MANAGEMENT OF MELON FLY (*Bactrocera cucurbitae* Coquillett) USING LOCAL ISOLATES *Beauveria bassiana* (Bals.) Vuill, *Paecilomyces lilacinus* (Thom.) Samson AND *Aspergillus candidus* Link: Fries

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

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2010

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DECLARATION

I hereby declare that this thesis entitled **"Management of melon fly (*Bactrocera cucurbitae* Coquillett) using local isolates *Beauveria bassiana* (Bals.) Vuill, *Paecilomyces lilacinus* (Thom.) Samson and *Aspergillus candidus* Link: Fries"** 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 melon fly (*Bactrocera cucurbitae* Coquillett) using local isolates *Beauveria bassiana* (Bals.) Vuill, *Paecilomyces lilacinus* (Thom.) Samson and *Aspergillus candidus* Link: Fries"** is a record of research work done independently by Ms. Amala, U. (2008-11-117) 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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ACKNOWLEDGEMENT

An opportunity to pay tributes to those who contributed to the preparation of this thesis is indeed joyful. I wish these words convey my gratitude and gratefulness to all who supported me to complete this endeavour successfully.

Let me place on record my profound feelings of gratitude and indebtedness to the Chairperson of the Advisory committee, Dr.Jiji.T., Professor, Department of Agricultural Entomology for suggesting the research problem, noteworthy guidance, constructive criticism during the course of investigation and in the execution of the thesis. I feel immense pleasure in expressing my thankfulness for her patience, expert advice, constant support and dedication amidst her busy schedule without which this piece of work would not have materialized.

I affirm a great deal of thanks to Dr. M. S. Sheela, Professor and Head, Department of Agricultural Entomology for her timely advice and critical suggestions for the scrutiny of the manuscript.

I greatly acknowledge the guidance rendered by Dr. A. Naseema, Professor, Department of Plant Pathology during the period of study. I specially thank for her overwhelming support, words of comfort, keen interest and friendly approach for the successful completion of the work in time.

I dedicate my heartfelt thanks to Dr. Arthur Jacob, Head, Instructional Farm for his kind co-operation and help rendered during the field experiment and at all stages of the study.

My grateful thanks to Dr. T. NalinaKumari, Professor and former Head, Department of Agricultural Entomology for her valuable guidance and sustained interest during the period of investigation.

My sincere gratitude to Dr. Anitha, Professor, Department of Agricultural Entomology for her timely help rendered throughout the period of work.

I submit my sincere respectful regards to Dr.HebsyBai, Dr.Sudharma, Dr.Nandakumar, Dr.Faizal, Dr.Prathapan, Dr.Krishnakumar, Dr.Devanesan, Dr.Premila, Dr.RejiRani for their expert teaching, kind treatment, moral support, timely and valuable guidance during the course of study.

I greatly acknowledge the help rendered by Dr.Vijayaraghavakumar, Professor and Head, Department of Agricultural Statistics and Mr.C.E.Ajithkumar, Junior Programmer for the help rendered during the statistical interpretation of the experimental data.

I always remember with much gratitude Dr.Sulochana, Dr.Girija, Dr.Mary, Dr.Santhakumari, Dr.Gokulapalan, Dr.Umamaheswaran, Dr.Joseph, Department of Plant Pathology for their constant co-operation and support rendered throughout the period of work.

I dedicate my sincere thanks to the non teaching staff of Department of Agricultural Entomology for their help.

I convey my heartiest gratitude to Soorya chechi for her care, affection and valuable assistance in the preparation of the manuscript. I am much grateful to her for her patience.

I take this opportunity to thank my seniors, Jomy chechi, Thania chechi, Anis chechi, Kavitha chechi and Arpitha chichi for their help, inspiration and company throughout the work.

Hearty thanks to Nisha chechi, Sridevi chechi, Sarika chechi, Remya chechi, Pravidha, Archana chichi, Saradha chichi for their care, affection and unfailing moral support.

My special thanks to my fellow friends Gayathri, Gayathri Karthikeyan, Jinsy, Abhijith, Golda, Praveena, Lekshmi, Sugeedha, Seethal, Suma, Uma, Manisha, Vaiju for their unconditional and ever willing help throughout the period of study.

I express my love and affection to my beloved juniors Kranthi, Shameena, Neena, Seena, Preetha chechi, Simi for their continous support and help

I am most indebted to my loving father, better half (Venkatesh), sister (Yaso) for their undying love, constant support, sacrifices, and blessings which enabled me to complete the work in time.

I am grateful to Indian Council of Agricultural Research for granting me the Junior Research Fellowship during the course of my study.

Above all, I bow before the Almighty for all the blessings showered on me.

Amala.U
(2008-11-117)

Dedicated to Lord
Narasimhar

CONTENTS

LIST OF ABBREVIATIONS

%	Percentage
° C	Degree Celsius
CD	Critical Difference
Cm	Centimetre (s)
cfu	Colony Forming Units
DAI	Days after Inoculation
DAT	Days After Treatment
et al.	And others
Fig.	Figure
SC	Suspension Concentrate
SL	Soluble Concentrate
G	Gram
kg	Kilogram
a.i.	Active ingredient
ha^{-1}	Per hectare
L	Litres
ml	Millilitre
mm	Millimetre
$/m^2$	Per metre square
PDA	Potato Dexrose Agar
spp.	Different species
viz.,	Namely
WAI	Weeks After Inoculation
WG	Water Dispersable Granules

Introduction

1. INTRODUCTION

Cucurbits are important vegetable crops belonging to the family cucurbitaceae. The melon fly, *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) is a serious pest of cucurbitaceous vegetable crops, causing substantial economic loss. The extent of crop loss varies between 30 to 100 per cent (Gupta and Verma, 1992; Saptoka et al., 2010). Stonehouse (2001) estimated a loss of Rs. 26,902 million due to melon fly infestation, in India.

Several management measures involving hydrolyzed protein bait spray (Pawar et al., 1991; Zaman, 1995), para-pheromone lures (Stonehouse et al., 2002 and 2002a; Verghese et al., 2006; Stonehouse et al., 2007), botanicals, field sanitation, bagging of fruits and chemical sprays (Akhtaruzaman et al., 2000) have been used for the management of the pest. However, no biological control measures are standardized yet, at field level. Use of pesticides needs to be minimized, as vegetables are mostly consumed fresh.

In this context, it is relevant to explore the scope of biological control of melon fly for implementing effective integrated pest management (IPM). Deuteromycetes fungi *viz., Beauveria bassiana* (Bals.) Vuill, *Metarhizium anisopliae* (Metsch.) Sorok. (Carswell et al., 1998; Maniania, 2002; Destefano et al., 2005 and Cossentine et al., 2010), *Paecilomyces fumosoroseus* (Wize) Brown & Smith (Castillo et al., 2000), *P. lilacinus* (Thom) Samson and *Aspergillus candidus* Link:Fries (Jiji et al., 2006a) have been known to cause widespread epizootics in fruit flies under laboratory and field conditions. Biocontrol of fruit flies using entomopathogenic fungi is an eco-friendly and cost-effective option, especially in the humid tropical climatic conditions of Kerala. It would be ideal to develop an IPM strategy after incorporating biological control methods also.

Under these conditions, the present study was undertaken to,

- screen the entomopathogenic fungi against melon fly pupae and adults for selecting the promising fungi.

- standardize the effective dosage of the promising entomopathogenic fungi under laboratory condition by conducting bioassay for selecting the most promising fungus.
- select the promising substrate for the mass multiplication of the most promising fungus.
- test the efficacy of the most promising fungus against pupae and adults of melon fly under trough and cage conditions.
- evaluate the fungus under field conditions and
- study the effect of the fungus on other pests and natural enemies.

Review of Literature

2. REVIEW OF LITERATURE

The Melon fly, *Bactrocera cucurbitae* Coquillett (Diptera : Tephritidae) is a serious pest of cucurbits causing threat to the cultivation of cucurbitaceous vegetables. The present work aims at the biological control of melon flies using entomopathogenic fungi. The literature related to these aspects are reviewed here.

2.1 MELON FLY, *Bactrocera cucurbitae*

Fruit flies are serious economic pest affecting horticultural production world wide. *B. cucurbitae* is an important pest of cucurbitaceous vegetables. The genus *Bactrocera* (Dacus) causes heavy damage to fruits and vegetables in Asia (Nagappan et al., 1971). Among these *B. cucurbitae* is a major threat to cucurbits (Shah et al., 1948). Among the cucurbits, especially in bitter gourd, *Momordica charantia Linn*, the melon fly damage is the major limiting factor in obtaining good quality fruits and yield. (Srinivasan, 1959; Lall and Singh, 1969; Mote, 1975 and Rabindranath and Pillai, 1986).

2.1.1 Host range of melon flies, *B. cucurbitae*

Melon fly damages over 81 plant species. Doharey (1983) observed that melon fly infest over 70 host plants amongst which fruits of bittergourd (*Momordica charantia*), musk melon (*Cucumis melo*), snap melon (*C. melo. var. momordica*) and snakegourd (*Trichosanthes anguina* and *T. cucumeria)* were the most preferred hosts. Allwood et al. (1999) reported that the plants belonging to the family Cucurbitaceae were the most preferred hosts. Melon fly also infests other vegetables like ivy gourd (Narayanan and Batra, 1960), cantaloupe (Iwaizumi, 1994), grain legumes (White and Elson Harris, 1994), okra (Kumagai et al., 1996), tomato (Fontem et al., 1999), zingerone (Hong and Nishida, 2000), onion, fruits like mango, papaya, guava, peach and orange.

2.2 LABORATORY REARING OF MELON FLY

2.2.1. Diets

Mitchell et al. (1965) described a rearing medium for oriental fruit fly *B. dorsalis* (Hendel) consisting of dehydrated carrot powder with brewer's yeast, sodium benzoate and hydrochloric acid. Tanaka et al. (1969) developed a low cost rearing medium for *B. dorsalis* using methyl–p-hydroxy benzoate, sodium yeast type 200, concentrated hydrochloric acid, wheat sorts, gelgard and water. Yeast was the main nutritional constituent in the diet used to mass rear the adults and larvae of fruit flies (Schroeder et al., 1972; Cangussu and Zucoloto, 1992; Placido Silva et al., 1997 and Rohlfs and Hoffmeister, 2005).

Tsitsipis and Kontos (1983) used soy hydrolysate as protein source to maintain laboratory colony of olive fruit fly. Tzanakakis (1989) evaluated the performance of various nutritive and bulking components used in larval diets including groundnut corn cobs, eucalyptus pulp, soy hydrolysate, yeast hydrolysate, roasted peanuts, sucrose and chickpea seeds. Fay and Wornoayporn (2002) reported that the larvae of Mediterranean fruit fly *Ceratitis capitata* reared in a substrate enriched with wheat products resulted in increased pupal weight and pupal efficacy.

Protein ingestion increased the egg production in fruit flies (Saha et al., 1996). Verghese and Jayanthi (2002) evaluated the suitability of three fruit hosts for the laboratory rearing of oriental fruit fly, *B. dorsalis*. Different fruits like banana (var. Robusta & Elakki), guava (var. Allahabad safed) and papaya (var. Solo) were used for the study. He reported that all the host fruits could be used for the large scale rearing of *B. dorsalis*. Results indicated that fruit fly colonies reared on banana fruits showed highest pupal recovery (77.39% in Robusta and 69.17% in Elakki).

Saha et al. (2007) reported that proteose peptone and sugar supplied to the adults of *B. cucurbitae* in the ratio of 1:4 produced twice as many eggs compared to those supplied with yeast and sugar in the ratio of 1:3. He also found that sweet gourd (*Cucurbita maxima D.*) as the best natural host for the laboratory rearing of *B. cucurbitae* in terms of pupal recovery and adult emergence (88%). Hanife (2008) developed a modified agar based cost effective diet for small scale laboratory rearing of olive fruit fly *B. oleae.* He reported that 77% of the larvae reared on that diet achieved a higher pupal weight.

2.2.2. Biology of melon flies, *B.cucurbitae*

Biology of melon flies were studied by many workers (Agarwal et al., 1987; Liquido et al., 1989; Bhagat and Koul, 1999 and Weems and Heppner, 2001). Adult flies are yellowish brown in colour measuring 0.5 inches long with a wingspan of 0.5 to 0.6 inches. Oviposition occurs about ten days after emergence and may deposit up to 1000 eggs in the soft tender fruit tissues by piercing them with the ovipositor. The eggs are slender white measures 0.5 inches in length inserted in the fruits in bunches of 1 to 33. They hatch in 2 to 3 days. The larvae have three instars and are cylindrical, narrow, elongate and somewhat curved downwards at the end with mouth hooks at the head. Larval period lasts from 6 to 11 days. Pupation occurs in soil. The puparium measures 1/4 inches long – elliptical, dull white to yellowish brown in colour. Pupal period varies between 9 to 11 days. The total life cycle was completed between 12 to 28 days according to the individual species, host and weather conditions.

2.3 SCREENING OF FOOD BAITS

Fruit flies need sugar and protein source to survive and mature. They are highly attracted towards high quality sugar and protein sources. In Bait Application Technique (BAT), adult flies were attracted and killed by spot spray of bait or bait traps mixed with insecticides (Roessler, 1989). Sasidharan et al.

(1991) evaluated the superior performance of plantain fruit over jaggery, molasses and honey.

Banana traps (Palayamkodan + Carbofuran 3G) placed in coconut shells at the rate of two traps /three plants provided better protection against fruit files in snakegourd (Sivakumar, 2001). Meat based baits was also found effective against melon fruit flies (Stonehouse et al., 2002b). A bait of 20% jaggery solution showed superior performance in the trap catch of melon flies (Jiji et al., 2005). Boiling jaggery increased the bait's attractiveness when mixed with banana food baits containing banana + jaggery (50:50) which also served better in the trap catch of melon flies (Thomas et al., 2005).

Food bait containing a mixture of Rasakadali + jaggery + carbofuran 3G placed at a spacing of 2.5 x 2.5m attracted more files in cucurbits. Heating jaggery at 80^{o}C served to increase the keeping quality of food baits and increased the duration of effective trap catch of fruit files (Vidya, 2005).

2.4 PEAK TIME OF ACTIVITY OF MELON FLY

Jiji et al. (2006a) reported the peak time of activity of oriental fruit fly *Bactrocera dorsalis* (Hendel) infesting mango fruits by using parapheromone methyl eugenol traps. The maximum catch of fruit flies (16.8) was observed during the early morning hours (6-8 a.m.).

2.5 MANAGEMENT OF MELON FLY USING ENTOMOPATHOGENIC FUNGI

Entomopathogenic fungi comprise a diverse group of pathogens that have been recorded from all major taxa of arthropods (Roberts and Humber, 1981). The use of microbial control agents including entomopathogenic fungi an alternative to chemical control as a component of IPM strategies is being widely explored for the management of wide range of fruit fly pests (Dimbi et al., 2003; Lux et al.,

2003; Yee and Lacey, 2005 Mochi et al., 2006 and Quesada – Moraga et al., 2006).

Entomopathogenic fungi which infect their host through their cuticle holds greater potential as biocontrol agents for various species of fruit flies (Pell et al., 1993; Vega et al., 1995). It has been demonstrated that fungi may cause sub lethal reproduction effects on target fruit fly population that have important implication for the population dynamics of the host (Fargues et al., 1991; Arthurs and Thomas, 2000; Blanford and Thomas, 2001 and Mulock and Chandler, 2001)

Various Deuteromycetes fungi v*iz., Beauveria bassiana* (Bals.) Vuill, *Metarhizium anisopliae* (Metsch) Sorok, *Paecilomyces fumorosoroseus* (Wize) Brown Smith were known to cause widespread epizootics in various species of fruit flies under laboratory and field conditions (Barson et al., 1994; Watson et al., 1996; Reithinger et al., 1997). Jiji et al. (2006b) reported the pathogenicity of three fungal isolates of *B. bassiana* (ITCC 6063), *P. lilacinus* (Thom) Samson (ITCC 6064) and *Aspergillus candidus* Link:Fries (ITCC 5428) on melon fruit fly, *B. cucurbitae*.

2.5.1 *Beauveria bassiana* (Bals.) Vuill

B. bassiana is a deuteromycetes fungus that occur naturally in soil acting as a pathogen on various insect species causing white muscardine disease. The mycoinsecticides based on *B. bassiana* have been used to control various insect pests (Babu et al., 2001; Sharma, 2004).

2.5.1.1 Spore character

B. bassiana usually grows as a white mould producing many dry powdery conidia in distinct white balls. Each spore ball consist of a cluster of conidigenous cells, which are short, ovoid and terminate in a narrow spiral extension called rachis (Li et al., 2001). The rachis elongates after each conidium resulting in a

long zig zag extension. The conidia are single celled, haploid and hydrophobic (Rehner and Buckley, 2005).

2.5.1.2 Pathogenicity

Magnano Di San Lio and Vacante (1989) reported that the adults of Mediterranean fruit fly, *Ceratitis capitata* exposed to host fruits treated with the strains of *B. bassiana* showed less numbers of ovipunctures than the untreated fruits. The results indicated that the strains emitted repellent volatile cues (eg. Peramine) (Rowan et al., 1990; Daisy et al., 2002). Benuzzi and Santopolo (2001) reported that *B.bassiana* when tested against the adults of *C. capitata* acted mainly by contact and also by producing toxins like beauvericin, bassianolide and destruxin.

Anagnou – Veroniki et al. (2005) evaluated the pathogenicity of *B. bassiana* strain ATCC 74040 against the cherry fruit fly, olive fruit fly (Benuzzi et al., 2007; Ladurner et al., 2008). Konstantopoulou and Mazomenos (2005) reported that two strains of *B. bassiana* and *B. brongniarti* were more pathogenic to the adults of Mediterranean fruit fly *C. capitata*. The levels of mortality due to these two species were 97.4% and 85.6%, respectively. A new strain of *B.bassiana* was isolated from Bhindi leaf roller, *Sylepta derogata* and its cross infectivity to *B.cucurbitae* and *B.dorsalis* was reported by Jiji et al. (2006b).

2.5.1.3 Bioassay

Munoz (2000) evaluated sixteen strains of *B. bassiana* against the adults of Mediterranean fruit fly, *C. capitata* and observed the mortality levels between 20% and 78.7%. De La Rosa et al. (2002) achieved a mortality per cent between 82 and 100 in the laboratory spraying against the adults of *Anastrepha ludens Loew.* with *B. bassiana* at a spore concentration of 1.0×10^8 conidia/ml. The adults of *A. ludens* when exposed to a spore concentration of 1.0×10^8 conidia /ml showed a mortality per cent of 98.7% with an LC_{50} value of 2.67×10^7 conidia/ml

(Toledo et al., 2007). Ali et al. (2008) reported the susceptibility of adults of *C. capitata* to *B.bassiana* with a mortality of 100 per cent at 3 x 10^7 conidia/ml, followed by 82.5 per cent at 3 x 10^6 conidia/ml. He also observed the mouldiness in the dead insects in the range of 72.5% to 77.5% at 3 x 10^6 conidia/ml and 3 x 10^7 conidial/ml.

2.5.2 *Paecilomyces* spp.

P. lilacinus is a soil fungus with a good potential for biological control of insect pests. This species has been described as being as effective as the commonly used nematicides (Kerry, 1990 and Schenck, 2004) and also controls green house insects and mite pests (Fielder and Sosnowska, 2007).

2.5.2.1 Spore character

P. lilacinus forms a dense mycelium that gives rise to conidiospores. The conidiospores bear phialides from the ends of which spores are formed in long chains. Colonies consist of a basal felt with a floccose overgrowth of aerial mycelium at first white in colour but when sporulating changes to various shades of vinaceous brown. Conidia are in divergent chains, ellipsoid to fusiform in shape, smooth walled to slightly roughened in nature (Samson, 1974).

2.5.2.2 Pathogenicity

P. fumosoroseus strain CG 260 isolated from the tephritid *Anastrepha* sp was highly virulent to the Mediterranean fruit fly under laboratory conditions stressing that the isolation of fungi from naturally infected hosts was a good practice in search of a isolate with a potential as biological control agents (Watson et al., 1996; Vanderberg and Frank, 1997). Daniel and Wyss (2009) evaluated the pathogenicity of different strains of *P. fumorosoroseus* against the larvae and adults of western cherry fruit fly *Rhagoletis cerasi.*Younger flies showed higher susceptibility to the strains compared to older flies with a mortality range between

90-100% along with a strong influence on the fecundity and fertility of adult and a conidial suspension of *P. fumosoroseus* resulted in 21% mycosis in the pupal stage. Jiji et al., (2006) reported the occurrence of *P.lilacinus* on melon fly *Bactrocera cucurbitae* and its cross infectivity on oriental fruit fly *B.dorsalis.*

2.5.2.3 Bioassay

Jiji et al. (2006b) reported that the adults of melon fly *B. cucurbitae* when sprayed with *P. lilacinus* at $1.0x10^9$ spores/ml caused 96.67 percent and 100 per cent cumulative mortality in fruit flies on second and third day, respectively. Ali et al. (2008) reported the susceptibility of adults of Mediterranean fruit fly to conidial suspension of *P. fumosoroseus* and recorded a mortality percent of 70 at $3x10^7$ conidia per ml and 30per cent at $3x10^5$ conidia per ml. He also observed the mouldiness of the dead flies at 52.5 per cent at $3x10^7$ conidia/ml.

2.5.3 *Aspergillus* spp.

Aspergillus is a genus of few mould species found throughout in nature world wide. These group of fungus commonly grow as moulds on the surface of a substrates. Some of the species were commonly identified as pathogens of animals and insects.

2.5.3.1 Spore character

Fungal colonies of *A. candidus* appear dense with surface texture granular to floccose with white mycelium. Conidial heads appear densely packed and produce small amount of pale yellow orange exudates. Conidiospores are borne from the surface of hyphae with smooth to quite roughened walls bearing phialides over the entire surface (Kilch and Pitt, 1988).

