos 0.03 %	500 ml	69.82 (8.42)
12	Control (Water)	500 ml	8.33 (3.05)
	CD (0.05)		(1.19)

LAF – Leaf axil filling
B. bassiana spore load – 1.8×10^7 spores ml^{-1}
M. anisopliae – Spore load – 1.6×10^6 spores ml^{-1}
Figures in parentheses are values after $\sqrt{x + 1}$ transformation

Table 21. Mortality of grubs of *O. longicollis* by curative treatments in banana plants

Sl. No.	Treatments	Quantity/ plant	Percentage mortality
1	LAF of spore suspension of *B. bassiana*	500 ml	46.66 (6.90)
2	LAF of *B. bassiana* in rice bran	300 g	35.00 (6.00)
3	Stem injection of *B. bassiana*	10 ml	15.00 (6.00)
4	LAF of spore suspension of *M. anisopliae*	500 ml	20.00 (4.58)
5	LAF of *M. anisopliae* in rice bran	300 g	15.00 (4.00)
6	Stem injection of *M. anisopliae*	10 ml	10.00 (3.32)
7	LAF NeemAzal T/S 0.4 %	500 ml	60.00 (7.81)
8	LAF Chlorpyriphos 0.03 %	500 ml	72.50 (8.57)
9	Control (Water)	500 ml	13.33 (3.79)
	CD (0.05)		(2.59)

LAF – Leaf axil filling

B. bassiana spore load – 1.8×10^7 spores ml^{-1}

M. anisopliae – Spore load – 1.6×10^6 spores ml^{-1}

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

Discussion

5. DISCUSSION

Banana is one of the important fruit crops in the tropics. It is the cheapest, plentiful and nourishing of all the fruits. One of the major constraints, which restrict the production and productivity of the crop is the high incidence of pests and diseases. Among the pests, the pseudostem weevil *Odoiporus longicollis* Oliv. is the most destructive and has become very serious in the southern districts of Kerala.

Chemical control is effective against this pest. However, chemicals which are being used indiscriminately by farmers leading to environmental pollution and health hazards. Hence, alternative methods of control have to be evolved. Use of entomopathogenic fungi is a safe pest control strategy against this pest, and has great potential in Kerala, as the weather conditions are suitable for the growth and development of the fungi.

5.1 SCREENING OF ENTOMOPATHOGENIC FUNGI

The present study was conducted to screen four commonly available entomopathogenic fungi *viz.*, *Metarhizium anisopliae, Beauveria bassiana, Paecilomyces lilacinus* and *Nomuraea rileyi* for their pathogenicity to grubs and adults of *O. longicollis*.

Of the four fungal pathogens tested, *M. anisopliae* and *B. bassiana* were found pathogenic to the grubs of *O. longicollis*. The pathogenicity of *M. anisopliae* and *B. bassiana* to grubs and adults of *O. longicollis* was earlier reported by several workers (Anitha *et al.*, 1998; Padmanaban *et al.*, 2001; Yue *et al.*, 2003).

M. anisopliae treated grubs fed normally in the initial phase of infection, but the appetite diminished 72 h after inoculation. The fungus usually takes time to acclimatize with the environment and to multiply. This initial period of time may be the time taken by the fungus to surmount the insect's natural barrier to infection (Hall, 1976). Larvae died

six days after inoculation of the fungus. Immediately after death the body of the larvae became very soft and flexible owing to the heavy growth of mycelium in the larval body. It became hard, stiff and mummified. Mycelial growth was visible all over the cadaver which later turned green. Similar symptoms were observed by Anitha (2000).

The isolate of *B. bassiana* was also found pathogenic to the grubs of pseudostem weevil. The development of mycoses observed in the infected larvae was described in 4.1.3. This was similar to the earlier reports of Easwaramoorthy and Santhalakshmi (1993), Gurusubramanian *et al.* (1999) and Simon *et al.* (2003). The affected grubs became sluggish and turned pinkish in colour. Growth of the fungus on the cadaver was visible as white fluffy fungal mat (Plate 2).

The development of muscardine disease was described by several scientists. According to Ferron (1981), after crossing the integument the muscardine develops in the haemocoel in the presence of cellular defensive reactions of the host. Plasmatocytes surround the mycelium as a pseudotissue or granuloma. *Beauveria* and *Metarhizium* produce toxins which erode these granuloma and allow blastospores to invade the haemocoel. Several toxic cyclodepsipeptides, such as destruxins A, B, C and D and desmethyl destruxin have been isolated from *M. anisopliae* (Suzuki *et al.*, 1971) and also beauvericin has been isolated from *B. bassiana*. These toxins kill the host by inciting progressive degeneration of the host tissues due to loss of the structural integrity of membranes and then dehydration of the cells by fluid loss (Zacharuk, 1971).

The non infectivity of *P. lilacinus* and *N. rileyi* may be due to the specificity of microorganism. Burdeos and Villacarlos (1989) were of the opinion that the difference in virulence of the entomopathogenic fungi was due to their specificity. Resistance of the host insect to certain isolates of the pathogen may be another reason for the negative results of

pathogenicity tests (Fargues, 1976; Feng *et al.*, 1994). Often a strain presents no activity on a host while it causes a high mortality rate on other insects of the same family (Danfa and Valk, 1999). Khan *et al.* (1990) reported pathogenicity of *P. lilacinus* against *Cylas formicarius*. *N. rileyi* found effective against *Aracanthus* sp. (Sosa *et al.*, 1994).

The differential susceptibility of the pseudostem grubs to the different fungal pathogens may be due to the inherent variation in the susceptibility of the host to the fungal pathogens. The biochemical interactions in the infection process which may be specific to host pathogen interaction might have contributed to the differential susceptibility (Devaprasad *et al.*, 1989).

None of the fungi were pathogenic to the adult weevil. The non pathogenicity of fungi to adult weevil, may be due to the presence of hard elytra which acted as a barrier to fungal invasion (Cohen, 1987). Rajendran (2002) reported that *M. anisopliae* was not much potent on the adults of egg plant spotted beetle. The fungi *M. anisopliae* and *B. bassiana* which were found to be effective against the grubs were selected for further investigations.

