PRODUCTION AND EVALUATION OF THE FUNGUS *Fusarium pallidoroseum* (Cooke) Sacc. AS A BIOPESTICIDE AGAINST PEA APHID *Aphis craccivora* Koch.

By

REJI RANI O. P.

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2001


Dedicated

to my

Beloved Father


DECLARATION

I hereby declare that this thesis entitled **"Production and evaluation of the fungus *Fusarium pallidoroseum* (Cooke) Sacc. as a biopesticide against pea aphid *Aphis craccivora* Koch."** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellayani,
5-6-2001.

REJI RANI. O. P.
(96-21-04)

CERTIFICATE

Certified that this thesis entitled **"Production and evaluation of the fungus *Fusarium pallidoroseum* (Cooke) Sacc. as a biopesticide against pea aphid *Aphis craccivora* Koch."** is a record of research work done independently by **Mrs. Reji Rani. O.P.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Susamma Mathai

Vellayani,
5-6-2001.

Dr. SUSAMMA MATHAI
(Chairperson, Advisory Committee)
Professor of Entomology,
College of Agriculture, Vellayani.

Approved by

Chairperson :

Dr. SUSAMMA MATHAI
Professor
Department of Entomology
College of Agriculture, Vellayani

Susamma Mathai
27/8/01

Members :

1. **Dr. K. SARADAMMA**
   Professor and Head
   Department of Entomology
   College of Agriculture, Vellayani

27/8

2. **Dr. THOMAS BIJU MATHEW**
   Associate Professor
   Department of Entomology
   College of Agriculture, Vellayani

27/8/01

3. **Dr. (Mrs.) P. SARASWATHI**
   Professor and Head
   Department of Agricultural Statistics
   College of Agriculture, Vellayani

27/8/01

4. **Dr. C. K. PEETHAMBARAN**
   Professor and Head
   Department of Plant Pathology
   College of Agriculture, Vellayani

27/8/01

External Examiner :

27/8/2001 S. EASWARAMOORTHY
Principal Scientist & Head i/c
Division of Crop Protection
Sugarcane Breeding Institute
Coimbatore

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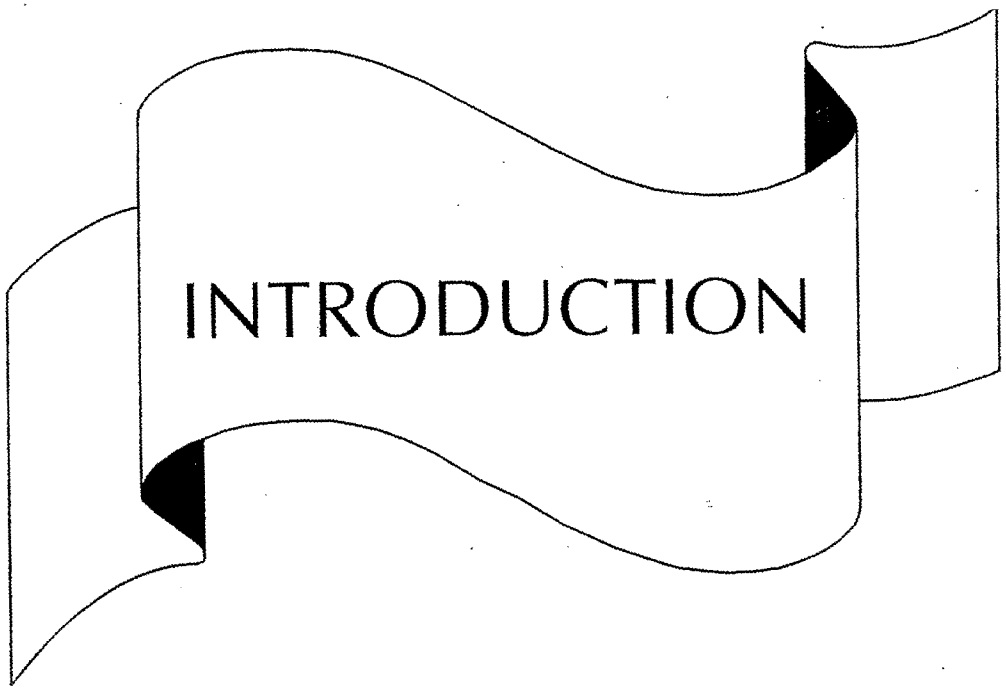

INTRODUCTION

1. INTRODUCTION

The black pea aphid *Aphis craccivora* Koch. is a major pest of cowpea *Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdc., and many other pulse crops like black gram, green gram, lablab, ground nut, peas, beans etc. It also acts as a vector of viral diseases like rosette, mottle, stunt and stripe (Singh, 1984; Porter *et al.*, 1984). Singh and Allen (1980) reported *A. craccivora* as a major pest of cowpea in Asia causing 40 per cent reduction in yield.

Being soft bodied and slow moving, the insect is amenable to chemical control. But the highly polyphagous nature of the pest and round the year availability of many of the leguminous crops often result in continuous abundance of this insect in all agroecosystems. When the persistent effect of insecticides in the treated plot fades out the pest re-establishes and multiplies faster in the absence of natural enemies and assumes more serious levels. At sublethal levels many of the insecticides are known to induce resurgence of aphids. These limitations necessiate repeated and frequent application of insecticides for the control of *A. craccivora* and the technology often becomes economically nonviable.

On crops like cowpea, pest reaches the peak population at the bearing stage of the crop. Studies conducted at College of Agriculture, Vellayani under the All India Coordinated Research Project on Pesticide Residues revealed that the insecticide residue in this vegetable is seven times beyond the Maximum Residue

Limit (MRL) (Mathew *et al.*, 2000). This menace has been widely reported and accepted as a serious drawback of chemical control particularly for vegetable and fruit crops. Moreover, chemical pesticides, previously the major control measure against pest outbreaks, are increasingly unacceptable due to concern about their environmental effects. Consequently in recent years there has been an increased interest in research in biological control using pathogens which enables more viable and ecologically sound technologies for management of the pest.

Fungal infections are common and widespread and often decimate insect population in spectacular epizootics. Charnley (1989) recorded more than 700 species of fungi as pathogenic to insects. The main factor limiting the use of insect pathogenic fungi as biological control agents is their requirement for high humidity and temperature for germination, growth and sporulation. Fungi could provide inexpensive and long lasting control of insect pests only in areas of frequent rainfall and humidity (Dresner,1949). Kerala being such an area offers considerable scope for the use of entomogenous fungi in pest management programmes.

Hareendranath *et al.* (1987) reported *Fusarium pallidoroseum* (Cooke) Sacc. as a potent fungal pathogen of pea aphid *Aphis craccivora*. Preliminary studies have proved the effectiveness of the fungus against this pest (Faizal, 1992; Sunitha, 1997) and the scope for evolving an ecologically sound pest management programme. Development of a good mycoinsecticide using this fungus relies on its laboratory virulence, field performance, productivity, stability on storage, formulation and its ecological and mammalian safety. Considering the above parameters, present investigation was undertaken to get detailed information on the following aspects.

1. Basic research on the morphology of the fungus

2. Amenability of the fungus to mass production, utilizing easily available and cost effective substrates.

3. Standardization of cost effective and easily available carrier materials for formulating the spores.

4. Assessment of shelf life of the fungus at different storage temperatures.

5. Identification of a suitable packing material for the mycoinsecticide which can retain the spore qualities.

6. Compatibility of the entomopathogen with commonly used botanical insecticides. It's compatibility with commonly used insecticides and fungicides have been studied earlier.

7. Assessment of time and method of application of the mycoinsecticide under field conditions.

8. Evaluation of safety of the fungus to crop plants, natural enemies, productive insects and pollinators, it's cross infectivity to other aphids, insect pests and mites.

9. Evaluation of its safety to higher animals.

10. Detection, isolation and determination of efficacy of the toxins present in *F. pallidoroseum* if any.


REVIEW OF
LITERATURE

2. REVIEW OF LITERATURE

The public pressure to reduce the use of pesticides in agriculture is a comparitively recent phenomenon. The outcome of such pressure resulted in giving more and more emphasis to nonchemical methods of pest management. One such management system is the use of biopesticides which implies the use of a living organism or its products for the management of pests. The use of biological tactics in pest management is in its infancy. Eventhough several biological methods have been developed against an array of insect pests, commercial exploitation of this is limited to very few cases. One of the widely researched field in biological control is the use of entomopathogenic fungi in restricting the development of insect pest. Nearly 700 species of fungi under 90 genera, belonging to Entomophthorales and Deuteromycetes are known to be as entomopathogens (Charnley, 1989). Entomopathogenic fungi belonging to 14 genera namely *Beauveria*, *Metarhizium*, *Verticillium*, *Hirsutella*, *Erynia* (*Zoophthora*), *Nomuraea*, *Aspergillus*, *Aschersonia*, *Paecilomyces*, *Tolypocladium*, *Leptolegnia*, *Culicinomyces*, *Coelomomyces* and *Lagenidium* have been extensively studied (Moore and Prior, 1993). The genus *Fusarium*, better known as a plant pathogen, consists of certain species which are very effective in managing certain insect pests and weeds. During the recent past, extensive investigations were carried out using this genus, against verteberate and nonverteberate organisms (Parry, 1995).

2.1 Taxonomic position of the genus *Fusarium*

Genus *Fusarium* was originally grouped under the class Deuteromycotina, sub-class Hyphomycetidae, order Moniliales and family Tuberculariaceae (Alexopoulos and Mims, 1983). The present classification is as follows

Kingdom	-	Fungi
Phylum	-	Ascomycota
Class	-	Pyrenomycetes
Order	-	Hypocreales
Family	-	Nectriaceae
Genus	-	Gibberella

Under the anamorphic stage of genus Gibberella comes *Fusarium* (Alexopoulos *et al.*,1996).

2.2 Host range of entomopathogenic *Fusarium*

A wide range of insects are found to be attacked by *Fusarium* spp. (Table).

2.3 Cross infectivity of entomopathogenic fungi

Sunitha (1997) tested the cross infectivity of *F. pallidoroseum* isolated from the pea aphid *Aphis craccivora* and reported that it was not infectious to other aphid species tried viz. *A. gossypii*, *A. malvae* and *Toxoptera aurantii*.

Host range of entomopathogenic *Fusarium*

Sl.No.	Fungal pathogen	Insect host	Crop	Reference
1.	*F. acridiorum*	*Schistocerca gregaria*	Polyphagous	Kunckel d' Hercularis and Langlois (1891)
2.	*F. episphaeria*	Scale insect	Citrus	Reinking (1921)
3.	*F. cocophila*	Scale insect	Citrus	"
4.	*F. parasiticum*	Scale insect	Citrus	Teodoro (1937)
5.	*F. juruanum*	Scale insect	Coconut	"
6.	*F. aleurodes*	*Dialeurodes citri*	Citrus	Steinhaus (1949)
		Dialeurodes citrifolia		
7.	*Fusarium* sp.	*Monochamus notatus*		Steinhaus and Marsh (1962)
		Coccus viridis		
8.	*Fusarium* sp.	*Eurygaster pacifica*		"
		Chilo zonellus		"
		Platynota rostrana		"
		Carpocapsa pomonella		"
9.	*Fusarium* sp.	*Macrosiphum pisum*	Pea	Rachvelishrilli (1965)
10.	*F. episphaeria cocophila*	*Aonidiella aurantii*	Coconut	Gabriel (1968)
		Chrysomphalus aonidium		"
		Coccus viridis		"
11.	*Fusarium* sp.	*Xyloterinus politus*		MacLean and Giere (1968)
12.	*F. citrculatum*	Cerambycid beetles		Madelin (1968)
13.	*F. oxysporum*	*Coccus viridis*		Viswanathan (1972)
14.	*Fusarium* sp.	*Chilo partellus*		Atwal *et al.* (1973)
15.	*F. larvarum*	*Aedeges piceae*		Smirnoff (1973)
16.	*Fusarium* sp.	*Eurygaster integriceps*		Popov and Illiesu (1975)
17.	*F. equiseti*	*Melanagromyza hibisci*	Bhindi	"
18.	*F. solani*	*Scolytus scolytus*	Elm	Barson (1976)
19.	*F. gibbosum* var. *bullatum*	"		"
20.	*F. javanicum*	"		"

Sl.No.	Fungal pathogen	Insect host	Crop	Reference
21.	*F. oxysporum* var. *orthoceras*	Forest pests		Kalvesh (1976)
22.	*F. sambueinnum* var. *minus*	"		"
23.	*F. semitectum*	"		"
24.	*F. moniliformae* var. *subglutinans*	*Henosepilachna vigintioctopunctata*		Jacob *et al.* (1978)
25.	*F. oxysporum*	*Nilaparvatha lugens*	Rice	Kuruvilla (1978)
26.	*F. oxysporum*	*Ostrina nubilalis*	Maize	Lynch and Lewis (1978)
27.	*F. oxysporum*	*Melanitis leda ismene*	Rice	Nayak and Srivastava (1978)
28.	*F. moniliformae* var. *subglutinans*	*H. vigintioctopunctata*		Beevi (1979)
29.	*F. equiseti*	*Nephotettix virescens*	Rice	Devanesan *et al.* (1979)
30.	*F. equiseti*	*Coccidohystrix insolita*	Brinjal	Gopinathan *et al.* (1982)
31.	*F. moniliformae*	*Mylabris pustulata*		Beevi and Jacob (1982)
		Aulacaphora sp.		"
32.	*F. semitectum*	*Myzus persicae*		Nagalingam and Jayaraj (1986)
33.	*F. coccophilum*	*Hemberlosia rapex*	Tea	Devnath (1987)
34.	*F. pallidoroseum*	*Aphis craccivora*	Cowpea	Hareendranath *et al.* (1987)
35.	*F. solani*	*Cossus cadambae*	Teak	Mathew and Ali (1987)
36.	*F. subglutinans*	*Melanaspis glomerata*	Sugarcane	Raghavendran *et al.* (1987)
37.	*F. oxysporum*	,,		"
38.	*F. tricinctum*	*Sitobion avenae*		Ozino *et al.* (1988)
39.	*Fusarium* sp.	*Orthezia praelonga*	Coffee	Martins *et al.* (1989)
40.	*Fusarium* sp.	*Heteropsylla cubana*		Villacarolas and Robin (1989)
41.	*Fusarium* sp.	*Bemisia tabaci*		Fransen (1990)
42.	*Fusarium* sp.	*Orthezia praelonga*	Tea	Ye *et al.* (1991)
43.	*F. pallidoroseum*	*Cnaphalocrocis medinalis*	Rice	Manisegarame and Letchoumanane (1996)
44.	*Fusarium* sp.	*Laevicaulis alte*	Neem	Seemakumar *et al.* (1998)
45.	*Fusarium* sp.	*Oxya nitidula*	Rice	Mahalakshmi (1998)
46.	*Fusarium* sp.	*Ceroplastes destructor*		Lo and Chapman (1998)
		C. cinensis		"
47.	*F. solani*	*Odoiporus longicollis*	Banana	Anitha *et al.* (1999)
48.	*F. oxysporum*	*Planococcus* sp.	Pepper	Devasahayam and Koya (2000)

Beevi and Jacob (1982) reported that *F. moniliformae* var. *subglutinans* isolated from *Henosepilachna vigintioctopunctata* was pathogenic to *Aulacophora* and *Mylabris pustulata* also. Camargo *et al.* (1985) observed that a particular strain of *B. bassiana* and *M. anisopliae* was pathogenic to the curculionids *Anthonomus grandis* and *Cosmopolites sordidus* as well as to the scolytid *Hypothenemus hampeii*. Diaz *et al.* (1986) reported that *B. bassiana* which caused 100 per cent mortality of sweet potato weevil *Cylas formicarius* was also pathogenic to *Chalcodermus ebeninus*, *Metamasius sericeus* and *Cosmopolites sordidus*. *B. bassiana* isolated from a species of scarabid genus *Ligyrus* and pyralid *Diatraea saccharalis* and *Metarhizium anisopliae* isolated from *Ligyrus* and from the cercopid *Deois flavopicta* showed infectivity to *Cosmopolites sordidus* ten days after treatment (Busoli *et al.*,1989). *B. bassiana* derived from an aphid species was found to infect another hop aphid *Pherodon humulii*, as reported by Dorschner *et al.* (1991). Two isolates of *Metarhizium*, FM8 and FM12, that are highly pathogenic to larvae of *Plutella xylostella* and *Crocidolomia binotalis* showed little or no pathogenicity to the larvae of *Spodoptera litura* and aphids *Myzus persicae* and *Lipaphis erysimi* (Santiago, 1994).