2.5.3.2 Pathogenicity

A. parasiticus was reported causing high mortality in dipteran insect, *Culex gelidus*. A sudden decline in oviposition and highest adult mortality of dipteran insect, *Anopheles gambiae* exposed to *Aspergillus* sp. was recorded in the larval stages. The infectivity of some species of *Aspergillus viz., A. parasiticus* and *A. flavus* on the larvae of mosquitoes was reported. (Govindarajan et al., 2005).

According to Hajek and St. Leger (1994) the fungus *A. clavatus* exhibited a proteolytic, lipolytic and chitinolytic mechanism that acted on larval cuticle (Crisan, 1971; Lacey et al., 1988; Domnas and Varner, 1991 and Silva et al., 2004). A number of secondary metabolites like tryptoquivaline and tryptoquivalone (Clardy et al., 1975; Buchi et al., 1977) cytochalasin (Demain et al., 1976 and Lopez -Diaz and Flannigan, 1997) and Patulin (Varga et al., 2003) have been noted to cause pathological effects on the immature stages of phytophagous insects.

Jiji et al. (2003) reported the occurrence of *A.candidus* from *B.dorsalis* infesting guava. Pathogenicity of *A. flavus* on greater wax moth, *Galleria melonella* was studied by Scully and Bidochka (2005). Seye et al., (2009) reported that larvae of mosquito *C. quinquefasciatus* treated with *A. clavatus* could pupate and produce adults. The resulting adults displayed fungal germination on their abdominal extremities on seven days after inoculation.

2.5.3.3 Bioassay

Aflatoxin produced by *A. flavus* has got a significant insecticidal, larvicidal, chemosterilising properties against several insect pest and mite species (Moore et al., 1978; Gaston and Llewellyen, 1980). Of all the types of aflatoxin produced, Aflatoxin ATB, is mostly associated with insecticidal activity (Al-Adil

et al., 1972). The genus *Aspergillus* being truely entomopathogenic many species of the fungus caused secondary infection in the insects (Nnakumusana, 1985).

Wicklow et al. (1988) showed that non aflatoxigenic strains of *A. flavus* was found toxic to maize insect *Carpophilus hemipterus*. Drummond and Pinnock, (1990) reported around 20% of non aflatoxigenic isolates of *A. flavus* from naturally infected sugarcance mealybug, *Saccharicoccus sacchari*.

Gopal et al. (2000) obtained natural isolates of *A. flavus* from lacewing bug *Stephanitis typica* (nymphs & adults) and coconut black headed caterpillar *Opisinia arenosella* (larvae) and *Proutista moesta* (adults) and tested under *in vitro* conditions. Ponnama et al., 2000 reported 80% mortality of *S. typica* within three days, 80-90 percent mortality in *O.arenosella* within 3-4 days and *Proutista moesta* died within four days.

Jiji et al. (2006b) reported that adults of melon fly sprayed with *A. candidus* at 1.25 x 10^9spores/ml caused 63.33%, 83.33% and 100% cumulative mortality with an LC_{50} value of 1.29 x 10^8, 1.22 x 10^7 and 2.27 x 10^6 spores/ml on the third, fourth and fifth day, respectively.

A. candidus mass cultured on inexpensive substances like grain seeds at 10 per cent concentration was found pathogenic to scale insect *Coccus hesperidum* under glass house conditions (Samsinakova and Kalalova, 2006).

2.6. MASS PRODUCTION OF ENTOMOPATHOGENIC FUNGUS

Several liquid media like Potato Dextrose Broth (Rangaswami, 1972), Richard's medium, Semiselective medium (Mitchell et al., 1987) were evaluated for the mass production of *P.lilacinus*. The nutrients carbon and nitrogen and their proper balance in the media play a vital role in the growth of the fungus and sporulation. The addition carbon source from amino acids like glycine, glutamine,

serine, alanine supported the growth of the fungus by which more biomass production could be achieved (Griffin, 1981)

Samson et al. (1988) reported the mass mycelial production of *P. fumosoroseus* at submerged system. Nutritional factors determine the growth of the fungus (Carlile and Watkinson, 1994). Calderon et al., (1995) found that semi selective media recorded the highest fungal biomass (19.82g) and spore load (32.8×10^7 spores /g).

Gopalakrishnan et al. (1999) reported sorghum as the ideal cereal for the mass production of *P. farinosus* with a spore production of 10.37×10^8 spores /ml and biomass production (0.71g). The blastospore production by *P. fumosoroseus* was considered as the desirable quality for the virulence and shorter time necessary for the infection to occur (Lopez et al., 2000). Sandoval et al. (2001) evaluated the performance of blastospore production of *P. fumosoroseus* in two different liquid media, a basal salt medium supplemented with cassamino acids and glucose and a medium containing peptone of collagen and glucose.

Tincilley et al. (2001) reported that carrot waste was found to be the cheapest and best suitable media for large scale production of deuteromycetes fungi. Brand et al. (2003) selected a low cost substrate saw dust for the spore production of *P.lilacinus* in solid state fermentation. Use of liquid media showed good results in the production of *P. fumosoroseus* (Jackson et al., 2004). He reported a spore production of $5x10^8$ conidia/ml of *P. fumosoroseus* obtained in a fermenter with a medium consisting of glucose, ammonium nitrate with yeast extract added as the nitrogen source. Leena et al. (2003) evaluated the mass production of *P. farinosus* and *P.lilacinus* on sugarcane molasses, spent wash and other agricultural industrial wastes. Sugarcane press mud supported the growth as well as greater spore production of both *P. farinosus* and *P.lilacinus* compared to other agro industrial by products and wastes tested.

Robl et al. (2009) reported that Brazilian strain Endo 69 of *P. fumosoroseus* showed higher spores production and mycelial growth on Refuse potato (RP) medium. The RP media contained amino acids such as lysine, methionine and cysteine, several minerals and growth promoting factors that contributed to higher mycelial growth of the fungus.

2.7. BIO EFFICACY OF ENTOMOPATHOGENIC FUNGI ON THE ADULT FRUIT FLIES

Castillo et al. (2000) reported that *P. fumosoroseus* strain CG 260 when evaluated against the adults of melon fly *C. capitata* established a LC_{50} value of 2.2 x 10^5 conidia/fly, six days after treatment, followed by a LC_{50} value of 6.1 x 10^3 conidia/fly, ten days after treatment. He observed that the speed of mortality of the host was positively correlated with the conidial concentration (Fargues and Rodriguez-Rueda, 1980). The adult females exposed to fungal treatment with *P. fumosoroseus* showed reduced fecundity with an effect ranging from 8.0% at 1 x 10^2 conidia/fly to 66.1% at 1 x 10^6 conidia/fly.

Dimbi et al. (2003) evaluated the bioassay of some isolates of *B.bassiana* towards the adults of three African fruit flies *C.capitata* Weidemann, *C.rosa var fasciventris* Karsch and *C.cosyra* (Walker) and demonstrated that the fungus could easily be incorporated into the baited auto inoculative device to disseminate the disease into the fruit fly population. He observed a mortality of 67% to 100% in a dosage range of 1.2 x 10^5 to 1.0 x 10^6 condia/fly. Roy et al. (2007) observed that the adult flies treated with the fungus produced semiochemicals that attracted male flies. He found that the attractiveness of male by females would evidently benefit the horizontal transmission of the fungus.

Almeida et al. (2007) reported the application of isolates of entomopathogenic fungus *B. bassiana* (IBCB 66) at 5.0 x10^8 conidia/ml in natural

and sterilised soil reduced Mediterranean fruit fly *C.capitata* emergence upto 80%. Mahmoud (2009) performed the contact bioassay of entomopathogenic fungi *B.bassiana* and *Lecanicillium lecanii* against the adults of olive fruit fly *Bactrocera oleae* under *in vitro* conditions in cages. The bioassay was performed by applying a dose of entomopathogenic fungi applied as topical application at $1x10^8$ conidia/ml on the adults maintained inside the cages. Infection by fungal pathogens was reported to give behavourial modifications such as reduced mating competitiveness and attractiveness to the opposite sex (Schaechter, 2000).

2.8. BIO EFFICACY OF ENTOMOPATHOGENIC FUNGI ON PUPAE OF FRUIT FLIES

The potential of using entomopathogenic fungi for the control of soil dwelling life stages of fruit flies has been demonstrated by Zimmermann (1994) and Rath et al. (1995) and Jackson et al. (1999). Carneiro and Salles (1994) reported that *P. fumosoroseus* isolate CG 260 caused 20 per cent mortality in *Anastrepha fraterculus* (Weid.) pupae 20 days after application on the third instar larvae with a value LC_{50} of 1.2 x 10^6 conidia/ml. According to the author, pupae of *A. fraterculus* when treated at a spore concentration of 10^8 conidia/ml caused cent percent mortality with lesions on the body or mycelium emerging out of the natural extremities.

The entomopathogenic fungi survived better under soil conditions (Gaugler et al., 1989). Entomopathogenic fungi may be used against prepupating larvae and puparia in the soil which provides a favourable environment for fungal microbial control (Jackson et al., 2000). Application of conidia of the fungus to the soil had been suggested as a method to use entomopathogenic fungi for the control of fruit flies. The method of soil application was found as a best way to infect newly emerged adults (Ekesi et al., 2005).

Alves et al. (2004) evaluated the effectiveness of the fungi *B. bassiana*, *P. fumosoroseus* and *Verticillium* sp. on Mediterranean fruit fly *C. capitata* via immersion of pupae and pre pupae and topical application of a suspension containing 1 x 10^8 conidia/ml in adults with an efficiency of 90 per cent in pupae and 60 per cent in adults. Mochi et al. (2006) verified the pathogenicity of the fungus *M. anisopliae* to Mediterranean fruit fly *C. capitata* larvae, pre pupae and pupae under laboratory conditions which was evident by mycosis in the pupal stage and reduced adult emergence.

2.9. FIELD APPLICATION OF ENTOMOPATHOGENIC FUNGI

Garcia et al. (1989) reported that the application of entomopathogenic fungi to the soil was a viable alternative fruit fly management option in orchards. Lezama-Gutirrez et al. (2000) tested the virulence of *M. anisopliae* on the adults of *A. ludens* in both laboratory and field conditions. In field cage experiments, application of *M. anisopliae* on the soil at 2.5x 10^6 cfu/ml reduced adult emergence by 33% in loam soil and 49% in sandy loam soil. Soil application of *M. anisopliae* under field condition for the management of Mediterranean fruit fly *C. capitata* revealed that the soil temperature not exceeding 26°C under tree canopies provided a congenial environment for the persistent action of the fungus (Studdert et al., 1990; Quintela et al., 1992).

Ekesi et al. (2007) found that exposures of the target fruit fly species to dry conidia of *M. anisopliae* and *B. bassiana* resulted in a mortality ranging from 9% to 100% within five days of post inoculation. It was also observed that the most pathogenic isolate of *M. anisopliae* was deployed in the baiting stations and evaluated for two successive generations in small scale mango orchards. Results indicated that the effectiveness of the baiting stations equipped with the pathogen did not differ from the insecticide (malathion). A 70% reduction in fruit fly population was achieved in treated small plots.

Monchan et al. (2008) evaluated the efficacy of *P.fumosoroseus* against the second instar larvae of diamond back moth, *Plutella xylostella* under laboratory conditions. Later he formulated the fungus into wettable powder at a concentration of 1 x 10^9 conidia/g and evaluated under field conditions. Results revealed that *P. fumosoroseus* at 1.25 x 10^{13} conidia/ml/ ha effectively controlled the larvae of diamond back moth.

Radoslav et al. (2008) evaluated the efficacy of *P. fumosoroseus* (PreFeRal WG) at 0.1% and 0.2% against the three important pests of black cherry aphid, *Myzus persicae*, cherry weevil *Rhynchites auratus* and fall webworm *Hyphantia cunea* under field conditions. He observed that PreFeRaL WG applied at 0.2% showed a satisfactory effect with 79.7% efficacy at 3 DAT and 83% in the following days and increase of its concentration could result in the improvement of the efficacy of this insecticide.

Daniel and Wyss (2009) evaluated the performance of two mycoinsecticides Naturalis L. (*B. bassiana*) and PreFeRaL WG (*P. fumosoroseus*) against the adults of cherry fruit fly, *Rhagoletis cerasi* L. at 5.75 x10^4 cfu/ml. The results indicated a significantly reduced number of damaged fruits with 69-74% efficacy in *B. bassiana* and 27% efficacy in *P. fumosoroseus*.

2.10. CHEMICAL CONTROL OF FRUIT FLIES

Gupta and Verma (1982) reported that fenitrothion (0.025%) in combination with protein hydrolysate (0.25%) reduced fruit fly damage to 8.7% as compared to 43.3% damage in untreated control. Application of carbofuran granules at 1.5 kg ai/ha at the time of sowing, vining and flowering gave 83.35% protection to bittergourd against *B.cucurbitae* (Thomas and Jacob, 1990). Reddy (1997) reported triazophos to be the most effective insecticide against *B.cucurbitae* in bittergourd. Application of molasses + malathion + water in the

ratio 1: 0.1:100 provided good control over melon fly (Akhtaruzaman et al., 2000).

Application of 0.2% malathion + 1% sugar as coarse droplets on the ventral surface of leaves during the fruiting period reduced the infestation in cucurbitaceous vegetables (KAU, 2007). Spraying lambdacyhalothrin reduced the number of melon fruit fly pupae and increased the quality of harvested cucumbers (Oke, 2008). Verghese et al., 2006 reported that an additional cover spray of Deltamethrin 2.8 EC@ 0.5 ml/l along with methyl eugenol traps + field sanitation provided 100 per cent control of oriental fruit fly *B. dorsalis* in mango. Waseem et al. (2009) reported that acephate 75 SP at 375 g ai/ha, spinosad 45 SC 54 ml/ha, Imidachloprid 17.8 SL 53.4 ml/ha recorded 8.00%, 9.00% and 10.33% less fruit damage in cucumber due to *B.cucurbitae.*

Materials and Methods

3. MATERIALS AND METHODS

The laboratory and field experiment on the management of melon fly, *Bactrocera cucurbitae* Coquillett using the local isolates of entomopathogenic fungi *viz*, *Beauveria bassiana* (Bals) Vuill, *Paecilomyces lilacinus* (Thom) Samson, *Aspergillus candidus* Link: Fries were conducted in the Department of Entomology and Instructional Farm, College of Agriculture, Vellayani, respectively.

3.1 STANDARDISATION OF EFFECTIVE DOSAGE OF ENTOMOPATHOGENIC FUNGI

The entomopathogenic fungi namely *Beauveria bassiana* (ITCC 6063), *P. lilacinus* (ITCC 6064), *A. candidus* (ITCC 5428) were screened against the pupae and adults of melon flies and their effective dosage of the promising fungus were standardized under laboratory conditions (Plate 1). The fungal cultures were obtained from the Department of Agricultural Entomology, College of Agriculture, Vellayani. The isolates were the findings of ICAR *ad hoc* project on biological control which was in operation in the Department of Agricultural Entomology, College of Agriculture, Vellayani during the year 2004-2007. In order to screen the entomopathogenic fungi against the pupae and adults, *in vitro* rearing of melon fly *B. cucurbitae* was carried out.

3.1.1 *In vitro* rearing of melon fly, *B. cucurbitae*

For rearing the melon fly under *in vitro* conditions different dietary constituents consisting of yeast along with honey, sugar, jaggery, sucrose and water at different combinations were evaluated.

3.1.1.1 Standardisation of diets

The experiment was conducted in Completely Randomized Design (CRD) with four replications. The adult flies were maintained inside the wooden cages of size 50 x 50 x 50 cm (Plate 2). Twenty adult flies with male and female in the ratio 1:1 were used per replication. They were supplied with different diets kept in petriplates (3 numbers) containing diet solution at the rate of 4 ml per plate. The diet was replaced regularly at an interval of two days (Plate 3). Four bittergourd fruits per replication were kept inside the cages for oviposition and the fruits were replaced at an interval of 4, 8, 12, 16, 20, 24 days. The following were the treatments used for the study.

T_1 - Diet preparation in water (100 ml) containing Honey (20 ml) + Yeast (10g)

T_2 - Diet preparation in water (100ml) containing Sugar (20g) + Yeast (10g)

T_3 - Diet preparation in water (100 ml) containing Jaggery (20g) + Yeast (10g)

T_4 - Diet preparation in water (100 ml) containing Sucrose (20g) + Yeast (10g)

T_5 - Water (100 ml) + Yeast (10g)

The observations on the number of surviving adult flies were recorded at 4, 8, 12, 16, 20, 24 days after treatment (DAT) and the survival percentage of the flies were calculated using the formula,

$$\text{Survival percentage} = \frac{\text{Number of surviving insects}}{\text{Total number of insects released}} \times 100$$

The number of eggs per oviposition in each treatment was also recorded by destructive sampling at 4, 8, 12, 16 DAT.

3.1.1.2 Selection of suitable diet

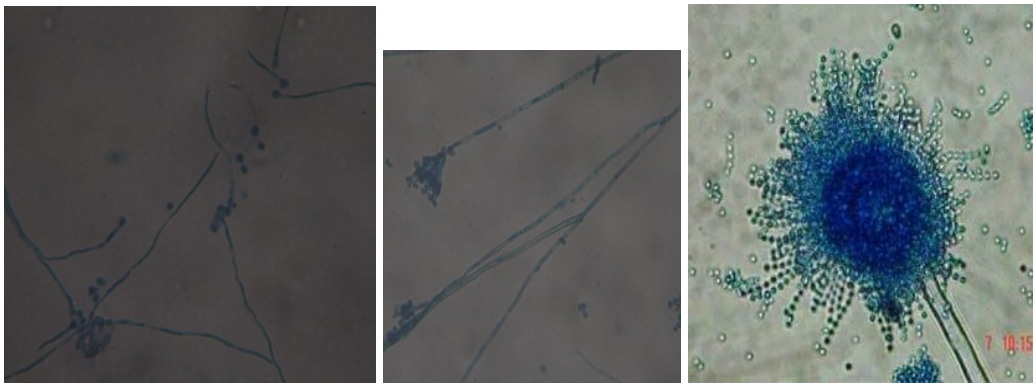
The dietary composition that gave the highest survival percentage of adult flies and maximum number of eggs per oviposition was used for the rearing of melon flies for the further studies.

3.1.1.3 Rearing Procedure

(i) Rearing of adults

The fruit fly infested bittergourd fruits were collected from the fields and introduced into the troughs containing soil for pupation. For this, uniform sized glass troughs of size 30 cm diameter was used (Plate 4). The troughs were filled with soil to a depth of 4 cm and moistened by sprinkling water. The troughs were covered using a muslin cloth and fastened using a rubber band and kept aside for three days. The troughs were constantly examined for the emergence of adult flies.

On the day of adult emergence, the troughs were kept inside the cages and the adults were allowed to move inside the cages. The flies were supplied with a diet standardised as in experiment 3.1.1.2. The diet was replaced at an interval of three days. Two petriplates containing sterile distilled water were kept inside the cages to meet the water requirement of the flies. The troughs were taken out of the cages and kept for further emergence of adults. The emerged adults were introduced into the cages. This process was continued until the adult emergence was stopped. The adults inside the cages were supplied with five bitter gourd fruits for oviposition.



B. bassiana **(ITCC 6063)** ***P. lilacinus*** **(ITCC 6064)** ***A. candidus*** **(ITCC 5428)**

Plate 1. Entomopathogenic fungi selected for the study

Plate 2. Cage for rearing of adults of melon fly

Plate 3. Diets supplied to the adults in cages

Plate 4. Troughs used for *in vitro* studies

(ii) Rearing of Pupae

The infested fruits from the experiment 3.1.1.3 containing mature maggots were collected regularly at an interval of five days and kept in soil maintained in the troughs (30 cm diameter) to a depth of 4 cm for pupation. The soil in the troughs was kept moist by sprinkling water over it and covered with a muslin cloth and fastened. The pupae were collected from the soil at an interval of three days and used for the further pathogenicity studies.

3.1.2 Screening of Entomopathogenic fungi

The entomopathogenic fungi *B. bassiana, P. lilacinus, A. candidus* were screened against the pupae and adults of melon flies.

3.1.2.1 Pathogenicity studies

The entomopathogenic fungi *B. bassiana, P. lilacinus, A. candidus* were tested for their pathogenicity on the pupae and adults of melon flies under *in vitro* conditions.

3.1.2.1.1 Pupae

Five mm fungal discs taken from seven days old well sporulated cultures of the test fungi maintained in PDA was used for the study. The spore suspension was prepared in sterile distilled water and filtered through a muslin cloth. For this, glass troughs of 30cm diameter was filled with sterile soil (autoclaved at 1.02 kg/cm^2 for one hour) to a depth of 4 cm. The soil in the troughs were drenched with the spore suspension at the rate of 30 ml per trough of the test pathogen. One trough was maintained for each entomopathogen under study. Soil drenched with 30 ml of sterile water served as control. The troughs were covered using a muslin cloth and fastened. Ten numbers of one day old pupae were introduced into each of the troughs.

Observations on the nature of symptom development and time taken for the development of disease symptom were recorded at 2 DAT.

3.1.2.1.2 Adults

Five mm fungal discs taken from seven days old sporulating cultures of the test fungi maintained is PDA was used for the study. The spore suspension was prepared in sterile distilled water and filtered through a muslin cloth. The adult flies were released into the cages. Ten numbers of one day old adult flies were used for the study. The flies were sprayed with the spore suspension using an atomiser and supplied with diet as in 3.1.1.2. Insects sprayed with sterile water served as control. Fruits sprayed with fungal cultures were also kept.

Observations on the nature of symptom development and time taken for the death of the insects were recorded at two DAT.