5.2 AGE SUSCEPTIBILITY OF GRUBS

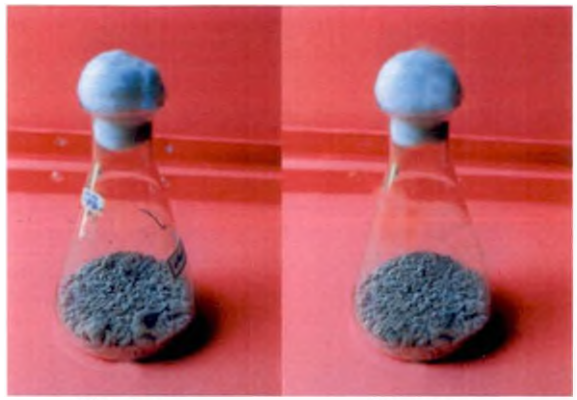
Comparison of percentage mortality of grubs revealed that susceptibility of grubs to *M. anisopliae* was high in first and second instars, whereas it was low in third, fourth and fifth instars. Similar results were reported earlier for *M. anisopliae* on *O. longicollis* (Anitha, 2000) and *Plocoederus ferrugineus* L. (Saminathan *et al.*, 2004).

B. bassiana also caused infection in the first and second stage grubs with 99.99 per cent mortality. The overall mortality of fourth and fifth instar stages were comparatively lower indicating that older grubs were relatively more tolerant to infection.

The results clearly showed that the susceptibility of pseudostem borer larvae decreased with increase in the larval age. Similar observations were reported by Devaprasad *et al.* (1989) on *S. litura* and Sivasankaran *et al.* (1990) on *Chilo infuscatellus*. The decreased mortality of older larvae may be due to the insufficiency of spore load or it may be due to variation in chemical constituents resulting in progressive hardening of the cuticle and increased humoral defense mechanism to the microbial infections (Devaprasad *et al.*, 1989). Higher susceptibility of the younger larvae to fungal infection observed in this study is a positive factor in the management of *O. longicollis*. As the younger larvae are present near the outer sheath and older larvae towards the interior of the stem, it is advantageous to get contact of the fungi to the highly susceptible stages of the pest. Maximum mortality of 99.99 per cent was recorded in first and second instar grubs due to *B. bassiana* on eighth day after inoculation, while *M. anisopliae* recorded 60.48 per cent mortality of second instar grubs on tenth day after inoculation (Fig. 1). This clearly indicates that *B. bassiana* is more effective than *M.anisopliae* mycopathogen, recording the higher mortality within a shorter period.

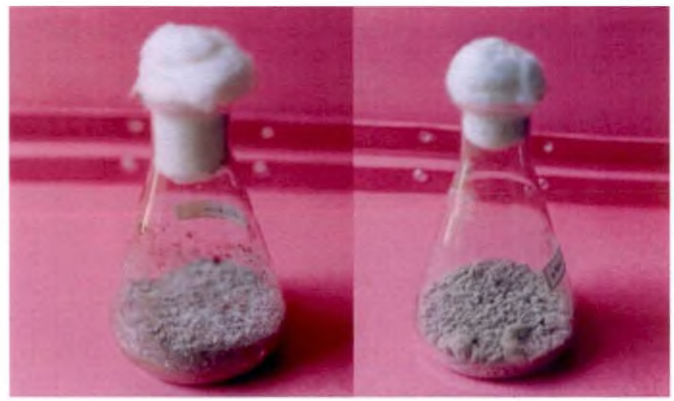
5.3 DETERMINATION OF EFFECTIVE DOSES

In the present study, a correlation between the doses of fungi and the response of *O. longicollis* grubs in terms of mortality was established and LC_{50} values were calculated using probit analysis and the results are presented in para 4.3. Death of second instar grubs of *O. longicollis* inoculated with *M. anisopliae* was noticed from six days after inoculation. More than fifty per cent mortality was noticed from ninth day onwards. The study also revealed that the mortality increased with increase in spore concentration. Patel *et al.* (1988) also observed similar results against *Agrotis segetum* (Schiff). When the spore concentration was below 1.6 x 10^5 fifty per cent mortality was not reached even ten days after inoculation.



