BIOCONTROL OF COWPEA APHID *Aphis craccivora* (Koch) USING ENTOMOPATHOGENIC FUNGI

By

S. SARANYA
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THESIS

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Department of Agricultural Entomology

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

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DECLARATION

I, S.Saranya (2007-11-113) hereby declare that this thesis entitled " Biocontrol of cowpea aphid, *Aphis craccivora* (Koch) using entomopathogenic fungi**"** is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

S. Saranya

Vellanikkara

CERTIFICATE

Certified that this thesis, entitled **"Biocontrol of cowpea aphid, *Aphis craccivora* (Koch) using entomopathogenic fungi"** is a record of research work done independently by **Miss S. Saranya** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Dr. R. Ushakumari

(Chairperson, Advisory Committee)
Associate Professor
Department of Agricultural Entomology
College of Horticulture
Kerala Agricultural University
Thrissur, Kerala

Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee of **Miss S. Saranya** a candidate for the degree of **Master of Science in Agriculture**, with major field in **Agricultural Entomology**, agree that the thesis entitled **"Biocontrol of cowpea aphid, *Aphis craccivora* (Koch) using entomopathogenic fungi"** may be submitted by **Miss S. Saranya** in partial fulfilment of the requirement for the degree.

Dr. R. Usha kumari

(Chairperson, Advisory Committee)
Associate Professor
Department of Agricultural Entomology
College of Horticulture
Vellanikkara

Dr. Sosamma Jacob
(Member, Advisory committee)
Professor and Head
Department of Agrl. Entomology
College of Horticulture
Vellanikkara

Dr. Babu M. Philip
(Member, Advisory committee)
Professor and Head (BCCP&W)
Department of Agrl. Entomology
College of Horticulture
Vellanikkara

Dr. Sally K. Mathew
(Member, Advisory committee)
Professor and Head
Department of Plant Pathology
College of Horticulture
Vellanikkara

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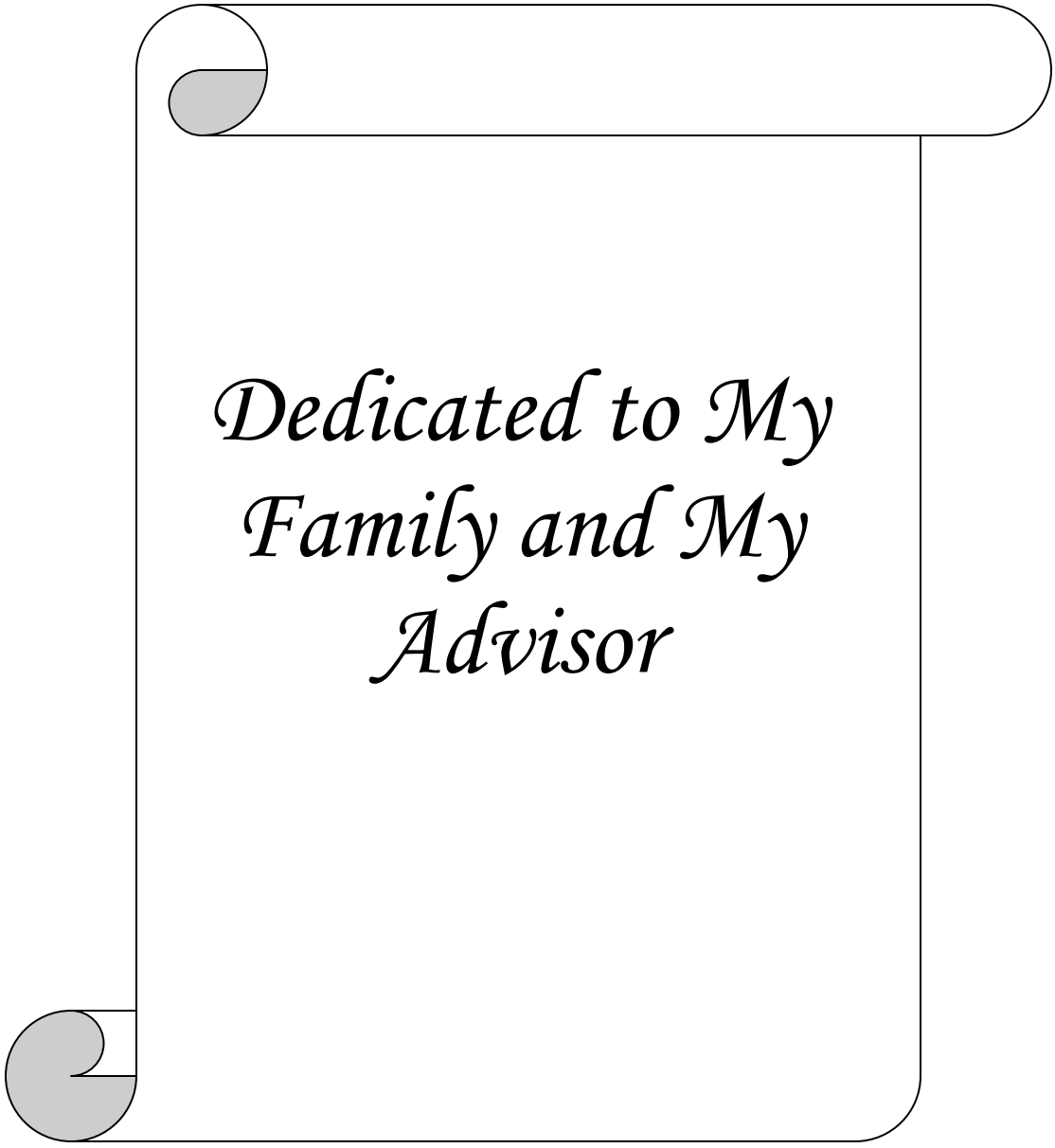
Dedicated to My
Family and My
Advisor

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Introduction

1. INTRODUCTION

Cowpea, *Vigna ungiculata* (L.) Walp. is the most common vegetable pulse crop extensively cultivated in Kerala throughout the year and has higher dietary and nutritional value. The growth and productivity of the crop is seriously hampered by a number of insect pests causing about 70 per cent yield loss. Among the different pests, cowpea aphid, *Aphis craccivora* (Koch) alone causes 20 to 40 per cent yield loss (Singh and Allen, 1980 and Koshy *et al.*,1987). It is a polyphagous pest and besides cowpea it attacks a large number of host plants *viz.*, lablab, pigeon pea, green gram, black gram, bengal gram, ground nut, beans, dolichos, glyricidia and other crops.

Both nymphs and adults of *A. craccivora* suck plant sap and cause serious damage right from the seedling to pod bearing stage. It also plays a vital role in the transmission of cowpea mosaic virus (Porter, 1984). Due to heavy infestation of aphids, young seedlings succumb to death, whereas the older plants shows symptoms such as stunting, crinkling and curling of leaves, delayed flowering, shrivelling of pods and finally resulting in yield reduction. Aphids reproduce by parthenogenesis and it occurs throughout the year. Dry period following heavy rain is the most favourable condition for the build up of aphid population. It attained peak during September to April and low during May to August in Kerala (Mathew *et al.*,1971).

For the immediate control of pests, farmers often resort to frequent application of insecticides. Indiscriminate application of insecticides results in various ill effects like development of resistance, resurgence, secondary pest outbreaks, residues in the harvested produce, health hazards and inimical effects on non-target organisms.

In recent years, microbial control of insect pests is becoming popular as insect pathogens such as bacteria, viruses, fungi and nematodes serve as potential bioagents in pest management. Among the different microbial agents, entomopathogenic fungi (EPF) are gaining importance in pest control. Fungi unlike bacteria or viruses directly infect through insect cuticle and do not require ingestion for infection and thereby it offers great

potential for the management of sucking pests (Ramanujam, 2004). Charnley (1989) recorded more than 700 species of fungi as pathogens to insects. They can be easily mass cultured on artificial media without affecting their virulence at a cheaper cost. They are highly species specific with minimal impact on non target organisms. EPF requires high humidity and temperature for their germination, growth and sporulation. This is the important limiting factor for the success of EPF as biocontrol agent.

Entomopathogenic fungi particularly *Beauveria* sp. and *Metarhizium* sp. are of considerable importance because of their ability to infect a wide array of insects. *Verticillium lecanii* (Zimm.) Viegas is being extensively used for the control of sucking pests. Hareendranath *et al.* (1986) identified *Fusarium pallidoroseum* (Cooke) Sac. as a promising biocontrol agent in controlling cowpea aphid in Kerala.

In Kerala, only scanty work has been done on EPF against *A. craccivora.* Hence, the present study was undertaken with the following objectives.

1. Survey for the collection, isolation and identification of local isolates of entomopathogenic fungi on *A. craccivora.*
2. Estimation of Median Lethal Concentration (LC_{50}) of EPF *viz.*, *B. bassiana*, *M. anisopliae*, *V. lecanii* and *H. thompsonii* against *A. craccivora* by bioassay studies.
3. To evaluate the bioefficacy of the above EPF against *A. craccivora* in field conditions by pot culture experiment.

Review of literature

2. REVIEW OF LITERATURE

Entomopathogenic fungi includes those genera of fungi that associate with insects and some other arthropods like spiders and mites. They are characterized by their ability to attach and penetrate host cuticle, multiply with in the host and ultimately resulting in the death of the host insect (Easwaramoorthy, 2003). It includes a number of genera infecting almost all orders of insects. The bioefficacy of the commonly occurring species of entomopathogenic fungi such as *Beauveria, Metarhizium, Verticillium, Hirsutella, Fusarium* and other fungi infecting aphids are briefly reviewed here.

2.1 *Beauveria bassiana* (Balsamo) Vuillemin

The white muscardine fungus *B. bassiana* occurs throughout the world and has the largest host range among the fungi imperfecti. It mainly infects insects belonging to Lepidoptera, Coleoptera, Hemiptera, Diptera and Hymenoptera (Easwaramoorthy, 2003).

2.1.1 Bioefficacy of *B. bassiana* against aphids

Beauveria bassiana is an important insect pathogen that attack wide array of hosts. Sukhova (1987) observed that application of *B. bassiana* formulation (Boverin) could produce 98 per cent control of *Trialeurodes vaporariorum* (West.) and *Myzus persicae* (Sulz.). at 26-28ºC and 80 to 90 per cent relative humidity (RH) in green house condition.

Feng *et al*. (1990) studied the virulence of an aphid derived isolate of *B. bassiana* against cereal infesting aphids. The LC_{50} values were $8.2x10^4$, $2.1x10^5$, $1.1x10^6$, $2.1x10^6$ and $3.3x10^6$ conidia ml^{-1} respectively for *Diuraphis noxia* (Mordvilko), *Schizaphis graminum* (Roandani), *Metapolophium dirhodum* (Walker), *Sitobion avenae* (Fabricius), *Rhopalosiphum maidis* (Fitcher) and *Rhopalosiphum padi* (L.). Droschner *et al.* (1991) obtained the LC_{50} value of $1.37x10^5$ conidia ml^{-1} for hop aphid, *Phordon hamuli* (Schrank).

Miranpuri and Khachatourians (1993) studied the impact of *B. bassiana* isolates at 10^8 spores ml^{-1}on *M. persicae* infesting canola plants. Six days after the first application, they had obtained 72 to 86 per cent aphid mortality.

Zaki (1998) stated that *B. bassiana* based biopesticide Naturalis at $2.3x10^7$ conidia ml^{-1} was effective in controlling *Aphis craccivora* Koch., and *Bemisia tabaci* (Gennadius), infesting cucumber. It caused 100 per cent mortality of both pests when sprayed at a concentration of 1g l^{-1}. Adult longevity, period of reproduction and fecundity were markedly decreased with increasing concentrations. The nymphs were more susceptible to the fungus.

Mathew *et al*. (1998) tested the efficacy of *B. bassiana, Beauveria brongniartii* and *Verticillium chlamydosporium* and *Metarhizium anisopliae* against banana aphid *Pentalonia nigronervosa* f. caladii Vander Goot. It was observed that all the fungi caused mortality to both apterous adults and nymphs ranging from 37.00 to 96.66 per cent and 32.80 to 75.40 per cent respectively. Among the different fungi *B. bassiana* showed the highest mortality of nymphs and adults.

Poprawski *et al*. (1999) conducted replicated field trials using *B. bassiana* based myco insecticide against the brown citrus aphid, *Toxoptera citricidus* (Kirkaldy) on citrus. They recorded 79.80 and 94.44 per cent mortlity at $2.5x10^{13}$ and $5x10^{13}$ conidia ml^{-1} respectively. Liu *et al*. (1999) estimated the LC_{50} values for six aphid derived *B. bassiana* isolates against *M. persicae* and it ranged between $1.2x10^4$ to $1.55x10^6$ conidia ml^{-1}.

Ekesi *et al.* (2000) reported that *B. bassiana* isolate CPD11 was highly pathogenic to cowpea aphid, *A. craccivora* causing 91 per cent mortality, at 7 days after treatment. At the highest concentration of $1x10^8$ conidia ml^{-1}, it produced the shortest LT_{50} value of 3.5 days and the estimated LC_{50} was $6.8x10^5$ conidia ml^{-1}.

Experiment conducted by Liu *et al*. (2000) showed that *B. bassiana* produced higher mortality of aphids at higher temperatures. At a concentration of 1x 10^6 conidia ml^{-1}, the LT_{50} of *M. persicae* at 21, 28, 16 and 11ºC was 3.7, 5.1, 9.2 and 18.7 days respectively. The cumulative mortality rate of the aphids at 21ºC was higher than at 28ºC.

Beauveria bassiana isolates were highly virulent against wheat aphid *S. graminum* and *Macrosiphum avenae* Fabricius and it was found that the optimum temperature and pH for the growth and development was 25-30ºC and 4.0-6.0 respectively (Zhang *et al.,* 2001). Pandey and Kanaujia (2003) conducted bioassay with four concentrations ($5x10^9$, $5x10^8$, $5x10^7$ and $5x10^6$) of *B. bassiana* on *Lipaphis erysimi* (Kalt.). Highest mortality of 98.33 per cent was observed in the higher concentration and the estimated LC_{50} was $7.17x10^7$ conidia ml^{-1}. Roy *et al.* (2005) demonstrated that *B. bassiana* infected aphids produced less alarm pheromone than uninfected aphids. Baverstock *et al.* (2006) found reduction in fecundity of *B. bassiana* infected pea aphid, *Acyrthosiphon pisum* (Harris) within 24 h of infection.

Laboratory experiment was conducted by Nirmala *et al*. (2006) to test the pathogenicity of *B. bassiana* isolates against different aphid species. At a concentration of $1x10^7$ spores ml^{-1}, the mortality ranged between 16.7 to 60.45 per cent in *A. craccivora*, 15 to 80.8 per cent in *Aphis gossypii* Glover and 6 to 50 per cent in *R. maidis*. The LC_{50} of *B. bassiana* isolate (Bba) on *A. gossypii* was $6.75x10^5$ spores ml^{-1}.

The pathogenicity of four isolates in each of *B. bassiana, M. anisopliae* and *V. lecanii* prepared as oil emulsion formulations were tested on the sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner under field conditions at Karnataka. Mycosis was observed with six isolates *viz., B. bassiana* Bb4 (10%), Bb5a (19.8%), Bb6 (19.8%) and *M. anisopliae*, Ma2 (4.7%), Ma3 (16.2%) and Ma4 (42.3%) (Nirmala *et al.*, 2007).

Shapiro *et al*. (2008) confirmed the pathogenicity of *B. bassiana* against pecan aphids *Monellia caryella, Melanocallis caryae* and *Monelliopsis pecanis.* At the

concentration of $1x10^8$ spores ml^{-1} the fungus produced 80 per cent mortality, after four days of treatment.

Six days old nymphs inoculated with conidial suspensions of *B. bassiana* (10^6 conidia ml^{-1}) were maintained at 18, 21, 25 and 28ºC and 85 per cent relative humidity. The fecundity of the infected aphids were dropped by 57, 73, 74 and 60 per cent. It was showed that the fungus *B. bassiana* had great potential for the suppression of *M. persicae* populations at 21 and 25ºC (He and Li, 2008).

Chen *et al.* (2008) reported the natural occurrence of *B. bassiana* causing nearly 95 per cent mortality of cabbage aphids and 97 per cent on wheat aphids. Zhang *et al.* (2008) identified the cuticle degrading protease (CDEP-1) from *B. bassiana* strain (Bb0062). The LC_{50} of the pathogen against the green peach aphid *M. persicae* was decreased with increasing CDEP-1 concentrations from 0 to 100 micro g ml^{-1}. Results confirmed that the CDEP-1 enhanced fungal virulence due to acceleration of conidial germination and cuticle penetration.

2.1.2 Bioefficacy of *B. bassiana* against other sucking pests

Field studies conducted at Lucknow, showed that application of *B. bassiana* at 4.8×10^6 conidia ml^{-1} reduced the mango mealy bug, *Drosicha mangiferae* (Green) population by 33.3 to 100 per cent in 10 days (Srivastava and Fasih, 1988). Tripathi *et al.* (1990) recorded the natural incidence of *B. bassiana* on mango hopper *Idioscopus clypealis* Leth and *Idioscopus nitidulus* (Walker) in Uttar Pradesh.

Puterka *et al.* (1994) found that *B. bassiana* at 10^7 conidia ml^{-1} against pear psyllid, *Cacopsylla pyricola* recorded 92.5 to 99.6 per cent mortality after 7 days. Application of *B. bassiana* at $6.5x10^{13}$ spores ha^{-1} was effective against brown plant hopper, *Nilaparvata lugens* Stal. which resulted in 57.9 per cent mortality, 10 days after treatment (Thuy *et al.* 1994).

Hu *et al.* (1996) tested the pathogenicity of *B. bassiana* against the soybean coreid bug, *Riptortus linearis* (F.) in the laboratory. It was observed that the fungus was pathogenic to third, fourth and fifth instar nymphs and adults of the pest. At $4.5x10^3$ to $4.5x10^5$ conidia ml^{-1}, there was significant linear relationship between the dose of the fungus and the mortality of the coreid bug. Benuzzi and Santopolo (2001) reported that *B. bassiana* based bioinsecticides were used in greenhouse crops to control whiteflies (*T. vaporariorum* and *B. tabaci*), aphids, thrips and the spider mite, *Tetranychus urticae* Koch.

A study was conducted by Patil and Naik (2004) to evaluate the effectiveness of mycopathogens on tea mosquito bug, *Helopeltis antonii* (Sign.). The percent mortality was initially low at 3 days after treatment but increased gradually on the fifth day, recording 87.50, 32.50 and 60.00 per cent mortality of *H. antonii* exposed to *B. bassiana*, *M. anisopliae* and *V. lecanii*, respectively. *H. antonii* adults were more susceptible than the nymphal stage.

Application of *B. bassiana* at two concentrations, $1x10^6$ and $1x10^8$ conidia ml^{-1} was found to significantly reduce the emergence of pod sucking bug, *Clavigralla tomentosicollis* Stal. (Ekesi *et al.*, 2002). *Beauveria bassiana* isolate MK 2001 at $1x10^7$ conidia ml^{-1} was highly pathogenic to adults of *Lygus lineolaris* which resulted 84 per cent mortality, at four days after treatment (Kouassi *et al.*, 2003).

Andalo *et al*. (2004) reported that the fungus *B. bassiana* (UEL114) at $1x10^8$ conidia ml^{-1} caused high mortality in adult females of coffee root mealy bug, *Dysmicoccus texensis* (Tinsley).

Twenty five native *B. bassiana* isolates were evaluated against fourth instar nymphs of sweet potato whitefly, *B. tabaci* and greenhouse whitefly, *T. vaporariorum* at a concentration of $1x10^7$ conidia ml^{-1}. All isolates were pathogenic on both whitefly species, with mortality ranging from 3 to 85 per cent. The LC_{50} value of the four most virulent isolates varied from $1.1x\ 10^5$ to $6.2\ x10^6$ conidia ml^{-1} and average survival time

(AST) of treated nymphs from 5.9 to 7.4 days. *Trialeurodes vaporariorum* was significantly more susceptible to all *B. bassiana* isolates than *B. tabaci* (Moraga *et al.*, 2006).

A field study conducted by Ghatak *et al*. (2008) showed that *B. bassiana* at 2.5 g l^{-1} against tea mosquito bug, *Helopeltis theivora* recorded 56.00 per cent mortality in West Bengal.

2.2 *Metarhizium anisopliae* (Metchinkoff) Sorokin

The green muscardine fungus *M. anisopliae* is a common and widely distributed fungus with a wide host range. Over 100 species of insects belonging to different insect orders are known to be infected by this fungus.

2.2.1 Bioefficacy of *M. anisopliae* against aphids

Butt *et al.* (1994) identified that isolates of *M. anisopliae* was highly pathogenic to oil seed rape aphid, in Europe. Field application of *M. anisopliae* (Metarizin) at 10 litres ha^{-1} and *B. bassiana (*Boverin) 4 litres ha^{-1} recorded the highest mortality (68-72%) and showed a significant reduction in damaged plants due to *M. persicae* in tobacco (Filipchuk *et al*., 1995).

Chandler (1997) tested the virulence of *M. anisopliae* against lettuce root aphid, *Pemphigus bursarius* (L.). The LC_{50} of the fungus at 10 days post inoculation was $2.45x10^{6}$ conidia ml^{-1}. Butt *et al*. (1998) reported that *M. anisopliae* strain producing destruxin toxin was highly pathogenic against *M. persicae* and *L. erysimi,* but harmless to bees. The strain without destruxin was found to be less virulent against aphids. Mathew *et al*. (1999) found that *M. anisopliae* was highly virulent against cardamom aphid *P. nigronervosa* causing 75.4 per cent mortality to both apterous adults and nymphs.

Pathogenicity of four isolates of *M. anisopliae* to apterous adult *A. craccivora* was evaluated in the laboratory. The isolates CPD 4 and 5 caused significantly higher mortality ranging between 64 to 93 per cent and 66 to 100 per cent respectively, at 7 days post treatment. At the highest concentration of $1x10^8$ conidia ml^{-1}, these isolates produced the shortest LT_{50} with 3.6 and 3.4 days respectively. The estimated LC_{50} values were $3.1x10^5$ and $2.7x10^5$ conidia ml^{-1} respectively (Ekesi *et al.*, 2000).