3.1.2.2 Maintenance of fungal cultures

The pathogens were isolated from the infected adults and pupae and maintained in PDA slants. The cultures were purified by hyphal tip method. The virulence of the pathogen was maintained by introducing them at monthly intervals into healthy pupae and adults of melon flies.

3.1.3 Bioassay of the entomopathogenic fungi on adults and pupae for the standardisation of effective dosage of entomopathogenic fungi

The promising fungal pathogens *viz., B. bassiana, P. lilacinus, A. candidus* were used for this experiment. The experiment was conducted under laboratory conditions to standardise the dosage of inoculum required for the effective killing of the pupae and adults of melon flies. The dosage was fixed based on the studies

conducted in the Department of Agricultural Entomology, COA, Vellayani (Jiji et al., 2006b). The following treatments were given

No.	Test Pathogen	Treatment / concentrations
1	*Beauveria bassiana*	T_1 - 2 x 10^3 spores / ml T_2 - 2 x 10^4 spores / ml T_3 - 2 x 10^5 spores / ml T_4 - 2 x 10^6 spores / ml T_5 - 2 x 10^7 spores / ml T_6 - Control (water spray)
2	*Paecilomyces lilacinus*	T_1 - 1.5 x 10^3 spores / ml T_2 - 1.5 x 10^4 spores / ml T_3 - 1.5 x 10^5 spores / ml T_4 - 1.5 x 10^6 spores / ml T_5 - 1.5 x 10^7 spores / ml T_6 - Control (water spray)
3	*Aspergillus candidus*	T_1 - 1.75 x 10^3 spores / ml T_2 - 1.75 x 10^4 spores / ml T_3 - 1.75 x 10^5 spores / ml T_4 - 1.75 x 10^6 spores / ml T_5 - 1.75 x 10^7 spores / ml T_6 - Control (water spray)

The spore suspension with the specified spore count was prepared in sterile water. The spore suspension was sprayed on healthy pupae and adults of

melon flies. Four replications were maintained for each fungus for each concentration. Suitable control treatments were kept for each fungus.

3.1.3.1. Bioassay of entomopathogenic fungi on pupae

The entomopathogenic fungi namely *B. bassiana, P.lilacinus, A. candidus* was screened against the pupae of melon flies under laboratory conditions. The experiment was replicated four times. Uniform sized glass troughs (30 cm diameter) filled with sterile soil to a depth of 4 cm was used for the study. The soil was drenched with 30 ml of spore suspension. Ten numbers of one day old pupae obtained from 3.1.1.3 were introduced into the troughs and troughs were covered with muslin cloth. Soil sprinkled with water served as control.

Observations on the pupal mortality count was taken each day after treatment for a period of seven days. The time taken for the death of insect was also recorded.

3.1.3.2. Bioassay of entomopathogenic fungi on adults

The entomopathogenic fungi namely *B. bassiana, P.lilacinus, A. candidus* were screened against the adults of melon flies under laboratory conditions. The experiment was replicated four times. The adult flies obtained from 3.1.1.3 was maintained inside the cages. Ten numbers of one day old adult flies were used. The adult flies were sprayed with ten ml of spore suspension using an atomiser. They were supplied with the diet as in 3.1.1.2. Adult insects sprayed with water served as control. Bittergourd sprayed with fungal cultures were also kept.

Observations on the adult mortality count was taken each day after treatment for a period of seven days. The time taken for the death of insect was also recorded.

3.1.3.3. Selection of promising fungal isolate

The observations recorded in experiment 3.1.3.1 and 3.1.3.2 was subjected to probit analysis (Finney, 1971). The LC_{50} value of the entomopathogenic fungi on each day after treatment was worked out.

3.2. MASS MULTIPLICATION OF THE PROMISING FUNGAL ISOLATE ON LOCALLY AVAILABLE SUBSTRATES

The promising fungal isolate identified in experiment 3.1.3.3 was used for the study. To evaluate the best medium for the growth of the fungus, five locally available solid and liquid substrates were used for the mass multiplication of the fungus. The experiment was conducted in CRD with four replications.

3.2.1 Solid substrates

The following solid substrates were used for the mass multiplication of the fungus.

T_1 - Rice bran

T_2 - Wheat bran

T_3 - Gingelly oil cake

T_4 - Coir pith

T_5 - Neem cake

Sixty gram each of the solid substrate added with 50 ml of sterile distilled water was taken in 250 ml conical flask. The substrate was sterilised in an autoclave at 121.1° C for 15-20 minutes at 1.02 kg/ cm^2 pressure. After sterilisation the substrate was artificially inoculated with five mm fungal disc

from well sporulating seven days old cultures maintained in Potato Dextrose Agar slants. Each treatment was replicated four times. After inoculation the flask was incubated at room temperature (28 ± 4° C). The conical flasks were shaken daily for the uniform growth of the fungus. The observations on the spore count and efficacy of the pathogen on the pupae and adults of melon fly in causing disease symptom were recorded.

3.2.1.1 Effect of solid substrate on sporulation of the promising fungal isolate

The spore count of the fungus grown on five solid substrates was estimated using a haemocytometer. One gram of the solid substrate was dissolved in 100 ml of sterile distilled water. This suspension was mixed well and filtered through a muslin cloth. The spore count of the fungus was taken using a haemocytometer at weekly intervals for a period of two months.

3.2.1.2 Effect of solid substrates on the efficacy of fungus

The efficacy of the promising fungus grown on different solid substrates was tested against the pupae and adults of melon flies. The experiment was conducted in CRD with four replications. The efficacy of the promising fungus grown on different solid substrates was tested at 1, 2, 3, 4 weeks after storage (WAS). The spore suspension was prepared in sterile water and filtered through a muslin cloth. The effective spore concentration of the fungus (1.5×10^7 spores / ml) obtained in experiment 3.1.3.3 was used for testing the efficacy of the fungi.

3.2.1.2.1 Effect on pupae

The soil along with the pupae was taken as in experiment 3.1.3.1 and drenched with 30 ml of spore suspension of the test pathogen grown on different solid substrates. Soil drenched with water served as control.

Observations on the mortality of the pupae on each day after treatment (DAT) was recorded for a period of one week. The percentage mortality of pupae was worked out.

3.2.1.2.2 Effect on adults

The adult flies maintained inside the cages as in 3.1.3.2 was sprayed with ten ml of the spore suspension using an atomiser. The flies were supplied with diet as in 3.1.1.2. Insect sprayed with water served as control.

Observations on the mortality of the adults on each day after treatment (DAT) was recorded at an interval of two days. The percentage mortality of adults was worked out.

3.2.2 Liquid substrates

The following liquid substrates were used for the study,

T_1 - Rice bran extract
T_2 - Wheat bran extract
T_3 - Coconut water
T_4 - Boiled rice water (Kanji water)
T_5 - Water

The extracts were prepared by boiling and filtering fifty gram of solid materials (Rice bran, wheat bran) with 400 ml of water for 10 minutes. In the case of boiled rice water, fifty gram of rice was cooked in 200 ml of water for 30 minutes and the extract was taken. From this 100 ml was taken in 250 ml conical flask and sterilised, inoculated and incubated as in experiment 3.2.1. The observations on the spore count and the efficacy of the pathogen on the pupae and adults was recorded as in 3.2.1.1.

3.2.2.1 Effect of liquid substrate on the sporulation of the promising fungal isolate

The spore count of the fungus grown on five liquid substrates was estimated using a haemocytometer. The inoculated liquid medium was shaken

well before taking the sample. One ml of liquid media was taken using a micropipette and dissolved in 100 ml of sterile distilled water and shaken well. The spore count was taken at weekly intervals for a period of one month.

3.2.2.2 Effect of liquid substrate on the efficacy of the fungus

The efficacy of the promising fungus grown on liquid substrates was tested against the pupae and adults of melon flies. The experiment was conducted in CRD with four replications. The efficacy of the fungus grown on different liquid substrates was tested at 1, 2, 3, 4 weeks after storage (WAS). The spore suspension was prepared in sterile distilled water and filtered through a muslin cloth. The effective spore concentration of the fungus (1.5×10^7 spores / ml) obtained in the experiment 3.1.3.3 was used for testing the efficacy of the fungus.

3.2.2.2.1 Effect on pupae

The effect of the promising fungus grown on different liquid substrates was tested on the pupae and the observations were recorded as in experiment 3.2.1.2.

3.2.2.2.2 Effect on adults

The effect of the promising fungus grown on different liquid substrates was tested on the adults and the observations were recorded as in experiment 3.2.1.2.

3.3. TESTING THE EFFICACY OF THE MOST PROMISING FUNGUS ON ADULTS UNDER CAGE CONDITIONS

The promising fungus identified in 3.1.3.3 mass multiplied in the promising substrate identified in 3.2.3 was used for this study. The experiment was conducted in CRD with four replications under cage conditions. Ten numbers of one day old adults were used per replication. The treatments were fixed based

on the LC_{50} value of the promising fungal isolate on the adults identified in 3.1.3.3. The following were the treatments

T_1 - 2.4×10^7 spores/ml

T_2 - 2.4×10^8 spores/ml

T_3 - 2.4×10^9 spores/ml

T_4 - Control (water spray)

3.3.1 Preparation of spore suspension

The spore suspension used for spraying on the adult flies was prepared as detailed in 3.2.1.1.

3.3.2 Testing the effect of the promising fungus on adults

The effect of the promising fungus on the adults based on LC_{50} values was carried out as detailed in 3.1.3.2.

Observations on the mortality count of adult flies was taken one DAT for a period of seven days and the percentage mortality was worked out.

3.4. TESTING THE EFFICACY OF THE MOST PROMISING FUNGUS ON PUPAE UNDER TROUGH CONDITIONS

The promising fungus identified in 3.1.2.3 mass multiplied in the promising substrate identified in 3.2.3 was used in this study. The experiment was conducted in CRD with four replications under trough conditions. The treatments were fixed based on the LC_{50} value of the promising fungus on the pupae as in 3.1.3.3. The treatments were as follows

T_1 - 1.3×10^7 spores/ml

T_2 - 1.3×10^8 spores/ml

T_3 - 1.3×10^9 spores/ml

T_4 - Control (water drench)

3.4.1 Preparation of spore suspension

The spore suspension needed for drenching the soil was prepared as detailed in 3.2.1.1.

3.4.2 Testing the effect of the promising fungus on pupae at different intervals after inoculation of soil

The troughs along with the soil was prepared as detailed in 3.1.3.1. Each treatment was replicated four times. The soil in the troughs were drenched with the spore suspension at the rate of 30 ml per trough. Ten numbers of one day old pupae were introduced into the soil on the 1, 3rd, 5th, 7th, 9th day of treatment of the fungus in each of the sets, respectively.

Observations on the number of infected pupae per trough seven days after introduction of the pupae were recorded.

3.5 TESTING THE EFFICACY OF PROMISING FUNGUS UNDER FIELD CONDITIONS

3.5.1 Field Trial

Before evaluating the promising fungus under field conditions, a preliminary study was conducted to document the peak time activity of melon flies. The experiment was conducted in Randomized Block Design (RBD) with three replications. In order to collect the flies, banana (var. Palayamkodan) jaggery traps were kept in plastic bottles of capacity 500 ml in each treatment plots. The traps were erected in the field during the early morning hours (6 a.m.). The following were the treatments used.

T_1 - Bait preparation in water (100 ml) containing banana (10g) + jaggery (10g) + 0.2 ml malathion + yeast (10g)

T_2 - Bait preparation in water (100 ml) containing banana (20g) + jaggery (10g) +0. 2 ml malathion + yeast (10g)

T_3 - Bait preparation in water (100 ml) containing banana (30g) + jaggery (10g) + 0.2 ml malathion +yeast (10g)

T_4 - Bait preparation in water (100 ml) containing banana (10g) + jaggery (10g) + 0. 2 ml malathion

T_5 - Bait preparation in water (100 ml) containing banana (20g) + jaggery (10g) + 0.2 ml malathion

T_6 - Bait preparation in water (100 ml) containing banana (30g) + jaggery (10g) + 0.2 ml malathion

T_7 - Control (water)

Observations on the count of adult flies was recorded in each treatment plot at an interval of two hours (6-8 am,8-10am,10-12am,12-2pm,2-4pm,4-6pm).

3.5.2 Evaluation of promising fungus under field conditions

Based on the *in vitro* studies conducted on the pupae as in experiment 3.3 and 3.4 the promising fungus was tested under field conditions. The field experiment was conducted in the Instructional Farm, College of Agriculture, Vellayani. The details of the experiments are as follows.

Design - RBD ; Crop - Bittergourd
Variety – Preethi ; Area - 400m^2
Spacing - 2 x 2 m ; Plot size - 4 x 4 m

The crop was raised under the pandal system during September – January 2010. Twenty five plots each of size 4 x 4m were maintained. The recommended POP of KAU was followed except for the plant protection measures which was given as per the treatments fixed in the study (KAU 2007).

The following were the treatments used

No	Concentration	Method of application	Quantity used
T_1	1.3 x 10^9 spores/ml	Soil drenching	170g in 17 L water per $40m^2$
T_2	2.4 x 10^9 spores/ml	Spraying	20g in 2 L water per $40m^2$
T_3	T_1+T_2	Soil drenching & Spraying	T_1+T_2
T_4	0.2% malathion + 1% jaggery	Spraying	8 ml malathion + 20g jaggery in 2 L water per $40m^2$
T_5	Control	Water spray	

The treatments were applied in the field twice at fruit initiation at fortnightly intervals during the fruiting stage. The treatments were applied during the peak time of activity of melon fly observed in 3.5.1.

3.5.2.1 Observations

Number of infested fruits per plant

For calculating the number of infested fruits per plant, five plots each of size 4 x 4m pandal area was selected in each replication and the percentage infestation was worked out.

$$\text{Percentage infestation} = \frac{\text{No. of infested fruits}}{\text{Total no. of fruits}} \times 100$$

Number of infected pupae

For calculating the number of infected pupae per plot, about one square metre area was marked in each treatment plot using a quadrant. The soil in the demarcated area was digged to a depth of 5-10 cm and the number of infested pupae was recorded.

Population of other pests

For calculating the population of other pests, number of larvae or adults in five leaves was counted at random from selected plants.

Population of natural enemies

For quantifying the population of natural enemies, the number of predators / parasitoids per plant was counted from ten randomly selected plants.

Mortality of other insects

For recording the mortality of other pests, four plants were randomly selected in each plot and mortality count of other pests was recorded.

3.5.3 Benefit Cost Ratio

The cost benefit ratio was worked out based on the premium price of Rs.15 per kg (market value of the organic produce) in the case of the treatments (soil drenching, spraying, soil drenchinng+spraying with *P.lilacinus*) whereas the cost benefit ratio of the treatment (0.2% malathion spraying) was worked out based on the normal market value of Rs.10 per kg.

3.5.4 Statistical Analysis

The observations were recorded and the data relating to each experiment was analysed by applying the Analysis of Variance (ANOVA) technique (Panse and Sukhatme, 1967).

Results

4. RESULTS

The results of the study entitled "Management of melon fly (*Bactrocera cucurbitae* Coquillett) using local isolates *Beauveria bassiana* (Bals.) Vuill, *Paecilomyces lilacinus* (Thom.) Samson and *Aspergillus candidus* Link: Fries carried out in the Department of Agricultural Entomology, College of Agriculture, Vellayani are presented here.

4.1 STANDARDISATION OF EFFECTIVE DOSAGE OF ENTOMOPATHOGENIC FUNGI

The fungi *viz., Beauveria bassiana*, *Paecilomyces lilacinus* and *Aspergillus candidus* were screened for their pathogenicity on the adults and pupae of melon fly and the effective dosages of the fungi were standardized by conducting bioassay studies (Table 3-9).

4.1.1 *In vitro* rearing of melon fly, *B. cucurbitae*

An experiment was conducted to standardize the *in vitro* rearing of melon fly *B. cucurbitae* for conducting laboratory studies (Table 1 and 2).

4.1.1.1 Effect of different diets on the survival of adults in cage conditions

On the fourth day a higher survival percentage of 98.14 (Table 1) was recorded in the case of adults of *B.cucurbitae* supplied with jaggery + yeast, followed by honey + yeast (91.50), sugar + yeast (74.58) and sucrose + yeast (66.92). The effect of all the above treatments were statistically on par. Water + yeast recorded a survival percentage of 30.09 on the same day.

Observations recorded on the eighth day revealed a higher survival percentage of adults supplied with jaggery + yeast (98.14), followed by honey + yeast (89.75) and these treatments were on par.

Adults supplied with sucrose + yeast and sugar + yeast recorded a survival percentage of 56.53 and 53.45, respectively and both the treatments were on par. Lower survival percentage of 13.97 was observed in the case of adults supplied with water + yeast on the same day.

On the 12th day, a significantly higher survival percentage of 97.98 was recorded in the case of adults supplied with jaggery + yeast, followed by 88.63 in honey + yeast and both the treatments were on par. Sucrose + yeast recorded a survival percentage of 60.45 on the 12th day which was on par with the survival percentage of 47.82 in sugar + yeast. Water + yeast recorded the least survival percentage of 17.77 on the same day.

On the 16th day, a higher survival percentage of 95.75 was recorded in the case of adults supplied with jaggery + yeast, followed by 76.02 in honey+ yeast and 63.87 in sucrose + yeast and the effect of the later two treatments were on par. Sugar + yeast recorded the least survival percentage of 11.77 and water + yeast recorded no survival.

A significantly higher survival percentage was recorded on the 20th day in the case of adults supplied with jaggery + yeast (85.36) which was on par with a survival percentage of 42.47 recorded in the case of adults supplied with honey + yeast (Table 1). Sucrose + yeast and sugar + yeast recorded a survival percentage of 17.77 and 10.87, respectively for the adults on the 20th day. Adults could not survive in the diet supplied with water + yeast on the same day.

Observations recorded on the 24th day revealed a significantly higher survival percentage in the case of adults supplied with jaggery + yeast (67.72) which was on par with a survival percentage of 34.91 recorded in the case of adults supplied with honey + yeast. Adult flies supplied with sucrose + yeast recorded a survival percentage of 8.76 on the same day. Adults supplied with sugar + yeast and water + yeast recorded no survival.

4.1.1.2 Fecundity of adults in different diets

The fecundity of adults supplied with different diets was recorded (Table 2). On the fourth day, adults supplied with jaggery + yeast recorded the maximum number of eggs (103.65) that differed significantly from all other treatments. The fecundity was 31.03 in the adults supplied with honey + yeast, followed by 14.44 in sucrose + yeast and 12.10 in sugar + yeast and these treatments were statistically on par.

On the eighth day, adults supplied with jaggery + yeast supported the maximum fecundity (57.37) which was on par with honey + yeast (39.96). The fecundity was 15.08 in the case of adults supplied with sucrose + yeast which was on par with the fecundity recorded in the case of adults supplied with sugar + yeast (8.79).

Observations recorded on the 12th day showed that the number of eggs laid was found maximum in the case of adults supplied with jaggery + yeast (46.33) that differed significantly from all other treatments (Table 2). The fecundity was 16.81 in the case of adults supplied with honey + yeast, followed by sucrose + yeast (10.83) and sugar + yeast (5.10).

Adults supplied with jaggery + yeast recorded the maximum number of eggs (62.20) on the 16th day that differed significantly from all other treatments. The fecundity was 10.49 in honey + yeast, followed by 5.76 in sugar + yeast and 3.54 in sucrose + yeast and all these treatments were on par. No oviposition was observed in the adults supplied with water + yeast, on any of the recorded days.

4.2 SCREENING OF ENTOMOPATHOGENIC FUNGI

4.2.1 Symptom of Infection

Pupae infected with *P.lilacinus* appeared blackened with visible white powdery mycelial growth from third day after treatment (Plate 5). Brownish white mycelial mat covered the entire pupae during the later

Table 1. Effect of different diets on the survival of adults in cage conditions

Treatments	Mean Survival Percentage of Adults (Days after treatment)					
	4	8	12	16	20	24
T_1 Honey (20ml) + Yeast (10g) in 100ml water	91.50 (73.05)	89.75 (71.33)	88.63 (70.30)	76.02 (60.68)	42.47 (40.67)	34.91 (36.22)
T_2 Sugar (20g) + Yeast (10g) in 100 ml water	74.58 (59.72)	53.45 (46.98)	47.82 (93.75)	11.77 (20.06)	10.87 (19.25)	0.00 (0.00)
T_3 Jaggery (20g) + Yeast (10g) in 100 ml water	98.14 (82.16)	98.14 (82.16)	97.98 (81.82)	95.75 (78.10)	85.36 (67.50)	67.72 (55.38)
T_4 Sucrose (20g) + Yeast (10g) in 100 ml water	66.92 (54.89)	56.53 (48.75)	60.45 (51.03)	63.87 (53.05)	17.77 (24.93)	8.76 (17.22)
T_5 Water (100ml) + Yeast (10g)	30.09 (33.27)	13.97 (21.95)	17.77 (24.93)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD(0.05)	17.65	23.16	27.26	25.12	28.67	39.14

Figures in parentheses are angular transformed values.

Table 2. Fecundity of adults in different diets

Treatments	Number of eggs (Days after treatment)			
	1-4 days	4-8 days	8-12 days	12-16 days
T_1 Honey (20ml) + Yeast (10g) in 100 ml water	31.03 (5.66)	39.96 (6.40)	16.81 (4.22)	10.49 (3.39)
T_2 Sugar (20g) + Yeast (10g) in 100 ml water	12.10 (3.62)	8.79 (3.13)	5.10 (2.47)	5.76 (2.60)
T_3 Jaggery (20g) + Yeast (10g) in 100 ml water	103.65 (10.23)	57.37 (7.64)	46.33 (6.88)	62.20 (7.95)
T_4 Sucrose (20g) + Yeast (10g) in 100 ml water	14.44 (3.93)	15.08 (4.01)	10.83 (3.44)	3.54 (2.13)
T_5 Water (100ml) + Yeast (10g)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
CD (0.05)	2.24	2.32	2.46	3.53

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

stage of infection. Infected adults became less active with reduced food intake, showing white mycelial growth during the initial phase of infection which later turned vinaceous brown in colour (Plate 8).