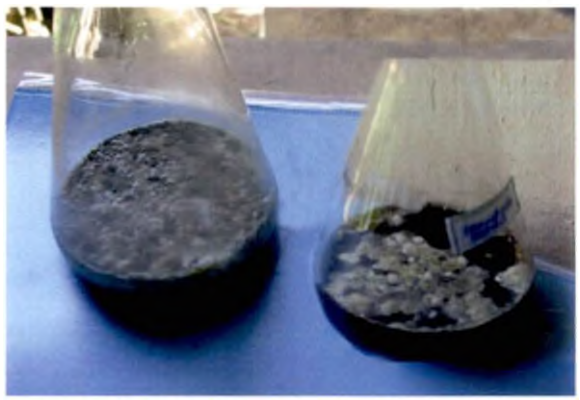
Rice bran Wheat bran

Plate 4. Growth of *M. anisopliae* in solid media



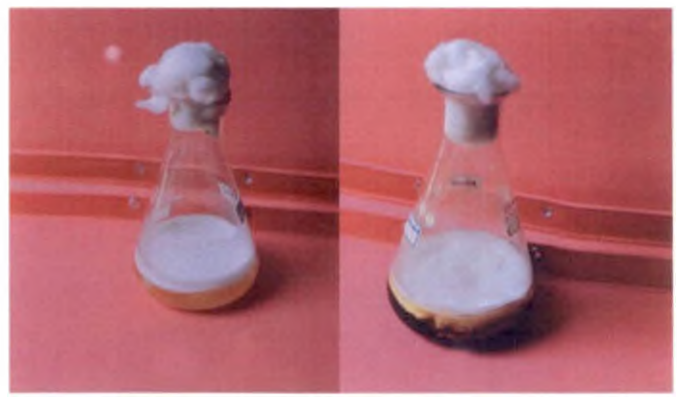
Rice bran Wheat bran

Plate 5. Growth of *B. bassiana* in solid media



Coconut water Rice bran extract

Plate 6. Growth of *M. anisopliae* in liquidmedia



Coconut water Rice bran extract

Plate 7. Growth of *B. bassiana* in liquid media

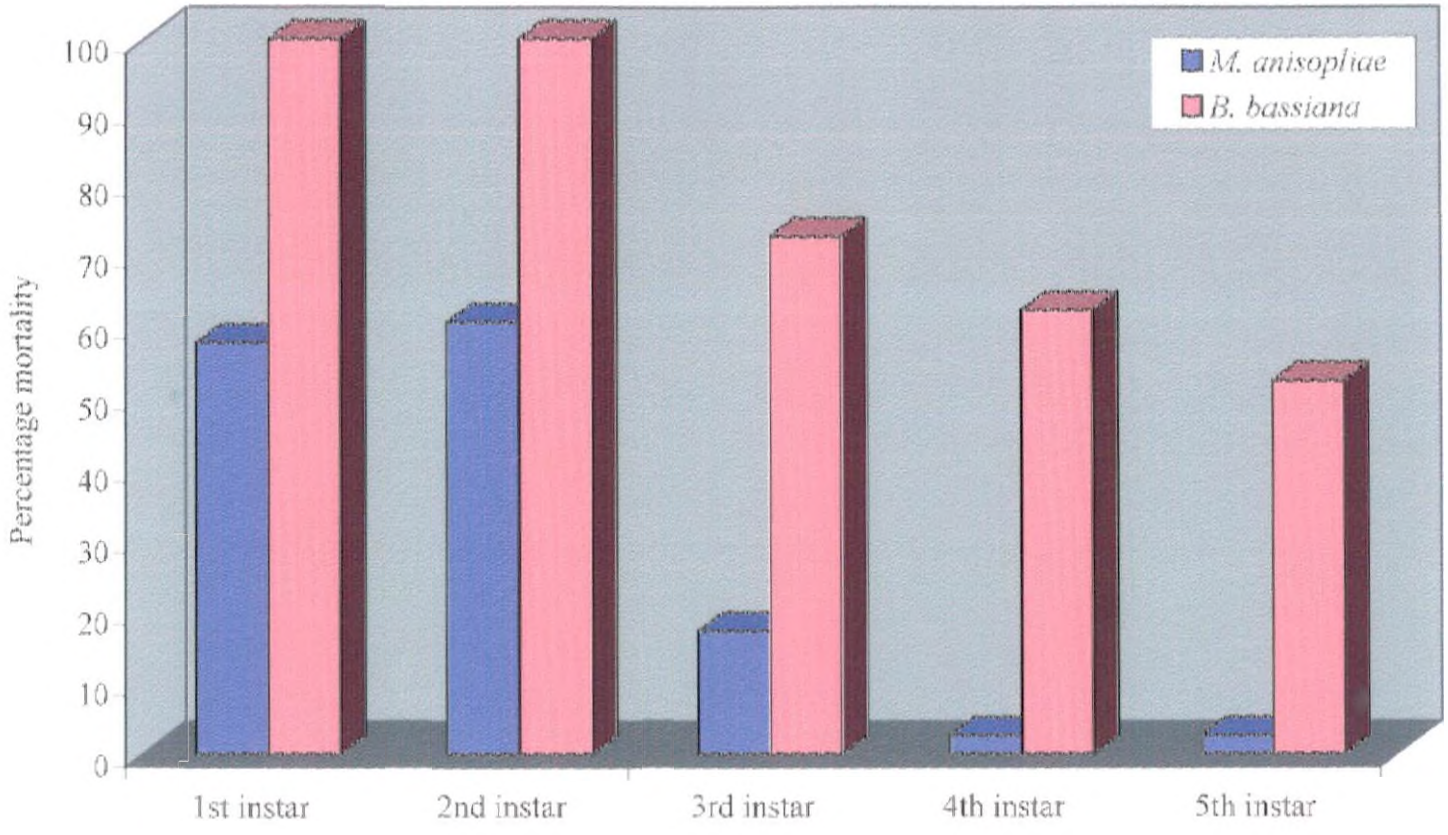


Fig. 1. Susceptibility of grubs of *O. longicollis* to entomopathogenic fungi

Bioassay of *B. bassiana* on second instar grubs of *O. longicollis* was carried out and the LC_{50} value was 1.6 x 10^5 spores ml^{-1} on eighth day after inoculation. The fungus caused 3.66 to 99.99 per cent mortality on eighth day after inoculation and maximum mortality was observed at a concentration of 1.8 x 10^7 spores ml^{-1}. The results showed that there is an increase in percentage mortality due to fungal infection, when there is an increase in conidial concentration. Similar results were obtained with *B. bassiana* in the case of *Heliothis armigera* (Gopalakrishnan and Narayanan, 1990) and *Chilo infuscatellus* (Sivasankaran *et al.*, 1990). In lower concentration the mortality was low and slow because the pathogen required more time for multiplication to reach the required spore concentration to infect the host.

The degree of pathogenicity varied among mycopathogens tested. *B. bassiana* was more virulent than *M. anisopliae*. From the results it is clear that *B. bassiana* is the most effective mycopathogen recording the lowest LC_{50} of 1.6 x 10^5 spores ml^{-1} when compared to *M. anisopliae* (3.9 x 10^6 spores ml^{-1}). This may be due to the host preference of the fungus. This is in corroboration with the findings of Khan *et al.* (1990) on *Cylas formicarius* (F.), Pandey (2003) on rice bug, *Heliothis armigera, Spodoptera litura* and *S. obliqua*.

5.4 MASS PRODUCTION AND SHELF LIFE

Large scale use of fungi for biological control requires mass production of inoculum. In the present study locally available cheaper substrates especially byproducts of agriculture were tested to find out suitable medium for the multiplication of the fungi.