2.4 Mass production of entomopathogenic fungi on naturally available substrates

Large scale use of fungi for biological control requires mass production of the inoculum. Low input mass production systems utilising agricultural or other waste materials may be of great value. Ease and low cost production and application are among the principal characteristics of a desirable pathogen (Butcher, 1958). Many attempts have been made in the past to mass produce entomopathogenic fungi in naturally available substrates.

2.4.1 Solid substrates

Different methods of mass production of entomopathogenic fungi have been tried on solid substrates with varying degrees of success.

Beevi (1979) reported sorghum and bajra to be the most suitable food source for mass production of *F. moniliformae* var. *subglutinans*, pathogenic to epilachna beetle. *F. oxysporum* pathogenic to brown plant hopper, *Nilaparvatha lugens* could be easily multiplied using green gram, wheat and sorghum (Kuruvilla and Jacob, 1981). Nagalingam (1983) reported that *F. semitectum* infecting *Myzus persicae* grew easily on a medium containing broken maize grain and black or red gram husk. Abundant sporulation of *F. subglutinans* was observed on sorghum grains by Raghavendran *et al.* (1987). Mathai *et al.* (1988) observed that *F. pallidoroseum* sporulated best when grown on wheat bran alone or when a combination of rice bran and tapioca bits were used as substrates, followed by wheat bran plus straw bits, rice bran and tapioca bits. They obtained only very poor growth on vegetable waste and tapioca stem peelings. According to Hareendranath (1989), broken maize grain was a good substrate for mass production of *F. pallidoroseum* followed by tapioca chips and jack seeds, while Faizal (1992) reported rice bran and wheat bran as the best substrates for maximum production of spores of the fungus.

Shands *et al.* (1958) could harvest a very good crop of spores of *Entomophthora aphidis* which was grown on cooked slices of potato. Sugarcane bagasse was found to be a good medium for conidial production of *Nomuraea rileyi*

(Holdom *et al.* 1986) while crushed sorghum grains containing one per cent yeast extract was reported to be suitable for mass production of *N. rileyi* (Devi, 1994).

York (1958) used rice bran for mass production of *B. bassiana* while Martigoni (1964) reported that wheat, cane or potato were good food source for the mass multiplication of *B. bassiana.* Bell (1974) obtained good growth of this fungus on wheat bran and Batista *et al.* (1989) cultured it on soaked rice bran. Barley was found to be a suitable medium for maximum conidia production of *B. brongniartii* by Aregger (1992). Narvaez *et al.* (1997) reported that certain isolates of *B. bassiana* sporulated well when cadaver of the host insect *Hypothenemus hampei* was used as the substrate. Puzari *et al.* (1997) reported that a mixture of rice hull, saw dust and rice bran in the ratio 75:25:100 was an ideal substrate for mass production of *B. bassiana.*

Metarhizium sp. could be multiplied using wheat, sugarcane or potato as food source (Martigoni, 1964). Bell (1974) obtained good growth of this fungus on wheat bran, while Aquino *et al.* (1977) used soaked rice grain as substrate for mass multiplication of *M. anisopliae*. Soaked rice bran could be used as a suitable substrate for mass production of *Metarhizium* sp (Batista *et al.*1989). Narvaez *et al.* (1997) reported that certain isolates of *M. anisopliae* sporulated well on rice substrates while certain other isolates sporulated well when cadaver of the host *Hypothenemus hampei* was used as the substrate. For large scale production of *M. anisopliae* sterile humid rice was found to be the best substrate (Alvarez *et al.*, 1997).

The fungus *Cephalosporium lecanii* pathogenic to coffee green bug *Coccus viridis* was cultured on moist sterile sorghum grains by Easwaramoothy and Jayaraj (1978). Use of ground almond mesocarp was found to be suitable for growth and sporulation of *Verticillium lecanii* (Lopez *et al.*, 1998).

2.4.2 Liquid substrates

Utilization of naturally available liquid substrates for mass production of entomopathogenic fungus was attempted by Batista *et al.* (1989). Manisekarame and Letchoumanane (1996) tried coconut water, par boiled rice water, par boiled rice gruel, raw rice water and raw rice gruel for mass production of *F. pallidoroseum*. They observed maximum biomass production in coconut water compared to other substrates.

Batista *et al.* (1989) observed better conidial production by *B. bassiana* in bran broth compared to rice and potato broth. Coconut water was found to yield maximum number of spores among various liquid media tried for mass production of *B. bassiana* and *Paecilomyces fumosoroseus*, against *Plutella xylostella* (Ibrahim and Low, 1993).

Dangar *et al.* (1991) used coconut water from copra making industry for mass production of *M. anisopliae* against rhinoceros beetle *Oryctes rhinoceros*.

2.5 Formulation of entomopathogenic fungi

Formulation of entomopathogenic fungi has been defined as the appropriate combination of ingredients required to make the fungus suitable for agricultural

applications (Soper and Ward, 1981). Success of any biocontrol agent, like chemical insecticides, depends on the type of formulation used. Formulation can be liquid or solid.

2.5.1 Dry formulations

The common dry formulations used for biocontrol include wettable powder, dust, granules and baits.

2.5.1.1 Wettable powder formulations

A wettable powder formulation of *F. pallidoroseum* with diatomaceous earth was developed by Faizal (1992) and Sunitha (1997). They found that the formulation at a concentration of $3.5x10^6$ spores ml^{-1} was as effective as aqueous suspension of the spore in controlling *Aphis craccivora*.

Ravensburg *et al.* (1990) observed that the wettable powder formulation of *V. lecanii* increased the viability of conidiophores 50 fold compared to its blastospore suspension in water. The half life of fungal spores of *V. lecanii* in wettable powder formulation could be extended by adding sugar or glycerol (10 to 25%) to the spray fluid (Kleespies and Zimmermann, 1994).

Zhang *et al.* (1992) developed a wettable powder formulation of *B. bassiana* against larvae of *Ostrinia furnacalis*. This formulation when sprayed at 50×10^9 spores g^{-1} resulted in 95 per cent death of the target insect.

2.5.1.2 Dust formulations

Faizal (1992) compared the efficacy of dust and wettable powder formulation of *F. pallidoroseum* against *A. craccivora* and found that diatomaceous earth based wettable powder was better than talc based dust formulation. The powder formulation of *B. bassiana* was effectively used against the fire ant *Solenopsis invicta* by Oi *et al.* (1994). They found that application of conidial powder in late autumn and early summer resulted in an infestation of 60 and 52 per cent respectively.

2.5.1.3 Granular or Pellet formulations

Schimazu *et al.* (1992) devised a novel method of application of *B. bassiana* for the control of *Minochamus alternatus* the host of pine wood nematode *Bursaphelenchus xylophilus* by implanting wheat bran pellets with the fungus, below the tree bark. Chiue (1993) prepared granules of *B. bassiana* using sand and wine derivatives for controlling *Ostrinia furnacalis*. Dry rice grain based inoculum of *B. bassiana* was formulated by Maniana (1993) for controlling the maize stem borer *Chilo partellus*.

Andersch (1992) patented the process of formulating *Metarhizium anisopliae* as pellets prepared by drying the fungus cells cultured and optimised in fermenters. The dried product named B10 1020 consists of granules with a diameter 0.5 - 1.0 mm. The tissue like cross linking of fungal hyphae in the granulate makes for extremely high resistance to mechanical stress, preventing the formation of dust. The fungus has high specificity for coleopterans. Prior *et al.*

(1991) found that the foliar application of *M. flavoviride* as granular formulation of maize grits containing conidia was effective for the control of *O. nubilalis*. The fungus was also formulated as granulates of dried mycelium against pea weevil *Sitona lineatus*.

2.5.1.4 Bait formulations

Zoebisih and Stimae (1990) formulated *B. bassiana* spores at 15 to 30 per cent as a bait using maize, wheat or vermicullite alone or mixed with soya oil. This formulation gave only 33.9 per cent mortality of *Solenopsis invicta* at the end of seven days. Lima (1992) reported that the conidia of *B. bassiana* formulated as bran bait gave good control of *Schistocerca gregaria*. Wright and Chandlier (1992) formulated a *B. bassiana* bait with cotton products, grand lure (boll weevil pheromone), sticker and ultraviolet protectant (Nufilm 7) for the control of cotton boll weevil *Anthonomus grandis*. Formulation of *M. flavoviride* into attractant bait resulted in prolongation of contact and increased the mortality of *S. gregaria*, the desert locust (Caudwell and Gatehouse, 1996).

2.5.2 Wet formulations

2.5.2.1 Water as carrier

Faizal (1992) formulated spores of *F. pallidoroseum* as an aqueous suspension and found that the formulation at a concentration of 3.5×10^6 spores ml^{-1} was effective in controlling *Aphis craccivora*. Similar results was also reported by Sunitha

(1997). According to her 92 per cent mortality of *A. craccivora* was possible when aqueous solution of *F. pallidoroseum* at a concentration of 7×10^6 spores ml^{-1} was sprayed on cowpea plants in the field.

The aqueous blastospore suspension of *B. bassiana* at a concentration of 2×10^{14} spores per hectare caused 85 per cent mortality of *Melolontha hippocastani*, a pest of Oak (Buchard and Trzebitzky, 1990). Badilla and Alves (1991) reported that the spore suspension of *B. bassiana* at a concentration of 4.5×10^{11} conidia per sugarcane piece resulted in 92.3 per cent mortality of sugarcane weevil *Sphenophorus levis*. The mineral oil-water emulsion of *B. bassiana* caused complete destruction of the grasshopper *Oedalus senegalensis* and *Diabolocatantops axillaris* when sprayed directly on pest (Lima *et al.*, 1991). Dorschner *et al.* (1991) observed the destruction of hop aphid *Phorodon humuli*, within three or four days when the spore suspension in water was sprayed at a concentration of 10^8 conidia per milli litre. The use of aqueous conidial suspension of *B. bassiana* was found to be effective in controlling *Chilo partellus*, the maize stem borer (Maniana, 1993). For the control of pear psylla *Cacopsylla pyricola*, the conidial suspension of *B. bassiana* in water was found to be very effective (Putreka *et al.*, 1994).

Sopp *et al.* (1989) prepared the blastospore suspension of *V. lecanii* to control the chrysanthemum aphid, under green house condition. The ultra low volume spray of the formulation, at a concentration of 2.5×10^3 spores ml^{-1} gave complete control of the pest. Aqueous conidial suspension of *V. lecanii* was used for soil drenching, with or without a wetting agent. The suspension could effectively kill the host insect in a loamy soil (Hirte *et al.*, 1994).

The fungi *Entomophthora thaxterina*, *E. virulenta* and *Basidiobolus sp.* were formulated as conidial suspension in water which could effectively control the aphids *A. fabae*, *A. pomi*, *Macrosiphum rosae*, *Hyalopterus pruni* and *Anuraphis subterrana* (Churdare, 1998).

2.5.2.2 Oil as carrier

Inglis *et al.* (1996) reported that the conidia of *B. bassiana* killed the grasshopper at a faster rate when oil was used as a carrier compared to water.

The oil based spray of *Metarhizium flavoviride* was formulated by Bateman *et al.* (1991) for the control of adult *Schistocerca gregaria* in field at 73 to 80 per cent relative humidity. Prior *et al.* (1991) formulated the conidia of *M. flavoviride* in oil diluents, suitable for controlled application using rotary atomiser for the control of *S. gregaria.* Bateman *et al.* (1991) demonstrated that, under low humidity conditions, the virulence of *M. flavoviride* against the locust *S. gregaria* can be increased substantially by formulating it in an oil, rather than in water. Hunt *et al.* (1994) demonstrated that the incorporation of sunscreens Eusolex 8021 and Parsol MCX into the oil formulations of *M. flavoviride* protected the conidia when exposed to simulated solar radiation for 2 h while the conidial germination was decreased when the exposure time was increased to 5 h. Use of sunscreens like oxybenzone to a ULV oil formulation of *M. flavoviride* did not increase the mortality of grasshopper *Krausella amabile* (Shah *et al.*, 1998).

2.6 Storage of entomopathogenic fungi

Shelf life is an important criterion to be taken into consideration while developing a biopesticide. There is no universal method for storing different biopesticides. Selection of a method must be based on the nature of the organism, carrier material, storage conditions, etc.

2.6.1 Carrier materials

2.6.1.1 Storage in water

Several bacterial and fungal cultures are maintained in submerged conditions for several months. However, water as a storage medium is not preferred because of problems of contamination, spore germination, dehabilitating metabolism of spores, release of metabolites, leaching of nutrients and adverse effect of concentration of spores.

Sporulation of the stored suspension of *F. pallidoroseum* was retained only upto 5 months after storage when it was stored in distilled water at room temperature (Sunitha, 1997). *Beauveria* and *Metarhizium* conidia when stored in water with or without wetting agents retained 80-90 per cent germination even after 12-18 weeks of storage (Moorhouse, 1990). Kleespies and Zimmermann (1994) found that *M. anisopliae* and *V. lecanii* conidia survived better in water plus 10-25 per cent glycerol, than in pure water.

2.6.1.2 Storage in oil

Stathers *et al.* (1993) reported that kerosene was better than soya oil and both were much better than ground nut oil for storing *M. flavoviride* conidia.

Spores of *M. flavoviride* in oil protected it from light and preserved its virulence (Hunt *et al.*, 1994, Welling *et al.*, 1994).

Improved survival of *M. flavoviride* was recorded when it was stored in groundnut or soya oil containing 0.01 pér cent antioxidants like butylated hydroxyanisole and 0.01 per cent butylated hydroxy toluene (Moore *et al.*, 1995).

According to Moorley-Davies *et al.* (1995) conidia of *M. flavoviride* survived better when stored as dry powder with silica gel than as an oil suspension. However, reverse effect was reported by Moore *et al.* (1996) which may probably be due to the uptake of moisture from air by the conidial powder because the containers used were inadequately air tight. The best procedure for oil sprays as suggested by Moore and Caudwell (1997) was to store the conidia as dry power with silica gel and then mix with oil as near as possible to the spray time. Burges (1998) reported that storage of *M. flavoviride* in diesel oil, odourless kerosene oil, shellsol K, aviation fuel, groundnut oil or soya oil did not make any consistent difference in shelf life.

2.6.1.3 Storage in other materials

F. pallidoroseum spores retained 75 per cent viability till four days of storage and thereafter a significant decrease was noticed in the virulence of the water, talc and diatomaceous earth formulations (Faizal, 1992).

Sunitha (1997) observed that viability of *F. pallidoroseum* formulation could be retained for 10 months, under refrigeration when stored as wettable powder using diatomaceous earth as carrier material.

The spores of *B. bassiana* formulated as wettable powder gave a spore germination rate of more than 85 per cent after 8 months of storage under refrigeration. The pathogenicity was maintained even after storage for one year at room temperature (Zhang *et al.*, 1992).

Moore *et al.* (1996) suggested that addition of sachets of silica gel pellets can improve the conidial survival of *M. anisopliae* by 33 per cent.

Cadaver storage of the fungus *Neozygites fresnii* was attempted by Steinkraus and Slaymaker (1994). The semidry cadaver of *A. gossypii* was stored at -15°C. The discharge of conidia from cadaver took one hour after rehydration. The cappiliconidia of the fungus remained infective for 14 days at 75 per cent or 100 per cent RH at 20°C and 25°C respectively.

2.6.2 Storage temperature

Sunitha (1997) observed that compared to room temperature, the shelf life of *F. pallidoroseum* spores could be doubled by storing the spores in diatomaceous earth under refrigeration. Further the virulence was reduced to 50 per cent at the end of 10 months when stored at room temperature, while at refrigeration 91.4 per cent of the spores retained the virulence. With the aqueous spore suspension of the fungus, the virulence was 78.3 per cent when stored under refrigeration.

Blachere *et al.* (1973) observed 85 per cent and 100 per cent reduction in the survival of *B. brongniartii* when it was stored for four and seven weeks

respectively, at 23°C. However when it was stored at 4°C, percentage of spores survived was 16, even after 8 months. According to Moorehouse (1990) survival of conidia of *Beauveria* was maximum when it was stored in water at 15-25°C. At the end of 12-18 weeks, 96-80 per cent germination was noticed. The viability of conidia of *B. brongniartii* was maintained at 98 per cent for two years when stored at 2°C (Aregger, 1992). Zhang *et al.* (1992) obtained a spore germination of more than 85 per cent after eight months when the spores of *B. bassiana* was stored at 10-20°C as wettable powder.