Yeo *et al*. (2003) found that *M. anisopliae* was highly virulent against black bean aphid, *Aphis fabae* Scopoli and *M. persicae* at 20 and 25°C. Pandey and Kanaujia, (2003) tested the virulence of *M. anisopliae* against mustard aphid, *L. erysimi* in the laboratory. At five days post inoculation, the highest and lowest mortality recorded was 98.33 and 76.66 per cent respectively, with an average LC_{50} value of 4.14×10^5 conidia ml^{-1}. Lin and Liu (2004) conducted bioassay with *M. anisopliae* isolate MA126 at $1x10^8$ conidia ml^{-1} and found that it showed more virulence against *M. persicae* than other isolates tested at 28°C.

The effects of the entomopathogenic fungi *B. bassiana* (IBCB 66), *M. anisopliae* (IBCB 121), on third instar nymphs of *A. gossypii* and *M. persicae* were evaluated in the laboratory at 25°C, 70±10 per cent relative humidity and 12 h photophase. The fungi were applied in suspension containing $1x10^6$ to $1x10^8$ conidia ml^{-1}. Both *B. bassiana* and *M. anisopliae* caused 100 per cent mortality at the seventh day after inoculation. *Myzus persicae* was more susceptible to both fungi than *A. gossypii* (Loureiro and Moino, 2006).

Nirmala *et al*. (2006) found that *M. anisopliae* isolates at the concentration of $1x10^7$ spores ml^{-1} recorded 20-60 per cent mortality in *A. craccivora,* at 10 days post inoculation. Laboratory studies conducted by Ramegowda *et al.* (2007) revealed that *Cladosporium oxysporum* Berk and Curt and *M. anisopliae* caused significant by the highest mortality of sugarcane woolly aphid, *C. lanigera*. The mortality increased gradually with increase in the concentration from $1x10^4$ to $2 x10^8$ conidia ml^{-1}.

Hesketh *et al*. (2008) studied the virulence of different isolates of *M. anisopliae* against *A. fabae*. At the concentration of $1x10^8$ spores ml^{-1}, for all the isolates the LT_{50} value ranged between 5.12 to 5.65 days.

2.2.2 Bioefficacy of *M. anisopliae* against other sucking pests

Gopalakrishnan and Narayanan (1989) reported that *M. anisopliae* suspension with $1.8x10^9$ conidia ml^{-1} was sufficient for controlling rice brown plant hopper, *Nilaparvata lugens* under field conditions.

Garcia *et al.* (1990a) reported that application of spore suspensions of *M. anisopliae* on adults of plant hopper, *Sogatodes oryzicola* (Muir) in rice seedlings caused significant reduction by recording 65 and 77 per cent mortality, within 15 days. Females were more susceptible to the pathogens than males.

Vyas *et al.* (1993) demonstrated that exposure of mango hopper, *Amritodus atkinsoni* (Leth.) for 75 minutes to an inert dust containing 1 billion spores g^{-1} of *M. anisopliae,* caused 100 per cent mortality after 96 hours. Reduced exposure times resulted in reduced mortality. Fungal spore suspensions in 0.1 per cent Tween 80, achieved greatest mortality within 24 hours.

Poprawski *et al.* (1994) assessed the toxicity of destruxin E (*M. anisopliae*) to nymphs of *Empoasca vitis* Goethe in the laboratory. Different concentrations of the toxin at 30, 100, 300 and 1000 ppm were sprayed on potato leaves and directly on the insects. After four days of treatment, the mortality rates were 87.5 per cent in nymphs sprayed directly and 93.8 per cent in nymphs exposed to treated leaves. Lowest LC_{50} of 46.4 and 38.2 ppm was obtained respectively for insects exposed to treated leaves and sprayed directly.

Puterka *et al*. (1994) evaluated the pathogenicity of *B. bassiana, M anisopliae, M. flavoviride, P. fumosoroseus* and *V. lecanii* against the nymphs of *Cacopsylla*

pyricola (Foerster), using a detached leaf bioassay. Among the different isolates *Metarhizium* sp. had significantly lower LC_{50} than the other isolates, and it ranged from $2.3x10^7$ to$11.7x\ 10^{10}$ conidia ml^{-1} after 7 days.

The pathogenicity of ten different isolates of *M. anisopliae* were tested in the laboratory against the fourth instar nymphs of *B. tabaci.* At the concentration of $1x10^7$ conidia ml^{-1}, five isolates of *M. anisopliae* produced 97 per cent mortality (Herrera *et al.*, 1999).

Rajak and Varma (2001) isolated the fungus *M. anisopliae* from sugarcane leaf hopper, *Pyrilla perpusilla* Walker in Andhra Pradesh. The fungus caused 83.33 to 90 per cent mortality in the field conditions. Priya *et al.* (2001) studied the pathogenicity of *M. anisopliae* on coconut coreid bug, *Paradasynus rostratus* Dist. The adults and nymphs of coreid bug (30 each) were sprayed with aqueous spore suspension of the fungus at $3.2x10^6$ spores ml^{-1}. All the treated nymphs were found dead after 5 days with mycelial growth on the surface and 96 per cent of the treated adults died after 10 days.

Ekesi *et al.* (2002) found that at a concentration of $1x10^8$ conidia ml^{-1}, *M. anisopliae* isolates CPD 5 and 12 were highly pathogenic to eggs of *C. tomentosicollis*, achieving 91-94 per cent mortality. Spray application of the fungus at two concentrations, $1x10^6$ and $1x10^8$ conidia ml^{-1}, significantly reduced the number of emerging bugs.

Bioassay carried out by Cannard *et al.* (2002) to evaluate the pathogenicity of five isolates of *M. anisopliae* and one isolate of *B. bassiana* against second instar citrus mealybug *Planococcus citri* at 26 ± 1°C and 85 ± 1 per cent RH under 24 hours dark period. All isolates exhibited pathogenicity. But *M. anisopliae* isolate FI-1248 was found to be the most virulent isolate in both water and oil suspensions with LC_{50} values of $6.4x10^5$ and $3.4x10^4$ conidia ml^{-1} respectively. Efficacy of *M. anisopliae* against *T. vaporariorum* and *B. tabaci* without additives was about 50 per cent, where as the fungus

formulated with sunflower oil (Biola) recorded the highest synergistic effect, reaching nearly 100 per cent control (Malsam *et al.* 2002).

Tounou *et al*. (2003) found that *M. anisopliae* at $1x10^7$ conidia ml^{-1} was very effective against green leafhopper, *Empoasca decipiens* Paoli. resulting in 97 per cent mortality, 7 days after application. Under laboratory conditions, *M. anisopliae* at a concentration of $5x10^6$ conidia ml^{-1} killed 66.7 to 100 per cent nymphs of the tobacco whitefly, *B. tabaci* within 3 days of treatment. When tested under field conditions, whitefly mortality ranged from 30.0 to 92.2 per cent on eggplants. In both conditions, significantly higher levels of mortality were obtained in coconut or soybean oil formulated conidia. Mortality rate was higher in formulated conidia compared to non-formulated conidia (Batta, 2003).

A study was conducted by Badilla *et al*. (2004) to evaluate the pathogenicity of five isolates of *M. anisopliae* and one isolate of *B. bassiana* on the adult and nymph stages of leaf hopper, *Perkinsiella saccharicida* Kirkaldy in green house and field conditions. They found that *M. anisopliae* was more effective against *P. saccharicida.*

Ramos *et al*. (2004) tested different isolates of *B. bassiana, M. anisopliae*, *Paecilomyces* sp. and *V. lecanii*, against third instar nymphs of *B. tabaci* biotype B. under controlled conditions. All the isolates were pathogenic to nymphs and caused 10 to 89 per cent mortality. The most pathogenic isolates were *B. bassiana* 447 and 969, *M. anisopliae* 1037, 816 and E9 causing 57, 59, 61, 68 and 89 per cent mortality, respectively.

Nguyen and Vo (2005) reported that isolates of *M. anisopliae* and *B. bassiana* were found to be effective for controlling rice ear head bug *Leptocorisa acuta* (Thunberg). Under field conditions, *B. bassiana* and *M. anisopliae* isolates caused 45.3 to 74.9 and 63.6 to 86.6 per cent mortality respectively, 10 days after treatment. *Metarhizium anisopliae* strains Ma1912, Ma1729 and Ma3605 were found to be pathogenic to the pink hibiscus mealy bug, *Maconellicoccus hirsutus* (Green) affecting

cotton crop. The strains were able to infect adults within 2 days after inoculation and showed 90 per cent mortality by 8th day (Ujjan and Saleem, 2007).

Haque and Ghosh (2007) noted that *M. anisopliae* (1x10^{8} conidia/ml), resulted in 37.35 to 43.13 per cent mortality of *C. insolita*. Panyasiri *et al.* (2007) tested the *M. anisopliae* isolates against citrus mealybug, *Pseudococcus cryptus* Comstock in greenhouses in Thailand. They found that the LC_{50} of *M. anisopliae* (KKU2) against *P. cryptus* was 2.35x10^{6} conidia ml^{-1}

2.3 *Verticillium lecanii* (Zimm.) Viegas

The white halo fungus *V. lecanii* is a wide spread hyphomycete fungus known primarily as a pathogen of homopteran insects mainly aphids and scales (Hall, 1981). Apart from these insects, it has been isolated from several other insect orders, spiders and mites. It is also a mycoparasite of rust and other phytopathogenic fungi such as powdery mildew.

2.3.1 Bioefficacy of *V. lecanii* against aphids

Easwaramoorthy and Jayaraj (1977) reported that the fungus *V. lecanii* was effective against chillli aphid *M. persicae.* Easwaramoorthy and Jayaraj (1979) tested the pathogenicity of *V. lecanii* on 10 species of sucking insects under field conditions and reported a mortality of 43.6, 69.0 and 75.4 per cent on *A. craccivora* Koch., *Pulvinaria psidii* Maskell and *A. gossypii* respectively. In the glass house conditions, *V. lecanii* spore suspension at 10^{8} spores ml^{-1} was highly effective against *Aphis fabae* Scopoli on sugarbeet and *Brachycaudus helichrysi* Kaltenbach, *Macrosiphoniella sanbornii* Gillette and *M. persicae* on cucumber (Khalil *et al*., 1983).

Hall (1984) identified that isolates of *V. lecanii* with large spores (6.7-8.4µm) had high epizootic potential in aphids compared to smaller spores (3.8-6.7 µm). Germination time was shorter for the large spore forms which resulted in higher epidemic levels of

disease. Khalil *et al.* (1985) observed that the fungus *V. lecanii* was highly effective against aphids at 25±2°C and 100 per cent RH.

Milner and Luton (1986) found that sporulation of *V. lecanii* isolated from *M. persicae* cadavers was delayed when the relative humidity was less than 100 per cent. Yokomi and Gottwald (1988) tested the virulence of three isolates of *V. lecanii* against three aphid species *viz., M. persicae, A. gossypii* and *Aphis citricola* Vander Goot. Mortality of aphids was observed from three days of post treatment and at concentrations of 10^6-10^7 conidia ml^{-1} with 100 per cent mortality after four days. Aphids were also susceptible at lower spore concentrations but required more time to obtain high mortality.

Feng *et al.* (1990) studied the virulence of *V. lecanii* against six species of cereal infesting aphids in the laboratory. The median lethal concentration (LC_{50}) values were $4.1x10^5$, $9.8x10^5$, $7.0x10^5$, $3x10^6$, $8.9x10^6$ and $6.9x10^6$ conidia ml^{-1} respectively for *D. noxia, S. graminum, M. dirhodum, S. avenae, R. maidis* and *R. padi.*

Fiume (1993) reported that application of methomyl followed by inoculation with *V. lecanii* and introduction of *Chrysoperla carnea* (Stephens) was the most effective treatment for *controlling M. persicae* and *A. gossypii* in green houses.

Karindah *et al*. (1996) tested six concentrations of *V. lecanii* (0, 10^2, 10^4, 10^6, 10^8 and 10^{10} conidia ml^{-1}) against *A. gossypii* in the greenhouse. They obtained the LC_{50} value of $2.7x10^4$ conidia ml^{-1} and the mortality was increased with increasing concentration. Bye and Charnley (1997) found that the presence of chymotrypsin (subtilisin) protease enzyme in *V. lecanii* plays a major role in cuticle degrading process and produced higher infectivity in *M. persicae.*

Askary *et al*. (1998) compared the activity of three strains of *V. lecanii* (DAOM 198499, 216596 and Vertalec) against potato aphid, *Macrosiphum euphorbiae* (Thomas) and cucumber powdery mildew, *Spherotheca fuliginea* (Schltdl.). Results revealed that strain 198499 had the best antagonistic property which reduced the disease incidence

upto 90 per cent. Although Vertalec showed good activity against aphids, it was that effective against powdery mildew. Mathew *et al.* (1999) observed the natural mycosis of *V. lecanii* on *Pentalonia nigronervosa* in Karnataka.

Bioassays were carried out by Safavi *et al*. (2002) to evaluate the commercial formulation of *V. lecanii* (Vertalec) against the second instar nymphs of pea aphid, *A. pisum* . They found that the concentrations ranging from 10^4-10^8 conidia ml^{-1} resulted in the LC_{50} value of $5.14x10^4$ conidia ml^{-1}, 12 days after treatment. Field application of *V. lecanii* spray suspensions containing $1x10^6$ spores ml^{-1} was effective in reducing the population of *L. erysimi* on Indian mustard, after ten days of treatment (Rana and Singh, 2002).

Ashouri *et al.* (2004) evaluated the pathogenicity of *V. lecanii* (DAOM 198499) against *M. persicae* under laboratory conditions. Six concentrations (0, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia ml^{-1}) treated against the third nymphal stage of *M. persicae* indicated significant mortality ranging from 17.77 to 100 per cent. The LC_{50} value for aphid mortality was $1.4x10^4$ conidia ml^{-1} and LT_{50} values at the concentrations, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia ml^{-1} were 10, 10, 9, 8 and 6 days respectively. The net reproductive rate of the aphid decreased significantly when the concentration increased. Koike *et al*. (2004) found that the *V. lecanii* isolates at $1x10^7$ spores ml^{-1} was much effective against aphids, whiteflies and powdery mildew in glass houses.

Application of *V. lecanii* at the concentration of $1x10^9$ spores ml^{-1} was very effective in controlling *Brevicoryne brassicae* (L.) infesting cabbage. It recorded only minimum number of surviving aphids (0.61 to 0.63 per three leaves) (Palande and Pokharkar, 2005).

Nirmala *et al.* (2006) tested the pathogenicity of four isolates each of *B. bassiana*, *M. anisopliae* and *V. lecanii* on *A. craccivora* at $1x10^7$ spores ml^{-1}. Among the twelve isolates tested, *V. lecanii* isolate V11 showed maximum mortality which was 74 per cent at 10 days post treatment. In green house conditions, *V. lecanii* at 25 g l^{-1} gave 35 to 65

per cent mortality of *A. gossypii* in cucumber. It was relatively less effective than neem products (Razvi *et al*., 2006).

Kim *et al*. (2007) reported that strains of *V. lecanii* and Vertalec have dual activity against aphids and powdery mildew. In the field conditions, Sahayaraj and Namasivayam (2007) tested the effect of *V. lecanii* against groundnut aphid, *A. craccivora* at $1x10^{10}$, $1x10^{8}$ and $1x10^{6}$ spores ml^{-1}. All the concentrations were found to be very promising and virulent to *A. craccivora.*

Bioassays were carried out with the commercial formulation of *V. lecanii*, Vertalec against woolly beech aphid *Phyllaphis fagi* L. using two different dosages $1x10^{6}$ and $2x10^{7}$ spores ml^{-1}. Both the nymphs and adults were susceptible to fungal infection at both dosages. After 14 days, 76 per cent of the nymphs were dead whereas only 53 per cent mortality was observed in adults after the same period (Iversen and Harding, 2007).

Pathogenicity of *V. lecanii* against cowpea aphid, *A. craccivora* was tested in the laboratory and field conditions by Armarkar and Agarkar (2007). The nymphs and adults were susceptible to all the concentrations ranging from 10^{2} to 10^{8} spores ml^{-1}. In the field conditions on the basis of effectiveness, economics and persistency, *V. lecanii* at 10^{8} spores ml^{-1} was found to be the best one.

Virulence of 25 isolates of entomopathogenic fungi belonging to *B. bassiana*, *V. lecanii*, *M. anisopliae* and *Paecilomyces fumoroseus* was investigated in the laboratory bioassays on cabbage aphid, *B. brassicae* at different temperatures (20, 25 and 30°C) and relative humidity (75, 85, 90 and 95%). Among three levels tested, aphid mortality was significantly higher at 25°C than 20 and 30°C. Aphid mortality decreased with decreasing relative humidity. Among the isolates *V. lecanii* isolates (V.1-1, V.1-2, V.1-6 and V.1-7) showed higher virulence to *B. brassicae.* In multiple dose bioassays, lowest LC_{50} was $1.2x10^{4}$ spores ml^{-1}, obtained from V.1-7 isolate (Derakshan *et al.*, 2007).

Roditakis *et al*. (2008) evaluated the effect of *Lecanicillium longisporum* (=*V. lecanii*) against the behaviour of green peach aphid, *M. persicae* in the laboratory. Results revealed that honey dew production of mycosed aphids was declined from two days post inoculation and reproductive rate was significantly reduced from fifth day of inoculation. Goettel *et al.* (2008) reported that hybrid strains of *V. lecanii* (Vertalec and Mycotal) were more effective against aphids, whiteflies, soyabean cyst nematode and cucumber powdery mildew. These hybrid strains were used to improve the host range of the pathogen.

Verticillium lecanii isolate CS625 was highly pathogenic to cotton aphid nymphs, when applied @ 10^8 conidia ml^{-1} and incubated at 25-30ºC with high humidity. The estimated LC_{50} was $5.85x10^5$ conidia ml^{-1} (Kim and Kim, 2008). Polyhouse studies conducted by Kadam *et al.* (2008) showed that *V. lecanii* at $6x10^5$ spores ml^{-1} recorded 93.44 per cent mortality of aphid *M. persicae* on gerbera, 14 days after treatment.

Bioefficacy of *V. lecanii* against *L. erysimi* on mustard was studied under laboratory conditions. Mustard leaves treated with the fungus at 4.0 g l^{-1} showed mortality of nymphs and adults which ranged from 65.50 to 86.50 per cent. It was found that the first and second instar nymphs were more susceptible than third and fourth instar nymphs (Parmar *et al.,* 2008).

2.3.2 Bioefficacy of *V. lecanii* against other sucking pests

Easwaramoorthy and Jayaraj (1977) reported that *V. lecanii* was effective against guava scale *P. psidii*. Srivastava and Tandon (1986) observed the natural incidence of *V. lecanii* on *I. clypealis* on mangoes in Uttar Pradesh, India. According to Russo *et al.* (1988), spraying with *V. lecanii* at 5×10^5 to 5×10^6 conidia ml^{-1} in the field on orange trees, infested with citrus black scale *Saissetia oleae* (Olivier), in Italy, resulted in 80 per cent reduction of its population. It was observed that the rate of infection has increased during winter.

Application of *V. lecanii* at 3.2×10^6 conidia ml^{-1} was effective against the nymphs of whiteflies, which recorded 78.8 per cent mortality after ten days of treatment (Nier *et al*. 1991). Saito (1993), was of the opinion that *V. lecanii* was as effective as buprofezin in controlling nymphs of *Bemisia tabaci* infecting tomatoes. The first incidence of *V. lecanii* infecting the mango leaf hopper, *I. nitidulus* and *Idioscopus nagpurensis* (Pruthi) was reported from Karnataka (Viraktamath *et al.*,1994).

Puterka *et al.* (1994) investigated the pathogenicity of *V. lecanii* on pear psyllid *Cacopsylla pyricola* (Foerster) in the laboratory. It was observed that the fungus at 10^7 conidia ml^{-1} caused mortality to nymphs ranging from 92.5 to 99.6 per cent after seven days.

Gindin *et al*. (1994) tested the methanol extract of *V. lecanii* against sweet potato whitefly *B. tabaci.* All stages of the whitefly were susceptible to the toxin. At 0.5 per cent crude toxin, the mortality rate of whiteflies was 88.0, 53.5, 53.2 and 37.0 per cent for nymphs, eggs, pupae and adults respectively.

According to Cavallazhi *et al.* (1998), *V. lecanii* @ $1.8\text{x}10^7$ conidia ml^{-1} was more effective in controlling soft scale *Philephedra tuberculosa* Nakahara and Gill in Colombia. It was also reported that second instar nymphs were most susceptible to the fungus.

Gindin *et al*. (2000) evaluated the pathogenicity of *V. lecanii* to different developmental stages of the silver whitefly *Bemisia argentifolii* Bellows and Perring. The mortality rate of nymphs, pupae and adults were 83 ± 2.4, 72.5 ± 13.1 and 52.6 ± 3.8 per cent respectively. Kulkarni *et al.* (2003) tested different concentrations (2, 3, 4, 5 and 6 g l^{-1}) of *V. lecanii* against *Ferrisia virgata* (Cockerell) and *Planococcus citri* (Risso) on pomegranate in Rahuri, Maharastra. All the concentrations were effective for controlling these mealybugs. However, on the basis of effectiveness, economics and persistency, concentration at 4 g l^{-1} was found to be the optimum for the management of mealybugs.

Application of the entomopathogenic fungus, *Lecanicillium muscarium* against second instar nymphs of sweet potato whitefly, *B. tabaci* produced significant mortality by giving 90 and 81 per cent in laboratory and green houses respectively (Cuthbertson and Walters, 2005).

Fatiha *et al.* (2007) conducted bioassay with four strains of *V. lecanii* (V20, V26, V07 and V17) against *B. tabaci* on egg plant. Results showed that the third instar nymph was more susceptible to the fungus. Among the fungal isolate V20 was the most virulent one having the least LC_{50} of $1.65x10^7$ spores ml^{-1}.