The two entomopathogenic fungi tested (*M. anisopliae* and *B. bassiana*) gave maximum growth and sporulation in rice bran and wheat bran when compared to other solid media and in these two substrates *B. bassiana* produced much higher number of spores when compared to *M. anisopliae* (Fig.2). Simon (2002) reported that rice bran was a better

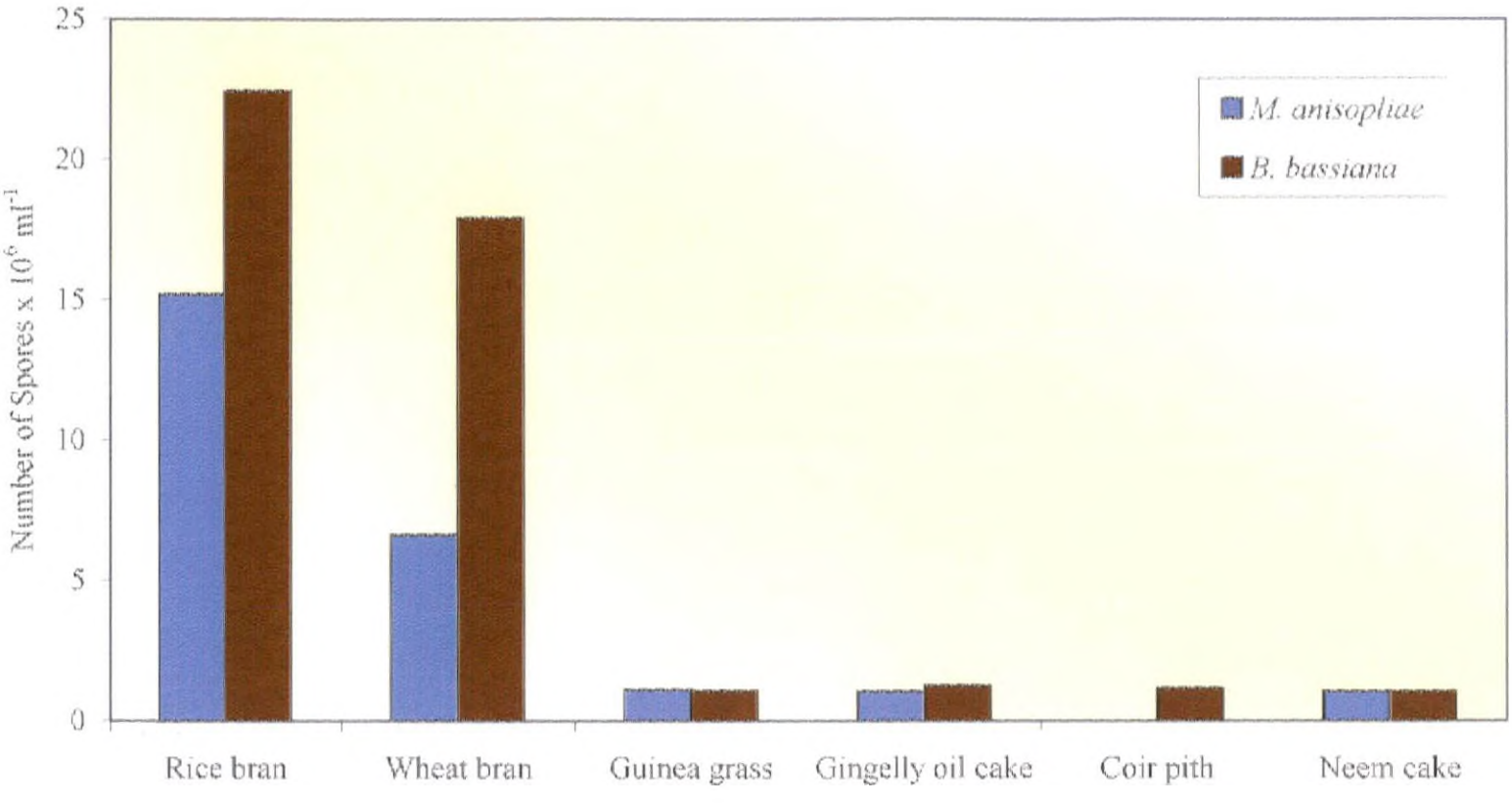


Fig. 2. Influence of solid substrates on the sporulation of entomopathogenic fungi

substrate for spore production of *B. bassiana*. Hussey and Tinsely (1981) reported wheat bran as a suitable substrate for the production of *B. bassiana*. The growth and sporulation of *B. bassiana* was found maximum in rice based solid media as reported by Ibrahim and Low (1993). Higher nutrient status in an available form in rice bran and wheat bran may be the reason why, it supported maximum fungal growth.

Slight mycelial growth was observed in guinea grass, gingelly oil cake and neem cake and the sporulation was also poor in these substrates. Suppression of fungi by neem cake may be due to the presence of inhibiting alkaloid azadirachtin (A to K) at high concentration. Rejirani (2001) reported inhibition of *Fusarium pallidoroseum* (Cook) by neem cake. Coir pith failed to support fungal growth and this may be due to the high cellulose and lignin content.

Among the six liquid media tested for mass production, coconut water and rice bran extract gave better growth and sporulation of the fungi (Fig. 3). Maximum spore count was obtained in coconut water. This finding is in agreement with earlier works (Danger *et al.*, 1991; Simon, 2002). The superiority of coconut water and rice bran extract for both mycelial growth and sporulation of *M. anisopliae* and *B. bassiana* could be due to qualitative variation of some cofactors or chemicals which are preferred by the fungi. Easy availability of common metabolites also can enhance the vegetative and reproductive growth of the fungi (Danger *et al.*, 1991). Differential responses of the fungus in biomass and conidial production on different media may be related to nutritional composition of the media (Churchill, 1982). Apart from the nutrient status, an ideal substrate for fungal growth should have proper particle size and surface area to volume ratio (Simon, 2002). The results of the study indicated that rice bran, wheat bran, coconut water and rice bran extract could be used for the mass multiplication of *M. anisopliae* and *B. bassiana*. These substrates are locally available in Kerala, so also coconut water, a