Pupae treated with *B.bassiana* became darkened with patches of white mycelial growth on its natural extremities (Plate 6). Infected adults became sluggish with cottony fluff of mycelial mat on its abdominal region during the later stage of infection (Plate 9).

Pupae infected with *A.candidus* became pale with white mycelial growth on its surface which later turned yellowish during sporulation (Plate 7). Infected adults became mummified and the body became stiff, hardened with yellowish white mycelial growth all over the body (Plate 10).

4.3 STANDARDISATION OF EFFECTIVE DOSAGE OF ENTOMOPATHOGENIC FUNGI

4.3.1 Effect of different doses of *P.lilacinus* on pupae of *B.cucurbitae*

Effective doses of the fungus *P.lilacinus* was tested on the pupae of melon fly (Table 3). On the second day after treatment, higher mortality percentage of 24.85 was recorded at $1.5x10^7$ spores/ml, followed by 18.63 at $1.5x10^6$ spores/ml and they were on par. Lower spore concentration of $1.5x10^5$ spores/ml and $1.5x10^4$ spores/ml recorded 10.27 and 0.65 per cent mortality, respectively.

On the third day after treatment a higher mortality rate (38.55%) was recorded at a spore concentration of $1.5x10^7$ spores/ml that differed significantly from all other treatments. The mortality percentage of 24.13 was recorded at a spore concentration of $1.5x10^6$ spores/ml which was on par with the mortality percentage of 14.45 at a spore concentration of $1.5x10^5$ spores/ml on the third day. A mortality percentage of 3.71 was recorded at a spore concentration of $1.5x10^4$ spores/ml, followed by 0.94 at $1.5x10^3$ spores/ml and both the treatments were on par.

Higher mortality percentage of 56.75 was recorded on the fourth day at $1.5x10^7$ spores/ml that differed significantly from all other treatments (Table 3). The mortality rate was found to be 34.86% at $1.5x10^6$ spores/ml that differed significantly from a mortality rate of 16.34% at $1.5x10^5$ spores/ml. A mortality percentage of 7.45 was recorded at a spore concentration of $1.5x10^4$ spores/ml, followed by 0.94 at $1.5x10^3$ spores/ml and they differ significantly from each other.

Observations recorded on the fifth day showed hundred per cent mortality at a spore concentration of $1.5x10^7$ spores/ml that differed significantly from all other treatments. The percentage mortality of 37.06 was recorded at a spore concentration of $1.5x10^6$ spores/ml. Mortality percentage of 23.67 was recorded at a spore concentration of 1.5×10^5 spores/ml that differed significantly from a mortality rate of 10.27% at 1.5×10^4 spores/ml.Lower mortality percentage of 5.71 was recorded at a spore concentration of 1.5×10^3 spores/ml.

On the sixth day after treatment a mortality percentage of 58.56 was recorded at a spore concentration of $1.5x10^6$ spores/ml which was on par with a mortality percentage of 25.89 at $1.5x10^5$ spores/ml (Table 3). Lower spore concentrations of $1.5x10^4$ spores/ml and $1.5x10^3$ spores/ml recorded a percentage mortality of 13.96 and 10.82, respectively.

Observations recorded on the seventh day after treatment showed a higher mortality percentage of 85.36 at $1.5x10^6$ spores/ml, followed by 36.84 at $1.5x10^5$ spores/ml and both these treatments were on par. Lower spore concentration of $1.5x10^4$ spores/ml and $1.5x10^3$ spores/ml recorded a mortality percentage of 22.03 and 12.14, respectively.

4.3.2 Effect of different doses of *B.bassiana* on the pupae of *B.cucurbitae*

Effective doses of the fungus *B.bassiana* was tested on the pupae of melon fly (Table 4). Mortality percentage was recorded from the third day onwards. Lower mortality percentage of 2.57 and 5.71 were recorded

at a spore concentration of $2x10^6$ spores/ml and $2x10^7$ spores/ml, respectively and both were statistically on par.

On the fourth day after treatment a higher mortality percentage of 15.77 was recorded at $2x10^7$ spores/ml, followed by 10.55 at $2x10^6$ spores /ml and both were on par. Lower spore concentrations of $2x10^5$ spores/ml and $2x10^4$ spores/ml caused a mortality rate of 5.71 per cent and 2.57 per cent and both were on par.

On the fifth day after treatment the spore concentration of $2x10^7$ spores/ml ,$2x10^6$ spores/ml and $2x10^5$ spores/ml recorded a mortality rate of 22.40 per cent, 20.24 per cent and 12.51 per cent, respectively and the effect of all these treatments were on par. On the same day mortality rates of 5.71 per cent and 0.65 per cent were recorded at a lower spore concentrations of $2x10^4$ spores/ml and $2x10^3$ spores/ml, respectively.

Observations recorded on the sixth day after treatment showed the highest percentage mortality of 37.32 at a spore concentration of $2x10^7$ spores/ml, followed by 28.71 at $2x10^6$ spores/ml and they were on par (Table 4). The mortality rate of 19.48 per cent was recorded at a spore concentration of $2x10^5$ spores/ml, 11.45 per cent at $2x10^4$ spores/ml and 2.57 per cent at $2x10^3$ spores/ml. There were significant difference among these treatments also.

On the seventh day after treatment the maximum percentage mortality of 98.29 was recorded at $2x10^7$ spores/ml that differed significantly from all other treatments. The spore concentration of $2x10^6$ spores/ml, $2x10^5$ spores/ml and $2x10^4$ spores/ml recorded mortality rates of 63.29 per cent, 32.26 per cent and 19.12 per cent, respectively and these treatments were on par. The least mortality percentage of 11.11 was recorded at a spore concentration of $2x10^3$ spores/ml on the same day. On all the recorded days, no mortality was observed in the control treatments.

4.3.3 Effect of different doses of *A.candidus* on pupae of *B.cucurbitae*

The results presented in the Table 5 shows the percentage mortality of pupae treated with different doses of *A.candidus*. The percentage mortality was recorded from fifth day onwards. Higher mortality of 29.71 was recorded at a spore concentration of $1.75x10^7$ spores/ml which was on par with a percentage mortality of 19.99 at $1.75x10^6$ spores/ml. The spore concentration of $1.75x10^5$ spores/ml and $1.75x10^4$ spores/ml recorded a mortality rates of 9.99 per cent and 9.44 per cent, respectively and both were on par.

A higher mortality percentage of 35.48 was recorded at $1.75x10^7$ spores/ml which was on par with a percentage mortality of 25.00 at $1.75x10^6$ spores/ml on the sixth day.

The spore concentration of $1.75x10^5$ spores/ml recorded a mortality rate of 14.22 per cent at $1.75x10^5$ spores/ml which was on par with 13.59 per cent at $1.75x10^4$ spores/ml. The least mortality percentage of 5.71 was recorded at a spore concentration of $1.75x10^3$ spores/ml on the same day.

On the seventh day after treatment a higher mortality percentage of 67.72 was recorded at a spore concentration of $1.75x10^7$ spores/ml that differed significantly from all other treatments (Table 5). The percentage mortality of 41.54 was recorded at a spore concentration of $1.75x10^6$ spores/ml followed by 30.25 at $1.75x10^5$ spores/ml and they were on par. A mortality percentage of 22.11 was recorded at a spore concentration of $1.75x10^4$ spores/ml which was on par with a percentage mortality of 12.51 at $1.75x10^3$ spores/ml, recorded on the same day.

4.3.4 Effect of different doses of *P.lilacinus* on adults of *B.cucurbitae*

The results presented in Table 7 shows the percentage mortality of 0.65 to 16.89 in adults treated with the increase in spore concentration

Table 3. Effect of different doses of *P.lilacinus* on the pupae of *B.cucurbitae*

Concentration, spores ml^{-1}	Percentage mortality (Days after treatment)					
	2	3	4	5	6	7
1.5×10^3	0.00 (0.00)	0.94 (5.56)	0.94 (5.56)	5.71 (13.82)	10.82 (19.20)	12.14 (20.39)
1.5×10^4	0.65 (4.61)	3.71 (11.11)	7.45 (15.84)	10.27 (18.69)	13.96 (21.94)	22.03 (27.94)
1.5×10^5	10.27 (18.69)	14.45 (22.34)	16.34 (23.84)	23.67 (29.11)	25.89 (30.59)	36.84 (37.37)
1.5×10^6	18.63 (25.57)	24.13 (29.42)	34.86 (36.19)	37.06 (37.50)	58.56 (49.93)	85.36 (67.50)
1.5×10^7	24.85 (29.90)	38.55 (38.37)	56.75 (78.88)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD(0.05)	6.50	7.71	5.87	16.84	20.42	30.69

Figures in parentheses are angular transformed values.

Table 4. Effect of different doses of *B.bassiana* on pupae of *B.cucurbitae*

Concentration, spores ml^{-1}	Percentage mortality (Days after treatment)				
	3	4	5	6	7
2×10^3	0.00 (0.00)	0.00 (0.00)	0.65 (4.61)	2.57 (9.22)	11.11 (19.47)
2×10^4	0.00 (0.00)	2.57 (9.22)	5.71 (13.82)	11.45 (19.78)	19.12 (25.93)
2×10^5	0.00 (0.00)	5.71 (13.82)	12.51 (20.71)	19.48 (26.19)	32.26 (34.61)
2×10^6	2.57 (9.22)	10.55 (18.95)	20.24 (26.74)	28.71 (32.40)	63.29 (53.08)
2×10^7	5.71 (13.82)	15.77 (23.40)	22.40 (28.25)	37.32 (37.66)	98.29 (82.49)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD(0.05)	8.53	8.99	12.39	5.83	11.39

Table 5. Effect of different doses of *A.candidus* on pupae of *B.cucurbitae*

Concentration, spores ml^{-1}	Percentage mortality (Days after treatment)		
	5	6	7
1.75×10^3	0.00 (0.00)	5.71 (13.82)	12.51 (20.71)
1.75×10^4	9.44 (17.89)	13.59 (21.63)	22.11 (28.05)
1.75×10^5	9.99 (18.43)	14.22 (22.15)	30.25 (33.27)
1.75×10^6	19.99 (26.56)	25.00 (30.00)	41.54 (40.13)
1.75×10^7	29.71 (33.08)	35.48 (36.56)	67.72 (55.38)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD(0.05)	8.17	7.31	10.21

Figures in parentheses are angular transformed values.

Plate 5. Symptom produced by *P.lilacinus* at 1.5 X 10^7 spores/ml in pupae

Plate 6. Symptom produced by *B.bassiana* at 2.0 x 10^7 spores/ml in pupae

from $1.5x10^3$ spores/ml to $1.5x10^7$ spores/ml on the second day after treatment. On the third day, the mortality rates were 20.25 per cent at $1.5x10^7$ spores/ml, 17.91 per cent at $1.5x10^6$ spores/ml, 14.02 per cent at $1.5x10^5$ spores/ml, 7.87 per cent at $1.5x10^4$ spores/ml and 3.23 per cent at $1.5x10^3$ spores/ml and all these treatments were equally effective .

On the fourth day, a higher mortality percentage of 40.27 was recorded at $1.5x10^7$ spores/ml, followed by 33.15 per cent at $1.5x10^6$ spores/ml and both were on par. At a spore concentration of $1.5x10^5$ spores/ml a percentage mortality of 20.03 was recorded. The spore concentration of $1.5x10^4$ spores/ml caused a mortality rate of 10.55 per cent which was on par with 5.71 per cent at $1.5x10^3$ spores/ml.

On the fifth day, a higher mortality percentage of 82.22 was recorded at a spore concentration of $1.5x10^7$ spores/ml that differed significantly from all other treatments. The spore concentration of $1.5x10^6$ spores/ml caused a mortality rate of 49.98 per cent which was on par with 20.33 per cent at $1.5x10^5$ spores/ml on the fifth day (Table 7). The spore concentration of $1.5x10^4$ spores/ml and $1.5\ x10^3$ spores /ml recorded a lower mortality rates of 11.80 per cent and 6.35 per cent, respectively and both were on par. On the sixth day after treatment a percentage mortality of 83.52 was recorded at a spore concentration of $1.5x10^7$ spores/ml, followed by 65.15 at $1.5x10^6$ spores/ml, 37.82 at $1.5x10^5$ spores/ml, 23.29 at $1.5x10^4$ spores/ml and 7.34 at $1.5x10^3$ spores/ml.

On the seventh day after treatment cent per cent mortality was recorded at a spore concentration of $1.5x10^7$ spores/ml which differed significantly from all other treatments. The spore concentration of $1.5x10^6$ spores/ml and $1.5x10^5$ spores/ml recorded a mortality rate of 69.13 per cent and 69.11 per cent, respectively and both were on par. Lower spore concentrations of $1.5x10^4$ spores/ml and $1.5x10^3$ spores/ml recorded mortality rates of 24.92 per cent and 8.76 per cent, respectively.

4.3.5 Effect of different doses of *B.bassiana* on the adults of *B.cucurbitae*

The percentage mortality of adults treated with different doses of *B.bassiana* was recorded (Table 8). On the third day after treatment, the mortality percentage recorded was 9.99 at $2x10^7$ spores /ml that differed significantly from all other treatments. The spore concentration of $2x10^6$ spores/ml and $2x10^5$ spores/ml recorded a percentage mortality of 2.56 and 0.65, respectively and both were on par. On the fourth day, a mortality percentage of 22.23 was recorded at $2x10^7$ spores/ml which differed significantly from all other treatments. The mortality rate of 12.98 per cent was recorded at $2x10^6$ spores/ml, followed by 12.52 per cent at $2x10^5$ spores/ml and 9.99 per cent at $2x10^4$ spores/ml and all these treatments were on par.

On the fifth day after treatment, the mortality rate of 50 per cent was recorded at a spore concentration of $2x10^7$ spores/ml that differed significantly from all other treatments. The percentage mortality of 24.58 was recorded at a spore concentration of $2x10^6$ spores/ml, followed by 16.31 at $2x10^5$ spores/ml, 11.90 at $2x10^4$ spores/ml and 7.17 at $2x10^3$ spores/ml and all these treatments were on par.

The maximum mortality percentage of 76.67 was recorded on the sixth day at a spore concentration of $2x10^7$ spores/ml that differed significantly from all other treatments (Table 5). A mortality rate of 28.32 per cent was recorded at a spore concentration of $2x10^6$ spores/ml, followed by 23.28 per cent at $2x10^5$ spores/ml, 14.99 per cent at $2x10^4$ spores/ml and 9.99 per cent at $2x10^3$ spores/ml on the same day.

On the seventh day after treatment a significantly higher mortality percentage of 76.67 was recorded at a spore concentration of $2x10^7$ spores/ml that was on par with a mortality percentage of 44.44 at $2x10^6$ spores/ml. A mortality percentage of 41.99 was recorded at $2x10^5$ spores/ml that differed significantly from a percentage mortality of 17.67 at $2x10^4$ spores/ml and 11.11 at $2x10^3$ spores/ml on the same day.

4.3.6 Effect of different doses of *A.candidus* on adults of *B.cucurbitae*

The percentage mortality of adults treated with varying doses of *A.candidus* was recorded (Table 9). *A.candidus* caused mortality of adults from third day onwards. A mortality percentage of 5.71 was recorded at a spore concentration of $1.75x10^7$ spores/ml, followed by 2.57 at $1.75x10^6$ spores/ml and both were on par. On the fourth day, a mortality percentage of 18.82 was recorded at $1.75x10^7$ spores/ml, followed by 18.11 at $1.75x10^6$ spores/ml, 9.99 at $1.75x10^5$ spores/ml and all these treatments were on par. The least percentage mortality of 2.57 and 0.94 was recorded at a spore concentration of $1.75x10^4$ and $1.75x10^3$ spores/ml on the fourth day.

Observations on the fifth day recorded a mortality percentage of 16.87 at a spore concentration of $1.5x10^7$ spores/ml, followed by 15.37 at $1.5x10^6$ spores/ml, 13.59 at $1.75x10^5$ spores/ml, 6.13 at $1.75x10^4$ and 2.57 at $1.75x10^3$ spores/ml and the effect of all these treatments were on par (Table 5). On the sixth day, mortality rates of 24.13 per cent was recorded at $1.75x10^7$ spores/ml, followed by 18.83 per cent at $1.75x10^6$ spores/ml, 15.30 per cent at $1.75x10^5$ spores/ml, 7.57 per cent at $1.75x10^4$ spores/ml and 6.13 per cent at $1.75x10^3$ spores/ml and the effect of all these treatments were on par.

On the seventh day, a significantly higher mortality percentage of 55.38 was recorded at $1.75x10^7$ spores/ml which was on par with a percentage mortality of 34.57 at $1.75x10^6$ spores/ml. At a spore load of $1.75x10^5$ spores/ml a mortality percentage of 25.82 was recorded which was on par with a percentage mortality of 11.51 at $1.75x10^4$ spores/ml. The least mortality rate of 6.89 per cent was recorded at $1.75x10^3$ spores/ml on the same day.

4.4 MASS MULTIPLICATION OF ENTOMOPATHOGENIC FUNGUS

The results of the mass multiplication of *P. lilacinus,* the most promising fungus identified under laboratory conditions are presented below.

Table 6. Comparative efficacy of the three entomopathogenic fungi on pupae of *B.cucurbitae*

Days after treatment	LC_{50} value spores ml^{-1}		
	P.lilacinus	*B.bassiana*	*A.candidus*
2	1.3×10^8	-	-
3	7.4×10^8	1.8×10^8	-
4	2.0×10^8	4.5×10^8	-
5	3.5×10^8	1.8×10^8	8.9×10^7
6	2.9×10^8	2.5×10^8	2.7×10^7
7	8.6×10^8	5.3×10^8	3.0×10^7

Table 7. Effect of different doses of *P.lilacinus* on the adults of *B.cucurbitae*

Concentration, spores ml^{-1}	Percentage mortality (Days after treatment)					
	2	3	4	5	6	7
1.5×10^3	0.65 (4.61)	3.23 (10.36)	5.71 (13.82)	6.35 (14.60)	7.34 (15.72)	8.76 (17.22)
1.5×10^4	2.57 (9.22)	7.87 (16.29)	10.55 (18.95)	11.80 (20.09)	23.29 (28.86)	24.92 (29.95)
1.5×10^5	6.13 (14.34)	14.02 (21.99)	20.03 (26.59)	20.33 (26.80)	37.82 (37.95)	69.11 (56.24)
1.5×10^6	10.83 (19.21)	17.91 (25.04)	33.15 (35.15)	49.98 (44.99)	65.15 (53.82)	69.13 (56.25)
1.5×10^7	16.89 (24.27)	20.25 (27.05)	40.27 (39.39)	82.22 (65.06)	83.52 (66.05)	100.00 (90.00)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD(0.05)	10.87	8.19	8.33	19.74	25.20	30.36

Figures in parentheses are angular transformed values

Table 8. Effect of different doses of *B.bassiana* on adults of *B.cucurbitae*

Concentration, spores ml^{-1}	Percentage mortality (Days after treatment)				
	3	4	5	6	7
2 x 10^3	0.00 (0.00)	0.00 (0.00)	7.17 (18.43)	9.99 (18.43)	11.11 (19.47)
2 x 10^4	0.00 (0.00)	9.99 (18.43)	11.90 (20.18)	14.99 (22.78)	17.67 (24.86)
2 x 10^5	0.65 (4.61)	12.52 (20.72)	16.31 (23.82)	23.28 (28.85)	41.99 (40.39)
2 x 10^6	2.56 (9.20)	12.98 (21.12)	24.58 (29.72)	28.32 (32.15)	44.44 (41.81)
2 x 10^7	9.99 (18.43)	22.23 (28.13)	50.00 (45.00)	76.67 (61.12)	76.67 (61.12)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD(0.05)	8.54	10.44	13.68	14.29	21.81

Figures in parentheses are angular transformed values.

4.4.1 Effect of solid substrates on the sporulation of *P.lilacinus*

The sporulation of *P.lilacinus* grown in different solid media was observed (Table 11) (Plate 11). Among the different solid media, rice bran and wheat bran recorded spore counts of $1.56x10^8$ spores/ml and $1.30x10^8$ spores/ml, respectively on the 7th day after inoculation and they were significantly different from each other. Gingelly oil cake recorded a spore count of $0.90x10^8$ spores/ml, followed by coir pith ($0.72x10^8$ spores/ml) and neem cake ($0.72x10^8$ spores/ml) and the effect of all these treatments were statistically on par.

On the 14th day, spore production was found at the maximum on rice bran ($2.68x10^8$ spores/ml) that differed significantly from wheat bran ($2.33x10^8$ spores/ml) and gingelly oil cake ($1.51x10^8$ spores/ml). Coir pith and neem cake recorded spore counts of $1.23x10^8$ spores/ml and $1.10x10^8$ spores/ml, respectively and both were on par.

On the 21st day, rice bran recorded the maximum spore count ($3.71x10^8$ spores/ml) that differed significantly from wheat bran ($2.87x10^8$ spores/ml) and gingelly oil cake ($2.15x10^8$ spores/ml). On the same day coir pith recorded a spore count of $1.58x10^8$spores/ml followed by neem cake ($1.46x10^8$ spores/ml) and both these treatments were on par.

On the 28th day, rice bran recorded a significantly higher spore count of $4.32x10^8$ spores/ml that differed significantly from wheat bran ($3.19x10^8$spores/ml) (Table 11). On the same day, gingelly oil cake recorded a spore count of $2.63x10^8$ spores/ml, followed by coir pith ($2.44x10^8$ spores/ml) and neem cake (2.16×10^8 spores/ml) and the effect of all these treatments were on par.