Studies conducted by Kadam *et al.* (2008) showed that, *V. lecanii* at 6×10^5 cfu ml^{-1} recorded 95.45, 91.67 and 82.40 per cent mortality of *T. vaporariorum*, *Thrips palmi* Karny and red spider mite, *T. urticae* respectively, at 14 days after treatment, under polyhouse conditions. It was more effective than dimethoate at 0.03 per cent. Prado *et al.* (2008) found that *V. lecanii* was very effective against coconut whitefly, *Aleurodicus cocoas* in Peru on Avacado trees. Scorsetti *et al*. (2007) conducted pathogenicity test with *V. lecanii* against the fourth instar nymphs of *T. vaporariorum* using the conidial suspension of 1×10^7 conidia ml^{-1}. The mortality per cent ranged between 26.6 and 76.6 per cent at 7 days post inoculation.

2.4 *Hirsutella thompsonii* (Fisher)

The genus *Hirsutella* sp. infects a number of different types of insects as well as mites and nematodes. *H. thompsonii* is a mite specific hyphomycete entomopathogenic fungi with narrow host range. So the literature on other *Hirsutella* species isolated from sucking pests are reviewed here.

2.4.1 Bioefficacy of *Hirsutella* sp. against aphids

Stainslaw (1985) reported *Hirsutella* sp. from different species of aphids viz., *Uroleucon cirsii* (L.), *U.tussilaginis* (Walk.) and *Microlophium eransi* (Theob.)

2.4.2 Bioefficacy of *Hirsutella* sp. against other sucking insects

Prasad (1961) reported the natural occurrence of *Hirsutella citriformis* on sugarcane leafhopper, *Pyrilla perpusilla* (Walker) in Bihar. It caused a maximum mortality of 84.7 per cent in adults and 87.8 per cent in nymphs.

Garcia *et al.* (1990b) described a new entomogenous fungus *Hirsutella cryptosclerotium* from the fruit mealy bug, *Rastrococcus invadans* Green collected from India. Ahmad (1993) isolated *Hirsutella citriformis* from *Heteropsylla cubana* Crawford in Malaysia. It was found that the adult psyllids were more prone to fungal infection than nymphs. The adult population had an average infection rate of about 20 per cent while nymphs had an infection rate of less than 2 per cent. This was the first report of an entomogenous fungus *Hirsutella versicolor* causing epizootic of *I. nitidulus* in mangoes in Malaysia (Lim and Chung, 1995).

Satpathi (2000) isolated the fungus *Hirsutella* sp. from green leafhopper, *Nephotettix virescens* (Distant) infesting paddy fields in West Bengal. Studies were carried out in Kannur, to test the pathogenicity of *H. citriformis* against *P. perpusilla*. Three concentrations (10^6, 10^7 and10^8 spores ml^{-1}) of the fungus along with 0.1 per cent glycerol were sprayed on the nymphs of *P. perpusilla.* The fungus caused 40 per cent mean mortality of nymphs at 10^6 spores ml^{-1} and it increased to 60 per cent at 10^8 spores ml^{-1} (Chitradevi *et al.*, 2003).

Five different hyphomycete entomopathogenic fungi were tested under field conditions for the control of brown plant hopper, *N. lugens.* Spore suspensions of *H. citriformis, M. anisopliae, M. flavoviridae, B. bassiana* and *Paecilomyces lilacinus* were applied at 1.5-2.0 kg ha^{-1}. Mortality due to these fungi ranged from 63 to 98 per cent, three weeks after application (Gopalakrishnan and Narayanan, 1989).

Pathogenicity of *Hirsutella verticillioides* to lace bug *Vatiga illudens* on cassava was evaluated in Brazil under laboratory conditions. In a controlled chamber (23±1º C and 85-90% RH), at $2x10^7$ conidia ml^{-1}, the average lace bug mortality reached 68 per cent, four days after inoculation (Junqueira *et al.*, 2005).

Smitha (2007) identified the natural occurrence of the entomopathogenic fungus, *Hirsutella* sp. on banana mealy bug, *Geococcus* sp. She had also found that the pathogen causing a nymphal mortality of 90 per cent at a concentration of 10^8 spores ml^{-1} and the estimated LC_{50} was $5.2x10^4$ spores ml^{-1}.

2.5 *Fusarium* sp. as biocontrol agents

The genus *Fusarium*, better known as a plant pathogen, consists of certain species which are very effective in managing certain insect pests and weeds. A wide range of insects are found to be attacked by *Fusarium* spp.

2.5.1 Bioefficacy of *Fusarium* sp. against aphids

In Georgia, pea aphid *A. pisum* was infected by *Fusarium* in addition to *Entomophthora* and *Tricothecium* (Rachvelisrivilli, 1965). Nagalingam and Jayaraj (1986) were the first to report *Fusarium semitectum* Berk and Rav (=*Fusarium pallidoroseum*) against *M. persicae* and *L. erysimi* from Coimbatore, India. They also reported that it was safe to all the instars of mulberry silkworm, honeybees and coccinellid beetles and also to plants like chilli, brinjal, tobacco and cabbage.

A fungal pathogen, *Fusarium pallidoroseum* (Cooke) Sacc. was first reported as pathogenic to pea aphid, *A. craccivora* from Kerala by Hareendranath *et al.* (1986). Pathogenicity tests conducted by spraying spore suspension prepared from nine day old culture of the fungus showed 100 per cent mortality of nymphs and adults in the laboratory.

Mathew *et al.* (1999) isolated five fungal species from *P. nigronervosa* which were identified as *F. oxysporum, V. lecanii, Penicillium fellutanum, Paecilomyces lilacinus* and *Aspergillus parasiticus.* Pathogenicity tests revealed that, all these fungus induced heavy mortality to both adults and nymphs of cardamom aphid.

Sunitha and Mathai (1999) studied the effectiveness of *F. pallidoroseum* against *A. craccivora* on cowpea under field conditions in Kerala. Yield data at 32 days after treatment showed that an increase in spore concentration from $0.875x10^6$ to $7x10^6$ spores ml^{-1} resulted in a corresponding decrease in the aphid population from 91.6 to 100 per cent. A consequent increase in cowpea yield from 246.34 to 265.22 g $plant^{-1}$ was recorded, together with an increase in number of pods from 29 to 33. The water suspension and diatomaceous earth wettable powder formulations at $7x10^6$ spores ml^{-1} achieved 100 per cent mortality at 12 and 16 days after treatment, respectively and it was found as effective as quinalphos 0.05 per cent.

Ganassi *et al.* (2000) reported that *Fusarium* strain having secondary metabolites fumonisin and beauvericin showed high insecticidal activity (>60%) against wheat aphid, *S. graminum.* Zhang (2001) isolated the *Fusarium lateritium* from citrus aphid, *Aphis citricidus* (Kirkaldy) in china. Bioassay results indicated that the fungus showed higher virulence to the nymphs of citrus aphid. The estimated LC_{50} was $2.71x10^7$ spores l^{-1} and the LT_{50} ranged between 2.21 to 6.34 days for the concentrations, $1x10^{11}$ to $1x10^7$ spores ml^{-1}. Under field conditions, the fungus caused mortality upto 91.80 per cent.

Mikunthan (2004) reported the pathogenicity of *F. semitectum* on nymphs and adults of sugarcane woolly aphid, *C. lanigera* and thrips *Scirtothrips dorsalis* Hood and mite *Polyphagotarsonemus latus* Banks in chilli. Field evaluation conducted by Nagarathana (2004) using *V. lecanii* and *Fusarium* sp. on *C. lanigera* proved to be highly effective causing 90 and 86.8 per cent mortality, respectively within 12 days after application.

Suresh (2005) reported the bioefficacy of *Fusarium solani* against *A. craccivora* nymphs under laboratory conditions. It was found that the concentration of $1x10^8$ conidia ml^{-1} gave 54.72 per cent mortality of aphids. Aswini (2006) assayed *F. semitectum* on *C. lanigera* under laboratory conditions. The LC_{50} value for adult aphid was $1.5x10^8$ spores ml^{-1}. The highest mortality (59.32%) by *F. semitectum* was at $2.7x10^9$ spores ml^{-1}.

Fusarium pallidoroseum at the concentration of $5.4x10^9$ spores ml^{-1} recorded 75.76 per cent mortality of sugarcane woolly aphid, *C. lanigera* in the laboratory. Nymphs were more susceptible to the fungus than the adults (Aswini *et al.*, 2007a.). In green house condition, combination of *F. pallidoroseum* ($2.7x10^9$ spores ml^{-1}) and chlorpyriphos (0.02%) recorded the highest mortality of 84.12 per cent followed by nimbicidine (0.03%) which recorded 75.76 per cent mortality against sugarcane woolly aphid (Aswini *et al*., 2007b).

Rooparani (2008) studied the efficacy of *F. semitectum* against *A. craccivora* under laboratory conditions. It was found that the highest mortality obtained at $4.2x10^9$ spores ml^{-1} was 89.20 and 64.66 per cent for the nymphs and adults respectively. In field conditions, the highest mortality was recorded in combination of *F. semitectum* $4.7x10^{14}$ spores ml^{-1} + oxydemeton methyl (0.018%) with 79.01 per cent mortality.

2.5.2 Bioefficacy of *Fusarium* sp. against other sucking pests

Viswanathan (1972) reported *Fusarium oxysporum* Schlecht. on coffee green scale, *Coccus viridis* (Green) which caused 90 per cent mortality within 10 days. The fungal pathogen *F. oxysporum* infectious to *N. lugens* was proved to be non pathogenic to cotton and tomato pants (Kuruvilla, 1978). Gopinath *et al.* (1982) recorded *Fusarium equiseti* as a fungal pathogen of the brinjal mealy bug, *Coccidohystrix insolita* (Green) for the first time in Kerala. Zheng *et al*. (1990) found that *F. pallidoroseum* was highly pathogenic to red wax scale *Ceroplastes rubens* Maskell.

Feng and Quing (1991) found that *Fusarium oxysporum* is a very common entomopathogen highly virulent against all the stages of aphid *B. brassicae*, whiteflies on citrus, and scarab larvae .

Microbial control of *C. insolita* infesting pigeonpea was studied under laboratory and field conditions at Junagadh, Gujarat, India. *Cladosporium cladosporioides* at $3.6x10^6$ and $2.7x10^6$ spores ml^{-1} recorded >70 per cent mortality of *C. insolita*, whereas *F. pallidoroseum* at $6.1x10^5$ spores ml^{-1} recorded >60 per cent mortality in laboratory condition. Under field conditions, *C. cladosporioides* at $3.6x10^6$ spores ml^{-1} resulted >67 per cent mortality. Similarly, *F. pallidoroseum* at $6.1x10^5$ spores ml^{-1} and *Alternaria alternata* at $5.6x10^4$ spores ml^{-1} resulted less than 32 per cent mortality (Borad and Bhalani, 1997).

Fusarium coccophilum was recorded as a fungal pathogen of the sugarcane whitefly, *Aleurolobus barodensis* (Maskell) in Maharashtra. The infection level varied from 2.2 to 12.8 per cent during rainy season (Sunil *et al.*, 2000). Tyson *et al*. (2005) recorded the infectivity of *Fusarium larvarum* and *F. coccophilum* against armoured scale insects in New Zealand.

The entomopathogenic fungi *F. semitectum* and *V. lecanii* were evaluated against spiraling whitefly *Aleurodicus dispersus* Russell. Under laboratory conditions, *F. semitectum* @ $4.2x10^9$ spores ml^{-1} recorded 75.21 and 64.40 per cent mortality of nymphs and adults respectively. Under field conditions, combination of *F. semitectum* at $6.2x10^{15}$ spores ml^{-1} + *V. lecanii* at $4.6x10^{14}$ spores ml^{-1} + Triazophos (0.03%) and *V. lecanii* at $4.6x10^{14}$ spores ml^{-1} + Triazophos (0.03%) were significantly superior over Triazophos alone (Aiswariya *et al.*, 2007).

Natural incidence of *F. pallidoroseum* was found on rice brown plant hopper *N. lugens*, causing 33.33 to 71.4 per cent mortality in Andhra Pradesh (Rao *et al.*, 2008).

2.6 Other fungal species

Field experiment with *Entomophthora aphidis* showed that conidial suspension of the fungus containing $6x10^6$ spores ml^{-1} killed 76 to 100 per cent of *A. gossypii*, *A. pomi*, *S. avenae* and *M. persicae* (Hussey and Tinsley, 1981). Abdel Bakey *et al*. (1998) recorded three species of entomopathogenic fungi (*Cladosporium uredinicola, C. cladosporioides* and *C. chlorocephalum*) which infects *Bemisia* sp., *A. gossypii* and *Empoasca* sp.

Laboratory and field experiments were conducted by Mejia *et al*. (2000) to examine the development, pathogenicity and incidence of *Neozygites fresenii* (Nowakowski) Remaudiere & Keller in *A. craccivora*. It recorded nearly 60 per cent reduction of aphids, after five days incubation. Moore (2002) reported that *C. oxysporum* as an effective biological control agent against black citrus aphid *Toxoptera citricidus* in laboratory. It also effective against citrus psyllid *Trioza erytreae* and citrus mealybug *Planococcus citri*. Abdel Bakey and Abdel Salam (2003) found that *Cladosporium* sp. was highly virulent against *A. gossypii, A. craccivora* and *B. argentifolii* in the laboratory.

Rachappa *et al.* (2007) identified the entomopathogenic fungi *C. cladosporioides*, *C. sphaerospermum*, *Cladosporium oxysporum* and *Aspergillus candidus* from ground nut pests in Northern Karnataka.

Survey conducted in the wheat producing regions of South Africa recorded five species of entomopathogenic fungi, including four entomophthorales (*Pandora neoaphidis* Remaudiere and Heenebert, *Conidiobolus thromboides* Drechsler, *C. obscurus* and *Entomophthora plonchiana* Cornu.) and one hyphomycete *B. bassiana*. *P. neoaphidis* was the most important pathogen recorded from wheat aphid *Diuraphis noxia* Kurdjmov. with 50 per cent mycosis under dry land conditions. Similarly it was the most prevalent species with in the population of *Metropolophium dirhodum* Walk. with 77 per cent mycosis under irrigated conditions (Hatting *et al.,* 2000).

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Biocontrol of cowpea aphid, *Aphis craccivora* (Koch) using entomopathogenic fungi" was conducted in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara during 2008 to 2009. The details of the materials used and the techniques adopted for the investigation are described below.

3.1 REARING AND MAINTENANCE OF COWPEA APHID, *A. craccivora*

Cowpea plants were raised in 30cm diameter earthern pots containing potting mixture with sand, soil and dried cowdung in the ratio of 1:1:1. The cowpea variety 'Bhagyalakshmi' obtained from the Department of Olericulture, College of Horticulture, Vellanikkara was used for rearing the aphids. The adult apterous aphids collected from the cowpea fields were inoculated on the trifoliate stage of cowpea seedlings. The pots were covered with netted cage and thereby the aphid colonies were kept free from natural enemies (Plate 1). Once in three weeks, new seedlings were raised and infested aphid twigs from the old plants were tied with them. Thus aphid culture was maintained throughout the period of study.

3.2 COLLECTION AND ISOLATION OF FUNGAL ISOLATES

A survey was conducted in the cowpea growing fields at Vellanikkara, Mannuthy, Chirakkekodu and Pandiparambu of Thrissur district during May 2008 to February 2009. The mycosed aphids were collected at fortnightly intervals. Dead aphids which were suspected to have fungal infection were periodically collected from the field and kept on moist filter paper in Petridish for further mycelial growth and sporulation.

3.2.1 Isolation of fungal pathogens from mycosed cowpea aphid

Dead aphids collected from farmers fields were kept in the humid chamber for two days to observe the mycelial growth. Once the fungal growth was visible externally,

Plate l. Maintenance of *Aphis craccivora* culture on cowpea plants

the cadavers were surface sterilized with 0.1 per cent mercuric chloride solution for one minute and then washed three times with sterile distilled water. The excess water was removed by keeping the mycosed aphids on Whatman no.1 filter paper. After drying, the dead specimens were carefully transferred using a sterile camel hairbrush to sterilised Petridishes containing Potato Dextrose Agar (PDA). Streptomycin was added to the media @ 0.16 g per 200ml before plating to avoid bacterial contamination. Petridishes with the cadavers were incubated at room temperature (28±1°C). After 48 h of inoculation, the fungi were transferred to PDA slants by hyphal tip method.

3.2.2 Pathogenicity tests

Pathogenicity of the fungi isolated from cowpea aphids was established by spraying the spore suspensions on healthy aphid colonies. The fungi were reisolated from the dead aphids and compared with the original culture.

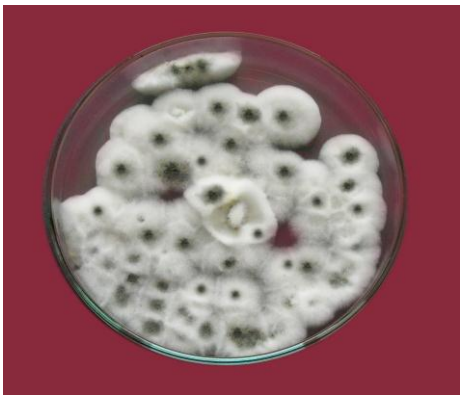
3.2.3 Identification

The new fungal pathogens isolated from cowpea aphid were identified at the Department of Plant Pathology, College of Horticulture, Vellanikkara and National Centre for Fungal Taxonomy (NCFT), New Delhi. Pure culture of the fungal isolates were maintained in the laboratory for further studies.

3.2.4 Maintenance of fungal isolates

Pure cultures of the entomopathogenic fungi *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii* and *Hirsutella thompsonii* obtained from the National Bureau of Agriculturally Important Insects (NBAII), Bangalore were reisolated after proving the Koch postulates. These fungi were then subcultured in Sabouraud's Maltose Agar enriched with one per cent yeast extract (SMA+Y) media (Annexure I) and incubated at room temperature for 10 days and stored in refrigerator (Plate 2). All the fungal isolates were subcultured once in three weeks. To maintain the virulence, after six

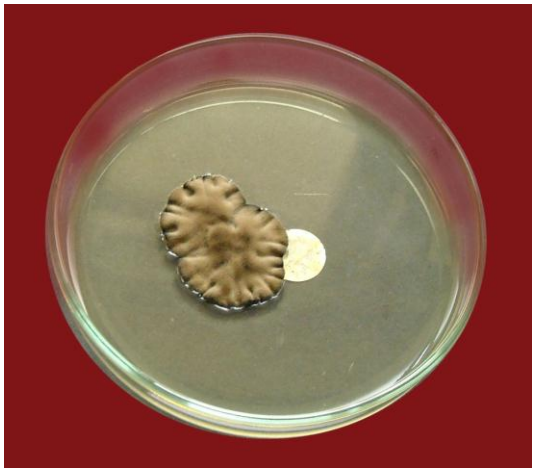
Beauveria bassiana



Metarhizium anisopliae

Verticillium lecanii

Hirsutella thompsonii



Cladosporium oxysporum

Plate 2. Fungal isolates used for the study

subculturing all the fungal isolates were subjected to pathogenicity test and again reisolated for further studies.

3.3 BIOASSAY STUDIES

3.3.1 Rearing of aphids

For conducting bioassay, aphids were reared in the laboratory by using the method of Yeo *et al.* (2003). Initially 20 adult apterous aphids were inoculated on fresh cowpea seedlings in the trifoliate stage. The inoculated aphids reproduced parthenogenetically, and the newly formed one day old first instar nymphs were reared on the same plant. After 24 h, the inoculated adult aphids were removed from the seedlings. The new generation of the aphids were used for the bioassay studies.

3.3.2 Preparation of spore concentrations of the fungal isolates

All the five fungal isolates were cultured in 100ml SMA+Y liquid medium in 250ml conical flask and incubated at room temperature for 10 days. After sporulation of the fungal isolates, it was ground in ordinary mixer and made into liquid spore suspension. This was filtered through double layered muslin cloth to remove the mycelial mat. The suspension was shaken thoroughly with a drop of teepol solution in order to disperse the spores in the solution. The spore count in the suspension was assessed by using a haemocytometer and was estimated using the formula suggested by Lomer and Lomer, (1996).

$$\text{Number of spores/ml} = \frac{X \times 400 \times 10 \times 1000 \times D}{Y}$$

Where,

X = Number of spores counted from 160 small squares

Y = Number of small squares counted

10 = Depth factor

1000 = Conversion factor from mm^3 to cm^3

D = Dilution factor

Based on the number of spores, all the cultures were adjusted to 1x 10^8 spores ml^{-1} from which the lower concentrations were prepared by serial dilution technique for bioassay studies.

3.3.3 Bioassay procedure

Cowpea seedlings were raised in small paper cups of size 7.5x7.5cm in the laboratory. Fifteen days old seedlings were used for the bioassay studies. Six different spore concentrations ($1x10^8$, $1x10^7$, $1x10^6$, $1x10^5$, $1x10^4$, $1x10^3$ spores ml^{-1}) were prepared for *B. bassiana, M. anisopliae, V. lecanii, H. thompsonii* and the local isolate. Each concentration was replicated three times. One day old adult apterous aphids were inoculated on the cowpea seedlings using a camel hairbrush @ 10 aphids per seedling. Totally 30 aphids were used for each treatment. After inoculation of aphids, the respective concentrations of all the fungal spore suspensions were sprayed on the seedlings using an atomizer. Aphids sprayed with 0.05 per cent teepol solution served as control. After spraying, seedlings were kept under belljar to avoid the escape of aphid population and to maintain the humidity (Plate 3). The mouth of the cup was closed with white paper, for the easy collection of dead aphids.

Plate 3. Bioassay of fungal isolates on *Aphis craccivora*

3.3.4 Observations

Mortality of aphids and nymphal production were recorded separately at 24 h interval up to seven days. Dead aphids were collected daily and placed in Petridish containing a moist filter paper and kept in humid chamber. The dead aphids which produced mycelial growth were considered for the mortality count.