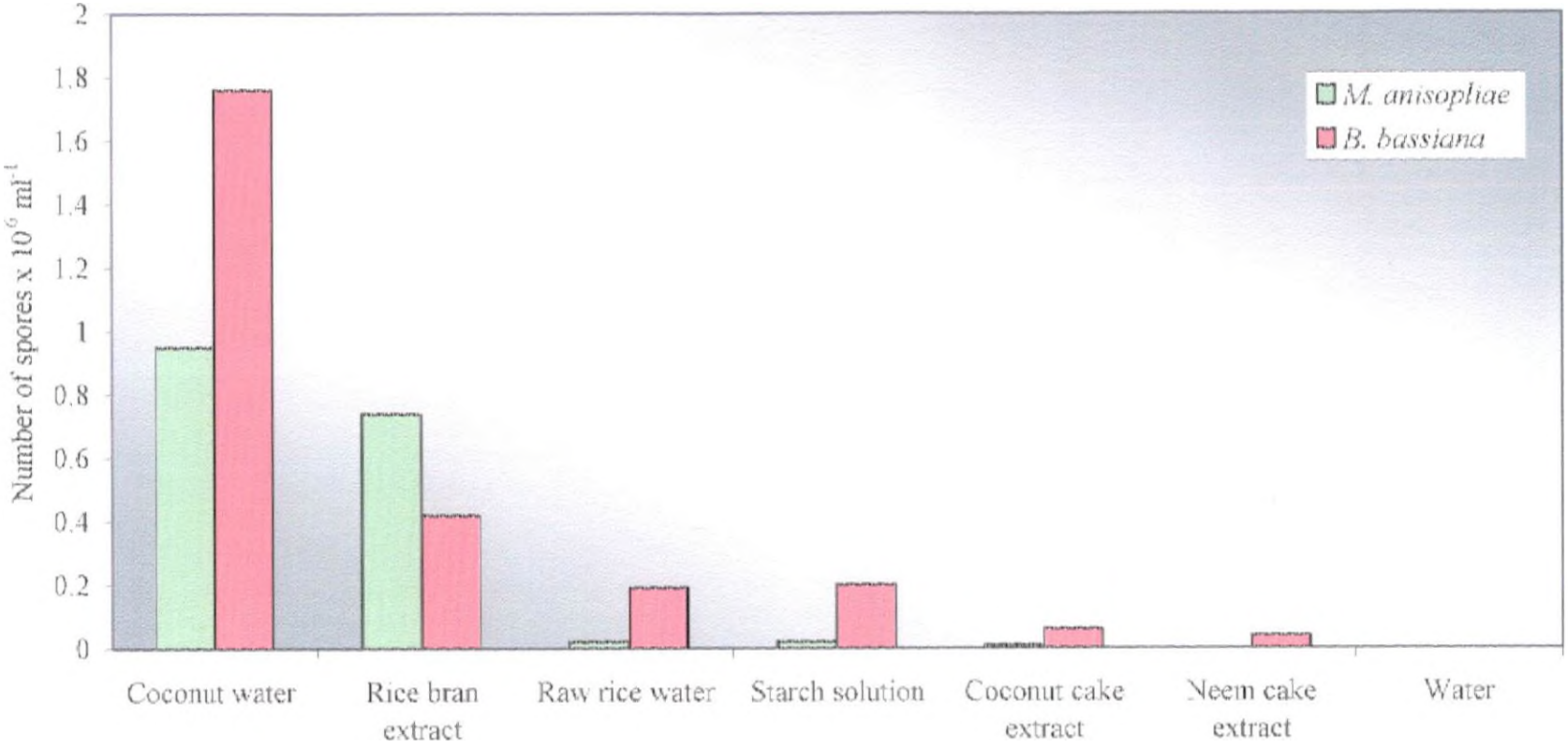


Fig. 3. Influence of liquid substrates on the sporulation of entomopathogenic fungi

byproduct of copra industry, which is currently wasted, could be used for large scale production of these fungi.

In the present study the sporulation of fungi in all the solid substrates was drastically reduced after first month of storage at room temperature. Similar results were obtained by Walstad *et al.* (1970). They observed that the spores of *M. anisopliae* and *B. bassiana* were survived only for 15 days at 21°C. The virulence of fungi in rice bran and wheat bran was tested on the basis of the per cent mortality of second instar grubs of *O. longicollis*, and the results showed a declining trend from third week onwards. The liquid substrates failed to sporulate after one month. As suggested by Soper and Ward (1981) the reduction in sporulation of fungus in liquid media may be due to the less stable nature of blastospores. However the virulence of the fungi in coconut water and rice bran extract was reduced second week after storage. Similar results were obtained by Simon (2002).

In rice bran and wheat bran, the spore count tend to decline below LC_{50} value of the fungi, on sixth week after storage for *B. bassiana* and fourth week after storage for *M. anisopliae*. In the case of liquid substrates the spore count recorded was below LC_{50} value from third week after storage for *M. anisopliae* and fourth week after storage for *B. bassiana*. These results indicated that the fungi grown in these media can be used for a period of one month for the management of grubs of *O. longicollis*.

Several factors which operate during the extended storage period of the fungi in different media will affect its survival. One of the major factors is the temperature fluctuation observed during the storage period. The spore production of *M. anisopliae* and *B. bassiana* were maximum at 25°C (Sharma *et al.*, 1998). Since the fungi were stored under fluctuating temperature conditions, the production and survival were affected. When stored under refrigerated conditions, fungi retained their viability for

extended periods (Filhos and Cardelli, 1988) as the biological activity was reduced to the minimum.

5.5 EFFECT OF PESTICIDES ON GROWTH AND SPORULATION

Often chemical pesticides have to be used along with bioagents. So it is necessary to obtain information on compatibility of fungi with agrochemicals utilized in a particular ecosystem. In the present study compatibility of *M. anisopliae* and *B. bassiana* with commonly used chemical pesticides in banana was studied by conducting Poison Food Technique.