The conidia of *M. anisopliae* retained 95-96 per cent survival after 130 days (18 weeks) when stored at 10-25°C in 0.01 per cent Triton X - 100 solution compared to 82-92 per cent germination of its blastospores in the same solution (Gillespie, 1986). Moorehouse (1990) also reported 80-96 per cent germination of *Metarhizium* conidia when stored at 15-25°C for 18 weeks. Andersch (1992) could store *Metarhizium* for two years without even using a carrier or additive, when the product was vacum packed and stored at low temperature. According to Bailey and Rath (1994) rapid drying of conidia of *M. anisopliae* at $\geq$ 30-35°C is critical for preservation of spore viability. The survival ability of *M. anisopliae* conidia was improved at 4°C when compared to storage at 20°C, when starch, Ringer's solution and glycerol (10-25 per cent) were used as carrier (Kleespies and Zimmermann, 1994). Moore *et al.* (1995) could extend the shelf life of *M. flavoviride* as powder by storing at low temperature. The germination percentage was 42 after four to five months at 25-37°C while it was 97 per cent at 10-12°C.

Chen *et al.* (1990) observed that the entomogenous fungus *Paecilomyces cicadae* isolated from the cicadid *Platylorria pieli* retained pathogenicity even

after one year when stored at room temperature. On the other hand only few blastospores of *P. fumosoroseus* survived after 24 hours of storage on silica gel, sand or diatomaceous earth at 20-23°C.

Germination of *V. lecanii* conidia was reduced to 50 per cent after storing for eight days at 2°C (Kanagaratnam, 1980), while the survival was improved at 2-4°C but not at -20 or + 20°C by addition of additives like 10-25 per cent glycerol, 10 per cent hydroxy ethyl starch, 25-100 per cent Ringer's solution, etc. (Kleespies and Zimmermann, 1994).

Li *et al.* (1993) found that *Erynia radicans* and *E. neoaphidis* could be stored at 4°C for several weeks but were largely destroyed by freezing. The semidry cadaver of *A. gossypii* infested by *N. fresenii* when stored at -15°C released the viable conidia after 1h. The cappiliconidia of the fungus remained infective for 14 days at 75-100 per cent RH, when stored at 20-25°C (Steinkraus and Slaymaker, 1994).

2.7 Effect of botanicals on growth and sporulation of fungi

In an integrated pest management programme, an entomopathogenic fungus can be recommended only after testing its compatibility with other pest control methods, viz. cultural, physical, mechanical and biological which includes the use of botanicals also. Several laboratory studies were conducted by various workers to test the inhibition of fungi by various botanicals.

It was found that leaf extract of aspen inhibited the growth as well as sporulation of the plant pathogenic fungus *F. roseum* (Grosjean, 1950). Alice (1984) found that the leaf extract of *Bougainvillea glabra* inhibited *Pythium monospermum* and leaf extract of *Catharnthus roseus* inhibited *F. moniliformae*. *F. moniliformae* was also found to be inhibited by the leaf extract of *Glyricidia maculata*. While studying the antifungal activity of some plant extracts on pathogenic fungi, Narayana Bhat and Sivaprakasam (1994) observed that *Azadirachta indica* caused 24.4 per cent inhibition on the growth of *Pythium aphanidermatum* when cold water extract of plant was used to poison the PDA to which the fungus was inoculated. When hot water extract was used the percentage inhibition was only 18.8 per cent. Senthilnathan and Narasimhan (1994) observed that *A.indica* leaf extract caused 44.9 per cent inhibition on mycelial growth of *Alternaria tenuissima* and 46.6 per cent inhibition on spore germination. They also found that the seed oil emulsion of *Azadirachta indica* caused 25.63 per cent inhibition of spore germination and 24.5 per cent inhibition of mycelial growth. Ganapathy and Narayanasamy (1994) observed that leaf extract of *A. indica* caused 95.1 and 91.15 per cent inhibition of growth of *Phaeoisiopsis personata* and *Puccinia arachidis*. They also found 7.8 and 11.78 per cent inhibition of these fungi respectively with leaf extract of *Thevetia nerifolia* and 52.46 and 43.53 per cent inhibition by *Bougainvillea spectallis* leaf extracts. The spore germination percentage recorded for these fungi were 4.34 and 7; 81.41 and 69.37; 41.95 and 42.96 respectively. Muthulakshmi and Seetharaman (1994) reported 27.24 per cent and 41.72 per cent inhibition of *Alternaria tenuis* by 5 per cent and 10 per cent leaf extract of bougainvillea respectively.

2.8 Field efficacy of entomopathogenic fungi

The field studies conducted by Sunitha (1997) using water suspension and wettable powder formulations of *F. pallidoroseum* revealed that the spray fluid at a concentration of $7x10^6$ spores ml^{-1} could cause cent per cent mortality of *Aphis craccivora* in cowpea within 12 days of treatment. She found that this treatment was as effective as application of 0.05 per cent quinalphos.

Beauveria bassiana at a concentration of 2×10^5 spores mg^{-1} when applied to banana plants at the rate of 4-16 g per plant reduced the attack of the pseudostem borer *Cosmopolites sordidus* (Ayala and Monzon, 1977). Buchard and Trzebiezky (1990) conducted a field study using *B. brongniartii* against adults of *Melolontha hyppocastani* on oak and obtained a decrease in larval density upto six fold after six weeks of treatment. Badilla and Alves (1991) found that *B. bassiana* isolates were effective against the sugarcane pest *Sphenophorous levis*. The fungus at a rate of 4.5×10^{11} conidia per piece of sugarcane could cause 92.3 per cent mortality of the pest. Lima *et al.* (1991) tested *B. bassiana* against *Oedalus senegalensis* and *Diabolocantops axillaris* under field conditions and obtained significant mortality of the insects when *B. bassiana* was sprayed directly on to the insects. Dorschner *et al.* (1991) found out that the aphid derived isolate of *B.bassiana* was effective against the hop aphid *Phorodon humulii*, under field conditions and the potential of the fungus for long term control was demonstrated by them. Bing and Lewis (1992) observed immediate suppression of *Ostrina nubilalis* in maize plants under field conditions by applying *B. bassiana*. Zhang *et al.* (1992) tested the wettable powder formulation of *B. bassiana* in the field

against the larvae of *Ostrinia furnacalis* and obtained 80 per cent control. Maniana (1993) conducted field experiments to evaluate the efficiency of dry rice grain based inoculam, soaked rice grain based inoculam and aqueous conidial suspension of *B. bassiana* against maize stem borer, *Chilo partellus*. Of these, dry rice grain based inoculam induced highest fungal infection two weeks after application. Santiago *et al.* (1994) found that *B. bassiana* was superior to *M.anisopliae* in the control of rice bug *Leptocorisa* sp. under field conditions. A single application of *B. bassiana* was as effective as the insecticide chlorpyriphos.

Rangaswami *et al.* (1968) reported for the first time the feasibility of microbial control of lepidopteran pests like *Helicoverpa* spp. with *Metarhizium*. Gopalakrishnan and Narayanan (1989) reported that at a concentration of 1.8×10^9 conidia ml^{-1}, *M. anisopliae* suspension was sufficient for controlling rice brown plant hopper *Nilaparvatha lugens* under field conditions. Bateman *et al.* (1991) successfully used oil based ULV sprays of *M. flavoviride* conidia to control adults of *Schistocerca gregaria*. Reduction of feeding by *S. gregaria* after applying *M.flavoviride* was also reported by Moore *et al.* (1992). Efficiency of dried mycelium granules of *M. anisopliae* was tested against *Sitona lineatus* by Verkleif *et al.* (1992). Khaderkhan *et al.* (1993) could get cent per cent mortality of *Semonotus japonicus* after 30 days by applying two grams of B10 1020, a granular formulation of *M. anisopliae*, around the trunk of Japanese cedar. They found that it was also effective against the termite *Odontotermes brunneus*. Baker *et al.* (1994) applied conidia of *M. flavoviride* isolate F. 1985 for the management of *Phaulacrididum vittatum* on pasture in Australia and observed marked reduction in the treated fields, one month after treatment. Santiago and

Madina (1994) reported *M. anisopliae* was not as effective as *B. bassiana* in controlling *Leptocorisa* sp, the rice bug. *M. anisopliae* alone or in combination with other pest control methods were equally effective in lowering the infestation of sweet potato weevil *Cylas formicarius* (Limon and Colting, 1995). Experiments conducted to standardise the field application of *M. anisopliae* in coconut plantations for the control of *Oryctes rhinoceros* revealed that the fungus cultured in maize grain when applied at a rate of five kg per 10 x 3 m heaps of rotting coconut debris resulted in cent per cent mortality of the grubs when applied at monthly intervals (Tey and Ho, 1996). The mortality rate got reduced to 88 and 71 per cent when applied at bimonthly and trimonthly intervals, respectively.

Squibbs (1934) reported that the Indian strain of *Cephalosporium lecanii* proved effective for controlling the scale insects of potted coconut seedlings. Evalakhova (1938) studied the control of mandarin orange scale *Ceroplastes sinensis* with *C. lecanii*. He observed effective control of the pest when fungal spores were applied as a dust to infested plants. Easwaramoorthy and Jayaraj (1978) used the same fungus for controlling coffee green bug *Coccus viridis* and they found that fortnightly application of the fungus at 16×10^6 spores ml^{-1} twice, resulted in 73.1 per cent mortality. 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*, *Sitobion avenae* and *Myzus persicae* (Hussey and Tinsley, 1981). Khalil *et al.* (1983) studied the effect of *V. lacanii* against aphid species *Brachycaudus*, *Macrosiphoniella sanbornii* and *Myzus persicae* on cucumber in glass house condition and found that the fungus was

highly effective against these aphids at 25 ± 2°C and 100 per cent relative humidity. The effectivity of the fungus against the aphids *A. gossypii*, *M. persicae*, *B. helichrysi* and *A. fabae* was reported by Grunberg *et al.* (1988) and against *A. gossypii* on chrysanthemum by Sopp *et al.*,(1989).

Devi (1994) identified the fungus *Nomuraea rileyi* against *Spodoptera litura* on *Ricinus communis*. The fungus could cause 100 per cent larval mortality after ninth day of spraying. A dose of $5x10^4$ hyphal bodies of *N. rileyi* caused 100 per cent mortality of *Spodoptera exigua* (Lopes and Boucias, 1994) and *S. frugiperda* (Lerana *et al.*,1994) within three to four days.

2.9 Safety of entomopathogenic fungi

Any entomogenous fungus to be a successful candidate in microbial control should be tested for its pathogenicity to commonly cultivated crop plants and other non target organisms like beneficial insects as well as to man and other vertebrates.

2.9.1 Safety to crop plants

The fungal pathogen *F. oxysporum* infectious to *Nilaparvatha lugens* was proved to be nonpathogenic to cotton and tomato (Kuruvilla, 1978). Nonpathogenicity of *F. moniliformae* variety *subglutinans,* infectious to epilachna beetles was proved on crop plants like cotton, tomato, bittergourd, brinjal and snake gourd (Beevi and Jacob, 1982). Nagalingam (1983) reported the

nonpathogenic nature of *F. semitectum* isolated from green peach aphid *Myzus persicae* to chillies, cabbage, brinjal and tobacco. Studies conducted by Hareendranath (1989) clearly proved the nonpathogenic nature of *F.pallidoroseum* isolated from black pea aphid *Aphis craccivora* to rice, bhindi, chillies and tomato. Manisegarame and Letchoumanane (1996) proved that *F. pallidoroseum* isolated from rice leaf roller *Craphalocrocis medinalis* was not pathogenic to crop plants like rice, tomato, chillies and bhindi. Nonpathogenic nature of the *F. pallidoroseum* isolated from *A. craccivora* to vegetables like bhindi, brinjal, amaranthus, tomato, chillies, snakegourd and bittergourd and medicinal palnts like adathoda, ocimum and notchi was proved by Sunitha (1997).

2.9.2 Safety to natural enemies

Nonpathogenicity to common predators and parasites associated with the environment of the host insect is an ideal attribute for any candidate pathogen. *F. semitectum* a fungal pathogen of *Myzus persicae* was safe to all instars of coccinellid predators of the aphid as well as to the hymenopteran parasitoids (Nagalingam and Jayaraj, 1986). Hareendranath (1989) reported *F. pallidoroseum* to be nonpathogenic to *Menochilus sexmaculata*, an efficient predator of pea aphid. Its safety to *Coccinella septumpunctata* and *Scymnus* sp. was proved by Sunitha (1997).

Sundara Babu *et al.* (1983) reported that topical application of *M. anisopliae* to larval and pupal parasites of *Opisina arenosella* did not cause mycosis. The fungus was also safe to predators like *Cyrtorhinus lividipennis*,

Coccinella arcuata and *Lycosa pseudoannulata* and parasitoids like *Trichogramma japonicum* and *Platygaster oryzae* (Rao, 1989). But pathogenicity of *Beauveria* to the predatory insects including coccinellids were reported by Magalhaes *et al.* (1988).

Parasites attacking *Helicoverpa* larvae and a spider was susceptible to the entomogenous fungus *N. rileyi* (Powers *et al.*, 1986). Both larvae and adults of *Chrysoperla carnea* when fed with nitrogen deficient diet were susceptible to entomogenous fungi (Donegan and Lightert, 1989).

2.9.3 Safety to beneficial insects

F. semitectum a fungal pathogen of *Myzus persicae* was found safe to all instars of mulberry silk worm and adult honey bees (Nagalingam, 1983). *F.pallidoroseum* was reported to be safe to Italian honey bee *Apis mellifera* and mulberry silkworm *Bombyx mori* (Sunitha, 1997) when applied at a concentration of 7×10^6 spores/ml.

Madelin (1966) and Aoki (1974) reported that *Metarhizium anisopliae* and *N. rileyi* infected silk worm. However its long spored taxon var. *major* was nonpathogenic to silk worm (Sundara Babu *et al.*, 1983).

2.9.4 Safety to mammals

Any intervention in an ecosystem will have an impact, so there is a need for risk assessment to determine the level of hazard involved in the exploitation of microorganisms as biological control agents in agriculture.

Muller Kogler (1965) reported that injection of *Paecilomyces farinosus* to rabbits did not adversely affect the animals. Scharffenberg (1968) observed that subcutaneous and intravenous injections of 0.5 ml of two per cent spore suspensions of *M. anisopliae* and *Beauveria bassiana* on rats did not cause any morbid changes. Investigation of the tissues showed the absence of fungal mycelium in the blood and organs, after a two month observation period. In inhalation experiments using the same, when rats were exposed to 4 mg of pure spore dust per cubic metre in a sealed box, no pathogenic or toxic effects, or allergic reactions were noted over two months. Oral treatments with the fungal bran was tested in three different groups of animals. The first group of animals fed with rich diet and fungal bran did not exhibit any weight loss and had normal appetite. In the second group fed with normal diet and fungal bran the test animals exhibited 30 per cent loss in weight and loss of appetite. In the third group fed with fungal bran alone 30 per cent animals died and the remaining ones suffered 40 per cent weight loss, diahrrhoea and complete loss of appetite. The surviving animals when supplied with normal diet, their appetite returned and regained normal weight by three weeks. Austwick (1980) observed that species of genera such as *Metarhizium* have conidia that are too large to lodge alveoli of the lungs and those of slime spored fungi such as *Verticillium* do not become airborne and did not pose a serious risk. Elkadi *et al.* (1983) carried out safety test in guinea pigs and mice with *M. anisopliae*. The fungus was administered by ingestion, inhalation, cutaneous and subcutaneous injection. Anatomical and histopathological examination showed that the fungus was neither toxic nor pathogenic. Jeevanand and Kannan (1995) evaluated the mammalian toxicity of *M. anisopliae* and *M. flavoviride*, biocontrol agents of the coconut pest *Oryctes rhinoceros*. The spore suspension of these fungi when given

orally and parentally to albino rats did not cause any change in appearance and behaviour even 21 days after administration.

2.10 Pathogenicity to mammals

Human allergic reactions like tiredness or weekness, neck or head pains and giddiness were reported by various scientists who worked with *B. bassiana* (Muller - Kogler, 1967). However, in most cases symptoms disappeared in a relatively short time. The serious risk of allergenic reactions are usualy noticed with sensitation through lungs especially with fungi with smaller conidia (< 3 μm in diameter) such as *Beauveria* and *Paecilomyces* species (Austwick, 1980). Lucia *et al.* (1984) studied the sensitivity of rabbit skin to culture extracts of *F. poae*. The depilated area of the flank of rabbits treated with *F. poae* showed cellular degenerative process. Rats fed with the same isolate developed internal haemmorhages. Intratracheal inoculation of live *F. oxysporum* microconidia in rabbits induced necrotic lesions and non-cellular lesions with hyphal growth of inoculated microconidia.