Mortality data was corrected with control mortality by using Abbott's formula (Abbott, 1925). The data was then analysed by probit analysis (Finney, 1971) and the Median Lethal Concentration (LC_{50}) and the Median Lethal Time (LT_{50}) values were computed by using the statistical computer programme, Statistical Package of Social Sciences (SPSS).

3.3.5 Statistical analysis

The per cent corrected cumulative mortality of each fungus was subjected to ANOVA test and the means were separated by Duncan's Multiple Range Test (DMRT).

3.4 POT CULTURE EXPERIMENT TO EVALUATE THE BIOEFFICACY OF DIFFERENT FUNGAL ISOLATES

A pot culture experiment was conducted to find out the efficacy of different fungal isolates against *A. craccivora.* The cowpea variety 'Bhagyalakshmi' was used for the experiment. The experiment was laid out in Completely Randomized Design (CRD) with 17 treatments and three replications. Each treatment consisted of three plants and three replications were maintained for each treatment.

The LC_{50} (T1-T5) and the dose which gave the shortest LT_{50} value (T10-T14) obtained from the laboratory bioassay studies and the recommended dose of commercial fungal formulations (T6-T9) except the local isolate were compared with the standard fungal culture *Fusarium pallidoroseum* (T15) and the commonly used chemical insecticide malathion 50EC (T16).

3.4.1 Details of the treatment

T1 - *B. bassiana* (Pure culture) @ $4.5x10^4$ spores ml^{-1}
T2 - *M. anisopliae* (Pure culture) @ $8.9x10^5$ spores ml^{-1}
T3 - *V. lecanii* (Pure culture) @ $2.5x10^4$ spores ml^{-1}
T4 - *H. thompsonii* (Pure culture) @ $2.5x10^4$ spores ml^{-1}
T5 - Local isolate @ $7.4x10^5$ spores ml^{-1}
T6 - *B. bassiana* (Biopower) @ 0.2%
T7 - *M. anisopliae* (Biomagic) @ 0.2%
T8 - *V. lecanii* (Biocatch) @ 0.2%
T9 - *H. thompsonii* (Mycar) @ 0.2%
T10- *B. bassiana* (Pure culture) @ 1x 10^8 spores ml^{-1}
T11- *M. anisopliae* (Pure culture) @ 1x 10^8 spores ml^{-1}
T12 - *V. lecanii* (Pure culture) @ 1x 10^8 spores ml^{-1}
T13 - *H. thompsonii* (Pure culture) @ 1x 10^8 spores ml^{-1}
T14 - Local isolate @ 1x 10^8 spores ml^{-1}
T15 - *Fusarium pallidoroseum* @ $7x10^6$ spores ml^{-1}
T16- Malathion 50EC @ 0.05%
T17- Control

Cowpea seeds were sown in earthern pots of size 30cm diameter containing potting mixture. Plants were maintained by following all the agronomic practices. Treatments were applied, when there was sufficient population build up of aphids in all the plants. Predators were removed daily, to know the treatment effect.

3.4.2 Culturing of fungal isolates

All the fungi were mass multiplied in coconut water in the laboratory for spray application (Plate 4). Three hundred ml of 50 per cent coconut water was poured in 500 ml bottle and autoclaved at 121°C for 20 minutes. After cooling, one ml of spore

Beauveria bassiana

Verticillium lecanii

Metarhizium anisopliae

Hirsutella thompsonii

Cladosporium oxysporum

Plate 4. Mass multiplication of fungal isolates in coconut water

suspension of fungal pathogens were inoculated into each bottle separately. They were incubated at room temperature for 15 days. After incubation, the bottles were kept in shaker for 10 minutes. From this 10ml of the spore suspension was drawn and transferred to 250ml conical flasks containing 100ml sterilized distilled water and 0.05 per cent Tween 80 solution. Spore count in each bottle was estimated using a haemocytometer.

The pathogenicity of the five entomopathogenic fungi was also tested under laboratory conditions before using in the pot culture studies. The different treatments were applied on the plants in the evening hours using a hand sprayer.

3.4.3 Observations

Observation on aphid population was recorded before and after the treatments. The post treatment counts were taken at 3, 5, 7, 10 and 14 days after treatment.

3.4.3.1 Estimation of aphid population

Population of *A. craccivora* was taken from top 5cm of the terminal shoot which includes the shoot, opened and unopened leaves, petiole and flower buds (Plate 5). Data on the mean number of aphid population were subjected to square root transformation and covariance analysis was computed using MSTATC (Gomez and Gomez, 1984). The per cent reduction of aphids over pre treatment count was worked out for each observation.

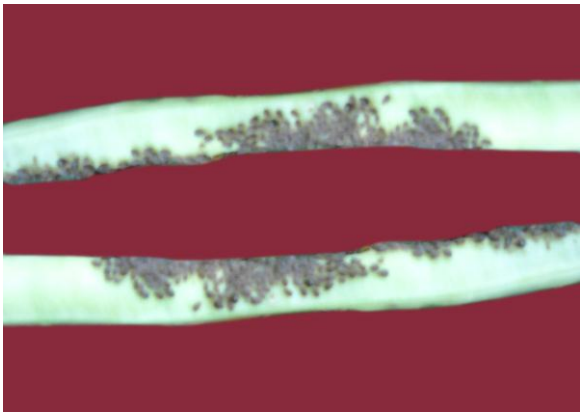
3.4.3.2 Observations on growth and yield of cowpea

Observations on biometric characters such as height of the plant, number of branches, shoots, leaves and yield characters such as number and weight of pods from different treatments were recorded separately and statistically analysed.

Terminal shoot

Flower

Leaves



Pods

Plate 5. Aphid infestation on different parts of cowpea plant

3.4.3.3 Statistical analysis

Data were subjected to ANOVA and the means were separated by Duncan's Multiple Range Test (DMRT).

3.4.4 Cost of protection

Cost of protection was worked out for each treatment which includes the cost of production of entomopathogenic fungi, cost of chemical insecticide and application charges (Appendix IV).

Results

4. RESULTS

The results obtained from the present study "Biocontrol of cowpea aphid, *Aphis craccivora* (Koch) using entomopathogenic fungi" are presented in this chapter.

4.1 COLLECTION AND ISOLATION OF FUNGAL ISOLATES

A survey was conducted in the cowpea growing fields at Vellanikkara, Mannuthy, Chirakkekodu and Pandiparambu of Thrissur district for the collection of mycosed aphids. Collection was done at fortnightly intervals during May 2008 to February 2009. During the period of survey, five local fungal isolates were collected from the field. These isolates were tested for its pathogenicity in the laboratory.

4.1.1 Pathogenicity of fungal isolates

The fungal isolates collected from the field were subjected to *in vitro* pathogenicity tests on *A. craccivora* as per the methodology described in 3.2.1. Mortality of aphids were observed from second day after inoculation of the respective fungal cultures. The fungal pathogen was then reisolated from the dead aphids on Potato Dextrose Agar (PDA) medium. Out of the five local fungal isolates, the one which was collected from Vellanikkara locality was found to be pathogenic to *A. craccivora*, by proving the Koch postulates. Other four cultures were not pathogenic to *A. craccivora.*

4.1.2 Identification of the fungi

The five fungal isolates obtained during the survey were identified from the Department of Plant Pathology, College of Horticulture, Vellanikkara and the National Centre for Fungal Taxonomy (NCFT), New Delhi. Among the five fungal isolates, the four non pathogenic ones were identified as *Fusarium* sp. and the pathogenic one as *Cladosporium oxysporum.* The natural incidence of pathogenic *C. oxysporum* on *A. craccivora* is the first report from Kerala.

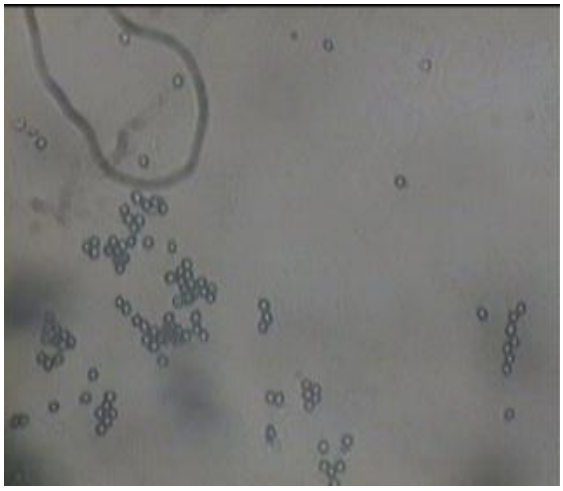
4.2 BIOASSAY STUDIES

The pure fungal isolates of *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Hirsutella thompsonii* obtained from the National Bureau of Agriculturally Important Insects (NBAII) and the identified local isolate *C. oxysporum* were subjected to bioassay against *A. craccivora.*

4.2.1 Symptoms and identification characters of the fungal isolates

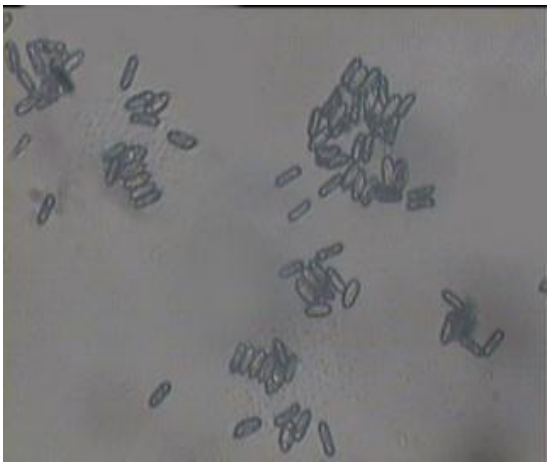
Fungal infected aphids were mummified and hard to touch. Mycelial growth was developed after 24 h of death. Initially, growth of the fungus was seen through the inter segmental membrane of abdomen, legs etc. and finally the entire cadaver was fully covered with fungal growth. The conidial characters of the respective fungus were confirmed by compound research microscope (Plate 6, 7 and 8). Symptoms and the conidial characteristics of the fungi under study are as follows.

Fungal species	Symptoms	Conidial characters
B. bassiana	Aphid body was fully covered with powdery white spores	Conidiophores single or branched bearing groups of clustered conidiogenous cells. Conidia smooth and round.
M. anisopliae	Initially a white mycelial mat was appeared all over the body and later turned to powdery green with sporulation.	Conidiophores short, hyaline, simple or branched. Conidia one celled, smooth, long, ovoid to cylindrical shaped.
V. lecanii	Adult aphid was completely embedded with white mycelial mat.	Conidiophores erect or little differentiated and conidia round to slightly ovoid.



Cadaver with white puffy growth **Conidia (40x)**

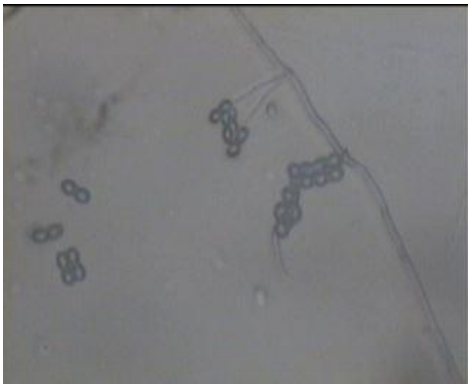
a) *Beauveria bassiana*



Mummified aphids with green spores **Conidia (40x)**

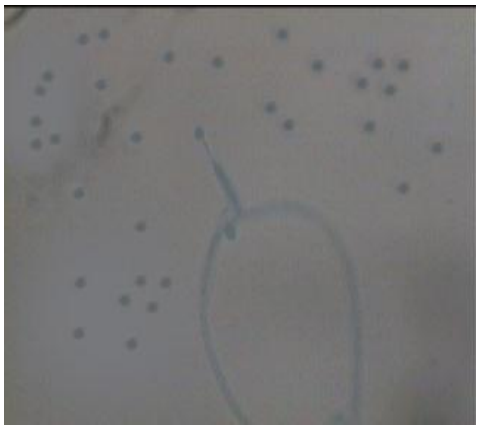
b) *Metarhizium anisopliae*

Plate 6. Symptoms and conidia of *Beauveria bassiana* and *Metarhizium anisopliae*



White mycelial strands with aphids **Conidia (40x)**

a) *Verticillium lecanii*

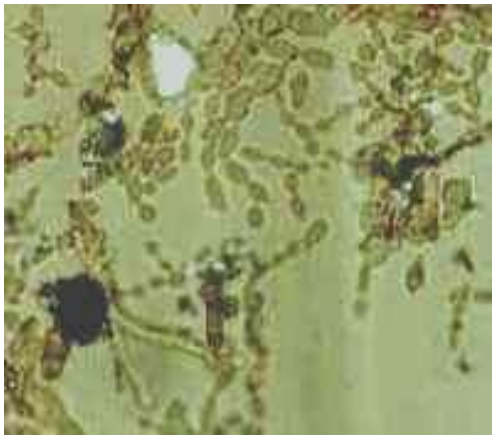


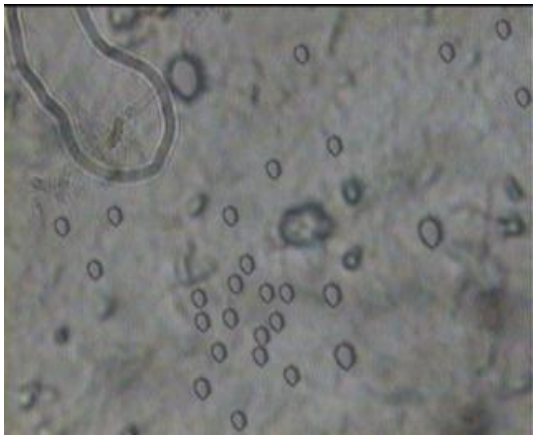
Olive green mycelial growth on aphids **Phialide and conidia (40x)**

b) *Hirsutella thompsonii*

Plate 7. Symptoms and conidia of *Verticillium lecanii* and *Hirsutella thompsonii*

Green mycelial masses on aphids





Conidiophore and conidia (40x) of *Cladosporium oxysporum*

Plate 8. Symptoms and conidia of *Cladosporium oxysporum*

H. thompsonii	Infected aphids showed grey mycelial growth	Conidiophores hyaline with a single phialide having a broad base and a narrow neck, bearing single spore.
C. oxysporum	Infected aphids exhibited dull green coloured spores.	Conidia round with a very small beak like constriction at the apex

4.2.2 Bioassay of the fungal isolates against *A. craccivora*

Bioassay of the five fungal isolates was conducted in the laboratory with six different concentrations namely 10^8, 10^7, 10^6, 10^5, 10^4 and 10^3 spores ml^{-1}. Ten numbers of one day old adult aphids, inoculated on 15 days old cowpea seedlings were treated with the respective concentrations of the fungal isolates. Observations were recorded separately on adult mortality and nymphal production at 24 h interval upto seven days after treatment. Mortality of aphids was estimated by counting the mycosed aphids.

4.2.2.1 Effect of fungal isolates on the mortality of adults of *A. craccivora*

Adult mortality per cent in different treatments was corrected with respect to control mortality. The data of corrected per cent mortality at different time intervals are presented in Table 1.

Table 1. Effect of fungal isolates on the mortality of adults of *A. craccivora* at different time interval

Fungal Isolates	Spores/ml	Per cent corrected mortality in hours						
		24	48	72	96	120	144	168
B. bassiana	10^8	3.33	10.00	26.66	43.33	73.33	86.66	96.66
	10^7	0.00	6.66	43.33	60.00	66.66	76.66	83.33
	10^6	0.00	6.66	33.33	40.00	53.33	66.67	70.00
	10^5	0.00	0.00	20.00	33.33	40.00	43.33	50.00
	10^4	0.00	0.00	6.66	20.00	33.33	36.66	40.00
	10^3	0.00	0.00	6.66	10.00	23.33	26.66	26.66
M. anisopliae	10^8	0.00	0.00	6.66	24.14	42.86	51.85	80.76
	10^7	0.00	0.00	6.66	13.79	17.86	33.33	61.53
	10^6	0.00	0.00	3.33	3.33	13.79	29.62	50.00
	10^5	0.00	0.00	3.33	6.89	10.34	29.62	38.47
	10^4	0.00	0.00	0.00	10.34	17.24	18.51	23.07
	10^3	0.00	0.00	0.00	3.33	6.89	14.14	19.23
V. lecanii	10^8	3.33	3.33	17.86	53.84	88.46	100.00	100.00
	10^7	0.00	0.00	32.14	50.00	76.96	92.30	100.00
	10^6	0.00	0.00	17.86	42.30	50.00	76.92	84.00
	10^5	0.00	0.00	21.43	32.14	46.15	57.69	60.00
	10^4	0.00	0.00	7.14	15.39	23.07	34.61	44.00
	10^3	0.00	0.00	0.00	11.54	23.07	26.92	28.00
H. thompsonii	10^8	3.33	13.33	35.71	60.71	80.70	96.50	100.00
	10^7	0.00	13.33	28.57	39.29	46.18	76.91	96.00
	10^6	0.00	3.33	7.14	21.43	26.94	61.53	80.00
	10^5	0.00	0.00	10.72	26.94	28.57	57.69	60.00
	10^4	0.00	0.00	7.14	14.29	23.07	42.30	44.00
	10^3	0.00	0.00	0.00	3.33	3.33	7.69	20.00
C. oxysporum	10^8	0.00	6.66	20.00	24.14	46.43	64.28	77.50
	10^7	0.00	3.33	16.66	13.79	37.93	57.13	60.71
	10^6	0.00	3.33	13.33	13.79	31.03	39.29	49.99
	10^5	0.00	3.33	10.00	10.34	27.58	35.71	39.29
	10^4	0.00	0.00	6.66	13.79	24.14	25.00	28.57
	10^3	0.00	0.00	6.66	3.45	10.34	14.29	17.86

Mortality of aphids was observed with in 24 h, at the highest concentration (10^8 spores ml^{-1}) of *B. bassiana, V. lecanii* and *H. thompsonii* and the mortality recorded was 3.33 per cent.

After 48 h of exposure, among the five fungal isolates *B. bassiana, H. thompsonii* and *C. oxysporum* showed aphid mortality at 10^8, 10^7 and 10^6 spores ml^{-1}. The per cent mortality ranged between 3.33 to 13.33 at 10^8 and 10^7spores ml^{-1} and 3.33 to 6.66 per cent at 10^6 spores ml^{-1}.

All the concentrations of *B. bassiana* and *C. oxysporum* recorded mortality of aphids after 72 h. The mortality of aphids in different concentrations ranged between 6.66 to 43.33 and 6.66 to 20.00 per cent in *B. bassiana* and *C. oxysporum* respectively. There was no mortality obtained in other fungal isolates namely *M. anisopliae*, *V. lecanii* and *H. thompsonii* at the lowest concentration (10^3 spores ml^{-1}). But in the highest concentration (10^8 spores ml^{-1}), they produced 6.66 to 35.71 per cent mortality.

At 120 h (5 days) after treatment the highest per cent mortality was obtained in the highest spore concentration of 10^8 spores ml^{-1} in *V. lecanii* (88.46%) followed by *H. thompsonii* (80.70%) and *B. bassiana* (73.33%). *Cladosporium oxysporum* and *M. anisopliae* showed only low mortality with 46.43 and 42.86 per cent respectively. At 10^7 spores ml^{-1}, *B. bassiana* and *V. lecanii* obtained more than 50 per cent mortality which was recorded as 66.66 and 76.96 per cent respectively. In the lowest concentration (10^3 spores ml^{-1}) *H. thompsonii, M. anisopliae* and *C. oxysporum* recorded the least mortality rate ranged between 3.33 to 10.34 per cent.

At 144 h (6 days) and 168 h (7 days) after treatment there was marked increase in the mortality of aphids. Among the five isolates, *V. lecanii* at 10^8 spores ml^{-1} showed cent per cent mortality on the 6^{th} day followed by *H. thompsonii* giving 96.50 per cent mortality.

All the fungal isolates in the highest spore concentration (10^8 spores ml^{-1}) produced high mortality per cent ranged from 77.50 to 100, after seven days of treatment. Among the five isolates *V. lecanii* and *H. thompsonii* gave cent per cent mortality. This was followed by *B. bassiana*, *M. anisopliae* and *C. oxysporum* with 96.66, 80.76 and 77.50 per cent respectively. A progressive reduction in the mortality of aphids was observed with decreasing concentrations. In the lower concentration of 10^3 spores ml^{-1} the mortality ranged from 17.86 to 28.00 per cent. It was found that the per cent mortality was directly proportional to the concentration of spores.

4.2.2.1.1 Cumulative mortality of *A. craccivora*

The corrected cumulative mortality per cent at seven days after treatment was analysed by ANOVA and the results are presented in Table 2.

The cumulative per cent mortality of *A. craccivora* obtained in all concentrations of the fungal isolates was found to be statistically on par. At the highest concentration of 10^8 spores ml^{-1}, *V. lecanii* and *H. thompsonii* produced cent per cent mortality and was found to be superior than the other fungal isolates. It was closely followed by *B. bassiana* causing 96.67 per cent mortality which was as effective as *V. lecanii* and *H. thompsonii.* In the next lower concentration also, *V. lecanii* ranked superior which produced cent per cent cumulative mortality of aphids. *Hirsutella thompsonii* and *B. bassiana* were found to be on par with *V. lecanii* producing 96.00 and 83.33 per cent mortality respectively.

4.2.2.1.2 Median Lethal Concentration (LC_{50})

Six different concentrations of the five fungal isolates were bioassayed against adult aphids. The per cent corrected cumulative mortality was subjected to Finney's method of probit analysis and the results are presented in Table 3.