All the insecticides and fungicides tested, inhibited the growth of the fungi. Compared to other chemicals NeemAzal showed least percentage inhibition of fungal growth (Fig 4). It has been reported earlier that five per cent neem oil had a significant inhibitory effect on conidial germination and sporulation of *M. anisopliae* and *B. bassiana* (Aguda *et al.*, 1988; Devaprasad *et al.*, 1989). Chlorpyriphos 0.03 per cent, copper oxychloride 0.4 per cent and mancozeb 0.3 per cent completely inhibited the growth of *M. anisopliae* and *B. bassiana*. This is in agreement with the findings by Todorova *et al.* (1998). They reported the inhibitory effect of *B. bassiana* with mancozeb. Simon (2002) reported complete inhibition of growth of *B. bassiana* by chloropyriphos 0.05 per cent and mancozeb 0.20 per cent.

In the light of present findings coupled with previous reports, it can be inferred that the combined use of fungi and chemicals may not be advantageous in the IPM programme.

5.6 FIELD EVALUATION

Field experiments were laid out to know whether the entomopathogenic fungi were as effective as chemical pesticides for managing the pest.

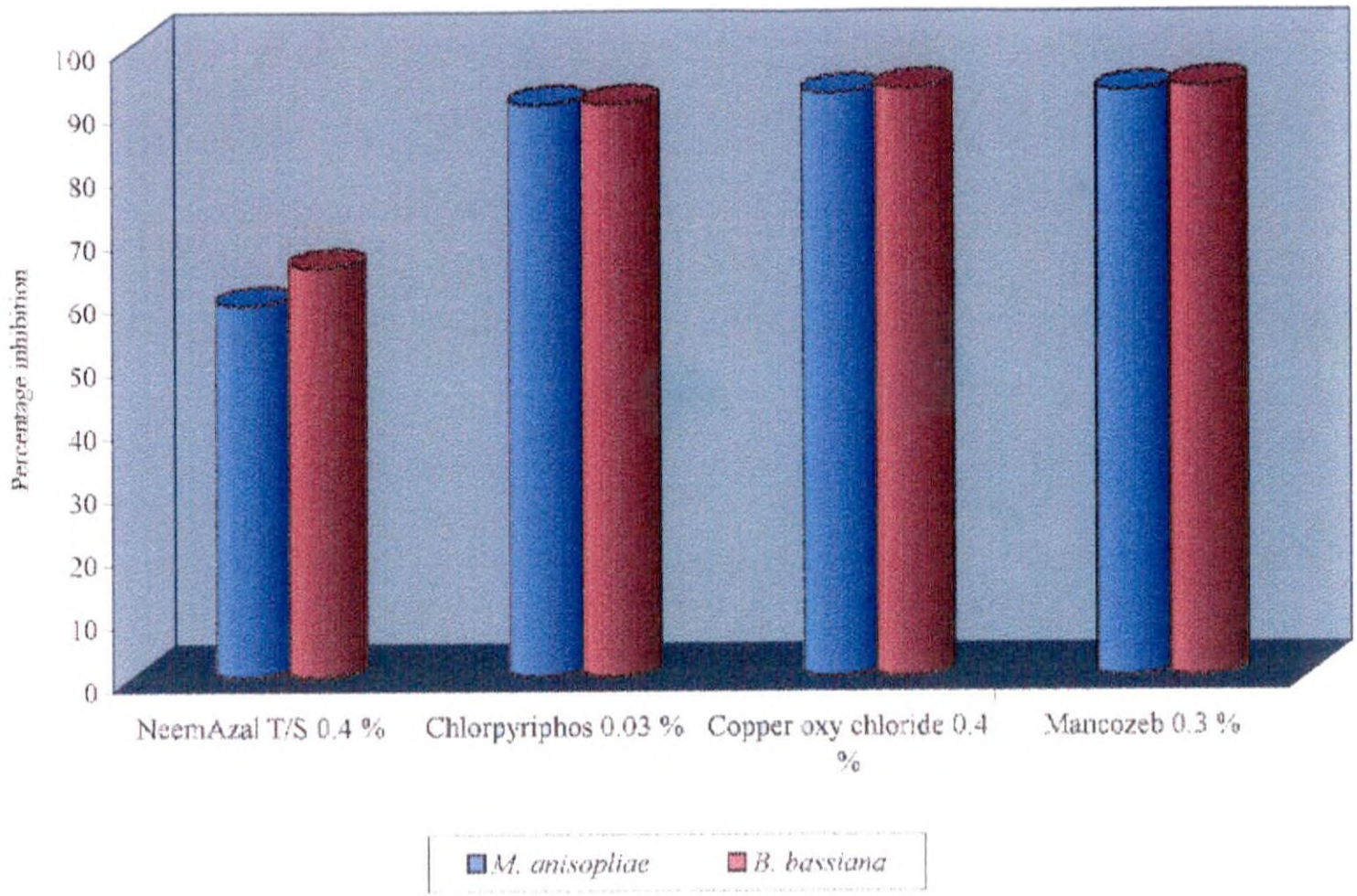
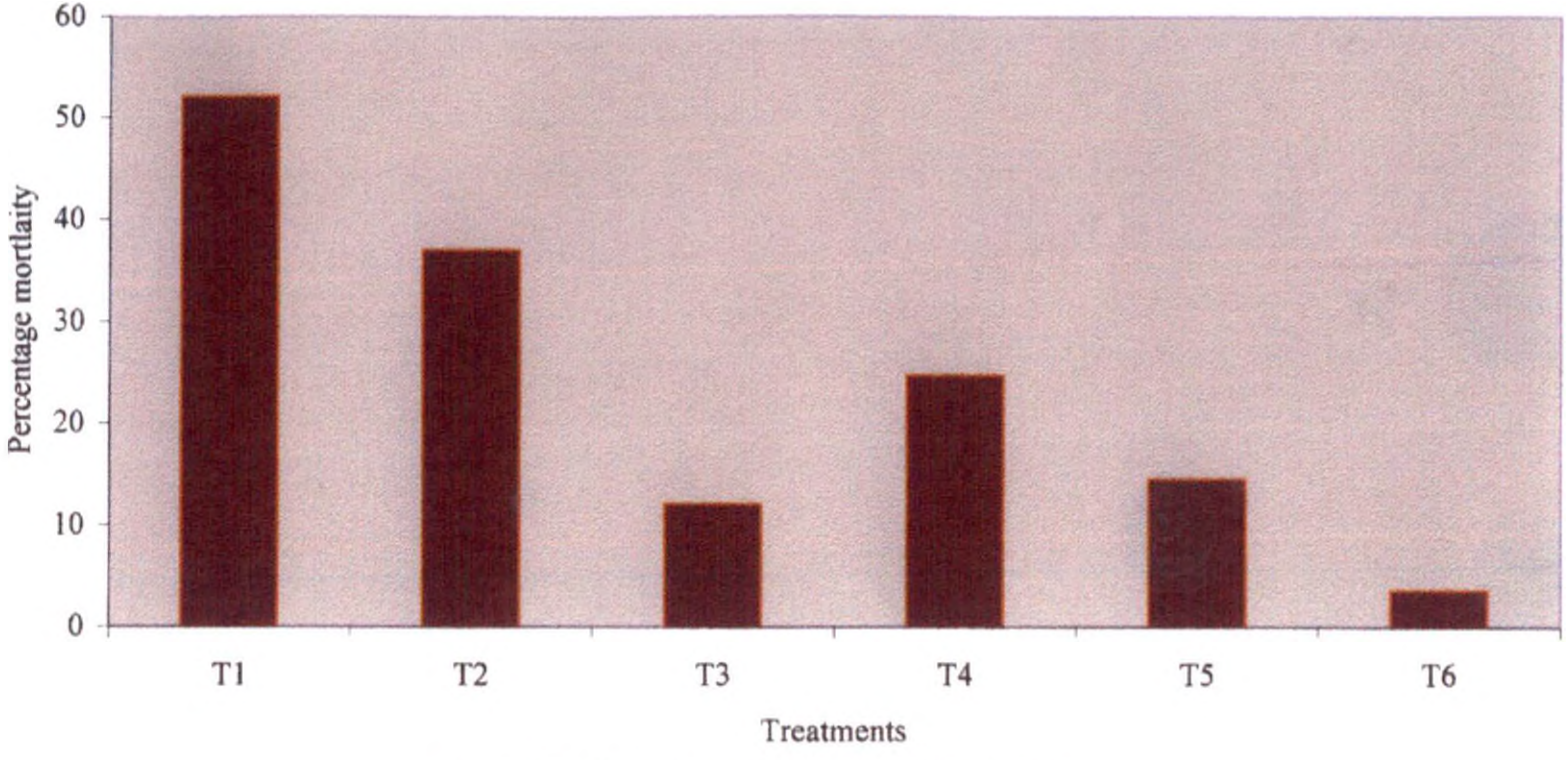