Ishibashi *et al.* (1986) studied the pathogenicities of *Cylindrocarpon tonkinense* and *F. solani* in the rabbit cornea. The fungal microconidia was inoculated at a rate of 10^{14} conidia per cornea. Corneal lesions were produced in both the eyes and the fungus invaded the anterior chamber on the fifth day after inoculation. Crude *Fusarium* toxins and its biological activities were studied by Qin *et al.* (1987). They isolated *F. semitectum* and *F. equiseti* from mouldy rice straw, collected from an area where sore foot disease occurred in cattle.

The culture extracts were tested on rabbits, mice and sheep. They caused skin reactions in all rabbits. After several applications oedema, haemorrhage and necrosis were produced. Eighty three per cent of treated mice died one hour to 12 days after oral or subcutaneous administration of the extract. In some animals, the tips of ears, tails and feet became oedematus, haemorrhagic and necrotic, while others showed haemorrhagic gastroenteritis. 54.5 per cent of treated sheep died 40 minutes to 20 days after extracts were administered orally or intraperitonially.

2.11 Toxins produced by entomopathogenic fungi

The importance of toxins to the virulence of an entomopathogenic fungus is difficult to evaluate because toxin production is preceeded by several activities of the fungus (Roberts, 1981).

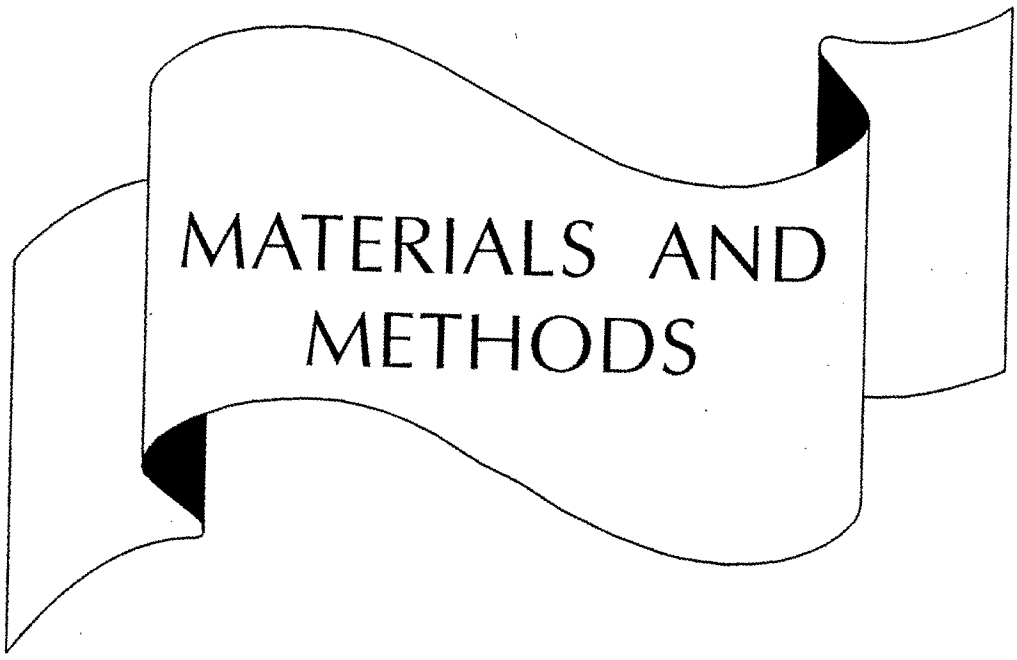
Two strains of *Fusarium solani*, one pathogenic to bark beetle *Scolytus scolytus* and the other to lobster *Homarus americanus* grown in liquid medium produced substances toxic to adult blow flies *Calliphora erythrocephala*, by intrahaemocoelic injection (Claydon *et al.*, 1977). The insecticidal activity was fully accounted for by the naphthazarin pigments fusarbin and anhydrofusarbin from both strains and javanicin and fusaric acid from the lobster strain.

Intrahaemocoelic injections of extracts from the fungus killed silkworm larvae into healthy larvae indicated that three entomopathogenic fungi *B. bassiana*, *M. anisopliae* and *A.ochraceous* produced significant amounts of toxic compounds within their host (Kodaira, 1961). *B. bassiana* produces a toxin, the desipeptide

beauvericin, which is toxic to mosquito larvae (Hamill *et al.*, 1969) and to cockroach (Vey and Quiot, 1975). Suzuki *et al.* (1977) isolated bassionolide from *B.bassiana* and *V. lecanii.* Both these fungi were isolated from dead silkworm larvae. Beauvericin produced by *B. bassiana* did not affect silkworm larvae at 1000 ppm when it was mixed with the artificial diet or injected at 100 μg/1.2 g larvae. Roberts (1981) suggested that some strains of *B.bassiana* and *B. brongniartii* synthesise toxic metabolites since the insect host declines rapidly following invasion of haemocoel. Hung and Boucias (1992) challenged larvae of beet army worm *Spodoptera exigua* with blastospores of *B.bassiana* and monitored the immune response *in vivo.* They recorded a fall in granulocyte, and fall in effective phagocytosis by haemocytes, suggesting the role of mycotoxins produced by the fungus in larval pathogenesis.

When Soxhlet extract of *Metarhizium* killed silkworm, were given as intrahaemocoelic injection to healthy silkworm they were killed indicating the presence of toxins in the extracts (Kodaira, 1961). The culture filtrate of *M.anisopliae* was toxic to wax moth larvae, *Galleria melonella* (Robert, 1966). Electron microscope studies of elaterid larvae infected by *M. anisopliae* revealed many intrastructural alterations in cells not invaded by the fungus presumably induced by fungal toxins (Zacharuk, 1971). Vey and Quiot (1975) found that the culture filtrates of *M. anisopliae* were toxic to *Oryctes rhinoceros* haemocytes *in vitro*, producing organelle changes. Six cyclodepsipeptides with the same five member amino acid skeleton of B alanine, alanine, valine, isoleucine, and proline were isolated from filtrates of *M. anisopliae* cultures by Suzuki *et al.* (1977). They found that the toxins produced by the fungus namely destruxins A and B

have an LD 50 of 0.15 - 0.3 μg/g in silkworm larvae at 24 h when administered by intrahaemococelic injection. Steyn (197 /) studied the biological activities of *Metarhizum* metabolite cytochalasins. These metabolites inhibited cytoplasmic changes in cultured mammalian cells, inhibition of cell movement and nuclear extrusion. Lin and Roberts (1986) found that *M. anisopliae* var.*anisopliae* is the major source of mycotoxin, destruxin A compared to *M. anisopliae* var.*major*. The biological effects of destruxin was investigated by Samuels *et al.* (1988). When a purified mixture of destruxin A (80 per cent), destruxin B (10 per cent), desmethyl destruxin B and destruxin A2 was applied to a semi isolated ventrolongitudinal muscle from larvae of *Manduca sexta,* produced a sustained dose dependent muscle contraction and depolarisation of muscle fibre membranes, which induced muscle contraction. Krasnoff *et al.* (1991) described the insecticidal properties of five efrapeptides extracted from broth culture of *Tolypocladium niveum* against the mosquito *Aedes sirensis*. Sloman and Reynolds (1993) described the method by which destruxin produced a significant reduction in ecdysteroid secretion by prothoracic gland of *M.sexta*. Parry (1995) demonstrated that the fungal metabolite, swinsonine, from *Metarhizium anisopliae* could inhibit the *in vivo* activity of α mannosidases which is involved in golgi body glycoprotein processing in mammals.


MATERIALS AND
METHODS

3. MATERIALS AND METHODS

3.1 Raising crops

The studies on the "Production and Evaluation of the fungus *Fusarium pallidoroseum* (Cooke) Sacc. as a biopesticide against *Aphis craccivora* Koch." was carried out in the Department of Entomology, College of Agriculture, Vellayani during 1997-2000. Seeds of cowpea, variety Malika which was used as the host plant of the pea aphid *A. craccivora* were obtained from the Instructional farm, College of Agriculture, Vellayani. The plants were raised in 30 cm diameter pots filled with potting mixture, following the recommendations of the package of practices of Kerala Agricultural University (1996). Plants were watered daily to keep them succulent thereby enhancing aphid multiplication. In order to get a continous supply of host plants, new seedlings were raised, once the test plants reached the reproductive stage. No insecticide was applied on the crop.

3.2 Rearing aphids

Cowpea plants naturally got infested with the aphids at 2-3 leaf stage. Whenever natural infestation was not observed, the plants were artificially inoculated with aphid colonies collected from other infested cowpea plants, in the evening hours. The colonies were kept free from natural enemies. When plants

reached its late reproductive phase and started drying, in order to get fresh infection, twigs from infested crops were tied to the new crop. Thus the aphid culture was maintained throughout the period of experiment.

3.3 Maintenance of *F. pallidoroseum* culture

F. pallidoroseum culture maintained at the Insect Pathology Laboratory, College of Agriculture, Vellayani was used for the present study. The culture was maintained on Potato Dextrose Agar (PDA) or rice bran. Thirty gram of rice bran moistened with 30 ml of water, sterilized at 1.1 kg cm^{-2} for 20 minutes was used as a standard medium for growing the fungus.

The virulence of the fungus was maintained by passing it periodically (once in a month) through *A.craccivora* and reisolating it afresh.

3.4 Effect of repeated subculturing on the infectivity of the fungus

To study the effect of repeated subculturing on the growth and virulence of the fungus, it was subcultured on PDA slants, every seventh day. Mycelial growth characteristics and virulence of the fungus was recorded after each subculturing. For this purpose the spore suspensions prepared from these subcultures were sprayed on to healthy aphids (50 numbers) colonised on tender twigs or pods of cowpea. The rate of mortality of the aphids were recorded at 24h interval for four days.

3.5 Measurement of conidia of the fungus

For measuring the conidial sizes, seven day old cultures of *F.pallidoroseum* grown on PDA and host extract were used. A bit of the culture was mixed with sterile water and a drop from this was placed on a glass slide and covered with cover glass. These were measured using ocular micrometer and drawn using a camera lucida at 400 x magnification. From each culture, 100 numbers each of macro and micro conidia were measured.

3.6 Mass production of *F. pallidoroseum* using naturally available substrates

3.6.1 Solid substrates

In order to find out the best solid medium for mass multiplication of the fungus, 13 naturally available substrates *viz.*, bran, sawdust, soil, unretted coirpith, retted coirpith, cowdung, pongamia leaves, cowpea leaves, cotton seed cake, gingelly cake, groundnut cake, coconut cake and neem cake were used as the food source. Mature, dried leaves of pongamia and cowpea were cut into small bits before sterilizing while the oil cakes and cowdung were powdered and bran, sawdust, soil and coirpith were used as such. Thirty gram each of the substrate was taken seperately in 250 ml conical flasks, plugged with cotton and sterilized at 1.1 kg cm^{-2} for 20 minutes in an autoclave. Enough water was added to the media to make it just wet. The sterilized substrates were then artificially inoculated with seven millimeter fungal disc of a seven day old culture of *F. pallidoroseum* under aseptic condition. Growth of the fungus was observed by visual comparison

and graded according to the method suggested by Mathai *et al.* (1992). In order to collect the spores, 150 ml of sterile distilled water was added to each of the conical flask and shaked vigorously for two minutes. The suspensions obtained from three replications were taken seperately and filtered through a muslin cloth. From this, a drop of the suspension was used for estimating the spore count using a haemocytometer.

3.6.2 Liquid substrates

Coconut water, boiled rice water (kanjivellam), raw rice water (kadi) and plain water were used as liquid substrates for growing the fungus. The above liquids, 125 ml each were taken seperately in 250 ml conical flasks, plugged with cotton and sterilized at 1.1 kg cm^{-2} for 20 minutes in an autoclave. After sterilization they were inoculated with seven day old culture bit of the fungus and incubated at room temperature. Each of the treatment was replicated thrice.

Growth of the fungus in the media was visually graded after seven days as described under 3.6.1. The mycelial weight of the fungus was also recorded. For this the media with the fungal growth was filtered through a pre weighed What man No.1 filter paper and oven dried at 60°C till two subsequent weights agreed. The intensity of sporulation was estimated on the seventh day using a haemocytometer. The spore count was analysed using CRD.

3.6.3 Influence of the substrates on virulence of the fungus

For this study the spores collected from the fungus grown on different substrates were used. To extract the spores, seven day old cultures were mixed

with 50 ml of water and seived through a muslin cloth. The spore count of the suspension was estimated and adjusted to $3.5x10^6$ spores ml^{-1} by adding enough quantity of water. The test insects were drawn from disease free culture of pea aphids. Wingless adults of uniform size were used for the experiment.

Spore suspension (20ml) prepared from each medium (both solid and liquid) was sprayed on healthy aphids colonised on fresh pods, using an atomiser. Before colonising the aphids, pods were cut into 6 cm bits and kept in sterile petri dishes. In order to keep the pods fresh, both the cut ends of the pods were covered with moist cotton wool. Each treatment was replicated thrice and for each replication 50 numbers of aphids were used. Aphid mortality was recorded daily upto the seventh day. Pea aphids sprayed with water served as the control. The relevant data was analysed using CRD.

3.7 Formulation of *F.pallidoroseum* using different carrier materials

Seven materials namely diatomaceous earth, fine charcoal, bran, sawdust, peat, vermicompost, leaf mold and semidry cadaver of *Aphis craccivora* were tried as the carrier materials for formulating *F. pallidoroseum* spores.

The different carrier materials were powdered and sieved through a 20 mesh sieve before mixing with the spores. Spores required were collected from seven day old cultures of the fungus grown on rice bran medium. For this enough water was added to the fungal growth in conical flasks and mixed thoroughly. The suspensions were then filtered through muslin cloth and the filtrate was

centrifuged at 5000 rpm for 15 minutes in a refrigerated centrifuge. The temperature was maintained at 10°C. Supernatant fluid obtained after centrifugation was discarded and the spore count in the sediment was counted using haemocytometer. The sediment was then resuspended in distilled water so as to get a spore load of 28×10^6 spores ml^{-1}.

This spore suspension (50 ml) was sprayed to 50 g of carrier material spread evenly on a paper to yield a formulation of concentration 7×10^6 spores ml^{-1}. Spraying was done uniformly using an atomiser, and mixed thoroughly and shade dried for 2-4 days and then oven dried at 35 to 40°C for 2 h before storing it in conical flasks.

3.8 Shelf life of *F. pallidoroseum* formulations

3.8.1 Effect of temperature

The formulations kept in conical flasks (125 ml) were stored at room temperature as well as under refrigeration. Asssessment of spore viability and virulence were done at monthly intervals.

3.8.1.1 Assessment of viability of spores

The spore suspension of the formulated material was prepared by suspending one gram of the material in four millilitre of sterile water to get a concentration of 7×10^6 spores ml^{-1}. This was then sieved through sterile muslin cloth. One drop of this suspension was inoculated in the centre of PDA medium in 9 cm petri dishes. The colony diameter was measured at the end of 7 days.The treatments were repeated at monthly intervals and observations recorded on every 24 h.

3.8.1.2 Assessment of virulence of spores

To treat the aphids with *F. pallidoroseum* at a concentration of 7×10^6 spores ml^{-1}, 25 mg each of the formulated material was dusted on healthy aphids of uniform age. Three replications were maintained for each treatment and each replication constituted 50 number of apterous aphids. Treatments were repeated at monthly intervals. Observations were made on aphid mortality at 24 h interval.

The mycelial colony diameter and cumulative per cent mortality of aphids were analysed using CRD.

3.8.2 Effect of packing materials

Based on the preliminary evaluation of viability and virulence, four materials *viz.* diatomaceous earth, charcoal, semidry cadaver of aphids and leaf mold were selected for evaluation of the packing materials and storage temperature. The spore formulation (10 g each) was packed in three types of packing materials viz. aluminium foil, polypropylene bags and glass vials and stored at room temperature as well as under refrigeration. Viability and virulence of the fungus were assessed at monthly intervals, as described under 3.8.1.1 and 3.8.1.2 and data analysed using CRD.