Table 2. Effect of fungal isolates on the cumulative mortality of *A. craccivora* at different concentrations

Fungal isolates	Cumulative mortality of aphids (%)					
	10^8	10^7	10^6	10^5	10^4	10^3
B. bassiana	96.66[a]	83.33[ab]	70.00[ab]	50.00[cd]	40.00[cde]	26.66[de]
M. anisopliae	80.76[ab]	61.53[bc]	50.00[cd]	38.47[cde]	23.07[de]	19.23[e]
V. lecanii	100.00[a]	100.00[a]	84.00[ab]	60.00[bc]	44.00[cde]	28.00[de]
H. thompsonii	100.00[a]	96.00[a]	80.00[ab]	60.00[bc]	44.00[cde]	20.00[de]
C. oxysporum	77.50[ab]	60.71[bc]	49.99[cde]	39.29 [cde]	28.57[de]	17.86[e]

Means in a column followed by same alphabet(s) is not different by DMRT at P=0.05

Table 3. Dose mortality response of fungal isolates against *A. craccivora*

Fungal isolates	Hetero geneit-y ($\chi 2$)	Regression equation	LC_{50} (spores ml^{-1})	95% Fiducial limits (spores ml^{-1})	Relative toxicity
B. bassiana	4.724	Y= 2.0507+0.44×	4.5×10^4	1.1×10^4 - 3.4×10^5	1.8
M. anisopliae	4.303	Y=2.7301+0.62×	8.9×10^5	4.1×10^5- 1.8×10^7	35.6
V. lecanii	4.303	Y=-2.7308+4.3×	2.5×10^4	1.5×10^4- 4.0×10^4	1.0
H. thompsonii	3.681	Y=-2.025+0.34×	2.5×10^4	1.5×10^4- 4.0×10^4	1.0
C. oxysporum	1.277	Y=-1.891+0.32×	7.4×10^5	2.6×10^5- 1.6×10^6	29.6

$\chi 2$- Chisquare; LC- Lethal Concentration

Table 4. Time mortality response of fungal isolates against *A. craccivora*

Fungal isolates	Median Lethal Time (Days)					
	10^8	10^7	10^6	10^5	10^4	10^3
B.bassiana	3.63	4.51	5.83	5.98	6.88	8.11
M.anisopliae	5.54	6.61	7.01	7.43	8.65	8.91
V.lecanii	3.90	3.96	4.86	5.65	7.25	7.87
H.thompsonii	3.64	4.52	5.84	5.98	6.89	8.12
C.oxysporum	5.24	5.96	6.75	7.34	8.19	10.17

The data on LC_{50} showed that *V. lecanii* and *H. thompsonii* produced 50 per cent mortality at the lowest concentration of 2.5×10^4 spores ml^{-1}, followed by *B. bassiana* with $4.5x10^4$ spores ml^{-1}. The isolates *C. oxysporum* and *M. anisopliae* recorded the higher LC_{50} values and their relative toxicity were 29.6 and 35.6 respectively.

Among the five fungal isolates, *V. lecanii* and *H. thompsonii* were the most virulent isolates with the lowest LC_{50} and relative toxicity. This was followed by *B. bassiana* with a relative toxicity value of 1.8. *Metarhizium anisopliae* and *C. oxysporum* recorded higher LC_{50} and relative toxicity values. Both these isolates showed lesser virulence compared to other isolates.

4.2.2.1.3 Median Lethal Time (LT_{50})

The time taken for 50 per cent mortality was estimated by Finney's method of probit analysis and is given in Table 4. The LT_{50} values for all the fungal isolates showed variation at different concentrations. The LT_{50} values consistently increased as conidia concentration decreased, but they differed between the fungal isolates. The LT_{50} values at 10^8 spores ml^{-1} for *B. bassiana, H. thompsonii, V. lecanii, C. oxysporum* and *M. anisopliae* were 3.63, 3.64, 3.90, 5.24 and 5.54 days respectively.

From the data, it was found that the spore concentration of the fungal isolates was inversely proportional to the LT_{50} values.

4.2.2.2 Effect of fungal isolates on the survival rate of *A. craccivora* nymphs

The effect of fungal isolates on the survival rate of newly born nymphs was observed at 24 h interval upto seven days after treatment (Appendix II). The survival per cent of nymphs at different time intervals over control are presented in Table 5. It is revealed that the survival rate of nymphs was considerably affected by the fungi at all the concentrations. All the fungal isolates showed reduction in number of nymphs, at 24 h of post inoculation. At 24 h itself, *V. lecanii* @10^8 spores ml^{-1} showed the minimum survival rate of nymphs (46.04%).

The nymphal production was markedly decreased in all the concentrations on 7^{th} day. The fungus, *V. lecanii* @10^8 spores ml^{-1} showed the lowest survival rate of nymphs (2.49%) followed by *H. thompsonii* and *B. bassiana* which recorded 7.96 and 9.64 per cent respectively. But the survival rate of nymphs in *C. oxysporum* was highest with 23.17 per cent, followed by *M. anisopliae* with 18.33 per cent survival of nymphs.

The rate of nymphal production was considerably higher at lower concentrations for all the fungal isolates. At 10^3 spores ml^{-1}, the lowest survival rate was observed in *B. bassiana* (34.75%) which was closely followed by *H. thompsonii* (37.61%). In all other isolates survival rate ranged between 79.23 to 90.24 per cent. The spore concentration of the fungal isolates was found to be inversely proportional to the survival rate of nymphs.

Among the five entomopathogenic fungi, *V. lecanii* at a concentration of 10^8 spores ml^{-1} was observed to have the highest influence on the survival rate of nymphs. In all the concentrations *H. thompsonii* and *B. bassiana* recorded low survival rates ranging between 7.96 to 37.61 per cent. Though *V. lecanii, M. anisopliae* and *C. oxysporum* were effective in the higher concentrations, it showed higher survival rate in the lower concentrations.

Table 5. Effect of fungal isolates on the survival rate of *A. craccivora* nymphs

Fungal Isolates	Spores/ml	Survival per cent of nymphs over control after inoculation (hours)						
		24	48	72	96	120	144	168
B. bassiana	10^8	88.48	111.47	57.03	35.14	29.58	13.65	9.64
	10^7	70.91	77.98	54.75	39.94	26.76	16.13	10.09
	10^6	70.30	65.60	50.95	44.73	35.21	24.32	16.37
	10^5	100.00	93.58	73.00	57.51	40.28	25.31	18.39
	10^4	89.09	82.57	83.27	61.98	49.58	38.21	27.58
	10^3	90.30	81.65	82.13	57.51	55.21	40.45	34.75
M. anisopliae	10^8	73.17	62.85	67.85	88.00	36.03	30.02	18.33
	10^7	79.51	97.77	115.93	111.22	46.88	58.89	56.82
	10^6	93.66	77.93	93.22	81.60	58.89	78.75	47.45
	10^5	111.22	83.80	115.04	9.86	78.75	82.22	63.75
	10^4	109.76	87.15	92.92	84.00	82.22	85.91	72.51
	10^3	105.37	46.09	71.39	85.33	85.91	82.22	79.23
V. lecanii	10^8	46.04	64.58	36.29	28.06	7.69	7.00	2.49
	10^7	68.30	97.92	52.74	52.54	48.72	26.05	13.93
	10^6	55.09	92.92	56.14	49.85	45.83	24.09	15.92
	10^5	60.38	75.83	45.43	72.54	61.22	45.66	35.82
	10^4	53.21	80.42	44.13	36.42	43.91	48.85	59.85
	10^3	75.85	68.33	68.41	84.78	1.60	94.12	85.82
H. thompsonii	10^8	88.82	57.87	47.62	27.84	20.90	13.62	7.96
	10^7	98.68	121.35	73.41	46.41	53.05	25.07	4.87
	10^6	85.53	50.56	40.87	55.39	50.80	35.42	14.38
	10^5	92.11	87.08	47.62	40.12	45.02	30.79	17.48
	10^4	91.45	73.03	40.48	44.91	49.84	39.51	38.27
	10^3	108.55	89.89	72.62	43.41	49.20	44.69	37.61
C. oxysporum	10^8	57.14	83.06	45.14	58.54	48.60	33.33	23.17
	10^7	76.02	100.81	52.14	66.46	54.75	39.35	29.67
	10^6	52.04	77.42	33.07	69.51	69.83	62.04	59.76
	10^5	63.78	152.42	84.05	107.32	86.03	58.80	47.56
	10^4	58.16	101.61	78.99	115.24	137.99	117.59	88.21
	10^3	98.47	95.97	75.10	95.12	102.23	94.91	90.24

4.3 POT CULTURE EXPERIMENT

A pot culture experiment was laid out to evaluate the efficacy of different fungal isolates against *A. craccivora* under field conditions (Plate 9). The LC_{50} and the dose, which gave the shortest LT_{50} value obtained from the bioassay studies of *B. bassiana, M. anisopliae, V. lecanii, H. thompsonii* and the local isolate *C. oxysporum* and the recommended dose of commercial formulations of these fungi except *C. oxysporum* were compared with the standard fungal culture *F. pallidoroseum* and commonly used chemical insecticide (Malathion 50EC) and control.

4.3.1 Bioefficacy of different fungal isolates against *A. craccivora*

The pre treatment count (PTC) of aphid population was recorded from the top 5 cm of the terminal shoot. The post treatment count was taken at 3, 5, 7, 10 and 14 days after treatment (DAT) by the same sampling method.

The mean data on pre and post count population of the aphids are presented in Table 6. The mean pretreatment count of aphids ranged between 77.11 to 251.77. The per cent reduction of aphid population over precount was calculated and given in Table 7.

As per the data given in Table 6 and Table 7, the plants treated with malathion (T16) recorded rapid reduction in population with 33.11, 9.16 and 2.77 aphids on 3^{rd}, 5^{th} and 7^{th} day respectively. The per cent reduction of aphids was increased from 3^{rd} to 10^{th} day which ranged between 68.86 to 100 per cent. But on the 14^{th} day, reinfestation of aphids was seen and population was again recorded (9.00 numbers) in this treatment.

There was remarkable reduction of aphid population in T15 (*F. pallidoroseum* @7x 10^6 spores ml^{-1}), T10 (*B. bassiana* @10^8 spores ml^{-1}) and T12 (*V. lecanii* @10^8 spores ml^{-1}) on 3^{rd}, 5^{th} and 7^{th} day. In these treatments, cent per cent mortality was observed on the 14^{th} day after treatment and were found to be more superior than malathion and all the other treatments.

Plate 9. Lay out of pot culture experiment

Table 6. Pre and post treatment population of *A. craccivora* from top 5 cm of the terminal shoot

Treatments (spores ml^{-1})	Mean population of *A. craccivora*					
	PTC	3DAT	5DAT	7DAT	10DAT	14DAT
T1 - *B.b* @$4.5x10^4$	101.11 (10.02) ^{ab}	95.67 (9.77)^{abc}	72.44 (8.51)^{abc}	32.33 (5.35)^{bcdefg}	20.44 (3.88)^{defg}	19.58 (4.46)^{defg}
T2 - *M.a* @$8.9x10^5$	196.11 (13.08)^{ab}	131.22 (10.68)^{bc}	81.77 (8.90)^{abc}	59.56 (7.66)^{defg}	72.89 (8.33)^{bc}	38.00 (6.13)^{bcde}
T3 - *V.l* @$2.5x10^4$	77.11 (8.72) ^{b}	60.67 (7.51)^{abc}	52.22 (7.15)^{abcde}	39.44 (6.11)^{bcdef}	30.22 (5.51)^{cdef}	23.44 (4.81)^{cdef}
T4 - *H.t* @$2.5x10^4$	251.77 (15.13) ^{a}	177.87 (12.87)^{a}	169.54 (12.00)^{ab}	95.43 (9.14)^{fg}	122.65 (10.82)^{ab}	103.98 (9.54)^{ab}
T5 - *C.o*@ $7.4x10^5$	161.88 (12.64) ^{ab}	141.66 (11.81)^{ab}	97.16 (9.88)^{abc}	63.00 (7.85)^{efg}	55.22 (7.43)^{bcd}	47.33 (6.78)^{bcd}
T6 - Biopower 0.2%	113.33 (10.29) ^{ab}	101.66 (9.92)^{abc}	36.56 (5.94)^{cde}	19.33 (4.41)^{cdefg}	20.00 (3.80)^{defg}	10.67 (2.94)^{defg}
T7 - Biomagic 0.2%	112.77 (10.56) ^{ab}	85.16 (9.21)^{abc}	70.23 (8.13)^{abcd}	49.56 (7.03)^{cdefg}	41.67 (6.38)^{cde}	43.00 (6.53)^{bcd}
T8 - Biocatch 0.2%	112.88 (10.63) ^{ab}	84.43 (8.71)^{abc}	30.47 (5.11)^{cde}	19.22 (3.57)^{defg}	8.22 (2.62)^{fg}	6.00 (1.91) ^{fg}
T9 - Mycar 0.2%	88.33 (9.35) ^{ab}	69.01 (8.17)^{abc}	61.44 (7.74)^{abcde}	76.22 (8.67)^{fg}	46.44 (6.84)^{cde}	78.55 (8.63)^{abc}
T10- *B.b* @$1x10^8$	170.11 (12.66) ^{ab}	128.44 (11.01)^{ab}	99.67 (9.05)^{abc}	60.00 (7.49)^{cdefg}	0.00 (0.71)^{g}	0.00 (0.71)^{g}
T11- *M.a* @$1x10^8$	144.22 (12.02) ^{ab}	108.55 (10.37)^{abc}	48.00 (6.87)^{bcde}	28.44 (5.31)^{bcdefg}	32.30 (5.66)^{cdef}	18.67 (3.78)^{cdef}
T12- *V.l* @ $1x10^8$	111.00 (10.61) ^{ab}	93.66 (9.66)^{abc}	43.88 (6.49)^{cde}	11.67 (2.46)^{fg}	1.89 (1.30)^{bcd}	0.00 (0.71)^{a}
T13- *H.t* @$1x10^8$	144.06 (11.96) ^{ab}	102.33 (10.06)^{abc}	108.00 (10.08)^{abc}	53.27 (7.20)^{cdefg}	16.89 (3.62)^{efg}	16.89 (3.62)^{defg}
T14- *C.o* @$1x10^8$	118.22 (10.86) ^{ab}	66.21 (8.16)^{abc}	71.93 (8.44)^{abcd}	35.29 (5.80)^{bcdefg}	55.67 (7.27)^{cde}	28.55 (5.26)^{cdef}
T15- *F.p* @7×10^6	113.44 (10.47) ^{ab}	99.00 (9.86)^{abc}	15.33 (3.42)^{de}	16.11 (3.42)^{efg}	3.00 (1.50)^{g}	0.00 (0.71)^{g}
T16-Malathion 0.05%	106.33 (10.06) ^{ab}	33.11 (5.77)^{c}	9.16 (2.70)^{e}	2.77 (1.46)^{g}	0.00 (0.71)^{g}	9.00 (2.21)^{g}
T17 - Control	162.88 (12.61) ^{ab}	169.22 (12.93)^{a}	152.78 (12.36)^{abc}	129.56 (11.36)^{efg}	145.56 (11.81)^{bcd}	119.22 (10.72)^{bcd}

Figures in the parentheses are square root transformed values($\sqrt{x+0.5}$)
Figures in each column with same alphabets form one homogenous groups

B.b- Beauveria bassiana; M.a – Metarhizium anisopliae;V.l- Verticillium lecanii;
H.t- Hirsutella thompsonii;C.o- Cladosporium oxysporum; F.p- Fusarium pallidoroseum
PTC- Pre treatment count; DAT-Days after treatment

Table 7. Per cent reduction of aphid population in different treatments at different periods

Treatments (spores ml^{-1})	Per cent reduction over pre count				
	3DAT	5DAT	7DAT	10DAT	14DAT
T1 - *B.b* @$4.5x10^4$	5.38	28.36	68.02	79.78	80.63
T2 - *M.a* @$8.9x10^5$	33.09	58.30	69.63	62.83	80.62
T3 - *V.l* @$2.5x10^4$	21.32	32.28	48.85	60.81	69.60
T4 - *H.t* @$2.5x10^4$	29.35	32.66	62.10	51.28	58.70
T5 - *C.o*@ $7.4x10^5$	12.49	39.98	61.08	65.89	70.76
T6 - Biopower 0.2%	10.30	67.74	82.94	82.35	90.59
T7 - Biomagic 0.2%	24.48	37.72	56.05	63.05	61.87
T8 - Biocatch 0.2%	25.20	73.01	82.97	92.72	94.68
T9 - Mycar 0.2%	21.87	30.44	13.71	47.42	11.07
T10- *B.b* @$1x10^8$	24.50	41.41	64.73	100.00	100.00
T11- *M.a* @$1x10^8$	24.73	66.72	80.28	61.40	80.20
T12- *V.l* @ $1x10^8$	15.62	60.47	89.49	98.30	100.00
T13- *H.t* @$1x10^8$	28.97	25.03	63.02	88.28	88.28
T14- *C.o* @$1x10^8$	43.99	39.16	70.15	72.68	84.21
T15- *F.p* @7×10^6	12.73	86.49	85.80	97.36	100.00
T16-Malathion 0.05%	68.86	91.39	97.39	100.00	92.07
T17 - Control	-3.89	6.20	20.46	10.63	26.81

B.b- Beauveria bassiana; M.a – Metarhizium anisopliae;V.l- Verticillium lecanii; H.t- Hirsutella thompsonii;C.o- Cladosporium oxysporum; F.p-Fusarium pallidoroseum.
DAT-Days after treatment

In T14 (*C. oxysporum* @10^8 sporesml^{-1}), 43.99 per cent reduction was observed in the population, three days after treatment. The population reduction in all the other treatments except T16 (Malathion) ranged between 5.38 to 33.09 per cent.

The treatments, T2 (*M. anisopliae* @$8.9x10^5$ spores ml^{-1}), T6 (Biopower), T8 (Biocatch), T15 (*F. pallidoroseum*), T10 (*B. bassiana* @10^8 spores ml^{-1}) and T12 (*V. lecanii* @10^8 spores ml^{-1}) gave more than 50 per cent reduction in population at five days after treatment. Among the treatments, T15 showed the maximum population reduction (86.49%) followed by T8 (73.01%), T6 (67.74%), T11 (66.72%), T12 (60.47%) and T2 (58.30%).

There was marked reduction in population of aphids in all the treatments at 7 days after treatment except T3 (*V. lecanii* @$2.5x\ 10^4$ spores ml^{-1}), T9 (Mycar) and T17 (control). The per cent reduction were found to be 48.85, 13.71 and 20.46 respectively. In all the other treatments, it ranged between 56.05 to 97.39 per cent.

Ten days after treatment there was 100 per cent population reduction in T16 (Malathion) and T10 (*B. bassiana* @10^8 spores ml^{-1}). This was closely followed by T12 (*V. lecanii* @10^8 spores ml^{-1}), T15 (*F. pallidoroseum* @$7x\ 10^6$ spores ml^{-1}) and T8 (Bio catch) showing more than 90 per cent reduction in population.

Results revealed that the treatments, *B. bassiana* and *V. lecanii* @10^8 spores ml^{-1} and *F. pallidoroseum* @$7x10^6$spores ml^{-1}, gave cent per cent mortality and were highly efficient in controlling aphid population at 14 days after treatment. This was followed by Biocatch, malathion and Biopower which recorded more than 90 per cent mortality The treatments *M. anisopliae*, *C. oxysporum* and *H. thompsonii* @ 10^8 spores ml^{-1} and the LC_{50} concentrations of *B. bassiana* and *M. anisopliae* also showed considerable reduction in aphid population ranging between 80.20 to 88.28 per cent. The treatment with the commercial formulation of *H. thompsonii*, T9 (Mycar) was less effective than all the other treatments and was inferior to control.

The adjusted means for the unequal pretreatment count, which was computed using covariance analysis were presented in Appendix III. It represents the theoretical interpretation of the effect of entomopathogenic fungi on aphid population. It also showed that *B. bassiana* and *V. lecanii* @10^8 spores ml^{-1} and *F. pallidoroseum* @$7x10^6$spores ml^{-1} were superior than all other treatments and were statistically on par.

4.3.2 Effect of different fungal isolates on growth characters of cowpea

Bio metric observations *viz.*, plant height, number of shoots and number of leaves, were recorded at the time of last harvest and the mean data are presented in Table 8.

4.3.2.1 Height of the plant

There was only meagre variation in the height of plants in different treatments. It ranged between 28.58 to 41.48cm. The maximum height was recorded in T12 (*V. lecanii* @10^8 spores ml^{-1}) and minimum in T5 (*C. oxysporum* @7.4x 10^5 spores ml^{-1}). The per cent reduction in the height was noticed in T4, *H. thompsonii* @$2.5x10^4$ spores ml^{-1} (3.48%) and T5, *C. oxysporum* @$7.4x10^5$ spores ml^{-1} (2.13%) and they were infereior to control.

4.3.2.2 Number of shoots

From the data it is revealed that the maximum number of shoots (25.17) were produced in T10 (*B. bassiana* @10^8 spores ml^{-1}) which was followed by T12 (23.44 numbers). When compared with the control, T10 and T12 recorded 67.80 and 56.27 per cent increase in number of shoots respectively. Malathion treated plants produced less number of shoots (17.44) than the above treatments and the per cent increase over control was 16.27. In all other treatments, total number of shoots ranged between 14.33 to 19.56.