Fig. 4. Percentage inhibition of fungi on PDA poisoned with different pesticides

To study the effect of prophylactic treatments, the plants were artificially infested with known number of grubs after the treatments application. The leaf axil filling of *B. bassiana* spore suspension @ 1.8 x 10^7 ml^{-1} gave 52.42 per cent mortality of grubs. Though the mortality per cent of grubs were higher in leaf axil filling of chlorpyriphos and NeemAzal, equally effective control was obtained with application of *B. bassiana* spore suspension. Treatment with *M. anisopliae* spore suspension (1.6 x 10^6 spores ml^{-1}) as leaf axil filling was also found effective but the mortality per cent was 34.83. Comparing the methods of application, leaf axil filling of spore suspension was more effective than stem injection or application of culture in rice bran (Fig. 5). Similar results were obtained with *M. anisopliae* in *O. longicollis* (Anitha (2000). According to Jayasree (1992) in stem injection, the lateral diffusion was found to be limited and moreover it caused damage to pseudostem also.

To study whether banana plants infested with the pseudostem weevil could be saved by curative application of entomopathogenic fungi, a field experiment was laid out. A known number of grubs were introduced into healthy plants before the treatments were applied. Plants curatively treated with leaf axil filling of *B. bassiana* caused 46.66 per cent mortality of grubs. The leaf axil application of chloropyriphos (0.03 per cent) and NeemAzal (0.4 per cent) resulted in higher mortality of grubs. Prophylactic treatments were found more effective compared to curative treatments. Similar results were obtained in the case of *N. rileyi* in soybean caterpillar (Ignoffo *et al.*, 1976).

Based on the present study it can be inferred that the entomopathogenic fungus, *B. bassiana* is an effective biocontrol agent for the management of *O. longicollis*. The fungus was effective against first and second instar grubs. Detection of infestation in the early stage and application of *B. bassiana* spore suspension as leaf axil filling can manage the pest effectively.



T1 LAF of spore suspension of *B. bassiana*
T2 LAF of *B. bassiana* in rice bran
T3 Stem injection of *B. bassiana*
T4 LAF of spore suspension of *M. anisopliae*
T5 LAF of *M. anisopliae* in rice bran culture
T6 Stem injection of *M. anisopliae*

Fig. 5. Mortality of grubs of *O.longicollis* by prophylactic application of entomopathogenic fungi

Summary

6. SUMMARY

Study was conducted to screen four entomopathogenic fungi and evaluate their efficiency against banana pseudostem weevil, *Odoiporus longicollis*. The commonly available entomopathogenic fungi *viz.*, *Metarhizium anisopliae*, *Beauveria bassiana*, *Paecilomyces lilacinus* and *Nomuraea rileyi* were tested for their pathogenicity to grubs and adults of *O. longicollis*.

Of the four fungal pathogens tested *M. anisopliae* and *B. bassiana* were found pathogenic to the grubs of pseudostem weevil. Comparison of the mortality of the grubs revealed that first and second instar grubs were highly susceptible to infection compared to the third, fourth and fifth instar grubs.