3.9 Effect of botanicals and quinalphos on the growth and sporulation of *F. pallidoroseum*

This experiment was carried out to find out whether the insecticide commonly used against aphids (quinalphos) and botanicals have any detrimental

effect on the growth and sporulation of *F. pallidoroseum*. The treatments used in this experiment were:

T_1 - 5 per cent leaf extract of *Bougainvillea spectabilis*

T_2 - 10 per cent leaf extract of *Bougainvillea spectabilis*

T_3 - 5 per cent leaf extract of *Hyptis sauveolens*

T_4 - 10 per cent leaf extract of *Hyptis sauveolens*

T_5 - 5 per cent leaf extract of *Thevetia nerifolia*

T_6 - 10 per cent leaf extract of *Thevetia nerifolia*

T_7 - 5 per cent leaf extract of *Azadirachta indica*

T_8 - 10 per cent leaf extract of *Azadirachta indica*

T_9 - 10 per cent seed oil emulsion of *Azadirachta indica*

T_{10} - 10 per cent seed oil emulsion of *Hydnocarpus wightiana*

T_{11} - 10 per cent soap solution

T_{12} - 0.05 per cent quinalphos

T_{13} - Control

Soap solution (10 per cent) was used as it is usually incorporated while preparing seed oil emulsions. Each treatment was replicated thrice.

3.9.1 Preparation of leaf extracts

For preparing leaf extracts, fresh leaves along with tender twigs were used. Fifty gram each of washed and shade dried leaf bits were blended and the slurry obtained was then strained through a muslin cloth.

3.9.2 Preparation of seed oil emulsions

Ten millilitre of saturated soap solution was mixed with 90 ml of seed oil to get a stock emulsion of seed oil. Ten millilitre of this stock emulsion was mixed with 90 ml of PDA to get a 10 per cent seed oil emulsion in PDA.

3.9.3 Growth and sporulation of the fungus on poisoned media

Quantity of botanicals necessary to yield the required concentration (mentioned under 3.9) were mixed with 100 ml PDA taken seperately in 250 ml conical flask and sterilized at 1.1 kg cm^{-2} pressure for 20 minutes. Sterilized media after cooling were poured aseptically into sterile petridishes. For studying the effect of quinalphos on the growth of *F. pallidoroseum* the insecticide was incorporated in 100 ml media after sterilization so as to get a 0.05% solution. These poisoned media were then inoculated with 7 mm fungal discs cut out from a seven day old culture using a sterile cork borer. The disc was placed in the centre of the petridish. The inoculated dish were incubated at room temperature. Each treatment was replicated thrice. Diameter of the fungal colony was measured daily, till the growth on control media covered the entire dish.

For estimating the effect of botanicals and insecticide on the sporulation of *F. pallidoroseum*, 7 mm fungal disc were cut out from seven day old growth of the fungus on poisoned food and one disc from each medium was mixed with 10 ml of sterile water for 5 minutes and the spores present in a drop of the suspension was estimated using haemocytometer.

The mycelial diameter and spore count were analysed using CRD.

3.10 Assessment of time and method of application of *F.pallidoroseum* formulations in the field

3.10.1 Details of the experiment

The experiment was conducted in 140 sq.m of field attached to the Instructional farm, College of Agriculture, Vellayani. The cultivation practices were carried out according to the package of practices of Kerala Agricultural University (1996). No plant protection chemicals were used on the plant. The experiment was laid out using randomised block design.

Treatments - 4 x 3 x 2

A. Carrier materials

1. Diatomaceous earth
2. Fine charcoal
3. Semi dry cadaver of *A. craccivora*
4. Leaf mold

B. Time of application

1. 3 weeks after planting
2. 6 weeks after planting
3. No application

C. Method of application

1. Spraying
2. Broadcasting

Number of replications - 3

Plot size - 1 m^2

Sampling unit - 15 cm terminal twig

Sampling frequency - Weekly

Observations on the aphid count before every treatment were recorded in the morning and treatments were applied in the evening. To provide adequate humidity in the field, plants were irrigated and wetted before treatment.

3.10.2 Preparation of spray fluid

To prepare the spray fluid at a concentration of 7 $x10^6$ spores ml^{-1}, 250 mg each of the formulated material (under 3.7) was added to one litre of tap water and sieved through a muslin cloth. Teepol @ 1 ml l^{-1} was added to the spray fluid as wetting agent. Spraying was done in the evening hours using a hand sprayer.

3.10.3 Estimation of aphid population in the field

Incidence of aphids on cowpea was recorded commencing from three weeks after planting. The population was recorded from three plants selected at random from each treatment. Aphid population on the 15 cm long terminal twig with unopened leaves and two opened leaves were taken at weekly intervals till the final harvest. Based on the intensity of infestation these twigs were classified into five classes following the method of Banks (1954). The classes were as follows

1. Zero (0) - no aphids

2. Very light (V) - From one aphid to a small colony confined to the very youngest leaves of the crown.

3. Light (L) - Several aphid colonies present on the stem and not only confined to the uppermost leaves.

4. Medium (M) - Aphids present in large numbers, not in recognisable colonies but diffuse and infesting a large proportion of leaves and stem.

5. Heavy (H) - Aphids present in large numbers very dense, infesting all the leaves and stem, the latter usually being black with aphids.

The collection of samples and estimation of aphid population were done following the method of Srikanth (1985). Ten number of shoots in each class were collected from the experimental field. The sample shoots were cut with a sharp blade ensuring that the number of aphids falling from the shoots was reduced to a minimum. These were then put in plastic containers with provision of ample aeration and were brought to the laboratory.

Each sample shoot was then transferred to a white paper and were gently tapped to dislodge the aphids. The mean number of aphids (all stages) per twig in each class was recorded as follows

Class	Number of aphids per sample										Mean number of aphids per class
	1	2	3	4	5	6	7	8	9	10	
V	64	29	46	38	55	16	9	60	32	20	36.70
L	89	75	120	150	95	103	130	158	98	212	123.00
M	362	286	403	195	205	220	260	396	200	177	270.40
H	475	605	562	498	623	612	535	572	680	784	594.60

3.10.4 Estimation of average yield

Yield was recorded from three randomly selected plants of each treatment for every alternate day commencing from 8 weeks after planting.

3.11 Biosafety evaluation of *F. pallidoroseum* to crop plants and other non target organisms

3.11.1 Crop plants

Certain strains of *F. pallidoroseum* were observed to cause diseases on plants. Such plants were selected to test whether the strain of *F. pallidoroseum* used in this study are pathogenic to them. The plants used for the study were

1. Amaranthus - *Amaranthus* sp
2. Banana - *Musa paradasiaca*
3. Drumstick - *Moringa olifera*
4. Mulberry - *Morus alba*
5. Passion fruit - *Passiflora edulis*
6. Pine apple - *Ananas comosus*
7. Sesamum - *Sesamum indicum*

These plants were raised in 30 cm diameter pots filled with potting mixture containing soil, sand and cowdung in the ratio 1:1:1. The plants were tested for pathogenicity by two methods.

3.11.1.1 Leaf inoculation

Three leaves of uniform age were selected from each plant and their upper surface was injured by pin pricks. Spore suspension (7×10^6 spores ml^{-1}) was smeared on the injured surface and covered with moist cotton wool. Plants injured as before, but without inoculum served as control. The plants were observed for symptom development for two weeks. Each treatment was replicated thrice.

3.11.1.2 Soil inoculation

Hundred millilitre of spore suspension at a concentration of 7×10^6 spores ml^{-1} was incorporated in the top 10 cm of the soil kept in pots just before planting. Three replications were maintained. Plants grown in uninoculated soil served as control. Plants were kept under observation for three months.

3.11.2 Natural enemies

Infectivity of *F. pallidoroseum* was tested on predators of *A. craccivora* viz. the coccinellids *Chilomenes sexmaculata, Coccinella septumpunctata and Micraspis crocea*; syrpids, *Ischiodon scutellare* and *Ischiodon sp*; hemerobid; the spiders *Pardosa pseudoannulata*, *Oxyopus* sp., *Tetragnatha maxillosa*; dragon flies and preying mantids.

The early instar gurbs (5 no. each) of the coccinellids, maggots of syrphids and larvae of hemerobids were taken seperately in petridishes and sprayed with the spore suspension @ $7x10^6$ spores ml^{-1}. The treated larvae were seperated

and provided with aphids as food. Adult coccinellids, spiders, dragon flies and praying mantids (five numbers of each species) were taken seperately in rearing jars and sprayed with the spore suspension. These were provided with aphids or scales as food. Insects sprayed with water served as the control. Three replications were maintained in each case. The treated insects were observed for symptoms of mycosis for its entire life cycle or for one week whichever is possible.

3.11.3 Productive insects and pollinators

The stingless bee *Trigona iridipennis* was collected from the field and fed with diluted honey in rearing jar. These were sprayed with *F.pallidoroseum* spores at a concentration of $7x10^6$ spores ml^{-1}.

Commonly observed pollinators *viz.* adults of *Bombus* sp, *Camponotus* sp., *Syrphus* sp. and *Xylocopa* were collected from the field and kept in rearing jars. These insects were sprayed with spore suspension @ $7x10^6$ spores ml^{-1}. The treated insects were fed with diluted honey. They were kept under observation for symptoms of mycosis. Each treatment constituted 15 number of insects.

3.11.4 Other insect species and mites

Insects belonging to the major insect orders *viz.*, Lepidoptera, Coleoptera and Hemiptera were reared in the laboratory and their mature stages were treated with *F. pallidoroseum* spore suspension @ 7×10^6 spores ml^{-1}. Insects sprayed with water served as control. The treated insects were observed for symptoms of mycosis. The insects used for this purpose are listed below.

Insect Order	Species
1. Lepidoptera	*Scirpophaga incertulas* *Cnaphalocrocis medinalis*, *Sylepta derogata, Leucinodes orbonalis*, *Psara basalis*, *Psara bipunctalis*, *Hymenia recurvalis* and *Spodoptera litura.*
2. Coleoptera	*Henosepilachna vigintioctopunctata*, *Oryctes rhinoceros*, *Cosmopolites sordidus, Odoiporus longicollis, Dicladispa armigera.*
3. Hemiptera	*Leptocorisa acuta*, *Menida histrio*, *Riptortus pedestris*, *Anchon pilosum*, *Nephotettix virescens, Sogatella furcifera, Pseudococcus lilacinus, Ferrisia virgatas.*
4. Orthoptera	*Oxya chinensis*, *Conocephalus pallidus, Hieroglyphus banian, Gryllotalpa africana*
5. Mites	*Aceria guerreronis, Tetranychus cinnabarinus*

Fifteen number of each species were used for the treatment.

3.11.5 Other aphids

Ten different aphid species were collected from the field and reared in the laboratory using their respective host plant twig. The aphid colonies were treated with the spore suspension at a concentration of 7×10^6 spores ml^{-1}. The treated insects were kept under observation for symptoms of mycosis for a period of two weeks. The aphid species used for this study were as follows.

Host plant		Aphid species
1. Brinjal	-	*Aphis gossypii*
2. Coccinea	-	*Aphis gossypii*
3. Hibiscus	-	*Aphis malvae*
4. Eupatorium	-	*Aphis spiraecola*
5. Guinea grass	-	*Hysteroneura setariae*
6. Sorghum	-	*Myzus persicae*
7. Banana	-	*Pentalonia nigronervosa*
8. Cashew	-	*Toxoptera odinae*
9. Pepper	-	Unidentified
10. Tulsi	-	Unidentified

3.11.6 Rabbits

The clinical and toxicological response of rabbits were studied after exposing them to spores of *F.pallidoroseum* by oral, dermal, subcutaneous, respiratory and intravenous routes. Acute (24 h) as well as chronic toxicity (8 weeks) were studied for each of these modes of exposure. The chosen concentration of spores was ten times that of the expected atmospheric contamination during the field application of the fungus.

Healthy New Zealand White rabbits of both sexes obtained from Government Livestock Farm, Kodappanakunnu, Trivandrum or their progeny weighing 750-1000 g, were used for the study. Rabbits were maintained in standard cages (2.5 ft x 2.5 ft) (Plate1) and fed a computed balanced ration containing

bengal gram, green gram, raw carrot, fresh fodder grass and cooked rice. Drinking water was made available *ad libedum* to the animals. Throughout the experiment, the animals were kept under the supervision of a named registered veterinary practioner.

3.11.6.1 Experimental design

Animals were randomly divided into 20 groups, 10 test groups and 10 control groups. Each group contained three animals. Animals in each group were exposed to the spores by anyone of the above mentioned routes. After administration of spores by various routes the biological and pathological response of animals were monitored and evaluated for a period of 24 hours for acute toxicity or eight weeks for chronic toxicity.

The experimental design was adopted from common procedure followed for safety evaluation of chemicals by Dikshith *et al.* (1989).

3.11.6.2 Experimental procedure

3.11.6.2.1 Oral administration

The spore suspension in distilled water (10 ml of spore suspension @ $7x10^7$ spores ml^{-1}) was given as an oral gavage using a three centimetre long blunt end needle and syringe (Plate2). Similar administration with distilled water was done in the control group.

3.11.6.2.2 Dermal application

The site chosen for dermal application of spores was mid abdominal region. The site was prepared by closely clipping the hairs from an area of 4 x 8 cm^2 marked for application of spores (Plate 3). The spore suspension (1ml) at a concentration of $7x10^7$ spores ml^{-1} was sprayed over the marked area using an atomiser. The control group was treated with distilled water.

3.11.6.2.3 Subcutaneous administration

The spores suspended in sterile normal saline ($7x10^8$ spores per 0.1 ml) was injected subcutaneously under the skin in the flank region. The control group was injected with 0.1 ml of vehicle.

3.11.6.2.4 Intranasal administration

7×10^8 spores suspended in 0.1 ml of pyrogen free distilled water was installed intranasally using a three centimeter long blunt-end needle and syringe (Plate 4). The control group received only 0.1 ml of the vehicle.

3.11.6.2.5 Intravenous administration

The spore suspension in sterile normal saline ($7x10^8$ spores per 0.1 ml) was injected into the marginal ear vein. The control group was injected with 0.1 ml of vehicle (Plate 5).

Plate 1. New Zealand White rabbits reared in cages (2.5 x 2.5 ft.)

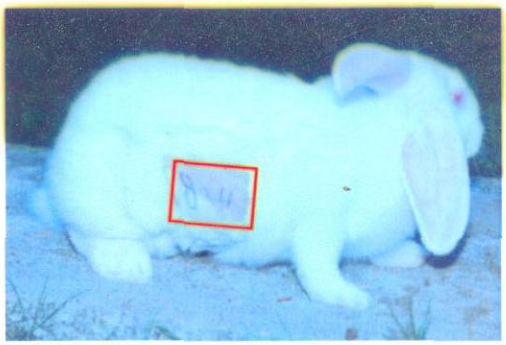


Plate 3. Dermal administration of *F.pallidoroseum* on rabbit

Plate 2. Oral administration of *F.pallidoroseum* on rabbit

Plate 4. Intranasal administration of *F.pallidoroseum* on rabbit

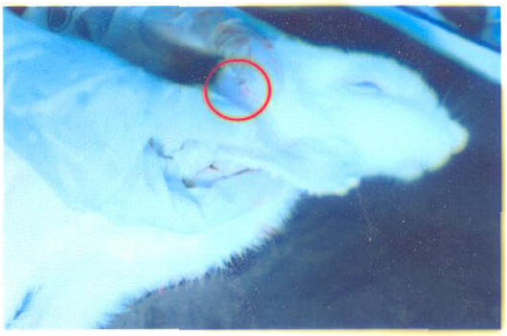


Plate 5. Intravenous administration of *F. pallidoroseum* on rabbit

In all the above routes of administration, only a single dose was given for acute toxicity studies. For chronic toxicity studies four repeated doses were given at fortnightly intervals.

3.11.6.2.6 Behaviour of animals

After administration of the spores animals were observed for any behavioural changes, every 15 minutes during the first hour, every 30 minutes during next four hours and at the end of 24 h in the case of acute toxicity studies and at every 24 hour interval in the case of chronic toxicity studies.

3.11.6.2.7 Clinical response of animals

The veterinary surgeon conducted clinical examinations of all animals whenever indicated by any behavioural changes or at the termination of experimental period. The temperature, pulse rate and respiration rates were recorded after 15 minutes, 1 h, 2 h, 3 h, 4 h and 24 h in acute toxicity studies and at weekly intervals in the case of chronic toxicity studies.

3.11.6.2.8 Determination of weight gain

The weight of the animals were recorded every week using a common balance.

3.11.6.2.9 Collection of blood and haematology

About 3 ml of blood was collected in vials containing calcium oxalate from marginal ear veins just before killing. Three samples were collected from

each experimental group. The blood samples were analysed for the haemoglobin content, white blood cells, packed cell volume, neutrophils, lymphocytes, easnophils, monocytes and basophils.

3.11.6.2.10 Killing and post mortem examination

The animals were stunned by a severe blow to the head and subsequently the jugular veins and the anterior vena cava were cut to bleed the animal, the bleeding was continued until death. All the dead animals were examined under the supervision of a veterinary pathologist. Samples of skin (at the site of application of spore in the case of dermal and subcutaneous group), liver, kidney, lung and heart were collected apart from any other organ with gross lesions for light microscopic studies. The internal organs such as lung, left and right kidneys, liver and heart were weighed soon after the post mortem examination.