Observations recorded on the 35th day showed a spore count of $3.97x10^8$ spores/ml in rice bran, followed by $3.73x10^8$ spores/ml in wheat bran and both were on par. Gingelly oil cake and coir pith recorded a spore count of $2.86x10^8$ spores/ml and $2.54x10^8$ spores/ml, respectively

and both were on par. Neem cake recorded a spore count of $1.26x10^8$ spores/ml on the same week.

On the 42nd day, the spore count of rice bran ($2.47x10^8$ spores/ml) was on par with wheat bran ($2.29x10^8$ spores/ml) (Table 11). Gingelly oil cake recorded a spore count of $1.88x10^8$ spores/ml which was on par with coir pith ($1.64x10^8$ spores/ml). Neem cake recorded the least spore count of $0.39x10^8$ spores/ml on the same week.

On the 49th day, the spore count of the fungus was higher in rice bran ($1.79x10^8$ spores/ml) which was on par with the spore count of the fungus in wheat bran ($1.25x10^8$ spores/ml). Gingelly oil cake recorded a spore count of $0.85x10^8$ spores/ml which was on par with coir pith ($0.72x10^8$ spores/ml). Neem cake recorded no spores.

Rice bran recorded a spore count of $1.13x10^8$ spores/ml that differed significantly from all other treatments on the 56th day after inoculation (Table 11). Wheat bran recorded a spore count of $0.89x10^8$ spores/ml which was on par with coir pith ($0.57x10^8$ spores/ml) and gingelly oil cake ($0.56x10^8$ spores/ml). No sporulation was observed in neem cake during the same week.

4.4.2 Effect of liquid media on the sporulation of *P.lilacinus*

The sporulation of *P. lilacinus* grown on different liquid media was observed (Table 12) (Plate 12). Among the different liquid media evaluated, rice bran extract recorded the highest spore count of $1.09x10^8$ spores/ml, followed by wheat bran extract ($0.95x10^8$ spores/ml), boiled rice water ($0.88x10^8$ spores/ml) and coconut water ($0.86x10^8$ spores/ml) on the seventh day after inoculation. The effect of all these treatments were statistically on par.

On the 14th day, rice bran extract recorded a higher spore count (1.83 $x10^8$ spores/ml) that differed significantly from all other treatments. Wheat bran extract recorded a spore count of $1.51x10^8$ spores/ml, followed by boiled rice water ($1.35x10^8$ spores/ml) and coconut water ($1.09x10^8$ spores/ml) and the effect of all these treatments were statistically on par.

Spore count was found to be the highest in the rice bran extract ($2.52x10^8$ spores/ml) that differed significantly from all other treatments in the 21st day after inoculation (Table 12). During the same period, wheat bran extract recorded a spore count of $2.06x10^8$ spores/ml, boiled rice water (1.89 $x10^8$ spores/ml) and coconut water ($1.82x10^8$ spores/ml) and the effect of these treatments were on par.

On the 28th day, rice bran extract recorded a spore count of $0.89x10^8$ spores/ml that differed significantly from all other treatments. Wheat bran extract recorded a spore count of $0.71x10^8$ spores/ml, followed by boiled rice water ($0.59x10^8$ spores/ml) and both were on par. Coconut water recorded the least spore count ($0.03x10^8$ spores/ml) on the same day. On all the recorded days, water recorded no spores.

4.4.3 Efficacy of *P.lilacinus* grown on solid media on pupae of *B.cucurbitae* at different intervals after storage

The efficacy of the *P.lilacinus* grown on solid media was tested on the pupae of *B.cucurbitae* (Table 13). One week after storage, the fungus grown on rice bran could cause a percentage mortality of 44.96 that differed significantly from a percentage mortality of 65.09 caused by the fungus two weeks after storage. The highest mortality of 75.17 per cent was recorded by the fungus grown on rice bran three weeks after storage which was on par with a percentage mortality of 75.96 caused by the fungus four weeks after storage.

Table 9. Effect of different doses of *A.candidus* on the adults of *B.cucurbitae*

Concentration, spores ml^{-1}	Percentage mortality (Days after treatment)				
	3	4	5	6	7
1.75×10^3	0.00 (0.00)	0.94 (5.56)	2.57 (9.22)	6.13 (14.34)	6.89 (15.22)
1.75×10^4	0.00 (0.00)	2.57 (9.22)	6.13 (14.34)	7.57 (15.98)	11.51 (19.83)
1.75×10^5	0.00 (0.00)	9.99 (18.43)	13.59 (21.64)	15.30 (23.03)	25.82 (30.54)
1.75×10^6	2.57 (9.22)	18.11 (25.18)	15.37 (23.08)	18.83 (25.72)	34.57 (36.01)
1.75×10^7	5.71 (13.82)	18.82 (25.71)	16.87 (24.25)	24.13 (29.42)	55.38 (48.09)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD(0.05)	8.54	9.87	16.06	17.22	13.59

Figures in parentheses are angular transformed values.

Plate 7. Symptom produced by *A.candidus* at 1.75x10^7 spores/ml in pupae

Plate 8. Symptom produced by *P.lilacinus* at 1.5x10^7 spores/ml in adults

Plate 9. Symptom produced by *B.bassiana* at 2.0x10^7 spores/ml in adults

Plate 10. Symptom produced by *A.candidus* at 1.75x10^7 spores/ml in adults

Table 10. Comparative efficacy of the three entomopathogenic fungi on adults of *B.cucurbitae*

Days after treatment	LC_{50} value spores ml^{-1}		
	P.lilacinus	*B.bassiana*	*A.candidus*
2	-	-	-
3	2.4×10^8	-	4.8×10^8
4	1.8×10^8	2.6×10^8	3.2×10^8
5	3.2×10^8	2.9×10^8	2.6×10^8
6	8.3×10^8	2.7×10^8	5.0×10^8
7	4.2×10^8	1.3×10^8	7.5×10^8

Table 11. Effect of solid substrates on the sporulation of *P.lilacinus*

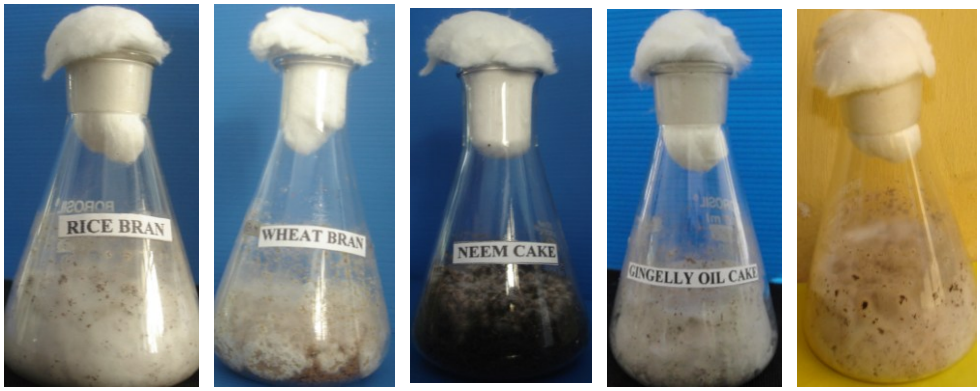
Solid substrates	Spore count x 10^8 spores / ml (DAI)*							
	7^{th}	14^{th}	21^{st}	28^{th}	35^{th}	42^{nd}	49^{th}	56^{th}
Rice bran	1.56 (1.60)	2.68 (1.92)	3.71 (2.17)	4.32 (2.31)	3.97 (2.23)	2.47 (1.86)	1.79 (1.67)	1.13 (1.46)
Wheat bran	1.30 (1.57)	2.33 (1.82)	2.87 (1.97)	3.19 (2.05)	3.73 (2.18)	2.29 (1.82)	1.25 (1.49)	0.89 (1.39)
Gingelly oil cake	0.90 (1.38)	1.51 (1.59)	2.15 (1.77)	2.63 (1.91)	2.86 (1.96)	1.88 (1.69)	0.85 (1.36)	0.56 (1.25)
Coir pith	0.72 (1.31)	1.23 (1.49)	1.58 (1.61)	2.44 (1.85)	2.54 (1.88)	1.64 (1.62)	0.72 (1.31)	0.57 (1.25)
Neem cake	0.72 (1.31)	1.10 (1.45)	1.46 (1.57)	2.16 (1.78)	1.26 (1.50)	0.39 (1.18)	0.00 (1.00)	0.00 (1.00)
CD(0.05)	0.08	0.07	0.08	0.08	0.09	0.11	0.08	0.67

* Day After Inoculation
Figure in parentheses are √x+1 transformed values.

Table 12. Effect of liquid substrates on the sporulation of *P.lilacinus*

Liquid substrates	Spore count x 10^8 spores / ml (DAI)*			
	7^{th}	14^{th}	21^{st}	28^{th}
Rice bran extract	1.09 (1.45)	1.83 (1.68)	2.52 (1.88)	0.89 (1.37)
Wheat bran extract	0.95 (1.39)	1.51 (1.59)	2.06 (1.75)	0.71 (1.31)
Boiled rice water	0.88 (1.37)	1.35 (1.53)	1.89 (1.70)	0.59 (1.21)
Coconut water	0.86 (1.36)	1.09 (1.44)	1.82 (1.68)	0.03 (1.01)
Water	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
CD(0.05)	0.09	0.09	0.08	0.06

* Day After Inoculation
Figure in parentheses are √x+1 transformed values.



a. **b.** **c.** **d.** **e.**

Plate 11. Growth of *P.lilacinus* in solid substrates

a. Rice bran

b. Wheat bran

c. Neem cake

d. Gingelly oil cake

e. Coir pith

a. **b.** **c.** **d.** **e.**

Plate 12. Growth of *P.lilacinus* in liquid substrates

a. Rice bran extract

b. Wheat bran extract

c. Coconut water

d. Starch solution

e. Water

The fungus grown on wheat bran caused a percentage mortality of 17.24 one week after storage that differed significantly from a percentage mortality of 34.91 caused by the fungus two weeks after storage. Three weeks after storage, *P.lilacinus* grown on wheat bran recorded a percentage mortality of 43.47 that differed from a significantly higher mortality percentage of 65.43 recorded during the fourth week after storage.

One week after storage, the fungus grown on gingelly oil cake recorded a percentage mortality of 12.22 that differed significantly from a percentage mortality of 19.48 recorded during the second week after storage (Table 13). In the third week, the fungus grown on gingelly oil cake recorded a percentage mortality of 29.49 which was on par with a percentage mortality of 42.47 caused by the fungus four weeks after storage.

P.lilacinus grown on coir pith recorded a percentage mortality of 3.81 one week after storage that was on par with a percentage mortality of 5.71 recorded during the second week after storage. Three weeks after storage, the fungus grown on coir pith recorded a percentage mortality of 11.19 that differed from a significantly higher mortality percentage of 39.99 caused by the fungus four weeks after storage.

The fungus grown on neem cake recorded a percentage mortality of 9.44 in the third week after storage which was on par with a percentage mortality of 19.48 caused by the fungus four weeks after storage.

4.4.4 Efficacy of *P.lilacinus* grown on liquid media on pupae of *B.cucurbitae* at different intervals after storage

The efficacy of *P.lilacinus* grown on liquid media on pupae of *B.cucurbitae* was tested (Table 14). One week after storage, *P.lilacinus* grown on rice bran extract recorded a percentage mortality of 12.22 that

was on par with a percentage mortality of 17.24 caused by the fungus two weeks after storage. The fungus grown on rice bran extract caused a percentage mortality of 29.99 three weeks after storage which was on par with a percentage mortality of 44.86 recorded during the fourth week after storage.

The fungus grown on wheat bran extract recorded a mortality of 7.47 per cent which was on par with a mortality of 17.24 per cent caused by the fungus two weeks after storage. Three weeks after storage, the fungus caused a mortality of 27.38 per cent which was on par with the mortality of 42.47 per cent caused by the fungus grown on wheat bran extract four weeks after storage.

One week after storage, the fungus grown on boiled rice water caused a percentage mortality of 5.71 which was on par with the mortality (16.75 %) caused by the fungus two weeks after storage (Table 14). *P. lilacinus* grown on boiled rice water recorded a percentage mortality of 24.83 three weeks after storage which was on par with the mortality of 36.96 per cent caused the fungus four weeks after storage.

P.lilacinus grown on coconut water recorded a percentage mortality of 2.57 one week after storage which was on par with a percentage mortality of 9.44 caused by the fungus two weeks after storage. Three weeks after storage, the fungus grown on coconut water caused a percentage mortality of 19.99 which was on par with the mortality of 33.67 per cent recorded by the fungus four weeks after storage. On all the recorded weeks, water recorded no mortality of pupae.

4.4.5 Efficacy of *P.lilacinus* grown on solid media on adults of *B.cucurbitae* at different intervals after storage

The efficacy of *P.lilacinus* grown on solid media was tested on the adults of *B.cucurbitae* (Table 15). The fungus grown on rice bran one

week after storage recorded a percentage mortality of 22.36 that differed significantly from a mortality percentage of 34.91 caused by the fungus two weeks after storage. Three weeks after storage, the fungus grown on rice bran recorded a percentage mortality of 44.96 that differed significantly from a higher percentage mortality of 72.62 caused by the fungus four weeks after storage.

The fungus grown on wheat bran recorded a percentage mortality of 19.48 one week after storage that was on par with a percentage mortality of 29.99 caused by the fungus two weeks after storage. Three weeks after storage, the fungus grown on wheat bran recorded a percentage mortality of 37.06 which differed significantly from a higher percentage mortality of 60.21 caused by the fungus four weeks after storage.

The fungus grown on gingelly oil cake recorded a percentage mortality of 16.75 one week after storage which was on par with a percentage mortality of 22.36 caused by the fungus two weeks after storage (Table 15). Three weeks after storage, the fungus grown on gingelly oil cake caused a percentage mortality of 27.38 that differed significantly from a per centage mortality of 47.49 caused by the fungus four weeks after storage.

One week after storage, fungus grown on coir pith recorded a percentage mortality of 14.63 which was on par with the mortality rates of 22.36 per cent and 27.13 per cent caused by the fungus on the second and third week after storage, respectively. Four weeks after storage, the fungus grown on coir pith recorded a significantly higher percentage mortality of 47.49 on the adults.

The fungus grown on neem cake recorded a percentage mortality of 7.47 one week after storage which was on par with a percentage mortality of 17.24 caused by the fungus two weeks after storage. Three weeks after storage, the fungus grown on neem cake caused a percentage

mortality of 27.13 which was on par with a percentage mortality of 42.47 caused by the fungus four weeks after storage.

4.4.6 Efficacy of *P.lilacinus* grown on liquid media on adults of *B.cucurbitae* at different intervals after storage

The efficacy of *P.lilacinus* grown on liquid media was tested on the adults of *B.cucurbitae* (Table 16). One week after storage, the fungus grown on rice bran extract caused a percentage mortality of 12.22 that was on par with a percentage mortality of 19.34 caused by the fungus two weeks after storage. Three weeks after storage, the fungus grown on rice bran extract caused a mortality rate of 29.74 per cent that was on par with a mortality rate of 37.45 per cent caused by the fungus four weeks after storage.

One week after storage, the fungus grown on wheat bran extract recorded a percentage mortality of 5.71 that differed significantly from a percentage mortality of 14.83 caused by the fungus two weeks after storage. Three weeks after storage, the fungus grown on wheat bran extract recorded a percentage mortality of 22.36 which was on par with a percentage mortality of 24.83 caused by the fungus four weeks after storage.

The fungus grown on boiled rice water recorded a percentage mortality of 0.65 one week after storage which was on par with a percentage mortality of 7.47 caused by the fungus two weeks after storage (Table 16). Three weeks after storage, the fungus grown on boiled rice water recorded a percentage mortality of 19.48 which was on par with a percentage mortality of 24.83 caused by the fungus four weeks after storage.

Two weeks after storage, the fungus grown on coconut water recorded a percentage mortality of 2.08. Three weeks after storage, the fungus grown on coconut water caused a percentage mortality of 13.53

Table 13. Efficacy of *P.lilacinus* grown on solid media on pupae of *B.cucurbitae* at different intervals after storage

WAS	Percentage mortality on 7 DAT				
	Rice bran	Wheat bran	Gingelly oil cake	Coir pith	Neem cake
1 WAS	44.96 (42.11)	17.24 (24.53)	12.22 (20.46)	3.81 (11.25)	0.00 (0.00)
2 WAS	65.09 (53.78)	34.91 (36.22)	19.48 (26.19)	5.71 (13.82)	0.00 (0.00)
3 WAS	75.17 (60.64)	43.47 (43.55)	29.49 (32.89)	11.19 (19.55)	9.44 (17.89)
4 WAS	75.96 (60.11)	65.43 (53.99)	42.47 (40.67)	39.99 (39.23)	19.48 (26.19)
CD(0.05)	9.11	7.69	10.43	15.45	14.88

WAS - Weeks After Storage
DAT - Days After Treatment
Figure in parentheses are angular transformed values.

Table 14. Efficacy of *P.lilacinus* grown on liquid media on pupae of *B.cucurbitae* at different intervals after storage

WAS	Percentage mortality on 7 DAT				
	Rice bran extract	Wheat bran extract	Boiled rice water	Coconut water	Water
1 WAS	12.22 (20.46)	7.47 (15.86)	5.71 (13.82)	2.57 (9.22)	0.00 (0.00)
2 WAS	17.24 (24.53)	17.24 (24.53)	16.75 (24.16)	9.44 (17.89)	0.00 (0.00)
3 WAS	29.99 (26.56)	27.38 (31.55)	24.83 (29.89)	19.99 (26.56)	0.00 (0.00)
4 WAS	44.86 (42.05)	42.47 (40.67)	36.96 (37.44)	33.67 (35.47)	0.00 (0.00)
CD(0.05)	7.88	10.50	13.89	12.83	0.00

WAS - Weeks After Storage
DAT - Days After Treatment
Figure in parentheses are angular transformed values.

Table 15 . Efficacy of *P.lilacinus* grown on solid media on adults of *B.cucurbitae* at different intervals after storage

WAS	Percentage mortality on 7 DAT				
	Rice bran	Wheat bran	Gingelly oil cake	Coir pith	Neem cake
1 WAS	22.36 (38.22)	19.48 (26.19)	16.75 (24.16)	14.63 (22.49)	7.47 (15.86)
2 WAS	34.91 (36.22)	29.99 (33.21)	22.36 (28.22)	22.36 (28.22)	17.24 (24.53)
3 WAS	44.96 (42.11)	37.06 (37.50)	27.38 (31.55)	27.13 (31.39)	27.13 (31.39)
4 WAS	72.62 (58.45)	60.21 (50.89)	47.49 (43.56)	47.49 (43.56)	42.47 (40.67)
CD (0.05)	5.18	9.23	10.17	10.08	10.57

WAS- Weeks After Storage
DAT- Days After Treatment

Figures in parentheses are angular transformed values

Table 16. Efficacy of *P.lilacinus* grown on liquid media on adults of *B.cucurbitae* at different intervals after storage

WAS	Percentage mortality on 7 DAT				
	Rice bran extract	Wheat bran extract	Boiled rice water	Coconut water	Water
1 WAS	12.22 (28.46)	5.71 (13.82)	0.65 (4.61)	0.00 (0.00)	0.00 (0.00)
2 WAS	19.34 (26.09)	14.83 (22.49)	7.47 (15.86)	2.08 (8.30)	0.00 (0.00)
3 WAS	29.74 (33.05)	22.36 (28.22)	19.48 (26.19)	13.53 (21.58)	0.00 (0.00)
4 WAS	37.45 (37.77)	24.83 (29.89)	24.83 (29.89)	22.36 (28.22)	0.00 (0.00)
CD (0.05)	7.27	8.88	12.39	17.36	0.00

WAS - Weeks After Storage
DAT - Days After Treatment
Figures in parentheses are angular transformed values

which was on par with a percentage mortality of 22.36 caused by the fungus four weeks after storage. On all the recorded weeks, water recorded no mortality of adults of melon fly.

4.5 EFFICACY OF *P.lilacinus* ON ADULTS IN CAGES

The percentage mortality of adults of melon fly treated with varying doses of *P.lilacinus* fixed on the basis of the LC_{50} value (Table 10) was recorded (Table 17). On the second day after treatment a higher percentage mortality of 72.62 was recorded at a spore concentration of $2.4x10^9$ spores/ml that differed significantly from all other treatments. At a spore concentration of $2.4x10^8$ spores/ml, a percentage mortality of 52.51 was recorded which was on par with a mortality rate of 23.33 per cent at $2.4x10^7$ spores/ml. Control treatment recorded the least mortality of 2.57 percentage on the same day.

On the third day, cent per cent mortality was recorded at a spore load of $2.4x10^9$ spores/ml that differed significantly from all other treatments. At a spore concentration of $2.4x10^8$ spores/ml the mortality rate of 69.12 per cent was recorded which was on par with the mortality rate of 42.37 per cent at $2.4x10^7$ spores/ml. Control treatments recorded a mortality percentage of 5.92 on the same day. On the fourth day, a higher mortality percentage of 85.34 was recorded at $2.4x10^8$ spores/ml that differed significantly from the percentage mortality of 56.02 at $2.4x\ 10^7$spores/ml. Control treatment recorded the least percentage mortality of 0.72 on the same day.

4.6 EFFICACY OF *P.lilacinus* ON PUPAE IN TROUGHS

The percentage mortality of pupae of melon fly treated with varying doses of *P.lilacinus* fixed on the basis of the LC_{50} value (Table 6) was observed (Table 18). On the first day after inoculation of the

fungus in the soil, a percentage mortality of 26.50 was recorded at a spore concentration of $1.3x10^9$ spores/ml that differed significantly from the percentage mortality of 12.51 recorded at $1.3x10^8$ spores/ml. The least percentage mortality of 2.50 was recorded at a spore concentration of $1.3x10^7$ spores/ml on the same day.