Table 8. Effect of different fungal isolates on growth characters of cowpea

Treatments (spores ml^{-1})	Height of the plant(cm)	% increase over control	Number of shoots	% increase over control	Number of leaves	% increase over control
T1 - *B.b* @4.5x10^4	32.36^{bc}	9.29	19.56^{abc}	30.40	66.50^{abc}	47.78
T2 - *M.a* @8.9x10^5	30.64^{bc}	3.48	17.28^{bc}	15.20	51.83^{abcd}	15.18
T3 - *V.l* @2.5x10^4	32.97^{abc}	11.35	19.22^{abc}	28.13	56.33^{abcd}	25.18
T4 - *H.t* @2.5x10^4	28.98^{bc}	-2.13	15.44^{c}	2.93	46.33^{cd}	2.96
T5 - *C.o*@ 7.4x10^5	28.58^{c}	-3.48	18.72^{abc}	24.80	56.17^{abcd}	24.82
T6 - Biopower 0.2%	33.52^{abc}	13.20	15.22^{c}	1.47	45.67^{cd}	1.49
T7 - Biomagic 0.2%	30.99^{bc}	4.66	15.33^{c}	2.20	50.33^{bcd}	11.84
T8 - Biocatch 0.2%	34.48^{abc}	16.45	18.72^{abc}	24.80	66.67^{abc}	48.16
T9 - Mycar 0.2%	32.69^{bc}	10.40	18.67^{abc}	24.47	56.00^{abcd}	24.44
T10- *B.b* @1x10^8	34.60^{abc}	16.85	25.17^{a}	67.80	70.67^{ab}	57.04
T11- *M.a* @1x10^8	33.17^{abc}	12.02	19.44^{abc}	29.60	47.00^{cd}	4.44
T12- *V.l* @ 1x10^8	41.48^{a}	40.09	23.44^{ab}	56.27	70.33^{ab}	56.29
T13- *H.t* @1x10^8	32.36^{bc}	9.29	14.33^{c}	-4.47	45.00^{d}	0.00
T14- *C.o* @1x10^8	36.17^{abc}	22.15	16.30^{c}	8.67	72.33^{a}	60.73
T15- *F.p* @7×10^6	37.43^{ab}	26.41	19.56^{abc}	30.40	71.33^{ab}	58.51
T16-Malathion0.05%	33.07^{abc}	11.69	17.44^{bc}	16.27	52.89^{abcd}	17.53
T17 - Control	29.61^{bc}	-	15.00^{c}	-	45.00^{d}	-

Figures in each column with same alphabets form one homogenous groups

B.b- Beauveria bassiana; M.a - Metarhizium anisopliae;V.l- Verticillium lecanii; H.t- Hirsutella thompsonii;C.o- Cladosporium oxysporum; F.p- Fusarium pallidoroseum

4.3.2.3 Number of leaves

Cladosporium oxysporum @10^8 spores ml^{-1} (T14) was found to be superior with respect to the production of number of leaves, recorded 60.73 per cent increase over control. This was followed by T15 *F. pallidoroseum* @7×10^6 spores ml^{-1} (58.51%), T10 *B. bassiana* @10^8 spores ml^{-1} (57.04%) and T12 *V. lecanii* @10^8 spores ml^{-1} (56.29%). The lowest number of leaves (45.00) was observed in the treatment *H. thompsonii* @10^8 spores ml^{-1} and it was found to be on par with control.

4.3.3 Effect of different fungal isolates on yield characters of cowpea

The data on yield characters *viz.,* number of pods and weight of pods were recorded separately and the per cent increase over control are given in Table 9.

4.3.3.1 Number of pods

Among all the treatments, T15 (*F. pallidoroseum* @ $7x10^6$ spores ml^{-1}) ranked superior (30.89 numbers) with respect to number of pods. An increase of 41.83 per cent over control was noticed in this treatment. The number of pods produced by all the other treatments were between 21.78 to 29.56 and the per cent increase over control ranged between 3.03 to 35.72. The treatments T2, T4 and T9 ranked inferior and was found to be on par with control.

Table 9. Effect of different fungal isolates on yield characters of cowpea

Treatments (spores ml^{-1})	Number of pods	% increase over control	Weight of pods (g)	% increase over control
T1 - *B.b* @ $4.5x10^4$	23.33^{c}	7.12	207.6^{ab}	21.12
T2 - *M.a* @ $8.9x10^5$	23.80^{c}	9.27	193.8^{ab}	13.07
T3 - *V.l* @ $2.5x10^4$	25.22^{bc}	15.79	208.9^{ab}	21.88
T4 - *H.t* @ $2.5x10^4$	22.78^{c}	4.59	190.5^{ab}	11.14
T5 - *C.o*@ $7.4x10^5$	26.56^{abc}	21.95	128.6^{c}	-24.97
T6 - Biopower 0.2%	26.67^{abc}	22.45	231.5^{ab}	35.06
T7 - Biomagic 0.2%	23.11^{c}	6.11	220.1^{ab}	28.41
T8 - Biocatch 0.2%	27.00^{abc}	23.97	221.6^{ab}	29.29
T9 - Mycar 0.2%	22.44^{c}	3.03	118.7^{c}	-30.75
T10- *B.b* @$1x10^8$	26.55^{abc}	21.90	248.7^{a}	45.10
T11- *M.a* @$1x10^8$	26.67^{abc}	22.45	226.6^{ab}	32.21
T12- *V.l* @ $1x10^8$	29.56^{ab}	35.72	252.2^{a}	47.14
T13- *H.t* @$1x10^8$	25.34^{b}c	16.35	190.8^{ab}	11.32
T14- *C.o* @$1x10^8$	25.29^{bc}	16.12	224.2^{ab}	30.81
T15- *F.p* @7×10^6	30.89^{a}	41.83	253.4^{a}	47.84
T16- Malathion 0.05%	26.50^{abc}	21.67	228.4^{ab}	33.26
T17 - Control	21.78^{c}	0.00	171.4^{bc}	0.00

Figures in each column with same alphabets form one homogenous groups

B.b- Beauveria bassiana; M.a - Metarhizium anisopliae; V.l- Verticillium lecanii; H.t- Hirsutella thompsonii;C.o- Cladosporium oxysporum; F.p- Fusarium pallidoroseum.

4.3.3.2 Weight of pods

The total weight of pods was also highest in T15, *F. pallidoroseum* @7x10^6 spores ml^{-1} (253.4 g $plant^{-1}$) followed by T12, *V. lecanii* @10^8 spores ml^{-1}(252.2 g $plant^{-1}$) and T10, *B. bassiana* @ 10^8 spores ml^{-1} (248.7 g $plant^{-1}$). These treatments were statistically on par and increased the yield upto 47.84, 47.14 and 45.10 per cent respectively. This was closely followed by T6 (Biopower), T16 (Malathion) and T14 (*C. oxysporum* @10^8 spores ml^{-1}) giving 35.06, 33.26 and 30.81 per cent increase over control. The yield increase in all the treatments except T9 and T5 was found to be statistically on par with T15. The lowest yield of 118.7 and 128.6 g $plant^{-1}$ was recorded in T9 and T5 respectively and was significantly lower than control.

In all the treatments T15 (*F. pallidoroseum* @7x10^6 spores ml^{-1}) recorded the maximum number and weight of pods followed by T12 (*V. lecanii* @ 10^8 spores ml^{-1}) and T10 (*B. bassiana* @ 10^8 spores ml^{-1}). The treatments with pure culture and commercial formulation of *H. thompsonii* (T4 and T9) was least effective as compared to other treatments.

The higher dose of *B. bassiana* and *V. lecanii* (10^8 spores ml^{-1}), and *F. pallidoroseum* (7x10^6 spores ml^{-1}) were found to be superior than other treatments causing cent per cent mortality at 14 days after treatment. Out of the four commercial formulations Biocatch (*V. lecanii*) and Biopower (*B. bassiana*) @0.2% gave more than 90 per cent population reduction.

From the study it is revealed that *B. bassiana* and *V. lecanii* @10^8 spores ml^{-1} were found to be as effective as the standard fungal culture *F. pallidoroseum* @7x10^6 spores ml^{-1}. These entomopathogenic fungi were found to be even more superior to the chemical insecticide, malathion based on the cumulative bioefficacy.

Discussion

5. DISCUSSION

The present study entitled "Biocontrol of cowpea aphid, *Aphis craccivora* (Koch) using entomopathogenic fungi" was undertaken with the following objectives.

1. Collection, isolation and identification of local isolates of entomopathogenic fungi (EPF) on *A. craccivora*
2. Estimation of LC_{50} of *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Hirsutella thompsonii* and local isolate in the laboratory.
3. Pot culture experiment to find out the bioefficacy of different fungal isolates against *A. craccivora* .

The results obtained from this study are discussed in this chapter.

5.1 COLLECTION AND IDENTIFICATION OF THE FUNGAL ISOLATES

A survey was conducted in the cowpea growing fields, at four locations of Thrissur district at fortnightly intervals, for the collection of entomopathogenic fungi. The mycosed aphids were collected and the fungi were isolated and subcultured in the laboratory. During the period of survey, five fungal isolates were collected from the field. All the fungal isolates were subjected to *in vitro* pathogenicity tests. Among the five isolates only one was found to be pathogenic to *A. craccivora,* and other four were non pathogenic. The pathogenic one was got identified as *Cladosporium oxysporum* and is the first report from Kerala. Abdel Bakey and Abdel Salam (2003) reported the pathogenicity of *Cladosporium* sp. against *A. craccivora.* Pathogenicity of *Cladosporium oxysporum* against sugarcane woolly aphid, *Ceratovacuna lanigera* was already reported by Ramegowda *et al.* (2007). Though *C. oxysporum* is an entomopathogenic fungus it also reported as a plant pathogen in pepper (Hammouda, 1992)

5.2 BIOASSAY OF THE FUNGAL ISOLATES AGAINST *A. craccivora*

Bioassay of the five fungal isolates *viz., B. bassiana, M. anisopliae, V. lecanii, H. thompsonii* and *C. oxysporum* was conducted against cowpea aphid, *A. craccivora.* It was tested at six concentrations ranging from 10^3 to 10^8 spores ml^{-1}.

Mortality of aphids was monitored at 24 h interval upto seven days. The cumulative mortality caused by different concentrations of the fungal isolates presented in Table 1 indicates that the mortality increased with increase in time interval (Fig. 1 to Fig. 5). But at the highest concentration of 10^8 spores ml^{-1}, *V. lecanii* and *H. thompsonii* recorded cent per cent mortality on 6^{th} and 7^{th} day respectively. Yokomi and Gottwald (1988) also reported cent per cent mortality of three aphid species *Myzus persicae, Aphis gossypii* and *Aphis citricola* at 10^6 -10^7 spores ml^{-1} after four days. Increased mortality due to *H. thompsonii* at 10^8 spores ml^{-1} was also reported by Smitha (2007). She obtained 90 per cent mortality of banana mealy bug, *Geococcus* sp. after three days of inoculation.

At the highest concentration of 10^8 spores ml^{-1}, *B. bassiana* and *M. anisopliae* also gave appreciable reduction in population showing 96.66 and 80.76 per cent respectively (Table 2 and Fig. 6). Ekesi *et al.* (2000) also got similar result with 91 and 93 per cent mortality of *A. craccivora* at 7 days post treatment. Loureiro and Moino (2006) obtained cent per cent mortality of *M. persicae* of *B. bassiana* and *M. anisopliae* @10^6 and 10^7 spores ml^{-1} respectively.

The local isolate, *C. oxysporum* recorded 77.57 per cent mortality @10^8 spores ml^{-1} against *A. craccivora*. At the same concentration, 93.33 per cent mortality was recorded against *C. lanigera,* ten days after treatment (Ramegowda *et al.,* 2007).

Mortality of the aphids was decreased with decrease in concentration. In the lower concentration, 10^3 spores ml^{-1} the mortality of aphids ranged between 17.86 to 28.00

Fig. 1 Mortality caused by different concentrations of *B. bassiana* against *A. craccivora*

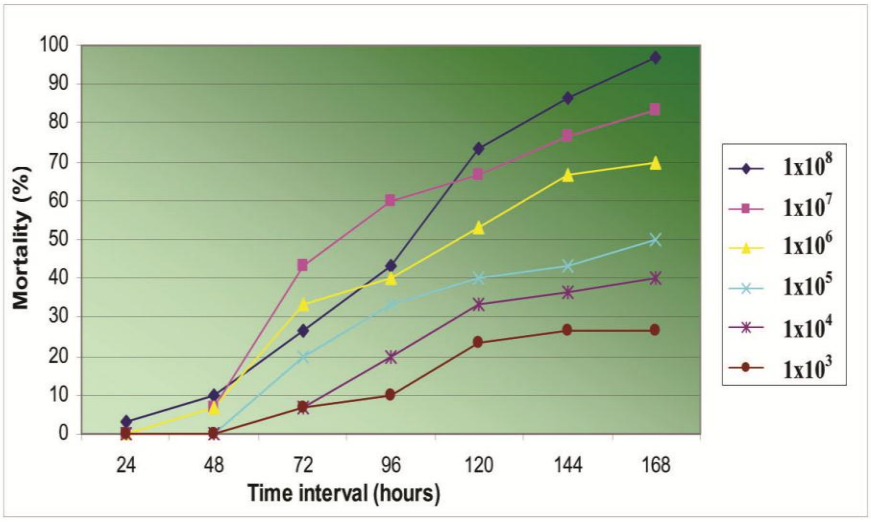


Fig. 2 Mortality caused by different concentrations of *M. anisopliae* against *A. craccivora*

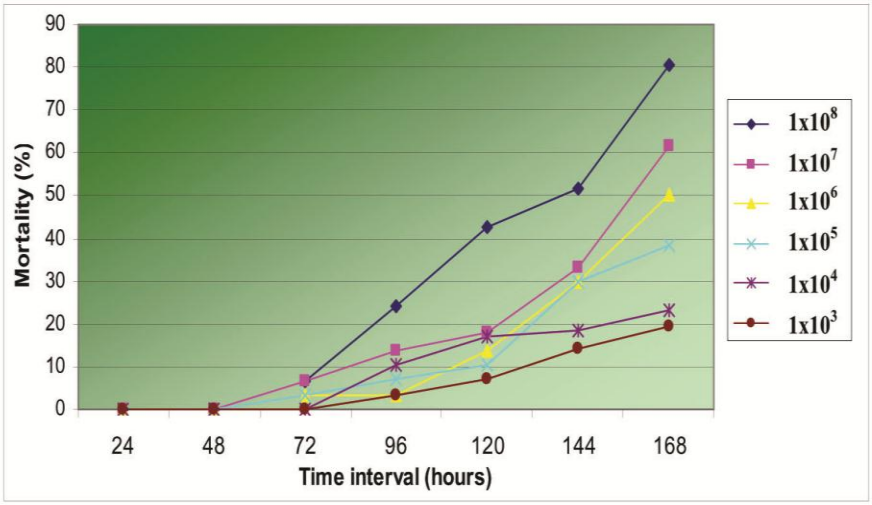


Fig. 3 Mortality caused by different concentrations of *V. lecanii* against *A. craccivora*

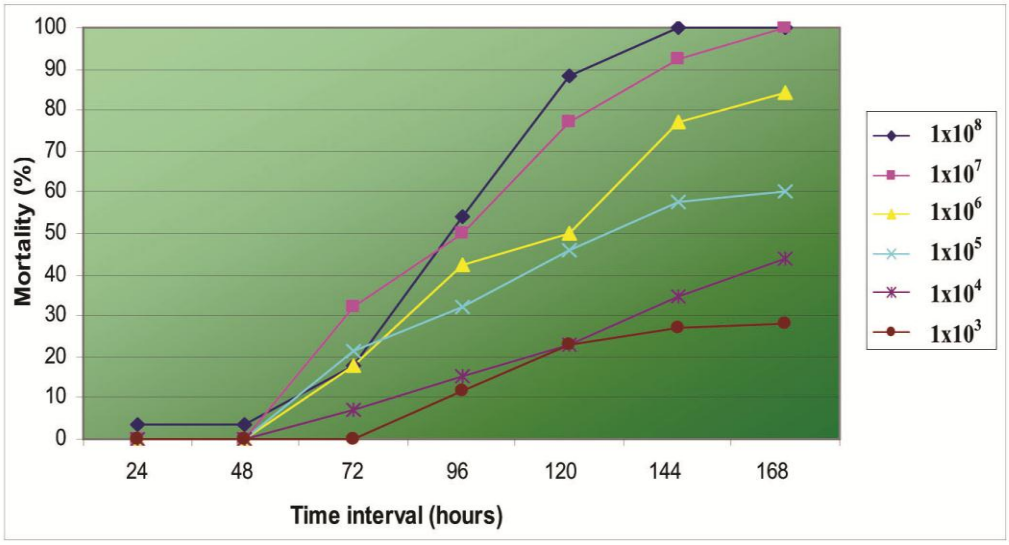


Fig. 4 Mortality caused by different concentrations of *H. thompsonii* Against *A. craccivora*

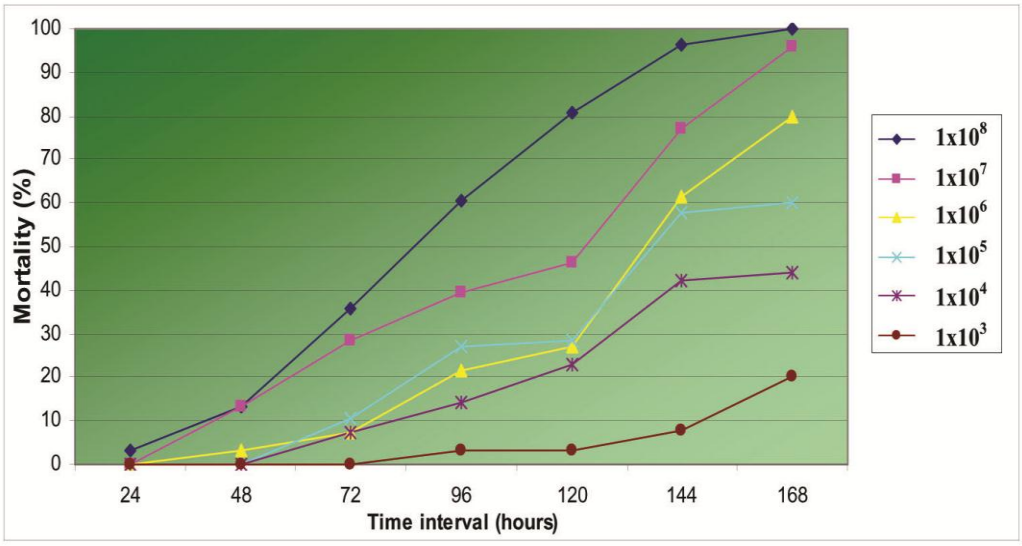


Fig. 5 Mortality caused by different concentrations of *C. oxysporum* against *A. craccivora*

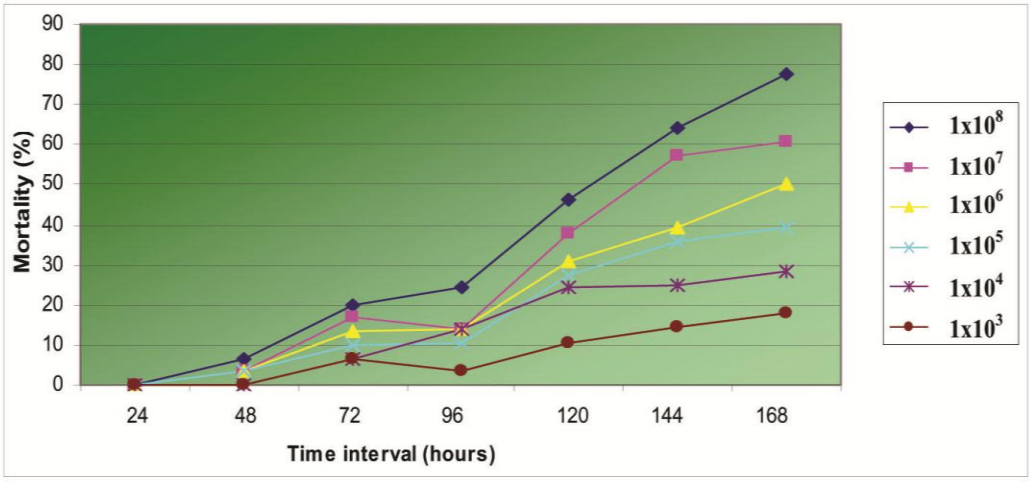
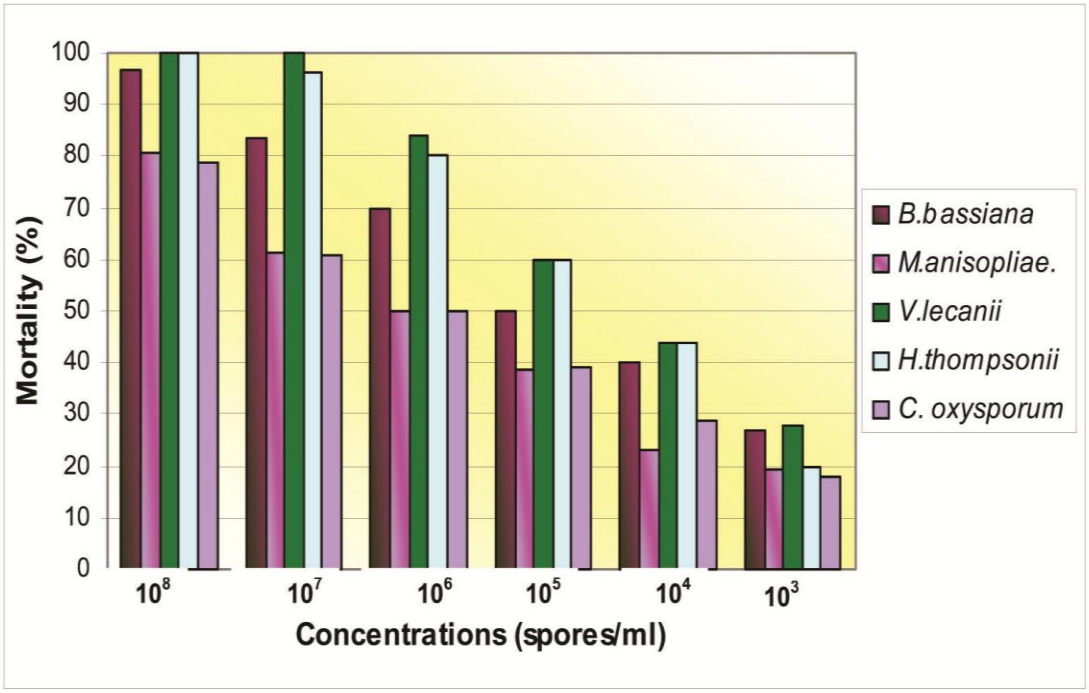


Fig. 6 Cumulative mortality caused by different fungal isolates on *A. craccivora*



per cent. When the spore concentration in the spray fluid is not adequate, the time taken for multiplication may be prolonged resulting in a reduced control of the aphids.