3.11.6.2.11 Histopathology

Histopathology examination was performed on all the samples collected during post mortem examination *viz.* skin, lungs, left and right kidneys, liver and heart. For this purpose tissues were fixed in neutral buffered formalin, routinely processed through alcohol and embeded in paraffin. Sections were cut at 5-7 mm thickness and stained with Harries haematoxyline and eosin and examined under a light microscope.

3.11.6.3 Statistical analysis

The relevant data were analysed using CRD.

3.12 Isolation, purification and efficacy of the toxin produced by *F. pallidoroseum*

The experiment was carried out following the method suggested by Mahadevan and Sridhar (1974).

3.12.1 Isolation of toxin

F. pallidoroseum was cultured in a 500 ml conical flask containing 100 ml of Czapek's liquid medium. Seven day old culture was shaken vigorously for two minutes and sieved through double layered muslin cloth to obtain the culture filtrate. The culture filtrate was centrifuged at 2000 rpm for 20 minutes under refrigeration. The sediment was discarded and the clear supernatant was collected. The pH of the culture filtrate was adjusted to 4 by adding 2 N hydrochloric acid. The filtrate (100 ml) was extracted repeatedly with equal volume of ethyl acetate at least four times in a seperating funnel, allowing 15 minutes for each extraction. All the ethyl acetate extracts were combined and evaporated to near dryness on a hot water bath. The residue was dissolved using 2 ml ethanol. The concentrated extract was spotted on a Whatman No.1 sheet using a microsyringe (5µl). The solvent mixture containing secondary butanol : formic acid : water (75:15:10 v/v) was used for developing the chromatogram. Chromatogram was developed descendingly for 10-12 h in a chromatographic sheet. The paper was dried under a hood for 14-16 h. The indicator bromophenol blue was sprayed on the paper, to detect the coloured spot. The rf value was noted.

3.12.2 Purification of toxin

One litre of culture filtrate was obtained from Czapek's liquid medium as described in para 3.12.1. The pH was adjusted with 5 N hydrochloric acid to 4. The filtrate was extracted repeatedly four times with ethyl acetate using 150 ml of the solvent for each extraction. A pinch of sodium chloride was used for each extraction for breaking the emulsion if any. The extracts were pooled, passing through anhydrous sodium sulphate. It was then concentrated to 10 ml by evaporating in a hot water bath.

To the concentrated extract (10 ml) 5 ml of water was added and pH adjusted to 8 using 0.1 N sodium hydroxide. This was again extracted repeatedly, four times with ethyl ether. The extract was pooled and dried using 20 g anhydrous sodium sulphate. The ether was evaporated from the extract keeping in a hot water bath. The purified material was then weighed in an electronic balance along with the container. It was then dissolved in the solvent (ethyl ether) and weight of the empty container was recorded. The difference in weight was recorded as the weight of the purified toxin. The concentrated solution of the toxin was again spotted on Whatman No. 1 chromatographic sheet as described under 3.12.1 to confirm the rf value.

3.12.3 Effect of fusaric acid

3.12.3.1 On mortality of *A. craccivora*

To study the effect of fusaric acid on aphids, purified fusaric acid was brought into solution in acetone. Stock solution of (1000 ppm) fusaric acid in acetone was prepared by dissolving 100 mg of purified fusaric acid in 100 ml of

acetone. From this stock solution, 500, 250, 100 and 10 ppm solutions were prepared by serial dilution technique.

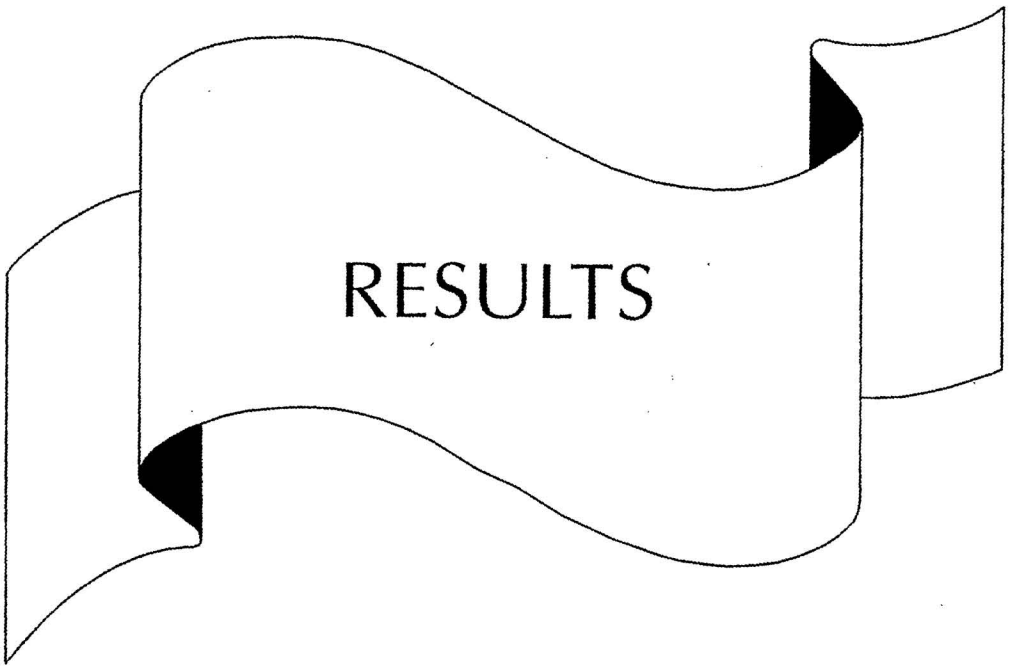
Adult apterous aphids (50 No.) were taken in a clean petridish and sprayed with 1 ml each of the five concentrations of fusaric acid. The treated aphids were colonised on tender cowpea twigs and kept under observation. Observation on mortality was recorded at fixed intervals till no aphids survived in the control.

3.12.3.2 On other aphid species

The aphid species tested for pathogenicity of *F. pallidoroseum* viz. *Aphis gossypii*, *A. malvae*, *A. spiraecola, Myzus persicae, Hystnoneura seteriae*, *Toxoptera odinae*, *Pentalonia nigronervosa,* two unidentified species collected from tulsi and pepper were treated with 1000, 500, 250, 100 and 10 ppm solutions of fusaric acid as described under 3.12.3.1 to find out its action on the treated insects.

3.12.3.3 On coccinellid predators of *A.craccivora*

Five number each of the adult coccinellid predators *Coccinella septumpunctata, Chilomenus sexmaculata* and *Micraspis crocea* were inactivated by keeping them at hypothermal conditions for 5 to 10 minutes. Five microlitre each of 1000 ppm, 500 ppm, 250 ppm, 100 ppm qand 10 ppm was applied topically to the thoracic tergites of the beetles at the ventral side using a microsyringe. The first, second and third instars and pupae were also treated as above, whereas the eggs were sprayed with these solutions using an atomiser. The treated insects were kept under observations for 72 h.


RESULTS

4. RESULTS

4.1 Mycosis of *Aphis craccivora* infected by *Fusarium pallidoroseum*

A. craccivora infected by *F. pallidoroseum* naturally or artificially, turned pale, sluggish and later developed a brown discolouration. Death of the aphids occurred within two to three days after infection. The cadaver was hard and mummified and seen firmly adhered to the host plant surface (Plate 6). Growth of the mycelium over the cadaver was observed four to five days after infection (Plate 7).

4.2 Morphological characters of *F. pallidoroseum*

The cultures *of F.pallidoroseum* were white during the initial days after inoculation and later developed a peach tinge on the under side of the culture. The aerial mycelium of the fungus was elongated, septate and branched. Conidiophores arise from the mycelium and bear the conidia on monophialide which are cylindrical in shape (Plate 9). The fungus produced two types of conidia elongated or curved macroconidia with both ends pointed and spherical or oval microconidia (Plate 10). The macroconidia were one to five septate when obtained from host insect and one to three septate when obtained from potato dextrose agar (PDA) culture. The microconidia always lack septa.

Plate 6. Symptom of *F. pallidoroseum* mycosis on *A. craccivora* (2 to 3 DAI)

Plate 7. Symptom of *F. pallidoroseum* mycosis on *A. craccivora* (4 to 5 DAI)

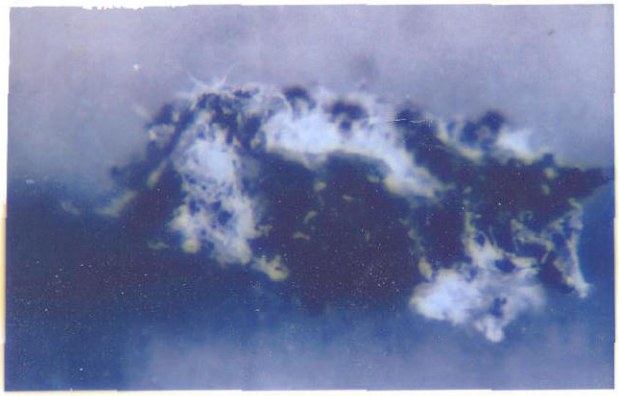


Plate 8. *A. craccivora* infected by *F. pallidoroseum*

4.3 Measurement of conidia of the fungus

The variation in mean length and breadth among the spores obtained from the host insect and PDA was tested using Students t test (Table 1). The average length of macroconidia from host was (25.67± 1.206 μm) nearly three times longer than that recorded from the growth noticed on PDA (9±2.19 μm). However the average breadth of microconidia recorded from the insect (2.15 μm) and PDA (2.19 μm) did not vary significantly (Fig.1 and 2).

Table.1 Mean length and breadth of *F. pallidoroseum* spores from host and PDA

Character	Macrospores			Microspores		
	Host	PDA	t value	Host	PDA	t value
Length (μm)	25.67 ± 1.21	9.0 ± 2.19	16.68**	7.41 ± 0.87	2.78 ± 0.23	5.41**
Breadth (μm)	2.15 ± 0	2.19 ± 0	0.04	2.59 ± 0	2.57 ± 0.21	1.00

** Significant at 1% level of significance

4.4 Effect of subculturing on growth characteristics of *F.pallidoroseum*

The morphological characters and sporulating ability of the fungus were altered on repeated subculturing on PDA (Table 2). The growth of the fungus on aphid was pale white and mycelia diffused and fluffy. Macroconidia were abundant and microconidia few. When cultured on PDA, the first subculture was white and

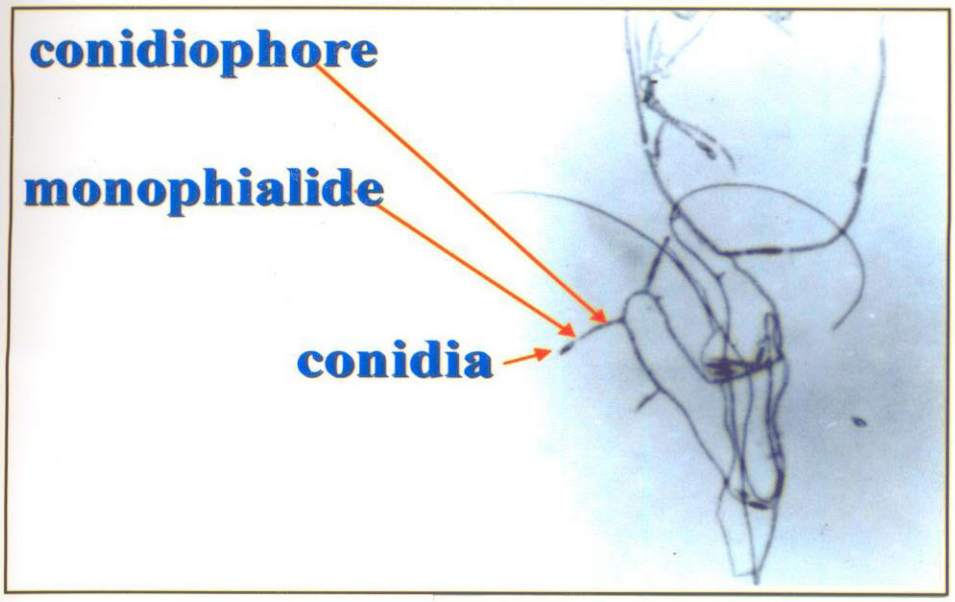


Plate 9. Mycelia and conidia of *F. pallidoroseum*

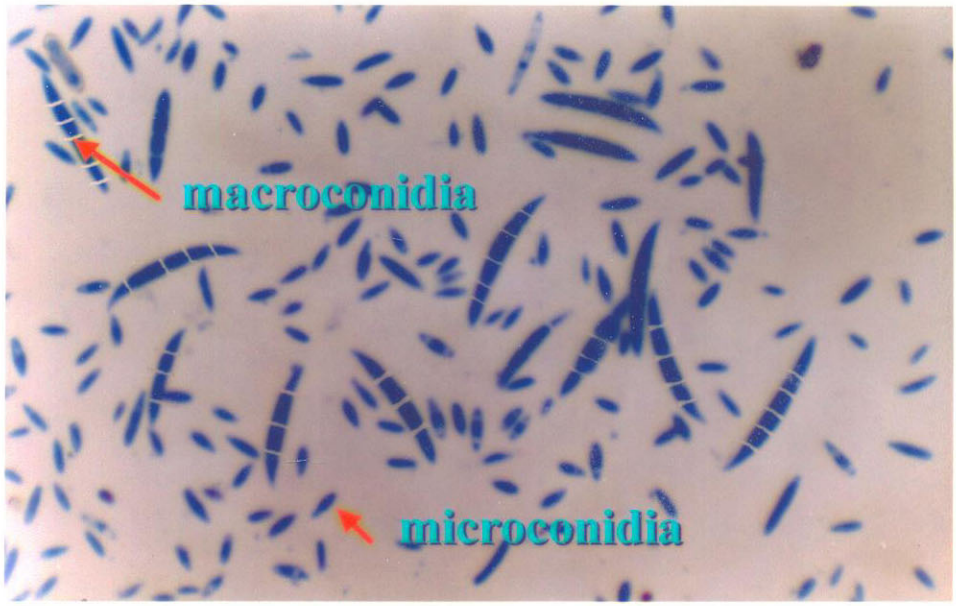


Plate 10. Macro and micro conidia of *F. pallidoroseum*

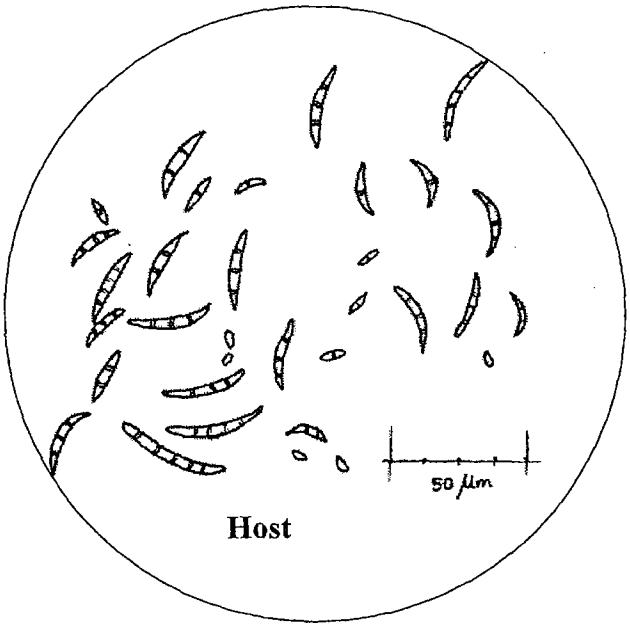


Fig. 1. Measurement of conidia of *F. pallidoroseum* from host (400x)

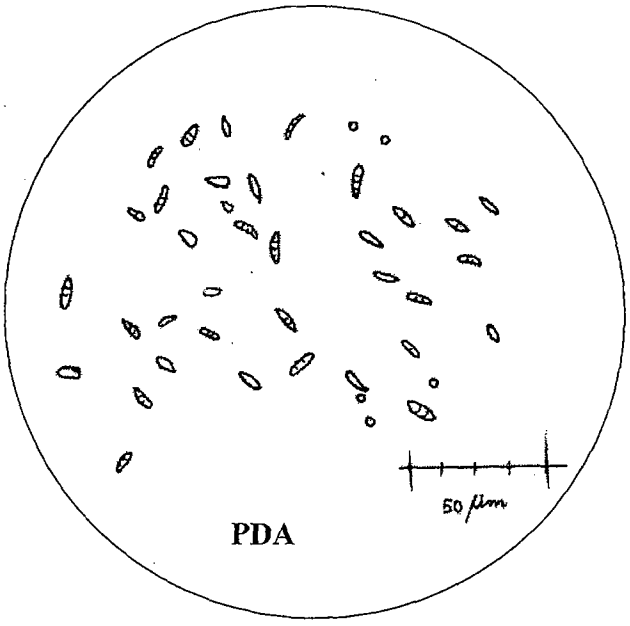


Fig. 2. Measurement of conidia of *F. pallidoroseum* from PDA (400x)