On the third day after inoculation of the fungus in the soil the mortality percentage of 92.45 was recorded at a spore concentration of $1.3x10^9$ spores/ml which was on par with a mortality rate of 72.28 per cent at $1.3x10^8$ spores/ml and 69.84 per cent at $1.3x10^7$ spores/ml (Table 18). Observations recorded on the fifth day after inoculation of the fungus showed a percentage mortality of 92.45 at $1.3x10^9$ spores/ml that was on par with 74.92 at $1.3x10^8$ spores/ml and 69.84 at $1.3x10^7$ spores/ml.

On the seventh day after inoculation of the fungus in the soil, a higher mortality percentage of 2.57 was recorded at $1.3x10^9$ spores/ml, followed by 44.62 at $1.3x10^8$ spores/ml and 32.39 at 1.3×10^7 spores/ml (Table 18). On the ninth day after inoculation of the fungus a mortality percentage of 11.59 recorded at $1.3x10^8$ spores/ml. On all the recorded days, control treatments recorded no mortality of the pupae.

4.7 PEAK TIME OF ACTIVITY OF MELON FLY, *B. cucurbitae* IN BITTER GOURD

Different food baits were screened to document the peak time activity of melon fly (Table 19) (Plate 13). The bait preparation in water (100 ml) containing banana (10g) + jaggery (10g) + 0.2 ml malathion + yeast (10g) (T_1) recorded the maximum catch of flies at 8-10 am (2.43), followed by 1.21 at 2-4 pm, 0.75 at 12-2 pm, 0.62 at 10-12 pm, 0.41 at 4-6 pm and 0.32 at 6-8 am. Bait preparation in water (100 ml) containing banana (20g) + jaggery (10g) + 0.2 ml malathion + yeast (10g) (T_2) recorded the maximum catch at 8-10 am (2.59) that was, followed by

1.76 (2-4 pm), 1.19 (12-2 pm), 0.99 (4-6 pm), 0.97 at (10-12 pm), 0.83 (6-8 am). Banana (30g) + jaggery (10g) + 0.2 ml malathion+ yeast (10g) in water (100 ml) of bait preparation (T_3) recorded the maximum trap catch of flies 3.66 at 8-10 am, 2.43 at 2-4 pm, 2.22 at 12-2 pm, 1.99 at 4-6 pm, 1.86 at 10-12 am and 1.78 at 6-8 am.

Maximum number of flies were trapped in (T_4) Banana (10g)+ jaggery (10g) + 0.2 ml malathion in water (100 ml) of bait preparation at 8-10am (1.74) followed by 10-12 am (1.43), 2-4 pm (1.30), 12-2 pm (0.99), 4-6 pm (0.66) (Table 19). The bait preparation in water (100 ml) containing banana (20g) + jaggery (10g) + 0.2 ml malathion (T_5) recorded the maximum catch at 8-10 am (1.97), 2-4 pm (1.62), 4-6 pm (1.54), 12-2 pm (1.47), 6-8 am (0.99) and 10-12 am (0.86). The number of flies trapped in (T_6) Banana (30g) + jaggery (10g) + 0.2 ml malathion in water (100 ml) of bait preparation was found at the maximum at 8-10 am (2.31), followed by 2-4pm (1.95), 12-2 pm (1.85), 4-6 pm (1.74), 6-8 am (1.51) and 10-12 am (1.12) (Table 19).

Water (100 ml) (T_7) recorded the minimum catch of flies at 8-10 am (0.98), 2-4 pm (0.33), 6-8 am (0.21), 4-6 pm (0.20), 12-2 pm (0.10). The bait preparation in water (100 ml) containing banana (30g) + jaggery (10g) + 0.2 ml malathion + yeast (10g) (T_3) was significantly different from all other treatments (Table 19). Banana (30g) + jaggery (10g) + 0.2 ml malathion in water (100 ml) of bait solution (T_6) was statistically on par with the bait preparation in water (100 ml) containing banana (20g) + jaggery (10g) + 0.2 ml malathion + yeast (10g) (T_2). The effect of the treatments banana (10g) + jaggery (10g) + 0.2 ml malathion in water (100 ml) of bait solution (T_4) was statistically on par with the bait preparation in water (100 ml) containing banana (30g) + jaggery (10g) + 0.2 ml malathion + yeast (10g) (T_1).

Maximum catch of adult flies was recorded during 8-10 am that differed significantly from other time periods. The mean catch of flies was similar during 2-4 pm and 12-2 pm and both were statistically on

par. The trap catch of adult flies recorded during 4-6 pm was on par with the trap catch during 10-12 am (Table 19).

4.8 FIELD EVALUATION OF ENTOMOPATHOGENIC FUNGUS

The results of the field studies conducted in bitter gourd to test the efficacy of the standardized dosage of *P. lilacinus* under laboratory conditions are given below.

4.8.1 Effect of *P. lilacinus* in the field on melon fly in bittergourd

The percentage infestation of bitter gourd fruits recorded at different intervals after application of the treatments was presented (Table 20) (Plate 14-18). One week after first application, the minimum percentage fruit damage (8.61) was recorded in soil drenching + spraying with *P.lilacinus* (T_3) which was on par with 0.2 per cent malathion spray (T_4) (14.63). Spraying and soil drenching with *P.lilacinus* recorded a percentage infestation of 18.24 and 19.07, respectively and the effect of both these treatments were on par. Control treatment recorded the maximum infestation (65.43).

Two weeks after first application, a very less infestation was recorded in soil drenching + spraying (1.76) with *P.lilacinus* that differed significantly from all other treatments. 0.2 per cent malathion spray and spraying with *P.lilacinus* recorded a percentage infestation of 6.35 and 7.01, respectively and the effect of both these treatments were on par. Soil drenching with *P.lilacinus* recorded an infestation level of 10.89 per cent. Control treatments recorded the maximum infestation (56.15%).

One week after second application, the minimum percentage infestation (2.09) was recorded in soil drenching + spraying with *P.lilacinus* that differed significantly from all other treatments. (T_4) 0.2% malathion spray recorded a percentage infestation of 7.13 which was on par with spraying of *P.lilacinus* (6.13%). Soil drenching with

Table 17. Effect of *P.lilacinus* on adults of *B.cucurbitae* in cages

Concentration, spores ml^{-1}	Percentage mortality (Days after treatment)		
	2	3	4
2.4×10^7	23.33 (28.88)	42.37 (40.61)	56.02 (48.46)
2.4×10^8	52.51 (46.44)	69.12 (56.24)	85.34 (67.49)
2.4×10^9	72.62 (58.45)	100.00 (90.00)	100.00 (90.00)
Control	2.57 (9.22)	5.92 (14.08)	0.72 (4.87)
CD (0.05)	9.87	7.18	26.26

Table 18. Effect of *P.lilacinus* on pupae of *B.cucurbitae* in troughs at different intervals after inoculation

Concentration, spores ml^{-1}	Percentage mortality at seven days after introduction of pupae				
	1 DAI	3 DAI	5 DAI	7 DAI	9 DAI
1.3×10^7	2.50 (8.66)	69.84 (28.26)	69.84 (28.26)	32.39 (20.59)	0.00 (0.00)
1.3×10^8	12.51 (14.94)	72.28 (28.69)	74.92 (29.14)	44.62 (23.37)	11.59 (14.69)
1.3×10^9	26.50 (19.11)	92.45 (32.01)	92.45 (32.01)	2.57 (9.22)	0.00 (0.00)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD(0.05)	1.3	18.11	18.78	7.45	5.19

DAI – Days After Inoculation

Figures in parentheses are angular transformed values.

Table 19. Peak time of activity of melon fly, *B.cucurbitae* in bittergourd

Treatments	Time					
	6-8 am	8-10am	10-12am	12-2 pm	2-4 pm	4-6 pm
T_1	0.32 (1.15)	2.43 (1.85)	0.62 (1.27)	0.75 (1.32)	1.21 (1.49)	0.41 (1.19)
T_2	0.83 (1.35)	2.59 (1.89)	0.97 (1.40)	1.19 (1.48)	1.76 (1.66)	0.99 (0.41)
T_3	1.78 (1.67)	3.66 (2.16)	1.86 (1.69)	2.22 (1.79)	2.43 (1.85)	1.99 (1.73)
T_4	0.00 (1.00)	1.74 (1.65)	1.43 (1.56)	0.99 (1.41)	1.30 (1.52)	0.66 (1.29)
T_5	0.99 (1.41)	1.97 (1.72)	0.86 (1.36)	1.47 (1.57)	1.62 (1.62)	1.54 (1.59)
T_6	1.51 (1.58)	2.31 (1.82)	1.12 (1.46)	1.85 (1.69)	1.95 (1.72)	1.74 (0.65)
T_7	0.21 (1.09)	0.98 (1.41)	0.00 (1.00)	0.10 (1.05)	0.33 (1.15)	0.20 (1.09)

CD (0.05) for treatments – 0.13
CD (0.05) for time – 0.12
Figures in parentheses are √x+1 transformed values.

T_1 - Bait preparation in water (100 ml) containing Banana (10g) + Jaggery (10g) + 0.2 ml Malathion + Yeast (10g)

T_2 - Bait preparation in water (100 ml) containing Banana (20g) + Jaggery (10g) + 0.2 ml Malathion + Yeast (10g)

T_3 - Bait preparation in water (100 ml) containing Banana (30g) + Jaggery (10g) + 0.2 ml Malathion + Yeast (10g)

T_4 - Bait preparation in water (100 ml) containing Banana (10g) + Jaggery (10g) + 0.2 ml Malathion

T_5 - Bait preparation in water (100 ml) containing Banana (20g) + Jaggery (10g) + 0.2 ml Malathion

T_6 - Bait preparation in water (100 ml) containing Banana (30g) + Jaggery (10g) + 0.2 ml Malathion

T_7 - Control (Water -100 ml)

P.lilacinus recorded a low infestation level of 14.95 per cent. Control treatments recorded the maximum infestation (55.56%).

Two weeks after second application, the minimum infestation was recorded in (T_3) soil drenching+ spraying (3.37%) with *P.lilacinus* which was on par with (T_4) 0.2% malathion spray (5.89%). Soil drenching and spraying with *P.lilacinus* recorded a percentage infestation of 7.92 and 8.05, respectively and both were on par. Control treatments recorded the maximum infestation (58.08%).

4.8.2 Effect of *P.lilacinus* on pupae in the field

The number of infected pupae per m^2 recorded at different intervals after application of the treatments during the fruiting period was observed (Table 21) (Plate 20). One week after first application, soil drenching with *P.lilacinus* recorded the maximum number of infected pupae (1.95) followed by soil drenching + spraying (1.26) with *P.lilacinus* and they were statistically on par. Two weeks after first application, soil drenching with *P.lilacinus* recorded greater number of infected pupae (2.30), followed by soil drenching + spraying with *P.lilacinus* (1.43) and they were on par.

One week after second application, soil drenching with *P.lilacinus* recorded the maximum number of infected pupae (3.49) that differed significantly from soil drenching + spraying (1.01) with *P.lilacinus*. Two weeks after second application, soil drenching with *P.lilacinus* recorded significantly higher number of infected pupae (2.88) that was on par with soil drenching + spraying (2.03) with *P.lilacinus*.

4.8.3 Incidence of other pests in the field

The population of other pests recorded at different intervals after the application of treatments during the fruiting period was observed (Table 22). The population of pumpkin caterpillar, *Diaphania indica* was

observed in the field. One week after first application, soil drenching+ spraying with *P.lilacinus* (T_3) recorded the minimum number of other pests (0.99) that differed significantly from all other treatments. The effects of spraying with *P.lilacinus* (1.18), 0.2% malathion spray (1.37) and soil drenching with *P.lilacinus* (2.17) were statistically on par. Control treatments recorded the maximum number of other pests (6.88).

Two weeks after first application, soil drenching + spraying with *P.lilacinus* recorded the minimum number of other pests (0.45), followed by 0.2% malathion spray (0.67) and both were on par. The effect of the treatments, spraying (0.99) and soil drenching (1.41) with *P.lilacinus* were statistically on par. Control treatments recorded the maximum number of other pests (7.59).

One week after second application, soil drenching + spraying with *P.lilacinus* recorded the minimum incidence of other pests (0.41) which was on par with 0.2% malathion spray (0.79) (Table 22). The effect of soil drenching (0.57) and spraying (0.58) with *P.lilacinus* were statistically on par. Control treatments recorded the maximum number of other pests (3.97).

Two weeks after second application, soil drenching + spraying with *P.lilacinus* recorded no population of other pests (0.00) which was on par with 0.2% malathion spray (0.31). The effect of spraying (0.39) and soil drenching (1.06) with *P.lilacinus* were statistically on par. Control treatments recorded the highest number of other pests (7.37).

4.8.4 Incidence of Natural Enemies in the field

The population of natural enemies was recorded at different intervals after the application of treatments (Table 23). Natural enemies like spiders, ladybird beetles, braconid parasitoids were observed in the plots. One week after first application, soil drenching + spraying with

Table 20. Effect of *P.lilacinus* in the field on melon fly in bittergourd

Treatments	Percentage of fruit fly infestation			
	One week after first application	Two weeks after first application	One week after second application	Two weeks after second application
T_1	19.07 (25.90)	10.89 (19.27)	14.95 (22.75)	7.92 (16.35)
T_2	18.24 (25.28)	7.01 (15.35)	6.13 (14.34)	8.05 (16.48)
T_3	8.61 (17.06)	1.76 (7.62)	2.09 (8.32)	3.37 (10.58)
T_4	14.63 (22.49)	6.35 (14.59)	7.13 (15.49)	5.89 (14.04)
T_5	65.43 (53.99)	56.15 (48.53)	55.56 (48.19)	58.08 (49.65)
CD(0.05)	9.29	14.39	9.13	15.28

Figures in parentheses are angular transformed values.

T_1 – Soil drenching with *P.lilacinus* ($1.3x10^9$ spores/ml)

T_2 – Spraying with *P.lilacinus* ($2.4x10^9$ spores/ml)

T_3 – Soil drenching + spraying with *P.lilacinus* ($1.3x10^9$ spores/ml + $2.4 x10^9$ spores/ml)

T_4 - 0.2% malathion + 1% jaggery spray

T_5 - Control

Plate 13. Banana – jaggery traps in the field

Plate 14. Effect of soil drenching with *P.lilacinus*

Plate 15. Effect of spraying with *P.lilacinus*

Plate 16. Effect of soil drenching+ spraying with *P.lilacinus*

Plate 17. Effect of 0.2% malathion + 1% jaggery spray

Plate 18. Control treatment

Table 21. Effect of *P.lilacinus* on pupae in field

Treatments	Number of infected pupae per m^2			
	One week after first application	Two week after first application	One week after second application	Two week after second application
T_1	1.95 (1.72)	2.30 (1.82)	3.49 (2.12)	2.88 (1.97)
T_2	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
T_3	1.26 (1.50)	1.43 (1.59)	1.01 (1.41)	2.03 (1.74)
T_4	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
T_5	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
CD(0.05)	0.54	0.59	0.57	0.64

Figures in parentheses are √x+1 transformed values.

T_1 – Soil drenching with *P.lilacinus* ($1.3x10^9$ spores/ml)

T_2 – Spraying with *P.lilacinus* ($2.4x10^9$ spores/ml)

T_3 – Soil drenching + spraying with *P.lilacinus* ($1.3x10^9$ spores/ml + $2.4 x10^9$ spores/ml)

T_4 - 0.2% malathion + 1% jaggery spray

T_5 – Control

P.lilacinus recorded the maximum number of natural enemies (1.79) that differed significantly from all other treatments . The number of natural enemies recorded were 0.59 in spraying, 0.36 in soil drenching with *P. lilacinus,* 0.16 in control treatments. The effect of all these treatments were statistically on par.

Two weeks after first application, soil drenching + spraying recorded the maximum number of natural enemies (3.04) that differed significantly from all other treatments (Table 23). The number of natural enemies was 1.29 in spraying with *P.lilacinus*, 0.76 in control treatments and 0.39 in soil drenching with *P.lilacinus* and all these treatments were statistically on par.

One week after second application, soil drenching + spraying with *P.lilacinus* recorded the highest incidence of natural enemies (2.05) which was on par with spraying (1.19) with *P.lilacinus*. Soil drenching (0.52) with *P.lilacinus* was on par with control treatments (0.15).

Two weeks after second application, a very high population of natural enemies was recorded in soil drenching + spraying (2.50) with *P.lilacinus* that differed significantly from all other treatments. The effect of spraying (1.06) and soil drenching (0.65) with *P.lilacinus* were statistically on par. On all the recorded weeks 0.2% malathion spray recorded no population of natural enemies.

4.8.5. Effect of *P.lilacinus* on other pests

The mortality of other pests recorded at different intervals after application of treatments was recorded (Table 24) (Plate 19). Egg mass of epilachna beetle, larvae of pumpkin caterpillar *Diaphania indica* were found infected with the fungus. One week after first application, soil drenching + spraying recorded a higher mortality of other insects (7.77) that differed significantly from all other treatments. Spraying with

Table 22. Incidence of other pests in the field

Treatments	Number of other pests per plant			
	One week after first application	Two weeks after first application	One week after second application	Two weeks after second application
T_1	2.17 (1.78)	1.41 (1.55)	0.57 (1.25)	1.06 (1.44)
T_2	1.18 (1.48)	0.99 (1.41)	0.58 (1.26)	0.39 (1.18)
T_3	0.99 (1.41)	0.45 (1.20)	0.41 (1.19)	0.00 (1.00)
T_4	1.37 (1.54)	0.67 (1.29)	0.79 (1.34)	0.31 (1.15)
T_5	6.88 (2.81)	7.59 (2.93)	3.97 (2.23)	7.37 (2.89)
CD(0.05)	0.44	0.48	0.59	0.29

Figures in parentheses are √x+1 transformed values.

T_1 – Soil drenching with *P.lilacinus* ($1.3x10^9$ spores/ml)

T_2 – Spraying with *P.lilacinus* ($2.4x10^9$ spores/ml)

T_3 – Soil drenching + spraying with *P.lilacinus* ($1.3x10^9$ spores/ml + $2.4x10^9$ spores/ml)

T_4 - 0.2% malathion + 1% jaggery spray

T_5 - Control

Table 23. Incidence of natural enemies in the field

Treatments	Number of natural enemies per plant			
	One week after first application	Two week after first application	One week after second application	Two week after second application
T_1	0.36 (1.16)	0.39 (1.18)	0.52 (1.23)	0.65 (1.28)
T_2	0.59 (1.26)	1.29 (1.52)	1.19 (1.48)	1.06 (1.44)
T_3	1.79 (1.67)	3.04 (2.01)	2.05 (1.75)	2.50 (1.87)
T_4	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.03 (1.01)
T_5	0.16 (1.09)	0.76 (1.33)	0.15 (1.07)	0.00 (1.00)
CD(0.05)	0.19	0.34	0.29	0.29

Figures in parentheses are √x+1 transformed values.

T_1 – Soil drenching with *P.lilacinus* ($1.3x10^9$ spores/ml)

T_2 – Spraying with *P.lilacinus* ($2.4x10^9$ spores/ml)

T_3 – Soil drenching + spraying with *P.lilacinus* ($1.3x10^9$ spores/ml + $2.4x10^9$ spores/ml)

T_4 - 0.2% malathion +1% jaggery spray

T_5 - Control

a. **b.**

a. Infected pupae of Snakegourd caterpillar
b. Infected egg mass of Epilachna beetle

Plate 19. Effect of *P. lilacinus* on other insects

Plate 20. Infected pupae of *B.cucurbitae* in the field

P.lilacinus and 0.2% malathion spray recorded a mortality of 1.86 and 1.51, respectively and they were on par. Soil drenching with *P.lilacinus* recorded a mortality of 0.85 and control treatment (0.29).

Two weeks after first application, soil drenching + spraying recorded the maximum mortality of other insects (8.11) that differed significantly from all other treatments. Mortality of other insects was recorded in 0.2% malathion spray (3.07), spraying (2.39) and soil drenching with *P.lilacinus* (1.52) and the effect of all these treatments were statistically on par. Control treatment recorded the least mortality of other insects (0.43).

One week after second application, soil drenching + spraying with *P.lilacinus* recorded the maximum mortality of other insects (7.79) that differed significantly from all other treatments (Table 24). Mortality of other insects was recorded in spraying (2.62) with *P.lilacinus*, followed by 0.2% malathion spray (1.83), soil drenching (1.43) with *P.lilacinus* and the effect of all these treatments were on par. Control treatment recorded the least mortality of other insects (0.25).

Two weeks after second application, soil drenching + spraying with *P.lilacinus* recorded the maximum mortality of other insects (6.98) that differed significantly from all other treatments. Mortality of other insects was recorded in spraying (1.07) with *P.lilacinus*, followed by 0.2% malathion spray (0.96), soil drenching (0.48) with *P.lilacinus,* control treatments (0.16) and the effect of all these treatments were on par.

4.9 YIELD DATA

Soil drenching+spraying with *P.lilacinus* (T_3) recorded the highest yield (16.62 tonnes per ha) that was on par with the yield recorded in 0.2% malathion spray (12.83 t/ha) (Table 25). Spraying and soil drenching with *P.lilacinus* recorded a yield of 8.61 and 7.21 t/ha,

4.9.1 Benefit Cost Ratio

Benefit : cost ratio for the different treatments was worked out (Table 26). The highest ratio was recorded in (T_3) soil drenching+ spraying with *P.lilacinus* (2.92).