5.2.1 Median Lethal concentration (LC_{50})

In the present study, a correlation between the spore concentration of fungal isolate and responses of the aphids in terms of mortality was determined and LC_{50} values were calculated using Probit Analysis.

The data presented in Table 3 shows the LC_{50} values and the relative toxicity of the five fungal isolates. Among the five fungal isolates, *V. lecanii* and *H. thompsonii* caused 50 per cent mortality at the lowest concentration of 2.5 $x10^4$ spores ml^{-1}. This was followed by *B. bassiana* ($4.5x10^5$ spores ml^{-1}), *C. oxysporum* ($7.4x10^5$ spores ml^{-1}) and *M. anisopliae* ($8.9x10^5$ spores ml^{-1}). It is understood that higher the LC_{50} values, higher will be the relative toxicity (Fig. 7). Low LC_{50} value of $1.2x10^4$ spores ml^{-1} for *V. lecanii* against *Brevicoryne brassicae* and $2.7x10^4$ spores ml^{-1} against *Aphis gossypii* was reported by Derakshan *et al.* (2007) and Karindah *et al.* (1996) respectively is in conformity with the present finding. LC_{50} value obtained in the present study was higher than that reported by Smitha (2007) for *Hirsutella* sp ($5.2x10^4$ spores ml^{-1}), Liu *et al.* (1999) for *B. bassiana* ($1.2x10^4$ spores ml^{-1}) and Chandler (1997) for *M. anisopliae* ($2.45x10^6$ spores ml^{-1}). The difference in the LC_{50} values might be due to the difference in the virulence of fungal isolates and the host species.

5.2.2 Median Lethal Time (LT_{50})

Time mortality response of the five fungal isolates given in Table 4 evidently shows the variation in LT_{50} values at different concentrations. It is understood from the present study that the LT_{50} values decreased with increase in concentrations (Fig. 8). At 10^8 spores ml^{-1}, low LT_{50} value was recorded by *B. bassiana, H. thompsonii* and *V. lecanii* as 3.63, 3.64 and 3.90 days respectively. Nirmala *et al.* (2006) also attained similar results for *B. bassiana* with LT_{50} value of 3.17 days. The LT_{50} value of 3.31 days

Fig. 7 Relative toxicity of different fungal isolates against *A. craccivora*

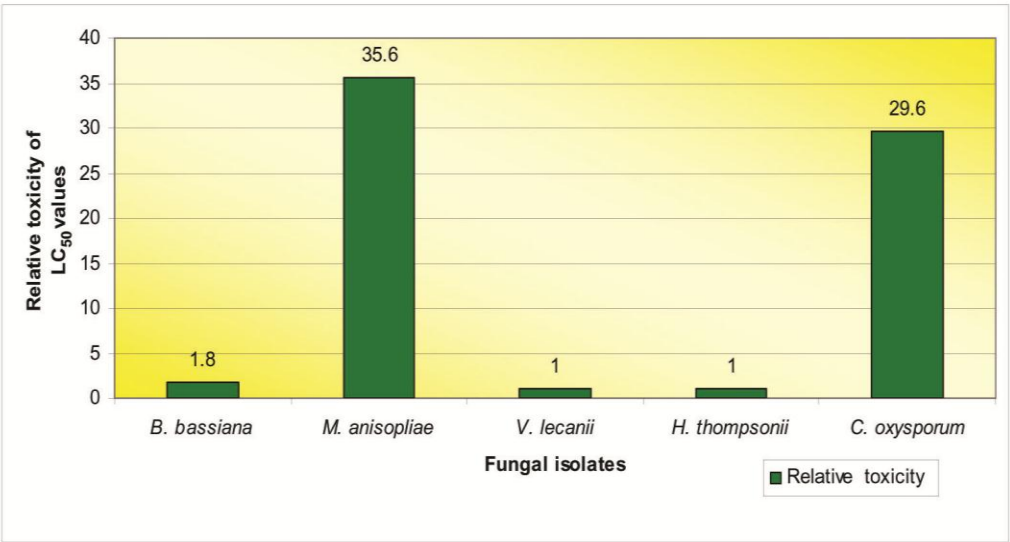
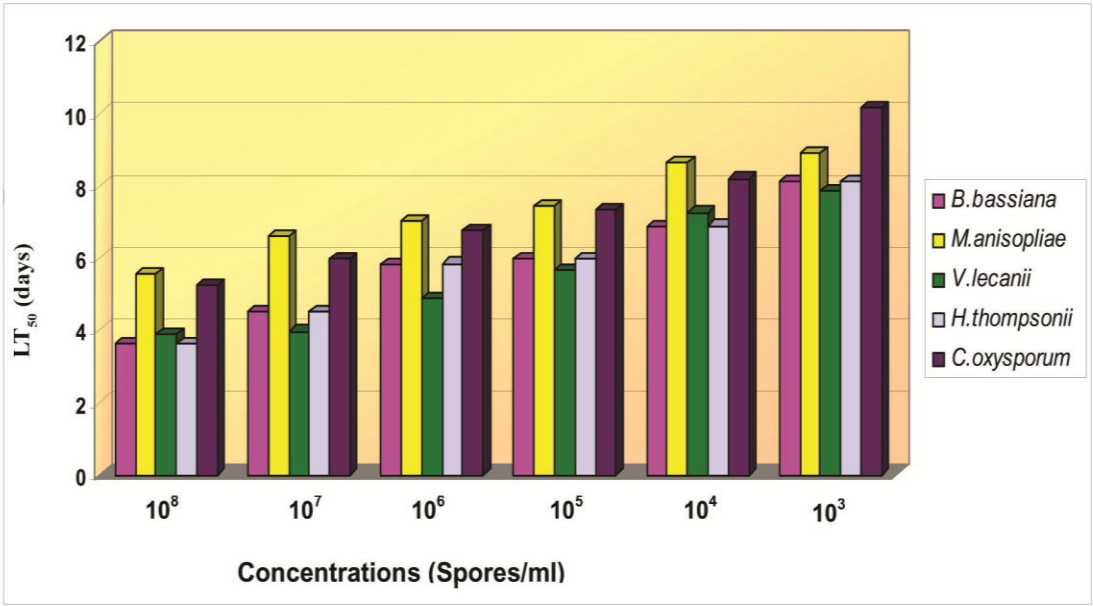


Fig. 8 Median lethal time (LT_{50}) of different fungal isolates against *A. craccivora*



obtained for *V. lecanii* against *Aphis fabae* by Hesketh *et al*. (2008) also agree with the present finding. *Metarhizium anisopliae* and *C. oxysporum* recorded higher LT_{50} values of 5.54 and 5.24 respectively.

Under laboratory conditions, *V. lecanii* and *H. thompsonii* were found to be more virulent recording cent per cent mortality within seven days after treatment. Other fungal isolates also showed promising result. The lowest LC_{50} and LT_{50} values of *V. lecanii, H. thompsonii* and *B. bassiana* indicate its higher virulence against *A. craccivora.*

5.2.3 Effect of fungal isolates on nymphal production of *A. craccivora*

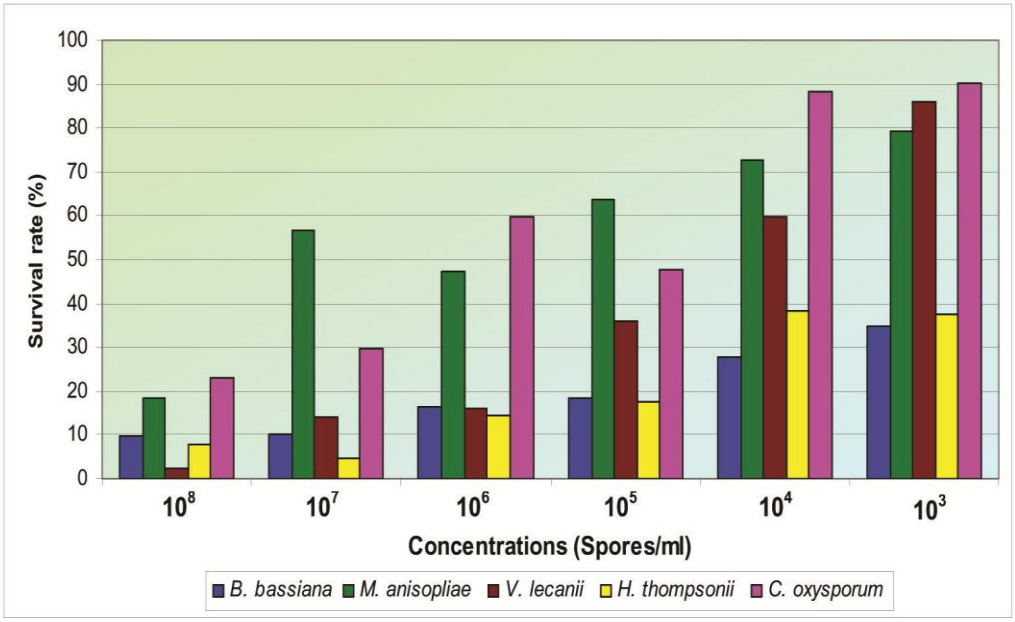
The nymphal production was observed upto seven days and the survival per cent over control was calculated and compared with different fungal concentrations (Table 5 and Fig. 9). It was observed that on the first day itself neonate production was less in the higher concentration of all the fungal isolates and the lowest survival rate was recorded by *V. lecanii* @ 10^8 spores ml^{-1} (46.04%). Baverstock *et al.* (2006) and Roy *et al.* (2005) observed reduced fecundity of aphids by *B. bassiana* within 24 h of inoculation.

It was observed that the reproductive potential of aphids was low in all the fungal treatments. The reduced fecundity may have been due to the weakened condition of adults due to the fungal infection.

On the seventh day (168 h) after inoculation, *V. lecanii* had the lower survival rate (2.49%), followed by *H. thompsonii* (7.96%) and *B. bassiana* (9.64%). Neonate skin was not much sclerotised and the penetration of the fungal spore is easier than adults. This may be the main reason for the reduction in the survival rate.

The present study clearly indicate that the entomopathogenic fungi influenced the neonate production of aphids. The higher concentration of all the fungal isolates exhibited considerable reduction ranging from 2.49 to 23.17 per cent. Out of the five isolates *B. bassiana* and *H. thompsonii* showed low survival rate in the lowest

Fig. 9 Effect of fungal isolates on survival rate of nymphs of *A. craccivora* at seven days of treatment



concentration ranged between (34.75 to 37.61%). Feng *et al*. (1990) and Liu *et al.* (2000) also ascertained that *B. bassiana* penetrates into the aphid cuticle within 4–6 h and causes a peak mortality in 3-4 days after inoculation.

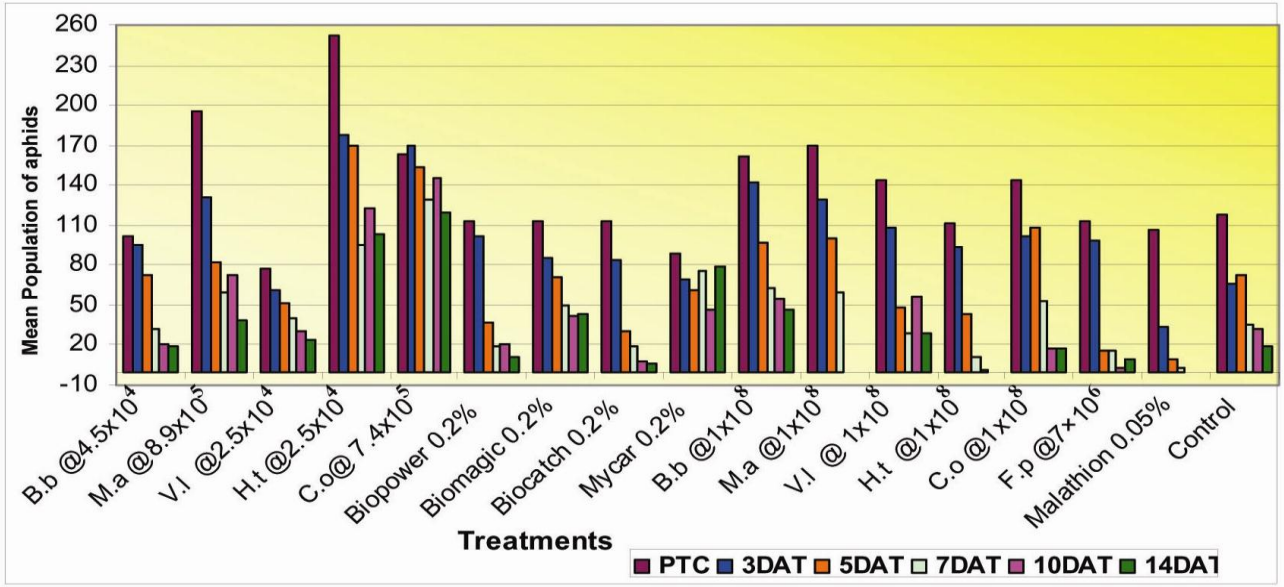
5.3 BIOEFFICACY OF DIFFERENT FUNGAL ISOLATES AGAINST *A. craccivora* BY POT CULTURE EXPERIMENT

A pot culture experiment was laid out to evaluate the efficacy of *B. bassiana, M. anisopliae, V. lecanii*, *H. thompsonii* and the local isolate *C. oxysporum* on *A. craccivora.* The LC_{50} and the dose which gave the shortest LT_{50} value obtained from the bioassay studies and the recommended dose of commercial formulations of these fungal isolates except *C. oxysporum* were evaluated in the pot culture studies under field conditions. These treatments were compared with the standard fungal culture *F. pallidoroseum* and a commonly used insecticide Malathion and control.

The pre treatment count of aphid population was taken when there was sufficient number of aphids in all the plants. During the period of study, the population build up of aphids was noticed at the time of flowering and pod formation stage. The aphid population varied between plants and it ranged between 77.11 to 251.77 numbers. Jagdish (2008) also reported that in all seasons the population of aphids reached peak during the pod formation stage.

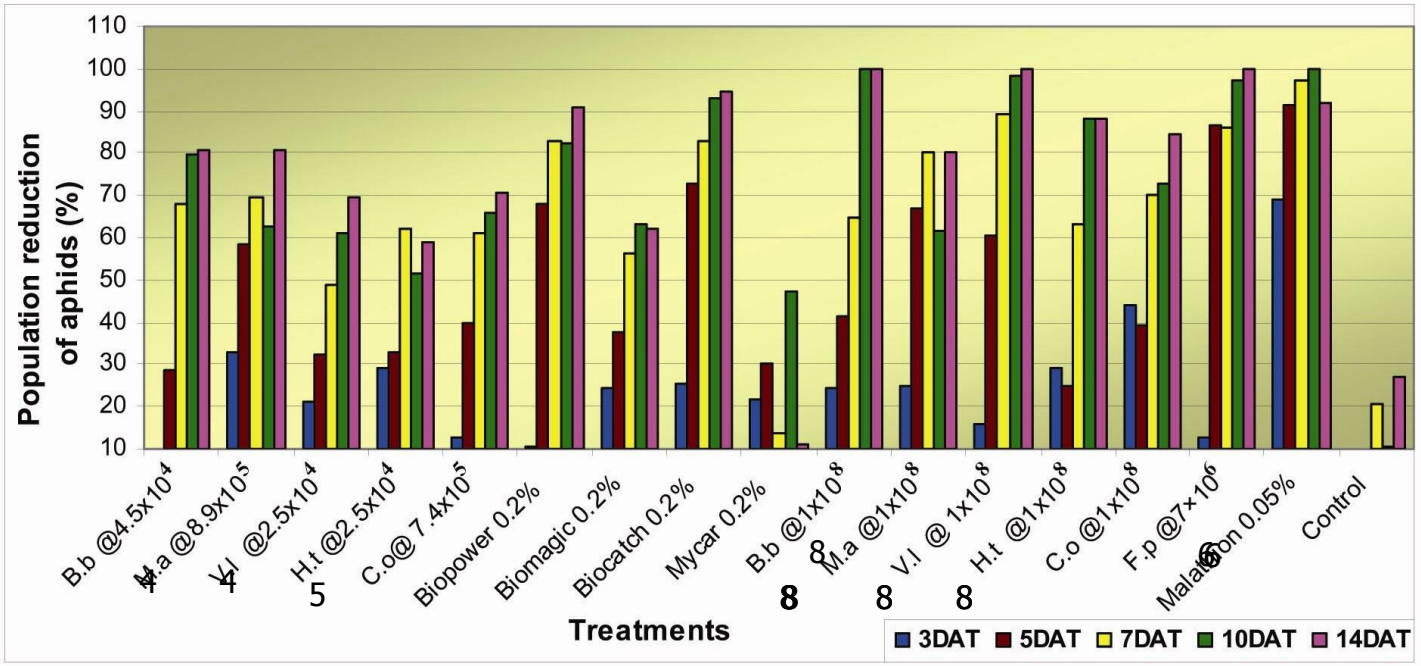
The per cent reduction in aphid population over pre count presented in Table 7 indicates that T10 (*B. bassiana* @10^8 spores ml^{-1}), T12 (*V. lecanii* @10^8 spores ml^{-1}) and T15 (*F. pallidoroseum* @$7x10^6$ spores ml^{-1}) recorded an increasing trend in population reduction on 3rd, 5th and 7th day (Fig. 10 and Fig. 11). *Beauveria bassiana* @10^8 spores ml^{-1} (T10) recorded cent per cent mortality on 10th day. On the 14th day, all the above three treatments resulted in cent per cent mortality and were found superior to all the other treatments. The effectiveness of *F. pallidoroseum* against *A. craccivora* on cowpea is in agreement with the findings of Sunitha and Mathai (1999). They reported that an

Fig. 10 Pre and post treatment population of aphids from top 5 cm of the terminal shoot



B.b- Beauveria bassiana; M.a - Metarhizium anisopliae; V.l- Verticillium lecanii; H.t- Hirsutella thompsonii; C.o- Cladosporium oxysporum; F.p- Fusarium pallidoroseum

Fig. 11 Per cent reduction of aphid population in different treatments at different periods



B.b- Beauveria bassiana; M.a - Metarhizium anisopliae; V.l- Verticillium lecanii; H.t- Hirsutella thompsonii; C. o- Cladosporium oxysporum; F.p- Fusarium pallidoroseum

increase in spore concentration from $0.875x10^6$ to $7x10^6$ spores ml^{-1} resulted in 91.6 to 100 per cent decrease in aphid population.

The result obtained with respect to the effect of *B. bassiana* in reducing the population of cowpea aphid is supported by the findings of Mathew *et al.* (1998). They reported the efficacy of *B. bassiana* on banana aphid *Pentalonia nigronervosa* which caused 37.0 to 96.66 per cent mortality of adult aphids and established that this fungus showed the highest mortality of nymphs and adults. Ekesi *et al.* (2000) also reported that *B. bassiana* isolate CPD11 was highly pathogenic to cowpea aphid, *A. craccivora* causing a mortality range of 58 to 91 per cent, seven days after treatment. Ramegowda *et al.* (2007) reported that *B. bassiana* was highly effective in controlling *C. lanigera* population at 14 days after treatment.

The efficiency of *V. lecanii* as an entomopathogenic fungus in reducing cowpea aphid population reported in this study is in conformity with the investigations of Sahayaraj and Namasivayam (2007). They reported that the *V. lecanii* @10^8 spores ml^{-1} was more effective in suppressing the *A. craccivora* in field conditions.

Compared to all the treatments there was rapid reduction of aphid population in T16 (Malathion 0.05%) on 3^{rd}, 5^{th} and 7^{th} day giving 68.86, 91.39 and 97.39 per cent respectively. Mortality reached cent per cent on 10 days after treatment. But on the 14^{th} day reinfestation of aphids was observed. This explains that action of chemical insecticide cease after a short period and again favoured the development of aphids. It was interesting to observe that *B. bassiana* which also recorded cent per cent mortality on 10^{th} day continued the same condition on the 14^{th} day without any reinfestation. The viable spores in the cadavers might have controlled or the fecundity of the aphids might have been influenced by the fungus.

Among the four commercial formulations tested *B. bassiana* (Biopower), *V. lecanii* (Biocatch) also showed promising results with 90.59 and 94.68 per cent control of aphids respectively. The present finding is in conformity with Zaki (1998) who found

that *B. bassiana* based biopesticide Naturalis caused cent per cent mortality @ 1g l^{-1}. A commercial formulation of *V. lecanii* @ 4.0 g l^{-1} showed 65.60 to 86.50 per cent mortality against mustard aphid, *Lipaphis erysimi* (Parmar *et al.*, 2008). The commercial formulation of *H. thompsonii* (Mycar) showed only poor response in reducing the aphid population.

At 10^8 spores ml^{-1}, T11 (*M. anisopliae*), T14 (*C. oxysporum*) and T13 (*H. thompsonii)* recorded mortality ranging between 80.20 to 88.28 per cent. At the LC_{50} dose, T1 (*B. bassiana*) and T2 (*M. anisopliae*) reduced the population upto 80 per cent. Although T3 (*V. lecanii*) and T4 *H. thompsonii* was highly effective in the laboratory conditions it was less effective in the field conditions giving only less than 60 per cent mortality. The poor performance of the fungus under field conditions might be due to the variatons in weather factors.

These observations clearly indicate that the concentration of spores in the fungal inoculum had a significant role in initiating epizootics and bringing effective check on the aphid population.

5.3.1 Effect of different fungal isolates on growth characters of cowpea

The effect of entomopathogenic fungi on growth characters presented in Table 8 and Fig. 12 suggests that the height of plant, number of shoots and number of leaves are not greatly influenced by the fungi.