Table 2 Effect of repeated subculturing on growth and sporulation of *F.pallidoroseum*

Substrates	Growth characteristics	Presence of macro and micro conidia	Percentage mortality of aphids	Time taken for mortality (days)
Aphid	Pale white, diffused growth of mycelia which developed a dark colour on the underside. Growth was slow.	Macroconidia abundant and microconidia very few.	100	2
PDA (No. of subcultre)				
1	White, fluffy growth of mycelia which turned pale pink or peach tint on the underside. Growth was quick.	Macroconidia abundant and microconidia few.	100	2
2	Pure white, cottony growth of mycelia which developed a peach tint on the underside. Growth was very quick.	Macroconidia more and microconidia less.	100	3
3	-do-	Macro and microconidia in equal proportion.	60	4
4	-do-	Macroconidia few and microconidia more.	35	4
5	-do-	Only microconidia	20	4
6	-do-	Only microconidia	8	4
7	-do-	Only microconidia	0	-

fluffy which turned pale pink or developed a peach tinge on the underside. Growth of the fungus was quick. Macroconidia were more and microconidia less. On the second subculture the mycelial growth was pure white and cottony and also developed peach tinge on the underside. The mycelial growth was quick, macroconidia were more and microconidia less. The appearance of third, fourth, fifth, sixth and seventh subcultures resembled the second. In the third subculture macro and microconidia were in equal proportion, while in the fourth, macroconidia were few and microconidia more. The fifth, sixth and seventh subcultures were found to possess only microconidia. The mortality rate of the test group of aphids and time taken for mortality also varied with repeated subculturing (Fig.3). The fungus cultured on aphids, when applied on test insects resulted in cent per cent mortality within two days. The first subculture also gave similar results but it took three days to cause mortality. When the third subculture was sprayed on aphids only 60 per cent mortality was recorded and it took four days for kill. There after the percentage mortality was greatly reduced. It was only 35 and 20 with fourth and fifth subculture respectively and only eight per cent mortality was recorded with sixth subculture. When the seventh culture was sprayed, none of the aphids were killed.

4.5 Mass production of *F. pallidoroseum*

4.5.1 Effect of solid substrates on growth and sporulation of the fungus

The mean mycelial growth of the fungus was maximum on cowpea leaves and cotton seed cake (Table 3) followed by pongamia leaves, bran and other oil seed cakes except neem cake. The fungus failed to grow on neem cake and cowdung while it's growth was meagre on coirpith, sawdust and soil (Plate 11).

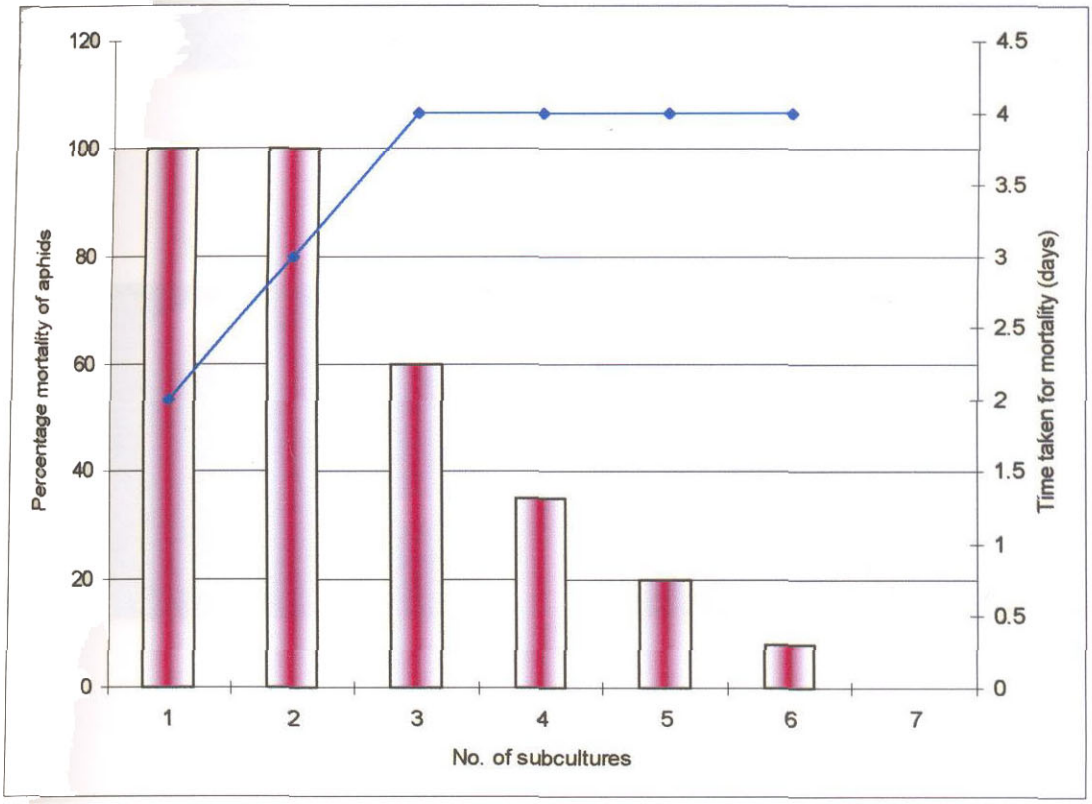
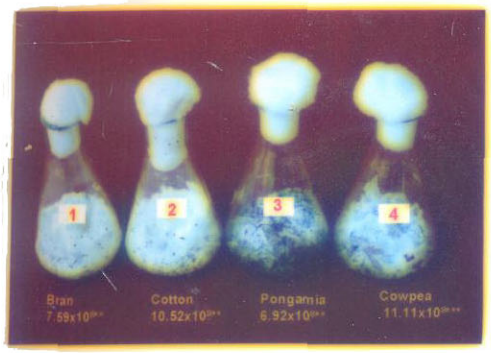
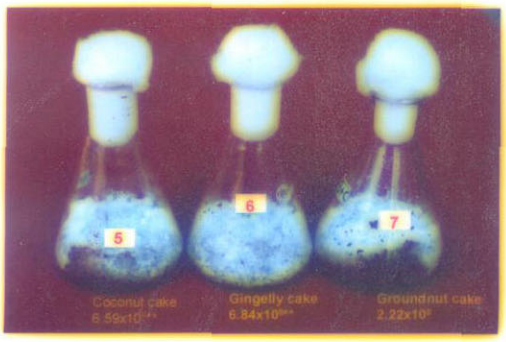
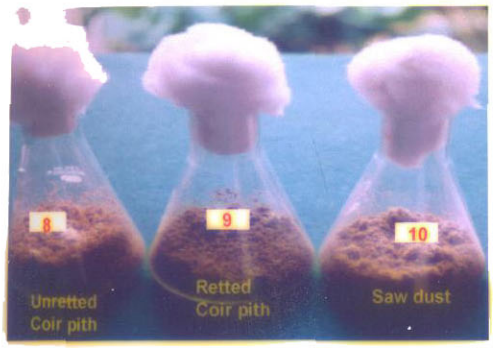
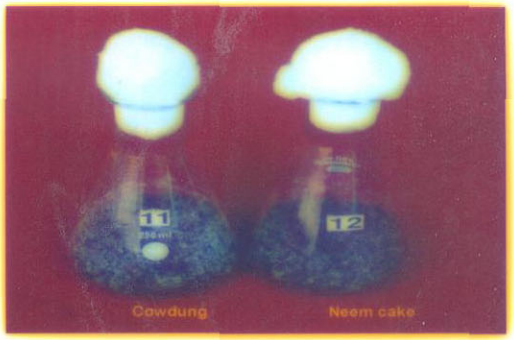


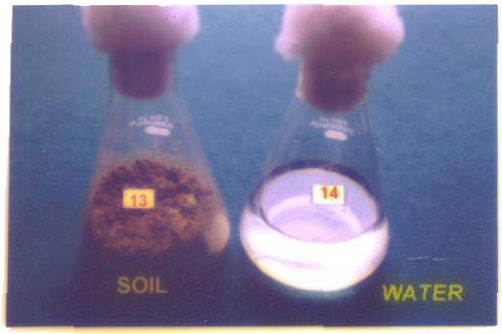
Fig. 3. Effect of subculturing of *F. pallidoroseum* on mortality of *A. craccivora*











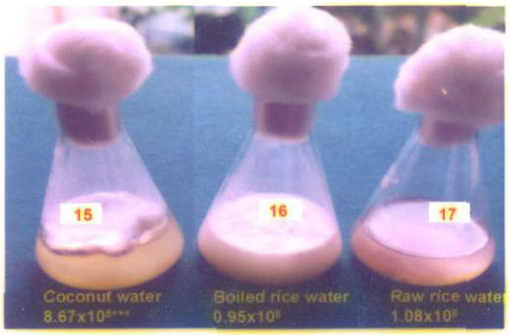


Plate 11. Growth of *F. pallidoroseum* on different solid and liquid substrates

Table 3. Mycelial growth and spore count of *F. pallidoroseum* in solid substrates

Sl No.	Substrates	Mycelial growth rate*	Spore count / ml x 10^8	Macro / Micro conidia
1	Bran	++	7.59 (2.93)	Macroconidia more
2	Saw dust	+	0.09 (1.04)	Microconidia more
3	Soil	+	1.23 (1.49)	-do-
4	Unretted coir pith	+	0.25 (1.12)	-do-
5	Retted coir pith	+	0.0004 (1)	-do-
6	Cowdung	0	0 (1)	No conidia
7	Pongamia leaves	++	6.92 (2.81)	Macroconidia more
8	Cowpea leaves	+++	11.11 (3.48)	-do-
9	Cotton seed cake	+++	10.52 (3.39)	-do-
10	Gingelly cake	++	6.84 (2.80)	-do-
11	Groundnut cake	++	2.22 (1.79)	Macro and microconidia in equal proportion
12	Coconut cake	++	6.59 (2.75)	Macroconidia more
13	Neem cake	0	0 (1)	No conidia

CD for comparison of spore count = 0.38

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

* +++ - Profuse growth
++ - Moderate growth
\+ - Slight growth
0 - No growth

When the spore count of the cultures at the end of the seven days in different substrates were recorded, it was noticed that cowpea leaves and cotton seedcake supported maximum number of spores ($11.11x10^8$ and $10.52x10^8$ spores ml^{-1} respectively). Its sporulating ability on bran, pongamia leaves, gingelly cake and coconut cake (7.59, 6.92, 6.84 and $6.59x10^8$ spores ml^{-1} respectively) was similar and significantly higher than that of groundnut cake ($2.22x10^8$spores ml^{-1}). The fungus failed to sporulate on neemcake and cowdung.

Population of macro and microconidia of the fungus vary on different substrates. Macroconidia were more on leafy substrates, bran and oil cakes (except ground nut cake) while the ratio of macroconidia to microconidia were 1:1 in groundnut cake and only microconidia were observed on sawdust, soil and coirpith.

4.5.2 Effect of liquid substrates on growth and sporulation of the fungus

Among the different liquid substrates tried the growth of the fungus was maximum in coconut water (Plate 11) with maximum biomass of mycelia. The mean mycelial weight recorded on dry weight basis was double from coconut water (40 mg) when compared to that recorded from boiled rice water (20 mg). In the case of raw rice water, yield was only 4.2 mg. The fungal growth was absolutely nil in plain water.

Spore count in coconut water ($8.67x10^8$ spores ml^{-1}) was significantly higher than that recorded from the other three substrates (Table 4). The spore count in raw rice water ($1.08x10^8$ spores ml^{-1}) was on par with that of boiled rice water ($0.95x10^8$ spores ml^{-1}).

Table 4. Mycelial growth and mean spore count of *F. pallidoroseum* in liquid substrates

Sl. No	Substrates	Mycelial growth rate*	Mean Mycelial weight (mg)		Spore count / ml x 10^8	Macro/Micro conidia
			Wet weight	Dry weight		
1	Coconut water	+++	162.70	40.00	8.67 (3.11)	Macroconidia
2	Boiled rice water	++	100.00	20.00	0.95 (1.39)	-do-
3	Raw rice water	+	22.00	4.20	1.08 (1.44)	Only microconidia
4	Plain water	0	0	0	0 (1)	No conidia

C D for comparison of spore count = 0.57
Figures in the parentheses are values after $\sqrt{x}$ + 1 transformation.

* +++ - *Profuse growth*
++ - *Moderate growth*
\+ - *Slight growth*
0 - No growth

Variation in the spore type was noticed when the fungus was grown on different liquid substrate also. Coconut water and boiled rice water supported many macroconidia while in raw rice water only microconidia were observed.

4.5.3 Virulence of *F.pallidodroseum* grown on different solid substrates

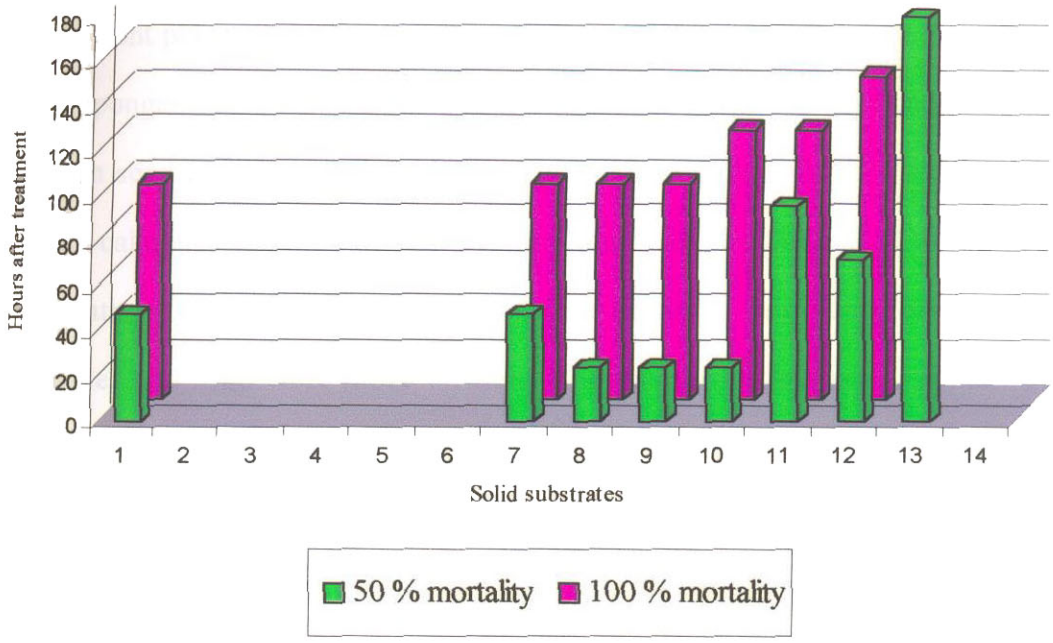
None of the spore suspensions did result in 50 per cent mortality of aphids, 24 h after treatment (Table 5). Two days after treatment more than 50 per cent mortality was observed with spore suspensions of cotton seed cake (79.34 per cent), bran (71.00 per cent), gingelly cake (63.55 per cent), pongamia leaves (56.69 per cent) and cowpea leaves (50.68 per cent). On the third day after

Table 5. Cumulative per cent mortality of aphids treated with spore suspensions from solid substrates

Sl No	Solid substrates	Cumulative per cent mortality at days after treatment						
		1	2	3	4	5	6	7
1	Bran	27.76 (31.78)	71.00 (57.39)	81.37 (64.40)	99.99 (90.00)	99.99 (90.00)	99.99 (90.00)	99.99 (90.00)
2	Saw dust	0 (0)	33.26 (35.20)	38.65 (38.43)	39.97 (39.20)	40.64 (39.59)	44.66 (41.92)	44.99 (42.13)
3	Soil	0.11 (1.91)	1.63 (7.33)	3.26 (10.39)	7.92 (16.34)	11.86 (20.14)	13.23 (21.32)	15.97 (23.55)
4	Coirpith (U)*	0 (0)	4.28 (11.93)	5.93 (14.08)	8.53 (16.97)	13.94 (21.91)	19.32 (26.07)	21.62 (27.70)
5	Coir pith (R)*	0.44 (3.82)	4.65 (12.45)	6.49 (14.75)	8.16 (16.60)	9.94 (18.37)	10.65 (19.04)	16.60 (24.04)
6	Cowdung	0.11 (1.19)	0.11 (1.19)	5.18 (13.16)	6.49 (14.75)	6.49 (14.75)	7.92 (16.34)	8.50 (16.95)
7	Pongamia leaves	25.58 (30.56)	56.69 (48.83)	97.89 (81.62)	100 (90)	100 (90)	-	-
8	Cowpea leaves	31.97 (34.42)	50.68 (45.37)	66.74 (54.76)	100 (90)	-	-	-
9	Cotton seed cake	23.29 (28.84)	79.34 (62.94)	83.06 (65.67)	100 (90)	-	-	-
10	Gingelly cake	20.00 (26.55)	63.55 (52.84)	74.87 (59.88)	98.70 (83.42)	99.99 (100)	-	-
11	Groundnut cake	18.63 (25.56)	35.28 (36.42)	47.95 (43.80)	71.57 (57.75)	100 (90)	-	-
12	Coconut cake	10.58 (18.98)	29.33 (32.77)	50.67 (45.36)	64.72 (53.54)	90.75 (72.26)	99.99 (90.00)	-
13	Neem cake	0	4.49 (12.22)	7.67 (16.07)	7.67 (16.07)	23.18 (28.76)	32.91 (34.99)	53.95 (46.90)
14	Control	0	0.22 (2.71)	0.68 (4.72)	5.51 (13.58)	14.57 (22.43)	14.57 (22.43)	16.35 (23.84)

CD for comparison of substrates = 5.79
Figures in the parentheses are values after angular transformation.