Table 24. Effect of *P.lilacinus* on other pests

Treatments	Mortality of other insects (per plant)			
	One week after first application	Two week after first application	One week after second application	Two week after second application
T_1	0.85 (1.36)	1.52 (1.59)	1.43 (1.56)	0.48 (1.21)
T_2	1.86 (1.69)	2.39 (1.84)	2.62 (1.90)	1.07 (1.44)
T_3	7.77 (2.96)	8.11 (3.02)	7.79 (2.96)	6.98 (2.82)
T_4	1.51 (1.59)	3.07 (2.02)	1.83 (1.68)	0.96 (1.39)
T_5	0.29 (1.13)	0.43 (1.19)	0.25 (1.12)	0.16 (1.08)
CD(0.05)	0.29	0.52	0.36	0.41

Figures in parentheses denote √x+1 transformed values.

T_1 – Soil drenching with *P.lilacinus* (1.3 x 10^9 spores/ml)

T_2 – Spraying with *P.lilacinus* (2.4x10^9 spores/ml)

T_3 – Soil drenching + spraying with *P.lilacinus* (1.3x10^9 spores/ml + 2.4x10^9 spores/ml)

T_4 - 0.2% malathion +1% jaggery spray

T_5 - Control

Table 25. Mean yield of bitter gourd per hectare for different treatments

Treatments	Yield (t/ha)
T_1	7.21
T_2	8.61
T_3	16.62
T_4	12.83
T_5	2.66
CD(0.05)	7.75

Table 26. Benefit – Cost ratio of different treatments taken as a package

Treatment	Cost (Rs.)	Gross income (Rs.)	Net profit(Rs.)	Benefit:Cost
T_1	64935	68438**	3503	1.05
T_2	60735	89340	28605	1.47
T_3	71685	209445	137760	2.92
T_4	76870	101750*	24880	1.32

* Price @ Rs.10 / Kg.

** Price @ Rs.15 / Kg.

T_1 – Soil drenching with *P.lilacinus*

T_2 – Spraying with *P.lilacinus*

T_3 – Soil drenching + spraying with *P.lilacinus*

T_4 - 0.2% malathion +1% jaggery spray

T_5 - Control

Discussion

5. DISCUSSION

Melon fly, *B.cucurbitae* is a major pest of cucurbitaceous vegetables, causing serious economic loss. Several management techniques involving semiochemicals, sanitation measures, bait sprays, chemical sprays are in practice for the management of this pest. However, no effective biocontrol measures are used. Vegetables being consumed as a fresh produce, it is necessary to produce residue less fruits. Biological control of fruit flies using entomopathogenic fungi being an eco-friendly, easily amenable, cost-effective method, it can well be integrated in the fruit fly integrated pest management. Entomopathogenic fungi have already been reported pathogenic to fruit flies (Saxena and Sinha, 1999; Jiji et al., 2003; Jiji et al., 2006 and 2006b and Quesada-Moraga et al., 2008). As this method provides long term control of the pest, there is ample scope of including it in melon fly IPM. The climatic conditions are also congenial for adopting this method in Kerala.

5.1. STANDARDISATION OF EFFECTIVE DOSAGE OF ENTOMOPATHOGENIC FUNGI

5.1.1 *In vitro* rearing of melon fly, *Bactrocera cucurbitae*

5.1.1.1 Standardisation of diets

Different dietary constituents were evaluated for the *in vitro* rearing of adults of melon fly, in the present study. Various constituents in combination with yeast supported the survival and fecundity of adults. Similar observations were reported by several workers (Schroeder et al., 1972; Placido-Silva et al., 1997; Aluja et al., 2001 and Rohlfs and Hoffmeister, 2005). Maximum survival percentage (98.14 on the 12^{th} day) of adults was recorded in jaggery (20g) + yeast (10g) diet. The

longevity of the adults was also found to be more (24 days) in jaggery + yeast diet. The higher survival percentage of adults in jaggery + yeast diet may be attributed to the diet preference of the host. Banana - jaggery food bait was found to be the preferred diet for the trap catch of melon flies under field conditions by many workers (Jiji et al., 2005; Thomas et al., 2005; Vidya, 2005).

Maximum fecundity of adults was also observed in jaggery + yeast diet. This higher fecundity may be due to the higher concentration of yeast (10g) in combination with preferred dietary constituent of melon fly (jaggery-20g). The quality of nutrients in the diet and the number of ovarioles determine the fecundity of adult flies (Khan et al., 2000). Yeast as a source of protein ingested by adults resulted in increased fecundity of adults. Similar results were obtained by Nakamori and Kakinohana (1980); Saha et al. (1996); and Khan et al. (1999). Honey (20 ml) + yeast (10g) diet was equally effective. Since jaggery + yeast diet was found to be more economical and effective in the maintenance of *in vitro* rearing colony of melon fly, this diet was selected for the further rearing.

5.1.2 Screening of Entomopathogenic fungi

The entomopathogenic fungi *viz., Paecilomyces lilacinus*, *Beauveria bassiana, Aspergillus candidus* were screened against the pupae and adults of melon flies. All of them were found pathogenic to *B. cucurbitae.* The pathogenicity of these entomopathogenic fungi was reported earlier by Jiji et al. (2006b).

5.1.2.1 Symptom of Infection

Pupae treated with *P. lilacinus* darkened, showing visible infection from two days after treatment. This may be due to the time period required for the fungus to multiply and cause infection. Pupae killed by the fungus showed no emergence of adults. As the days after

treatment advanced, complete mouldiness of the pupae was observed (Plate 5). Similar observations were recorded by Carneiro and Salles (1994). This may be due to the multiplication of the fungus on the dead host.

Adults treated with *P. lilacinus* became sluggish with mortality recorded from two days after treatment. Infected adults showed mycelial growth on abdominal extremities initially which later turned vinaceous brown at the time of sporulation (Plate 8). The results confirm the observations recorded by Ali et al. (2008).

B. bassiana treated pupae appeared distorted and turned pale, showing visible symptoms from three days after treatment. Infected pupae showed patches of white mycelial growth on its natural extremities (Plate 6). Nature of symptom development was found sparse over the infected pupae. This may be because of the fact that the death of the host is due to the toxins like beauvericin, bassinolide and destruxin produced by the fungus (Benuzzi and Santopolo, 2001).

Adults treated with *B. bassiana* became sluggish with reduced food intake and appeared normal during the initial phase of infection. Later, the body of the insect became stiff and hard with white fluffy mycelial growth all over the body (Plate 9).

Pupae treated with *A. candidus* appeared pale showing patches of white mycelial growth on the surface. During the later stage of infection yellowish mycelial growth was seen over the extremities of the pupae (Plate 7). This may be due to the sporulation of the fungus on the infected host.

Adults treated with *A. candidus* turned dark in colour and became sluggish. This may be due to the action of toxins and secondary metabolites produced by the fungus. Similar observations were recorded by Clardy et al. (1975), Moore et al. (1978), Gaston and Llewellyen (1980), Lopez-Diaz and Flannigan (1997) and Varga et al. (2003). Later

the dead adults became mummified with yellowish white mycelial mat all over the body (Plate 10).

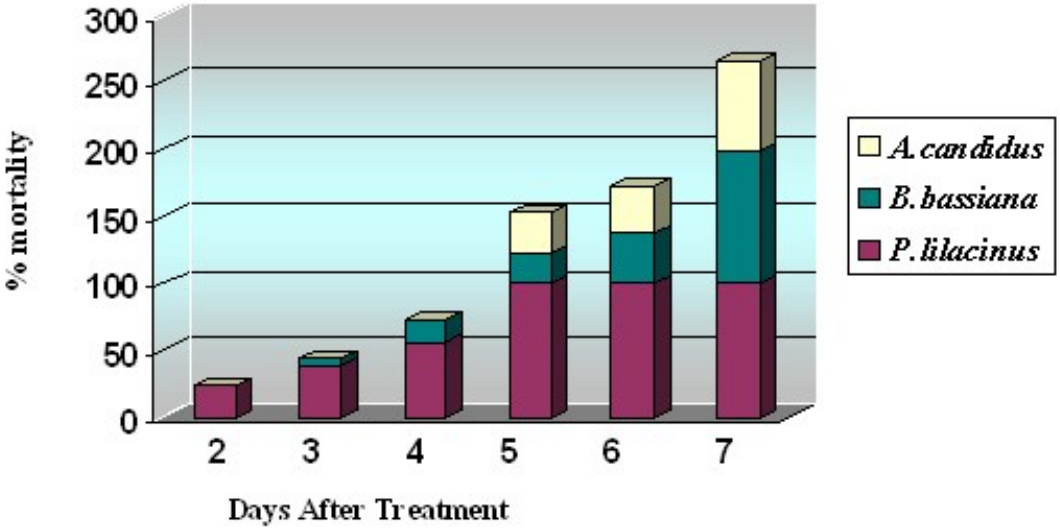
5.1.3 Standardisation of dosage of entomopathogenic fungi

Varying doses of the entomopathogenic fungi were evaluated against the pupae in troughs and adults in cages. Based on the percentage mortality, LC_{50} value of each of the fungi, each day after treatment was worked out. Death of the pupae was observed starting from two days after treatment in *P. lilacinus,* three days after treatment in *B. bassiana* and five days after treatment in *A. candidus*. In the case of adults mortality was observed two days after treatment in *P. lilacinus* and three days after treatment with *B. bassiana* and *A. candidus* (Fig 1&2).

Hundred percent mortality was observed in pupae and adults treated with *P. lilacinus* five days after treatment and seven days after treatment, respectively. This may be due to the high virulence and host suitability of the fungus. The fungus caused earliest mortality (two days after treatment) in pupae at a low LC_{50} value of 1.3 x 10^8 spores/ml and three days after treatment with a LC_{50} value of 2.4 x10^8 spores/ml in adults (Table 6 & 10).

B. bassiana recorded a mortality percentage of 98.29 in pupae and 76.67 in adults at a LC_{50} value of 1.8x10^8 spores/ml in pupae and 2.6 x 10^8 spores/ml in adults three days after treatment (Table 6 &10). The fungus took a slightly longer period to establish an infection on the host. *A. candidus* caused mortality of 67.72 per cent on pupae and 55.38 per cent on adults seven days after treatment showing LC_{50} values of 8.9 x 10^7 spores/ml five days after treatment on pupae and 4.8 x10^8 spores/ml and three days after treatment on adults (Table 6&10). *A. candidus* caused a lower percentage of mortality, compared to *P. lilacinus* and *B. bassiana*. This may be due to the lesser infectivity potential of the fungus. Similar observations were recorded by Jiji et al. (2006).

Fig 1. Comparative efficacy of three entomopathogenic fungi on pupae of *B.cucurbitae*

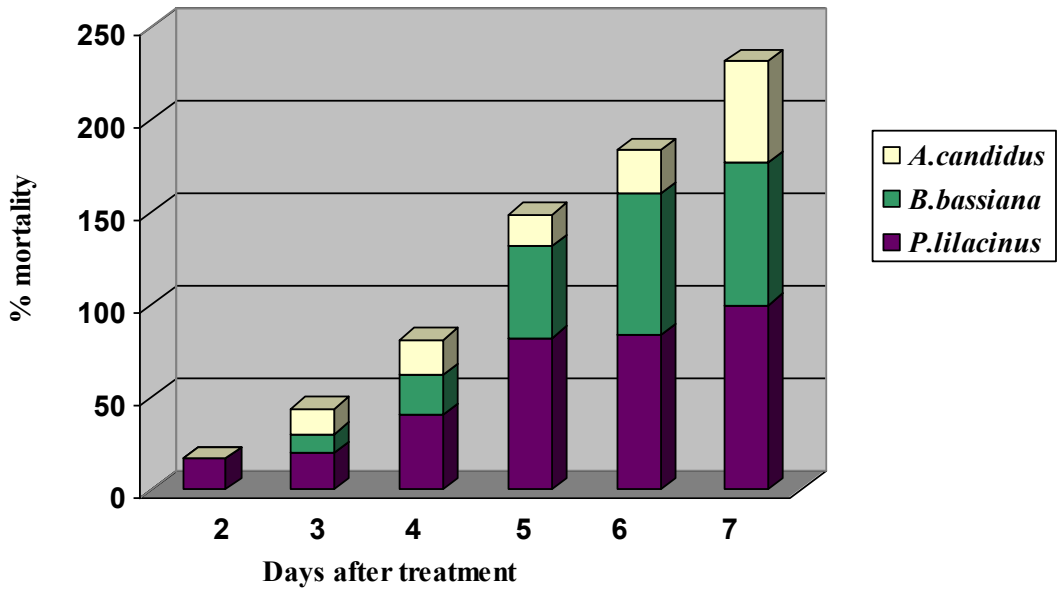


A.candidus - 1.75×10^7 spores/ml

B.bassiana - 2.0×10^7 spores/ml

P.lilacinus - 1.5×10^7 spores/ml

Fig 2. Comparative efficacy of three entomopathogenic fungi on adults of *B.cucurbitae*



A.candidus **- 1.75x10^7 spores/ml**

B.bassiana **- 2.0x10^7 spores/ml**

P.lilacinus **- 1.5x10^7 spores/ml**

Irrespective of the entomopathogenic fungi an increase in spore concentration resulted in higher percentage mortality of the pupae and adults. Similar observations were recorded by several workers (Mahmoud, 1997; Lux et al., 2003; Mahmoud, 2009). Similarly a lower spore concentration recorded a lower percentage of mortality. This may be due to the longer time taken by the fungus to multiply and cause an infection.

Since *P. lilacinus* treated pupae and adults recorded the highest percentage mortality at the lowest LC_{50} value, it was selected as the promising fungus.

5.2 MASS PRODUCTION AND SHELF LIFE OF *P.lilacinus*

For the evaluation of the entomopathogenic fungus under field conditions, mass multiplication of the fungus on suitable substrate was necessary. In the present study, different locally available solid and liquid substrates were used for the mass multiplication (Plate 11&12). Among the different solid media tested, rice bran recorded the maximum spore count on the 28^{th} day after inoculation. The result was in confirmity with the observations recorded by Sahayaraj and Karthick (2008). There was a gradual increase in spore count from the seventh day after inoculation.

The high spore count may be due to the higher nutrient status of the media that supported maximum growth and sporulation of the fungus (Fig 3). After four weeks there was a gradual decline in spore count. Similar observations were recorded by Ibrahim and Low (1993) who suggested rice bran as the suitable media for the mass culture of *B. bassiana.*

The spore count of *P. lilacinus* grown on all the media showed a decline in spore count twenty eight days after inoculation. This indicates that the fungus mass cultured on these media cannot be stored for more

Fig 3. Effect of solid substrates on the sporulation of *P.lilacinus*

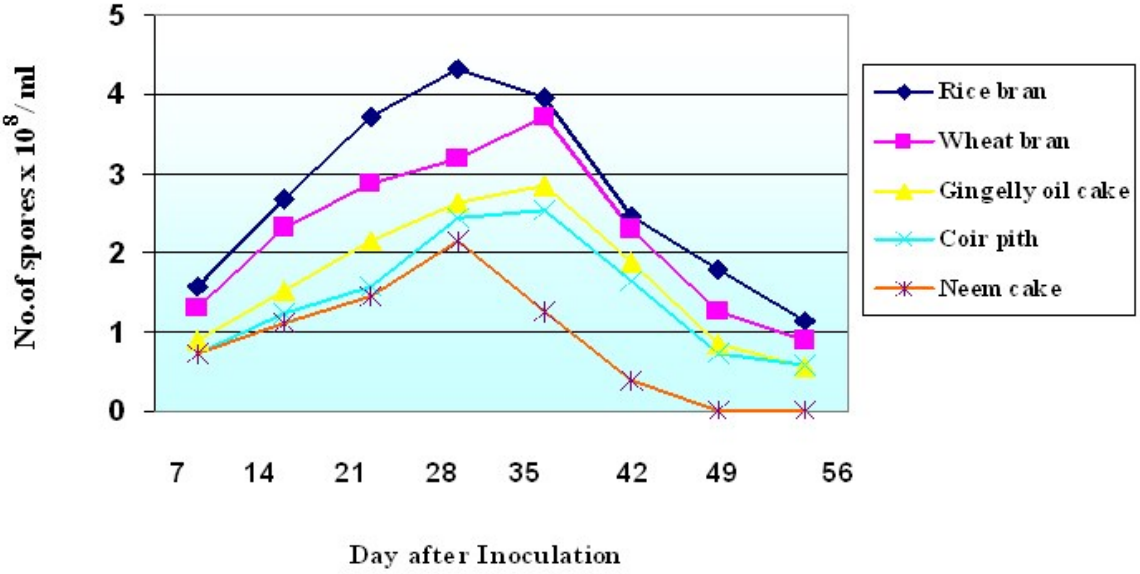
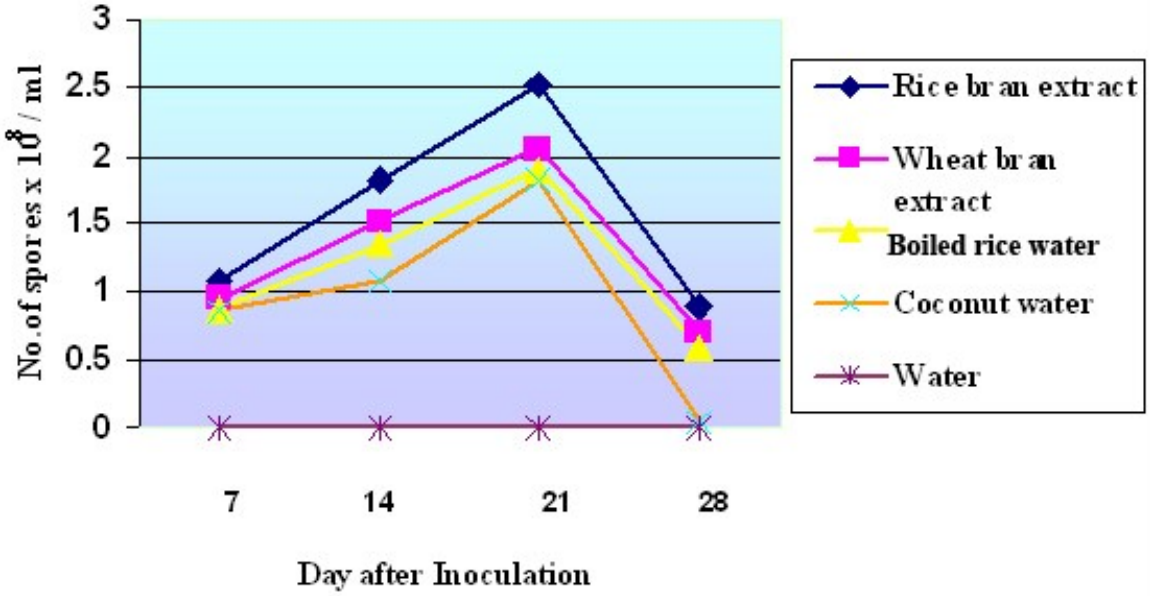


Fig. 4 Effect of liquid substrates on the sporulation of *P.lilacinus*



than four weeks at room temperature with an effective spore concentration. Neem cake recorded the least spore count less than the LC_{50} value of the pathogen on the 42nd day after inoculation.

Among the different liquid media used for the study, rice bran extract recorded the maximum spore count with a peak on the 21st day after inoculation (Fig 4). A gradual increase in spore count was recorded on the seventh day after inoculation. After the third week, the spore count decreased below the LC_{50} value of the pathogen. The results were in conformity with the observations recorded by Filho et al. (1989), Haraprasad et al. (2001) and Verghese et al. (2003) who evaluated rice broth as a media for the mass multiplication of deuteromycetes fungi.

The efficacy of *P. lilacinus* grown on solid and liquid media was tested on the pupae and adults of melon fly. Among the different solid substrates tested for their efficacy, the fungus cultured on rice bran recorded the maximum infectivity on the 28th day after storage. Similar observations were made by Griffin (1981). Among the different liquid substrates tested for their efficacy, the fungus grown on rice bran extract recorded the maximum infectivity on the 28th day after storage. This may be due to the maximum sporulation of the fungus on the fourth week.

From the observations it was concluded that rice bran was the suitable media for the mass multiplication of the fungus that yielded the highest spore count with high efficacy over the pupae and adults. The fungus grown on rice bran could be used for a period of one month for the management of melon fly. Similar observations were recorded by Sahi (2005) who mass multiplied *B. bassiana* for the management of banana pseudostem weevil, *Odoiporous longicollis*.

5.3 EFFICACY OF *P. lilacinus* ON ADULTS OF MELON FLY IN CAGE CONDITIONS

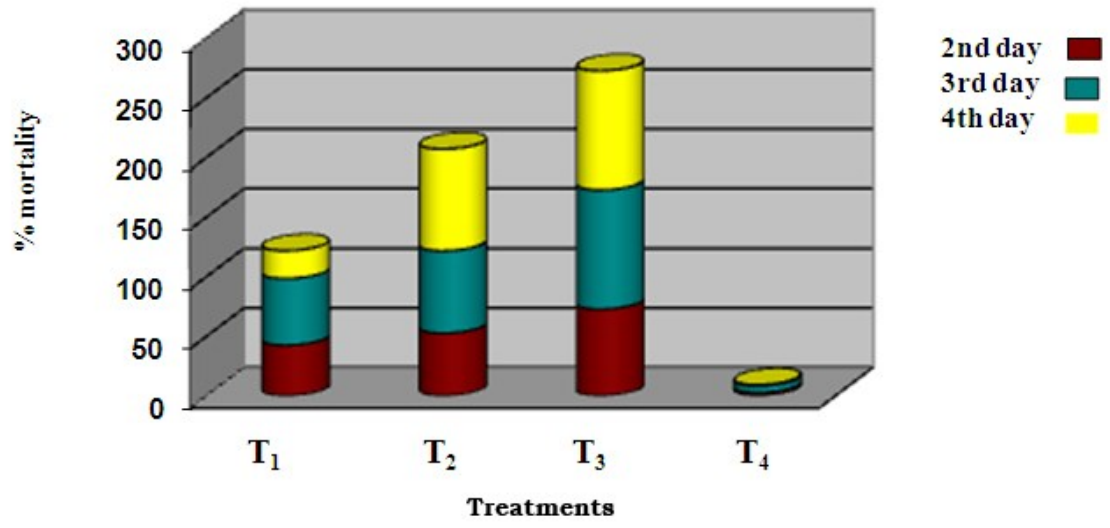
In this cage study, different doses of *P. lilacinus* based on the LC_{50} value (2.4 $x10^8$ spores /ml on third day after treatment) of the pathogen on the adults were tested. The highest mortality (100%) was recorded at a higher spore concentration of 2.4 x 10^9 spores /ml on the third day after inoculation (Fig 5). Similar results were obtained in the bioassay studies in olive fruit fly, *B. oleae* by Mahmoud (2009).