Considering the plant height, T12 (*V. lecanii* @10^8 spores ml^{-1}) was found to be superior to all the other treatments with 40.09 per cent increase in height over control. In the other treatments it ranged between 28.58 to 37.43 cm. With respect to the number of shoots, T10 (*B. bassiana* @ 10^8 spores ml^{-1}) produced maximum number of shoots (25.17) which was 67.80 per cent more than control. This difference might be due to the low population density of the aphids by the application of *B. bassiana.* This was closely followed by T12 (*V. lecanii* @ 10^8 spores ml^{-1}) where there was 56.26 per cent increase

Fig. 12 Effect of different fungal isolates on growth characters of cowpea

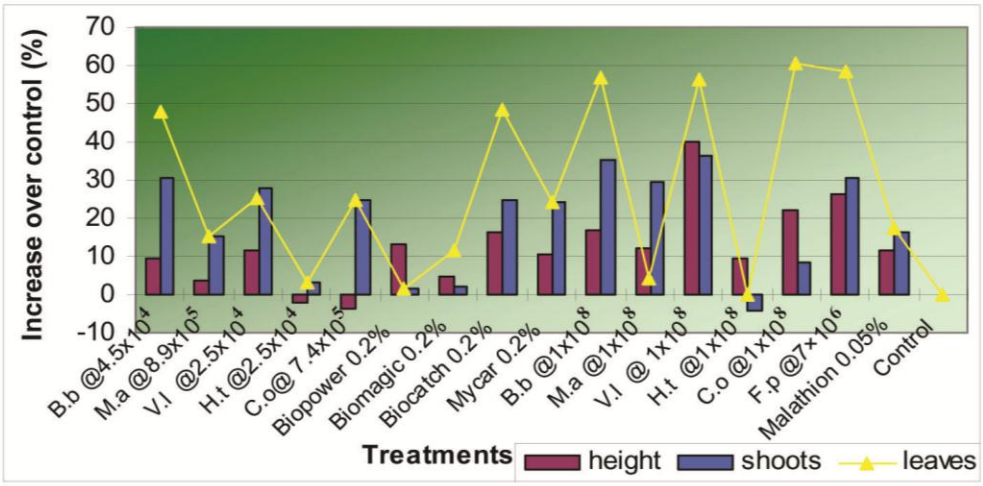
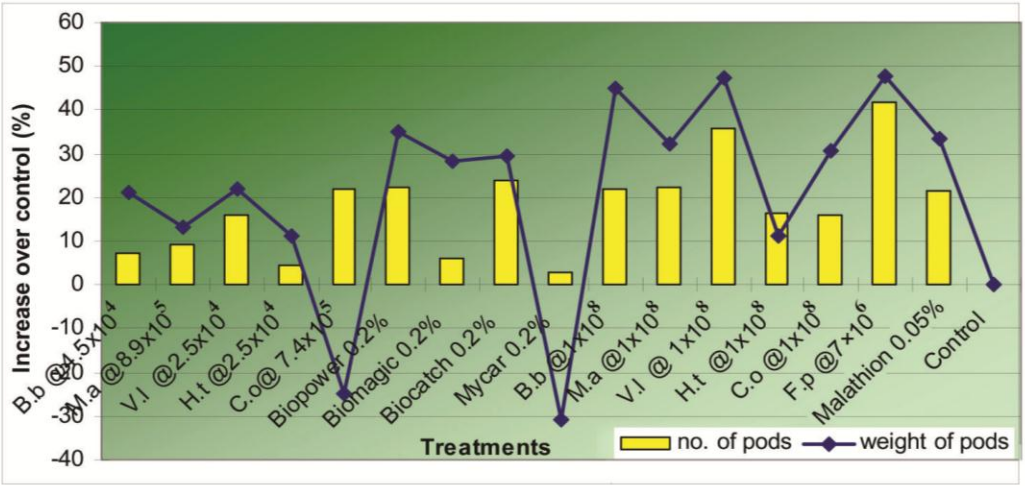


Fig. 13 Effect of different fungal isolates on yield characters of cowpea



B.b- Beauveria bassiana; M.a - Metarhizium anisopliae; V.l- Verticillium lecanii; H.t- Hirsutella thompsonii; C.o- Cladosporium oxysporum; F.p- Fusarium pallidoroseum

in the number of shoots. Promising reduction of aphid population caused by these fungi might be the reason for this. In all the treatments except T10 and T12, increase in number of shoots ranging from 15.00 to 19.56.

Regarding number of leaves T14 (*C. oxysporum* @ 10^8 spores ml^{-1}) was ranked superior producing maximum number of leaves (72.33), T15 (*F. pallidoroseum* @ 7×10^6 spores ml^{-1}), T10 (*B. bassiana* @10^8 spores ml^{-1}) and T12 (*V. lecanii* @10^8 spores ml^{-1}) were found to be statistically on par with T14.

5.3.2 Effect of different fungal isolates on yield characters of cowpea

The data on the number of pods and weight of pods presented in Table 9 and Fig. 13 clearly indicates that T15 (*F. pallidoroseum* @$7x10^6$ spores ml^{-1}) was superior with respect to number of pods which was 41.83 per cent more than control. This was closely followed by T12 (*V. lecanii* @10^8 spores ml^{-1}) and the per cent increase in number of pods was 35.72 per cent increase over control.

With respect to weight of pods also T15 was superior where the yield increase over control was 47.84 per cent followed by T12 and T10 with 47.14 and 45.10 per cent increase over control. T9 (Mycar) and T5 (*C. oxysporum* @7.9 $x10^5$ spores ml^{-1}) gave very poor yield which was inferior to control. Similar results as increase in yield was recorded earlier by Sunitha and Mathai (1999). They also observed proportionate yield increase with increase in spore concentration. Thus yield increase was 10 to 12 per cent higher than the malathion treated plants which suggests that entomopathogenic fungi can be substituted in place of chemical insecticide for the management of aphids.

A notable observation from the pot culture experiment was that *F. pallidoroseum* @ $7x10^6$ spores ml^{-1}, *V. lecanii* and *B. bassiana* @ 10^8 spores ml^{-1} were highly effective against *A. craccivora* and increased the yield from 45.10 to 47.84 per cent. The yield increase obtained by these fungal isolates was 10 to 12 per cent higher than the malathion treatment.

Summary

6. SUMMARY

The study entitled "Biocontrol of cowpea aphid, *Aphis craccivora* (Koch) using entomopathogenic fungi" was carried out in the Department of Entomology, College of Horticulture, Vellanikkara during 2008-2009. The salient findings of the present study are summarized below.

A survey was conducted at four locations of Thrissur district at fortnightly intervals. Mycosed aphids were collected from the field and isolation of fungus was done in the laboratory. During the survey five fungal isolates were collected. Among the five fungal isolates, the pathogenic one collected from Vellanikkara locality was identified as *Cladosporium oxysporum.* This is the first report from Kerala.

Laboratory bioassay studies were conducted with six different concentrations of the five entomopathogenic fungi (EPF) *viz., Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Hirsutella thompsonii* and the local isolate *C. oxysporum.* At the highest concentration of 10^8 spores ml^{-1}, cent per cent mortality was obtained with *V. lecanii* and *H. thompsonii* followed by *B. bassiana* (96.67%), *M. anisopliae* (80.76%) and *C. oxysporum* (77.50%). A progressive reduction in the mortality of *A. craccivora* was observed with the decreasing concentrations of EPF.

The lowest Median lethal concentration (LC_{50}) value of $2.5x10^4$ spores ml^{-1} was recorded by *V. lecanii* and *H. thompsonii* isolates, which showed higher virulence compared to other isolates. The LC_{50} values of *B. bassiana*, *M. anisopliae* and *C. oxysporum* were $4.5x10^4$, $8.9x10^5$ and $7.9x10^5$ spores ml^{-1} respectively. At the highest concentration (10^8 spores ml^{-1}) the Median lethal time (LT_{50}) values for *B. bassiana, H. thompsonii, V. lecanii, C. oxysporum* and *M. anisopliae* were 3.63, 3.64, 3.90, 5.24 and 5.54 days respectively. The LT_{50} values were found to be inversely proportional to the spore concentrations.

Fungal treatments showed considerable reduction in the survival rate of nymphs in all the concentrations. At the highest concentration (10^8 spores ml^{-1}) all the fungal isolates showed low survival rate of nymphs (2.49 to 18.33%). In the lower concentration (10^3 spores ml^{-1}), *H. thompsonii* and *B. bassiana* alone had the lower survivability (34.75 to 37.61%) whereas in all other isolates it was considerably high and varied from 79.23 to 90.24 per cent.

A pot culture experiment was laid out to evaluate the efficacy of different fungal isolates against *A. craccivora* under field conditions. The LC_{50} and and the dose, which gave the shortest LT_{50} value obtained from bioassay studies of *B. bassiana*, *M. anisopliae*, *V. lecanii*, *H. thompsonii* and the local isolate *C. oxysporum* and the recommended dose of commercial formulations of these isolates except the local isolate were compared with the standard fungal culture *F. pallidoroseum* and commonly used chemical insecticide (Malathion 50EC).

Among the different treatments, spray application of *B. bassiana* and *V. lecanii* @ 10^8 spores ml^{-1} and *F. pallidoroseum* @ $7x10^6$ spores ml^{-1} gave cent per cent mortality at 14 days after treatment and were found to be highly efficient in controlling the aphid population. Out of the four commercial formulations, Biocatch (*V. lecanii*) and Biopower (*B. bassiana*) @0.2 per cent gave more than 90 per cent population reduction and were found to be the next best treatments. The treatments *M. anisopliae*, *C. oxysporum* and *H. thompsonii* @ 10^8 spores ml^{-1} and the LC_{50} concentrations of *B. bassiana* and *M. anisopliae* also showed considerable reduction in aphid population ranging between 80.20 to 88.28 per cent.

Malathion (0.05%) and *B. bassiana* @10^8 spores ml^{-1} treated plants showed cent per cent mortality at 10 days after treatment. But on the 14th day of observation reinfestation of aphids was noticed in malathion treatment. In all the fungal treatments, with respect to time interval, there was subsequent reduction in aphid population.

Application of fungal isolates did not show any noticable effect on the growth character of the plant. The highest yield was obtained in plants treated with *F. pallidoroseum* @ $7x10^6$ spores ml^{-1} which recorded the maximum number and weight of pods followed by *V. lecanii* and *B. bassiana* @ 10^8 spores ml^{-1}. These treatments were statistically on par and increased the yield upto 47.84, 47.14 and 45.10 per cent respectively. The yield increase obtained by these fungal isolates was 10 to 12 per cent higher than the malathion treatment.

From the study it is revealed that *B. bassiana* and *V. lecanii* @10^8 spores ml^{-1} were found to be as effective as the standard *F. pallidoroseum* @ $7x10^6$ spores ml^{-1}. These entomopathogenic fungi were found to be even more superior to the chemical insecticide, Malathion based on the cumulative bioefficacy.

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* Originals not seen

Appendix

APPENDIX- I

SOLID MEDIA COMPOSITION

1. Sabouraud's maltose agar +yeast (SMA+Y)

Maltose - 40.00 g

Yeast extract -10.00 g

Bactopeptone -10.00 g

Agar - 15.00g

Distilled water- 1 litre

2. Potato Dextrose Agar (PDA)

Potato - 200g

Dextrose - 20 g

Agar - 20g

Distilled water-1 litre

APPENDIX II

Effect of fungal isolates on nymphal production of *A. craccivora*

Fungal Isolates	Spores/ml	Survival rate of nymphs at hours after treatment						
		24	48	72	96	120	144	168
B. bassiana	10^8	146	243	150	110	105	55	43
	10^7	117	170	144	125	95	65	45
	10^6	116	143	134	140	125	98	73
	10^5	165	204	192	180	143	102	82
	10^4	147	180	219	194	176	154	123
	10^3	149	178	216	180	196	163	155
	Control	165	218	263	313	355	403	446
M. anisopliae	10^8	150	225	230	330	156	130	90
	10^7	163	350	393	399	203	255	279
	10^6	192	279	316	306	255	341	233
	10^5	228	300	390	412	341	356	313
	10^4	225	312	315	315	356	372	356
	10^3	216	165	242	320	372	356	389
	Control	205	358	339	375	433	433	491
V. lecanii	10^8	122	155	139	94	24	25	10
	10^7	181	235	202	176	152	93	56
	10^6	146	223	215	167	143	86	64
	10^5	160	182	174	243	191	163	144
	10^4	141	193	169	122	137	103	120
	10^3	201	164	262	284	317	336	345
	Control	265	240	383	335	312	357	402
H. thompsonii	10^8	135	103	120	93	65	50	36
	10^7	150	216	185	155	165	92	22
	10^6	130	90	103	185	158	130	65
	10^5	140	155	120	134	140	113	79
	10^4	139	130	102	150	155	145	173
	10^3	165	160	183	145	153	164	170
	Control	152	178	252	334	311	367	452
C. oxysporum	10^8	112	103	116	96	87	72	57
	10^7	149	125	134	109	98	85	73
	10^6	102	96	85	114	125	134	147
	10^5	125	189	216	176	154	127	117
	10^4	114	126	203	189	247	254	217
	10^3	193	119	193	156	183	205	222
	Control	196	124	257	164	179	216	264

APPENDIX- III

Adjusted mean values of aphid population based on precount using covariance analysis

Treatments (spores ml^{-1})	Adjusted means of aphid population				
	3DAT	5DAT	7DAT	10DAT	14DAT
T1 - *B.b* @4.5x10^4	115.86 (10.61)^{ab}	84.25 (8.93)^{abc}	34.49 (5.29)^{bcdef}	26.38 (4.04)^{def}	23.24 (4.57)^{def}
T2 - *M.a* @8.9x10^5	94.76 (9.46)^{ab}	60.47 (8.30)^{abcd}	55.66 (7.77)^{abcd}	62.19 (8.08)^{abc}	31.40 (5.96)^{bcdef}
T3 - *V.l* @2.5x10^4	95.18 (9.23) ^{ab}	72.39 (7.99)^{abcd}	43.12 (5.97)^{bcde}	40.36 (5.85)^{cde}	29.69 (5.04)^{def}
T4 - *H.t* @2.5x10^4	108.22 (10.27)^{ab}	128.83 (10.74)^{ab}	87.99 (9.53)^{ab}	102.20 (10.28)^{ab}	91.38 (9.18)^{ab}
T5 - *C.o* @ 7.4x10^5	125.62 (10.89)^{ab}	87.42 (9.43) ^{ab}	61.28 (7.93)^{abcd}	50.50 (7.24)^{bcd}	44.42 (6.65)^{bcd}
T6 - Biopower 0.2%	114.57 (10.59)^{ab}	44.09 (6.26)^{bc}	20.71 (4.36)^{cdef}	23.79 (3.93)^{def}	13.00 (3.03)^{defg}
T7 - Biomagic 0.2%	98.41 (9.69)^{ab}	77.96 (8.36)^{abcd}	50.97 (6.99)^{abcd}	45.57 (6.48)^{cde}	45.39 (6.59)^{bcd}
T8 - Biocatch 0.2%	92.25 (9.13)^{abc}	35.01 (5.32)^{bcd}	20.05 (3.53)^{def}	10.51 (2.71)^{ef}	7.41 (1.96)^{fg}
T9 - Mycar 0.2%	96.82 (9.47)^{ab}	77.76 (8.37)^{abcd}	79.19 (8.56)^{abc}	54.61 (7.10)^{bcd}	83.59 (8.81)^{abc}
T10- *B.b* @1x10^8	107.49 (10.08)^{ab}	87.42 (8.60)^{abcd}	57.76 (7.57)^{abcd}	-6.15 (0.5165)^{f}	-3.79 (0.5792)^{g}
T11- *M.a* @1x10^8	103.04 (9.68)^{ab}	44.78 (6.63)^{abcd}	27.85 (5.36)^{bcdef}	54.04 (7.17)^{bcd}	33.56 (5.792)^{bcdef}
T12- *V.l* @ 1x10^8	107.97 (10.16)^{ab}	52.24 (6.73)^{abcd}	13.19 (2.41)^{ef}	6.09 (1.40)^{f}	2.58 (0.78)^{g}
T13- *H.t* @1x10^8	96.85 (9.59)^{ab}	104.79 (9.86)^{ab}	52.69 (7.24)^{ef}	15.28 (3.52)^{def}	22.89 (4.87)^{def}
T14- *C.o* @1x10^8	76.02 (8.43)^{bc}	77.84 (8.57)^{abcd}	36.36 (5.77)^{bcdef}	35.26 (5.72)^{cde}	28.81 (5.27)^{cdef}
T15- *F.p* @7×10^6	111.84 (10.39)^{ab}	22.84 (3.68)^{cd}	17.48 (3.38)^{def}	6.77 (1.60)^{f}	2.33 (0.78)^{g}
T16-Malathion 0.05%	50.20 (6.58)^{c}	17.15 (3.09)^{d}	4.60 (1.40)^{f}	5.017 (0.873)^{f}	12.09 (2.331)^{efg}
T17 - Control	152.58 (12.02)^{a}	143.05 (11.92)^{a}	127.77 (11.44)^{a}	140.67 (11.63)^{a}	116.211 (10.60)^{a}

Figures in the parentheses using square root transformed values($\sqrt{x+0.5}$)

Figures in each column with same alphabets form one homogenous groups

B.b- Beauveria bassiana; M.a - Metarhizium anisopliae;V.l- Verticillium lecanii; H.t- Hirsutella thompsonii;C. o- Cladosporium oxysporum; F.p- Fusarium pallidoroseum.

DAT- Days after treatment

Appendix IV

Cost of protection in different treatments

Treatments (spores ml^{-1})	Production cost of EPF cultures (Rs.)	Cost of fungal formulations / chemical (Rs.)	Application cost (Rs.)	Total cost (Rs.)	Cost per replication (Rs.)
T1-*B.b* @$4.5x10^4$	0.30	-	3.13	3.43	1.14
T2-*M.a* @$8.9x10^5$	0.30	-	3.13	3.43	1.14
T3-*V.l* @$2.5x10^4$	0.30	-	3.13	3.43	1.14
T4-*H.t* @$2.5x10^4$	0.30	-	3.13	3.43	1.14
T5-*C.o*@ $7.4x10^5$	0.30	-	3.13	3.43	1.14
T6-Biopower 0.2%	-	0.48	3.13	3.61	1.20
T7-Biomagic 0.2%	-	0.48	3.13	3.61	1.20
T8-Biocatch 0.2%	-	0.48	3.13	3.61	1.20
T9-Mycar 0.2%	-	0.48	3.13	3.61	1.20
T10-*B.b* @$1x10^8$	-	-	3.13	3.43	1.14
T11-*M.a* @$1x10^8$	0.30	-	3.13	3.43	1.14
T12-*V.l* @ $1x10^8$	0.30	-	3.13	3.43	1.14
T13-*H.t* @$1x10^8$	0.30	-	3.13	3.43	1.14
T14-*C.o* @$1x10^8$	0.30	-	3.13	3.43	1.14
T15-*F.p* @7×10^6	0.30	-	3.13	3.43	1.14
T16-Malathion 0.05%	-	2.12	3.13	5.25	1.75
T17-Control	-	-	3.13	3.13	1.04

B.b- Beauveria bassiana; M.a – Metarhizium anisopliae;V.l- Verticillium lecanii; H.t- Hirsutella thompsonii;C.o- Cladosporium oxysporum; F.p- Fusarium pallidoroseum

EPF- Entomopathogenic fungi

BIOCONTROL OF COWPEA APHID *Aphis craccivora* (Koch) USING ENTOMOPATHOGENIC FUNGI

By

S. SARANYA
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ABSTRACT OF THE THESIS

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Kerala Agricultural University, Thrissur

Department of Agricultural Entomology

COLLEGE OF HORTICULTURE

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ABSTRACT

The present study entitled "Biocontrol of cowpea aphid, *Aphis craccivora* (Koch) using entomopathogenic fungi" was undertaken to identify the effective local isolates of entomopathogenic fungi (EPF) and to evaluate the pathogenicity of pure cultures and commercial formulations of *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Hirsutella thompsonii* and local isolate on cowpea aphid, *Aphis craccivora.*

A survey was conducted at four locations of Thrissur district. During the survey, five fungal isolates were collected. Among the five isolates one pathogenic fungus, obtained from the Vellanikkara locality was identified as *Cladosporium oxysporum.* It is the first report from Kerala.

Laboratory bioassay studies were carried out with six different concentrations of *B. bassiana, M. anisopliae, V. lecanii, H. thompsonii* and *C. oxysporum* against the adults of *A. craccivora.* Among the five isolates tested, *V. lecanii* and *H. thompsonii* caused cent per cent mortality followed by *B. bassiana* with 96.66 per cent. It was revealed that *V. lecanii* and *H. thompsonii* showed higher virulence with the lowest LC_{50} value of $2.5x10^4$ spores ml^{-1}. At the highest concentration of 10^8 spores ml^{-1}, the LT_{50} values ranged from 3.63 to 5.96 days in the different fungal isolates which was found increasing along with the decreasing concentrations.

The survival rate of nymphs was considerably reduced with in 24 hours after treatment. At the highest concentration (10^8 spores ml^{-1}), all the isolates recorded less survival per cent of nymphs which ranged between 2.49 to 18.33 per cent. But even in the lower concentration (10^3 spores ml^{-1}), *H. thompsonii* and *B. bassiana* exhibited low nymphal survivability (34.75 to 37.61%) as compared to other isolates.

Based on the bioassay studies a pot culture experiment was conducted to evaluate the efficacy of different fungal isolates against *A. craccivora* under field conditions. Among the different treatments, *B. bassiana* and *V. lecanii* @ 10^8 spores ml^{-1} and

F. pallidoroseum @ 7x 10^6 spores ml^{-1} gave cent per cent mortality on 14^{th} day after treatment. This was followed by the commercial formulations, Biopower and Biocatch (0.2%) and the chemical insecticide Malathion (0.05%) which recorded more than 90 per cent mortality.

The highest yield was obtained in plants treated with *F. pallidoroseum* @ 7x10^6 spores ml^{-1} which recorded the maximum number and weight of pods followed by *V. lecanii* and *B. bassiana* @ 10^8 spores ml^{-1}. These treatments were statistically on par and were considerably increasing the yields upto 47.84, 47.14 and 45.10 per cent respectively.

From the study it is revealed that *B. bassiana* and *V. lecanii* @ 10^8 spores ml^{-1} were found to be as effective as the standard *F. pallidoroseum* @ 7x10^6 spores ml^{-1}. These entomopathogenic fungi were found to be even more superior to the chemical insecticide, malathion based on the cumulative bioefficacy.