Death of the second instar grubs of *O. longicollis* was noticed from sixth day after inoculation with *M. anisopliae*. The cadaver became hard and pale in colour. White mycelial growth appeared on the body, two days after death which later turned green. Grubs infected with *B. bassiana* died on fourth day after inoculation. The colour of the dead larvae changed from cream to pink. Within one week, the whole body surface was covered with white coloured powdery spores of the fungus.

Studies on the determination of the effective dose of the fungi showed that there is an increase in percentage mortality with increase in spore concentration. The LC_{50} value of *M. anisopliae* was 3.9×10^6 spores ml^{-1}, ten days after inoculation. For *B. bassiana* the LC_{50} value was 1.6×10^5 spores ml^{-1}, eight days after inoculation.

Rice bran and wheat bran were found suitable solid media for mass multiplication of fungi. Coconut water and rice bran extract were found suitable liquid media for large scale production of the *M. anisopliae* and *B.*

bassiana as they supported maximum mycelial growth and sporulation of the fungi.

The sporulation of the fungi both in solid and liquid media was found decreasing from third week after storage. Virulence of the fungi grown in coconut water, rice bran extract, rice bran and wheat bran were tested in terms of percentage mortality of grubs. The results showed that the per cent mortality decreased from third week of storage in case of solid substrates. The virulence of fungi in coconut water and rice bran extract decreased second week after storage.

Compatibility studies were conducted with two insecticides and two fungicides and the results showed that chlorpyriphos (0.03 per cent), copper oxychloride (0.4 per cent) and Mancozeb (0.3 per cent) completely inhibited the growth of fungi. The per cent inhibition was least in the case of Neem Azal (0.4 %).

To study the effectiveness of the fungi in field conditions, experiments were conducted with prophylactic and curative treatments. In prophylactic treatments, leaf axil filling of *B. bassiana* (1.8×10^7 spores ml^{-1}) was found as effective as leaf axil application of chlorpyriphos and Neem Azal. From the management trials, it was found that among the different methods of applications, leaf axil application of fungal spore suspension was superior compared to other methods of application.

Based on the results of present study, the entomopathogenic fungi, *B. bassiana* spore suspension @ 1.8×10^7 ml^{-1} as leaf axil filling can be recommended for the management of grubs of *O. longicollis*.

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*Original not seen

MANAGEMENT OF BANANA PSEUDOSTEM WEEVIL *Odoiporus longicollis* Oliv. USING ENTOMOPATHOGENIC FUNGI

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Abstract of the
thesis submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2005

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ABSTRACT

Investigation on "Management of banana pseudostem weevil, *Odoiporus longicollis* Oliv. using entomopathogenic fungi" was carried out in the Department of Agricultural Entomology, College of Agriculture, Vellayani. The study was conducted to screen four entomopathogenic fungi, to evaluate their biocontrol potential and to standardize the effective dose and method of application for the management of the banana pseudostem weevil.

Four entomopathogenic fungi *viz.*, *Metarhizium anisopliae, Beauveria bassiana, Paecilomyces lilacinus* and *Nomuraea rileyi* were tested against *O. longicollis*. Among them *M. anisopliae* and *B. bassiana* were found to infect *O. longicollis* grubs. Susceptibility of different stages of grubs to fungal infection was tested by using first, second, third, fourth and fifth instar grubs of *O. longicollis* and the results revealed that first and second instar grubs were more susceptible to fungal infection.

Determination of effective dose of fungi against second instar grubs of *O. longicollis* was carried out by spraying the grubs with different concentrations of *M. anisopliae* and *B. bassiana*. The LC_{50} values of *M. anisopliae* and *B. bassiana* were 3.9×10^6 spores ml^{-1} and 1.6×10^5 spores ml^{-1} respectively.

Evaluation of six naturally available solid media *viz.*, rice bran, wheat bran, guinea grass, gingelly oil cake, coir pith, and neem cake were carried out for mass multiplication of *M. anisopliae* and *B. bassiana*. The results revealed that both rice bran and wheat bran were suitable for the mass multiplication of these fungi. The spore production was 9.26×10^6 spores ml^{-1} and 8.71×10^6 spores ml^{-1} respectively for *M. anisopliae* on first week after inoculation. For *B. bassiana*, the spore count was 503.42×10^6 spores ml^{-1} and 320.32×10^6 spores ml^{-1} respectively on first week

after inoculation. Among the liquid media tried, coconut water and rice bran extract produced maximum mycelial growth and sporulation of fungi. For *M. anisopliae* the spore count was 7.77 x 10^7 spores ml^{-1} and 0.49 x 10^7 spores ml^{-1} respectively. *B. bassiana* recorded 440.03 x 10^6 spores ml^{-1} and 232.26 x 10^6 spores ml^{-1} respectively.

M. anisopliae and *B. bassiana* grown in rice bran and wheat bran recorded maximum virulence on two weeks after storage and decreased thereafter. However, coconut water and rice bran extract showed maximum virulence on first week after storage and then found decreasing.

Studies conducted to evaluate the compatibility of fungi with NeemAzal 0.4 per cent, chlorpyriphos 0.03 per cent, copper oxychloride 0.4 per cent and mancozeb 0.3 per cent revealed that all the pesticides tested inhibited the growth of *M. anisopliae* and *B. bassiana*. But the per cent inhibition was least in the case of NeemAzal 0.4 per cent.

Field experiments were conducted with *M. anisopliae* and *B. bassiana* with chlorpyriphos (0.03 per cent) and NeemAzal (0.4 per cent) as checks. The plants were artificially infested with known number of grubs. Two experiments were conducted, one with prophylactic treatments and the other with curative treatments. Three application methods *viz.*, leaf axil filling of spore suspension of *M. anisopliae* and *B. bassiana,* leaf axil filling of fungi in rice bran and stem injection of spore suspension were tested. Among these treatments leaf axil filling of *B. bassiana* spore suspension at 1.8 x 10^7 ml^{-1} as prophylactic treatment was most effective which gave 52.42 per cent mortality of grubs.