Treated adults showed a drastic reduction in food intake and fecundity. Similar observations were recorded by Castillo et al. (2000) in the *in vitro* efficacy of *P. fumosoroseus* (Wize) Brown & Smith on the adults of Mediterranean fruit fly, *C. capitata*. Reduced fecundity of the treated adults may be due to the reduced mating competitiveness and attractiveness to the opposite sex. Similar observations were recorded by Schaecter (2000). In the present study, the spore concentration 2.4 x 10^9 spores/ml caused cent per cent mortality on the third day. Control treatments recorded the minimum mortality. The mortality in the control may be due to the humidity generated by the water spray in the cage condition, thereby causing fungal infection.

5.4 EFFICACY OF *P.lilacinus* ON PUPAE OF MELON FLY IN TROUGH CONDITIONS

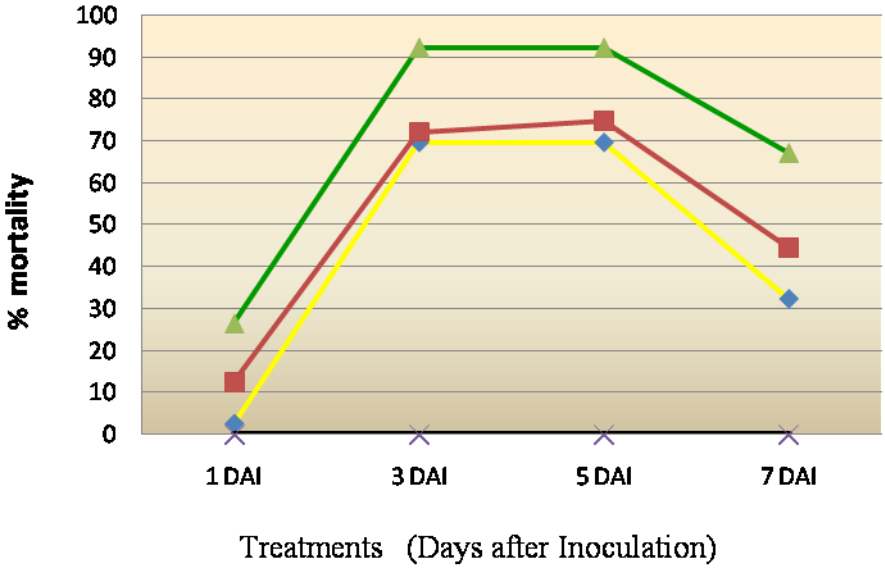
In the trough experiment, different doses of *P. lilacinus* based on the LC_{50} value (1.3 $x10^8$ spores/ml on second day after treatment) of the pathogen were tested. A steady increase in percentage mortality was recorded from one day after inoculation of the fungus in the soil (Fig 6). The lowest percentage mortality of the pupae was recorded one day after inoculation of the fungus in the soil. This may be due to the time taken

Fig 5. Effect of *P.lilacinus* on adults in cages



T_1 - $2.4X10^7$ Spores/ml
T_2 - $2.4X10^8$ Spores/ml
T_3 - $2.4X10^9$ Spores/ml
T_4 - Control

Fig 6. Effect of *P. lilacinus* (7 DAT) in troughs at different intervals after inoculation



T_1- 1.3X10^7 Spores/ml

T_2- 1.3X10^8 Spores/ml

T_3- 1.3X10^9 Spores/ml

T_4- Control

for the establishment of the fungus in the soil. The maximum percentage mortality was recorded on the third day after inoculation. The percentage mortality was found to be persistent upto the fifth day after inoculation. Similar observations were recorded by Alves et al. (2004) and Mochi et al. (2006). When pupae were introduced on the seventh and ninth day after inoculation of the fungus in the soil, a slight decrease in percentage mortality was recorded.

From the observations it can be concluded that the fungus applied in soil remains in an active state without suitable insect host. When pupae were introduced after a period of five days, though a significant percentage of mortality was observed, a decreasing trend was noticed. Similar observations about the survival of the entomopathogenic fungi were reported by Gaugler et al. (1989).

5.5. PEAK TIME OF ACTIVITY OF MELON FLY, *B. cucurbitae* IN BITTERGOURD

In the field evaluation of entomopathogenic fungus, the treatments were applied during the peak time of fruit fly activity. In order to assess the peak time of activity of fruit fly**,** different food baits containing banana + jaggery + malathion with and without yeast were screened for the trap catch of melon flies (Plate 13). Among the different treatments evaluated, T_3 bait preparation in water (100 ml) containing 30g banana, 10g jaggery, 0.2 ml malathion and 10g yeast attracted more number of melon flies.

Banana var. Palayamkodan has been used for the food bait traps of melon flies. Similar observations were recorded by Sivakumar (2001) and Vidya (2005). In the present laboratory study, it was observed that a higher concentration of yeast (10g) + jaggery (20g) in the diet encouraged higher fecundity and survival percentage of adults under *in vitro* conditions. Yeast as a source of protein was necessary for the

survival of adult flies (Saha et al., 1996). In this study, an improvement of this diet was made by adding banana in different proportions and evaluated under field conditions. A higher proportion of banana (30g) in combination with yeast (10g) might have made the trap more attractive to the melon flies.

Peak time activity of melon flies was recorded during the morning hours (8 -10 a.m.). Several congenial factors like moderate sunshine, microclimate prevailing inside the pandals during the morning hours may be reason for the high activity recorded during the period. Similar observations were recorded in the case of oriental fruit fly *B. dorsalis* (Hendel) by Jiji et al. (2006a).

5.6 FIELD EVALUATION OF ENTOMOPATHOGENIC FUNGUS

The entomopathogenic fungus *P. lilacinus* was evaluated under field conditions. The minimum percentage infestation of melon fly was recorded in the plots that received soil drenching + spraying with *P.lilacinus* (Fig 7) (Plates 14-18). As the treatments were applied during the morning hours (peak time of activity of fruit flies), adult flies could be exposed to the fungus spray. The mature maggots in the infested fruits that pupated in the soil could also be infected by *P. lilacinus* applied as soil drenching. Similar observations were recorded by Radoslav et al. (2008) in cherry. Efficacy of the fungus *P. fumosoroseus* for the management of Diamond back moth, *Plutella xylostella* was observed by Monchan et al. (2008).

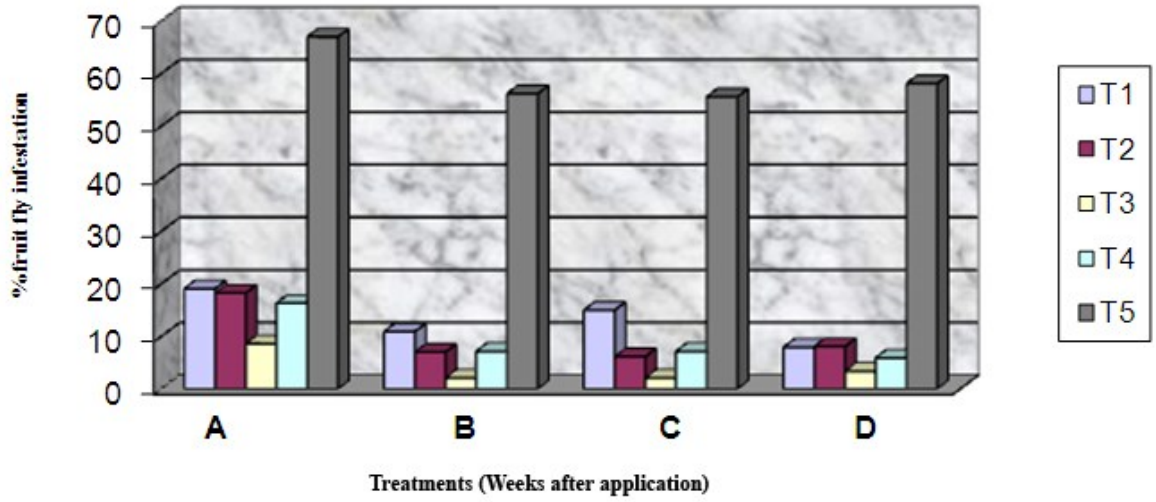
Other pests like pumpkin caterpillar *Diaphania indica* were observed in the plots It was observed that soil drenching + spraying treatment recorded lower incidence of pumpkin caterpillar. Higher mortality counts of other pests was also observed in the same treatment. Eggs of epilachna beetle and larvae of leaf feeders were also found infected with the fungus (Plate 19). This may be due to the reason that

the applied fungus caused slow death as it took more time to get infection. In malathion spray (0.2%), lower incidence of pumpkin caterpillar was noticed. Moreover, the number of dead insects observed in the treated plot was low. This may be due to the immediate death of insects caused due to the chemical spray, that later decayed after falling on the ground. This can be attributed to the contact action exhibited by the chemical on the foliage feeding insects. Effect of malathion on the pests of cucurbits has been reported by many workers (Butani and Verma, 1977; Reghupathy et al., 1997; Das and Isahaque, 1999).

Natural enemies like ladybird beetles, spiders, braconid parasitoids were observed in the field. In soil drenching + spraying with *P.lilacinus*, incidence of natural enemies was found higher. The application of the fungus has made the pests less active that might have supported the natural enemies as a food and host for their survival. The population was seen fluctuating during the initial periods *viz.,* one week after first spraying. Later a stable population was maintained. It is evident that application of entomopathogenic fungus being an ecofriendly measure causes less harm to non target individuals like natural enemies. Malathion spray (0.2%) recorded the least population of natural enemies. This may be due to the non selective toxic action of chemicals. Parasitoids and predators being highly active, showing maximum flight activity during the morning hours, were directly eliminated by the spraying of this chemicals with contact action. The hazardous action of chemical pesticides on natural enemies has been reported by several workers (Pfrimmer, 1964; Patel et al., 1976; Bindu, 1997; Nandakumar, 1999; Santhoshkumar, 1999 and Bernice, 2000).

Soil drenching with *P. lilacinus* recorded (Plate 20) the maximum number of infected pupae per m^2. The treatment applied from the period of fruit initiation paved way for the establishment of fungal inoculum in the soil. Pupae in the fallen infested fruits in due course got infected and adult failed to emerge from the infected pupae. This accounted for the

Fig 7. Effect of *P.lilacinus* in the field on melon fly in bittergourd



A - 1 week after first application
B - 2 weeks after first application
C - 1 week after second application
D - 2 weeks after second application

T_1 - Soil drenching with *P.lilacinus*
T_2 - Spraying with *P.lilacinus*
T_3 - Soil drenching+Spraying with *P.lilacinus*
T_4 - Malathion spraying
T_5 - Control

high count of infected pupae in soil. Similar results of pupal mortality was observed by applying dry conidia of *M. anisopliae* and *B. bassiana* in soil by Ekesi et al. (2007). A gradual increase in the number of infected pupae per m^2 was noticed in the soil drenching plots. This may be due to the time taken for the build up of fungal inoculum in the soil. Soil drenching + spraying with *P. lilacinus* was also equally effective. The other treatments *viz*., spraying with *P. lilacinus,* malathion spray and control recorded no infected pupae per m^2. This may be due to the emergence of adults from the pupae in the soil, as they did not receive any soil treatments. The result was in confirmity with the observations recorded by Garcia et al. (1989).

The highest yield was recorded in soil drenching+ spraying with *P.lilacinus*. Among the different treatments, malathion spray (0.2%) was the most expensive and this treatment recorded a yield of 12.83 t/ha (Table 26). Soil drenching+ spraying with *P.lilacinus* recorded a lower percentage infestation of fruit flies and maximum population of natural enemies. This treatment also recorded a low incidence of other pests and higher mortality of other pests. Hence soil drenching + spraying with *P.lilacinus* was found to be a viable option for the management of melon fly in bitter gourd.

The treatment soil drenching + spraying recorded more benefit due to the high yield as evident from the B:C ratio. Soil drenching with *P.lilacinus* recorded an yield of 7.21 t/ha, involving more cost compared to spraying with *P.lilacinus*. This may be due to the higher quantity of rice bran formulation needed for the soil drenching.

Though soil drenching + spraying with *P.lilacinus* was equally effective in terms of yield with malathion spray (0.2%), the former is better, as it is an eco – friendly, easily amenable method compared to malathion spray. All the other treatments could give profits since their benefit : cost ratio was greater than 1.00 which resulted in high yield due to low attack of fruit flies. Control treatment also recorded a lower yield.

No extra cost was incurred for plant protection measures in this treatment. The low yield may be due to the higher infestation by fruit flies.

Summary

6. SUMMARY

Bitter gourd (*Momordica charantia* L.) is an important cucurbitaceous vegetable. The melon fly*, B. cucurbitae* Coquillett is a serious pest of cucurbitaceous vegetables, affecting fruit quality, often causing great economic loss. Measures involving semiochemicals, field sanitation and chemical sprays are being used for managing the pest. However, use of pesticides needs to be minimized, as vegetables are mostly consumed fresh. In this context, it is relevant to explore the scope of biological control of melon fly using entomopathogenic fungi. Biocontrol of fruit flies using entomopathogenic fungi is an ecofriendly and cost-effective option. The present study attempted to manage the pest in bitter gourd, utilizing local isolates of entomopathogenic fungi under *in vitro* and *in vivo* conditions.

Three entomopathogenic fungi *viz*., *B. bassiana, P. lilacinus* and *A. candidus* were screened for their efficacy against the pupae and adults of melon flies. *In vitro* rearing of melon fly was done for the study. Dietary constituents comprising of honey, sucrose, jaggery, sugar and water in combination with yeast were evaluated. The best diet was selected and used for rearing the melon fly in the laboratory. Among the different diets evaluated for the *in vitro* rearing of the melon fly, bait preparation in 100 ml water containing 20g jaggery and 10g yeast supported the maximum survival percentage (97.68) and fecundity (103.65) of adult melon flies. Hence, jaggery + yeast diet was standardized as a suitable diet for the laboratory rearing of melon fly.

The most promising fungus was selected based on their pathogenicity. The bioassay of the fungi on the pupae and adults was conducted and the most promising fungus was selected. The studies were conducted by soil drenching

(pupae) and spraying (adults) using different spore concentrations of the fungi. Among the three entomopathogenic fungi screened *P.lilacinus* recorded the maximum mortality (100%) in pupae at a spore concentration of 1.5 $x10^7$ spores/ml seven days after treatment with a low LC_{50} value of 1.3 x 10^8 spores/ml, two days after treatment. In the case of adults, the highest mortality (100%) was recorded seven days after treatment with a low LC_{50} value of 2.4 x 10^8 spores /ml three days after treatment. Based on the maximum mortality per cent and low LC_{50} value, *P.lilacinus* was selected as the promising fungus for the further studies.

The promising fungus *P.lilacinus* was mass multiplied on locally available cheaper solid and liquid substrates *viz.,* rice bran, wheat bran, gingelly oil cake, coir pith, neem cake, rice bran extract, wheat bran extract, boiled rice water, coconut water and water. Among the different substrates, rice bran supported the maximum spore count (4.32 x 10^8 spores/ml) on 28^{th} day after inoculation. The fungus grown on rice bran also recorded the maximum efficacy causing 75.96 per cent mortality in pupae and 72.62 per cent in adults four weeks after storage. Based on the spore count and the efficacy, rice bran was selected as the promising substrate for the mass multiplication of *P.lilacinus*.

Based on the LC_{50} value of *P. lilacinus* on the adults and pupae, the fungus was tested for its efficacy on adults of melon flies under cage and trough conditions. Soil drenching with *P. lilacinus* @ $1.3x10^9$ spores /ml (30 ml per trough) resulted in 92.45 per cent mortality in pupae, five days after inoculation in soil, under trough conditions. Spraying with *P. lilacinus* @ $2.4x10^9$ spores/ml recorded cent per cent mortality in adults, three days after treatment, under cage conditions.

A field trial was conducted to assess the peak time of activity of melon fly. Food baits containing banana, jaggery and malathion in different combinations were used to document the peak time of activity of melon fly. Bait preparation in

water (100 ml) containing 30g banana, 10g jaggery, 0. 2 ml malathion and 10g yeast showed superior performance in the trap catch of melon flies with a peak in trap catch during 8-10 a.m.

The promising fungus was evaluated in bittergourd under field condition. Treatments *viz.,* soil drenching (*P. lilacinus*), spraying (*P. lilacinus*), soil drenching + spraying (*P. lilacinus*), 0.2% malathion spray and control were tried. The treatments were applied during the peak time of melon fly activity at fortnightly intervals twice during the fruiting period. Soil drenching with a spore concentration of 1.3 x10^9 spores/ ml (170 g *P.lilacinus* multiplied in rice bran in 17 litres of water per 40 m^2) + spraying with *P. lilacinus* at 2.4 x 10^9 spores / ml (20 g in 2 litres of water per 40 m^2) recorded the minimum percentage infestation of melon fly and significantly higher yield (16.6 t/ha) and a B: C ratio of 2.92 in bittergourd. Soil drenching with *P. lilacinus* recorded the maximum number of infected pupae in soil under field conditions. Lower incidence of pumpkin caterpillar was observed in soil drenching + spraying with *P. lilacinus*.. The same treatment supported the maximum incidence of natural enemies under field condition.

Based on the results of the present study, soil drenching (1.8 x 10^9 spores / ml) + spraying (2.4 x 10^9 spores/ml) using *P.lilacinus* twice during the fruiting period at fortnightly intervals can be recommended for the management of melon fly, *B.cucurbitae.*

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* Originals not seen

MANAGEMENT OF MELON FLY (*Bactrocera cucurbitae* Coquillett) USING LOCAL ISOLATES *Beauveria bassiana* (Bals.) Vuill, *Paecilomyces lilacinus* (Thom.) Samson AND *Aspergillus candidus* Link: Fries

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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

2010

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ABSTRACT

A study on "Management of melon fly, *Bactrocera cucurbitae* Coquillett using local isolates entomopathogenic fungi *viz*., *B.bassiana* (ITCC 6063), *P.lilacinus* (ITCC 6064), *A.candidus* (ITCC 5428)" was conducted in the Department of Agricultural Entomology, College of Agriculture, Vellayani.These fungi were screened against the pupae and adults of melon fly. The most promising fungus was selected based on its efficacy, mass multiplied for field application, standardized for field dosage and method of application.

Dietary constituents comprising of honey, sucrose, jaggery, sugar and water in combination with yeast were evaluated for the laboratory rearing of melon fly. Bait preparation in 100 ml water containing 20g jaggery and 10g yeast was the best suitable diet for *in vitro* rearing of melon fly.

The bioassay of the fungi on the pupae and adults was conducted and the most promising fungus was selected. The studies were conducted by soil drenching (pupae) and spraying (adults) using different spore concentrations of the fungi. Based on the percentage mortality and LC_{50} value*, Paecilomyces lilacinus* was identified as the most promising fungus for the management of melon fly.

The fungus was mass multiplied on locally available cheaper solid (rice bran, wheat bran, gingelly oil cake, coir pith, neem cake) and liquid substrates (rice bran extract, wheat bran extract, boiled rice water, coconut water and water). Rice bran recorded the maximum spore count of *P.lilacinus* on 28^{th} day after inoculation. The fungus cultured in rice bran, showed the maximum efficacy in causing pathogenicity on the pupae and adults of melon flies.

In vitro studies were conducted to evaluate the efficacy of the *P. lilacinus* on adults and pupae of melon flies under cage and trough conditions. Soil drenching with *P. lilacinus* @ $1.3x10^9$ spores /ml (30 ml per trough) resulted in 92.45% mortality in pupae, five days after inoculation in soil, under trough conditions. Spraying with *P. lilacinus* @ $2.4x10^9$ spores/ml recorded cent per cent mortality in adults, three days after treatment, under cage conditions.

Food baits containing banana, jaggery and malathion in different combinations were used to document the peak time of activity of melon fly. Bait preparations in water (100 ml) containing 30g banana, 10g jaggery, 0.2 ml malathion and 10g yeast, showed superior performance in the trap catch of melon flies, with the peak during 8-10 a.m.

Field experiment was conducted with the treatments *viz.,* soil drenching (*P. lilacinus*), spraying (*P. lilacinus*), soil drenching + spraying (*P. lilacinus*), 0.2% malathion spray and control. Soil drenching with *P. lilacinus* at a spore concentration of $1.3x10^9$ spores/ml (170 g *P. lilacinus* multiplied in rice bran in 17 litres of water per 40 m^2) + spraying with *P. lilacinus* at $2.4x10^9$ spores/ml (20 g *P. lilacinus* multiplied in rice bran in 2 litres of water per 40 m^2) recorded the minimum percentage infestation of melon fly. The same treatment recorded significantly higher yield (16.6 t/ha) and a B: C ratio of 2.92 in bittergourd. Soil drenching with *P. lilacinus* recorded the maximum number of infected pupae in soil under field conditions. Lower incidence of pumpkin caterpillar was observed in soil drenching + spraying with *P. lilacinus*.. This treatment supported the maximum incidence of natural enemies under field condition.