* U - Unretted
R - Retted



1 – Bran*
2 – Sawdust
3 – Soil
4 – Unretted coir pith
5 – Retted coir pith
6 – Cowdung
7 – Pongamia leaves*
8 – Cowpea leaves*
9 – Cotton seed cake*
10 – Gingelly cake*
11 – Groundnut cake*
12 – coconut cake*
13 – Neem cake
14 – Control

* More macroconidia

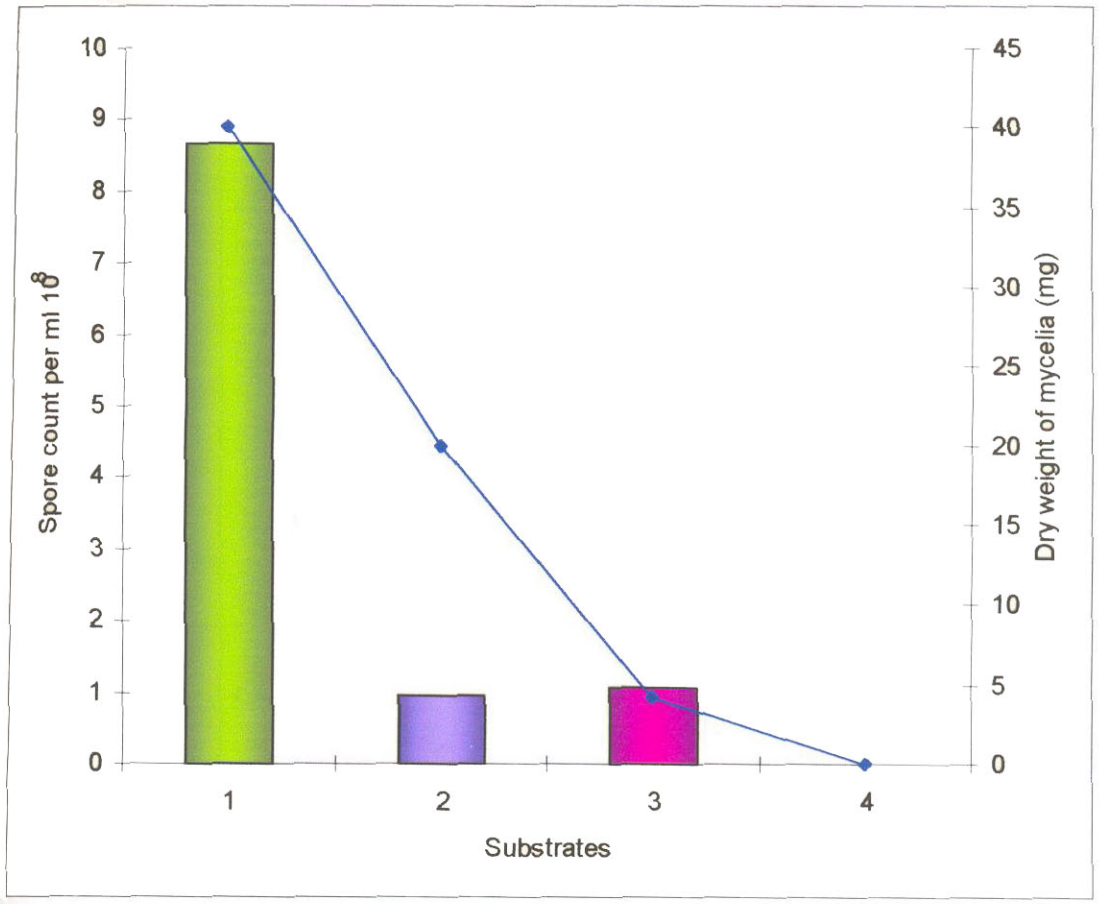
Fig. 4. Time taken by *F.pallidoroseum* spore suspensions from different culture media to cause 50 and 100 per cent mortality of *A.craccivora* - solid substrates

treatment culture filtrates of coconut cake also resulted in more than 50 per cent mortality (50.67). Four days after treatment ground cake resulted in 71.57 per cent mortality and by the end of seven days neem cake also resulted in 53.35 per cent mortality.

Cent per cent destruction of aphids was possible with spore suspensions of bran, pongamia leaves and cowpea leaves which took four days and with those of cotton seed cake, gingelly cake and groundnut cake, which took five days. Coconut cake also resulted in cent per cent mortality, by the end of the sixth day after treatment. In general, spore suspensions obtained from leafy substrates, oil seed cakes and bran were more virulent whereas those obtained from sawdust, soil and coirpith were least virulent against *A. craccivora*. Aphids were killed at a faster rate when spore suspensions containing macroconidia was used (Fig.4). It may be noted that macroconidia content was more in suspensions prepared from cultures of leafy substrates, bran and oil cakes (other than groundnut cake and neemcake) while microconidia dominated in suspensions prepared from cultures of sawdust, coirpith and soil.

4.5.4 Virulence of *F.pallidoroseum* grown on different liquid substrates

Spore suspensions prepared from coconut water culture alone contained more macroconidia and resulted in cent per cent mortality of aphids, three days after treatment (Table 6) while those from other liquid substrates failed to cause not even 50 per cent mortality of aphids even at the end of seven days after treatment (Fig.5).



1 - Coconut water 2 - Boiled rice water 3 - Raw rice water 4 - Plain water

Fig. 5. Time taken by *F.pallidoroseum* spore suspensions from different culture media to cause 50 and 100 per cent mortality of *A.craccivora* - liquid substrates

Table 6. Cumulative per cent mortality of aphids treated with spore suspension from different liquid substrates

Sl. No.	Liquid substrates	Cumulative per cent mortality - days after treatment						
		1	2	3	4	5	6	7
1	Coconut water	53.49 (46.99)	90.46 (72.27)	100	–	–	–	–
2	Boiled rice water	4.98 (12.89)	4.98 (12.89)	11.14 (19.49)	11.72 (20.01)	15.65 (23.29)	19.32 (26.06)	24.26 (29.49)
3	Raw rice water	1.00 (5.74)	1.00 (5.74)	5.97 (14.14)	7.53 (15.92)	7.82 (16.23)	10.48 (18.88)	10.83 (19.21)
4	Water	1.00 (2.71)	0.89 (5.42)	0.89 (5.42)	5.00 (12.92)	9.59 (18.04)	10.23 (18.65)	13.05 (21.17)
5	Control	0 (0)	0.22 (2.17)	0.68 (4.72)	5.51 (13.57)	14.56 (22.43)	14.56 (22.43)	16.35 (23.84)

CD for comparison of substrates = 7.84

Figures in the parentheses are values after angular transformation.

4.6 Shelf life of *F.pallidoroseum*

4.6.1 Effect of carrier materials on viability of the fungus

4.6.1.1 At room temperature

Survival ability of the fungus in the carrier material was studied by incubating the inoculated carrier materials at room temperature as well as under refrigerated condition, for a period of six months. The growth rate of the fungus at monthly intervals was tested by growing the fungus on PDA using samples drawn from the formulations at monthly intervals.

At the end of one month after storage (MAS) at room temperature, maximum inhibition of 53 per cent over the control was observed in the case of fungal spores stored in peat and vermicompost (Table 7). Inhibition was between 21 and 24 per cent in charcoal, bran and sawdust. When cadaver and leaf mold were used as carriers, the inhibition was only around 10 per cent. In diatomaceous earth, the inhibition was slightly more than that of cadaver (13.3 per cent). By the end of 5 MAS the spores stored in vermicompost and peat were totally inhibited and there was no growth at all. In the case of cadaver formulation only 43 per cent inhibition was noticed even at the end of six months. The percentage inhibition in saw dust and diatomaceous earth was around 60 while that with charcoal, leaf mold and bran, it ranged from 72-76 at 6 MAS. Eventhough at 1 MAS leaf mold formulation showed maximum viability and retained more than 50 per cent viability till 5 months of storage, at the end of 6 months it was found to be greately inhibited, percentage inhibition being 73. In general peat and vermicompost are unsuitable for storing spores while cadaver formulation was found to be the best.

Table 7. Effect of carrier materials on the viability of *F. pallidoroseum* formulations stored at room temperature

MAS	Diotomaceous earth		Charcoal		Leaf mold		Bran		Sawdust		Peat		Vermicompost		Cadaver		Control
	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)
1	7.70 (16.10)	13.48	6.97 (15.29)	21.60	7.93 (16.35)	10.80	6.83 (15.07)	23.20	6.77 (15.07)	23.90	4.10 (11.69)	53.90	4.13 (11.72)	53.50	7.97 (16.42)	10.40	8.90 (17.42)
2	6.97 (15.29)	22.50	6.57 (14.84)	27.00	7.00 (15.34)	22.22	6.33 (14.57)	29.68	6.03 (14.21)	33.00	2.96 (9.91)	67.00	2.93 (9.86)	67.44	7.60 (15.99)	15.58	9.00 (17.45)
3	6.47 (14.73)	28.10	5.83 (13.97)	35.22	6.33 (14.57)	28.79	5.41 (13.52)	38.47	5.46 (13.57)	38.58	2.15 (8.43)	75.80	2.03 (8.19)	77.16	7.03 (15.37)	20.92	8.89 (17.35)
4	5.50 (13.56)	38.00	5.10 (13.05)	43.33	5.87 (14.01)	34.77	4.06 (11.63)	54.88	5.03 (12.96)	44.10	0.93 (5.54)	89.00	1.33 (6.62)	85.22	6.33 (14.57)	29.66	9.00 (17.45)
5	4.10 (11.68)	54.00	3.03 (10.02)	66.33	4.90 (12.28)	45.55	3.10 (10.14)	65.50	4.70 (12.51)	47.77	0	100	0.89 (5.44)	100	5.83 (13.97)	35.22	9.00 (17.45)
6	2.93 (9.86)	67.00	2.00 (8.13)	76.56	4.06 (11.63)	72.77	2.45 (9.01)	72.65	3.27 (10.41)	63.50	0	100	0	100	5.10 (13.05)	43.05	8.96 (17.42)

MAS – Months After Storage
MCD – Mean Colony Diameter
PIOC – Percentage Inhibition Over Control

CD for comparison of treatment combinations = 0.43

Figures in parentheses are values after angular transformation

4.6.1.2 Under refrigeration

When stored under refrigeration the shelf life of the fungus was found to be improved (Table 8). As in the case of storage at room temperature, 1 MAS, formulations using semidry cadaver and diatomaceous earth showed maximum viability of spores followed by leaf mold. The percentage inhibition on colony diameter of the fungus was 14. Charcoal, bran and sawdust inhibited the spore germination only upto 22.2 per cent while maximum inhibition was recorded with peat and vermicompost formulations (33 per cent). When the period of storage increased, the spore viability was found to be gradually decreased in all the treatments and at the end of six months, vermicompost and peat showed maximum inhibition of spores, percentage inhibition being 67. The spores stored in bran and sawdust showed around 50-55 per cent reduction in viability while with charcoal it was 44 and leaf mold 34. Least inhibition was recorded with formulations stored in diatomaceous earth (27 per cent) and cadaver formulations (21 per cent).

It may be concluded that at both the storage temperatures, cadaver is the best carrier material followed by diatomaceous earth, leaf mold and charcoal. When storage under room temperature showed total inhibition of spores in peat and vermicompost, at five months after storage, the spore inhibition was only 67 per cent even at the end of 6 months under refrigeration. In all the formulations the rate of fall in viability was found to be reduced by refrigerated storage (Fig. 6).

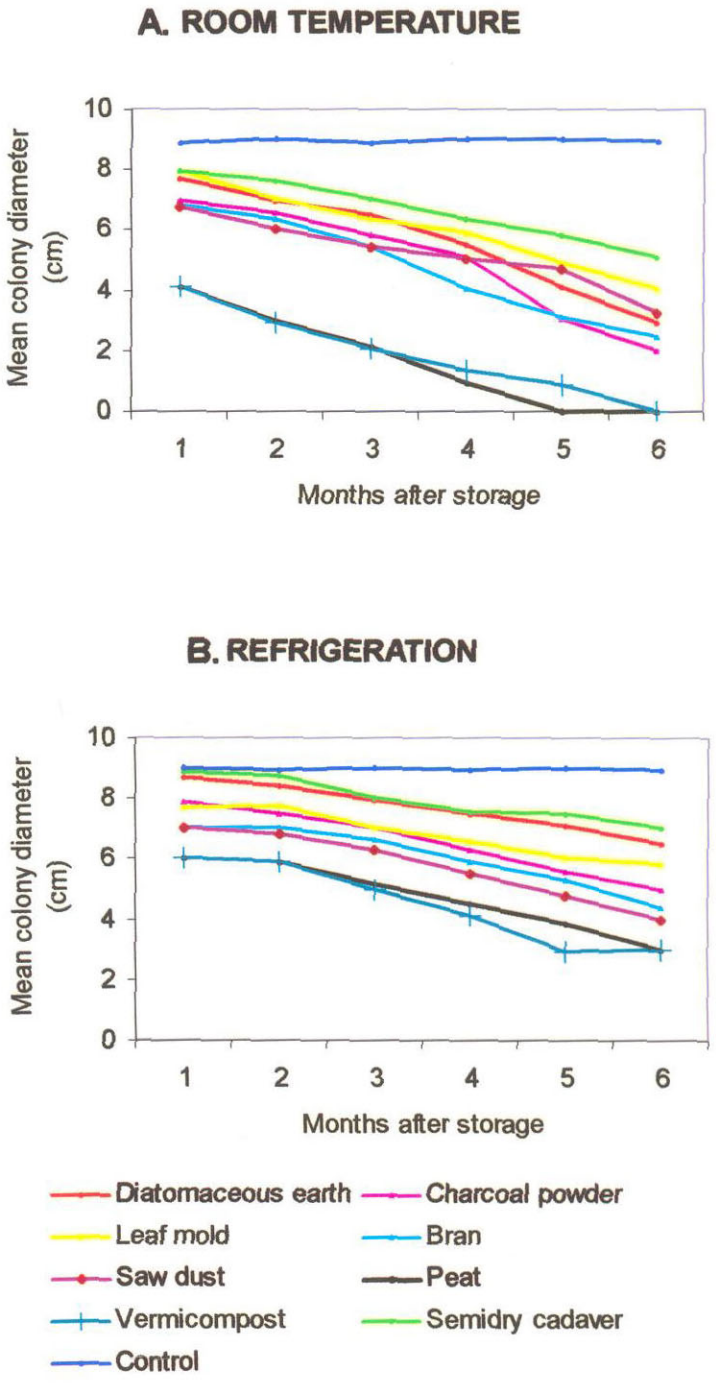


Fig. 6. Effect of carrier materials on viability of *F.pallidoroseum* spores stored at different temperatures

Table 8. Effect of carrier materials on the viability of *F. pallidoroseum* formulations stored under refrigeration

MAS	Diatomaceous earth		Charcoal		Leaf mold		Bran		Sawdust		Peat		Vermicompost		Cadaver		Control
	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)	PIOC	MCD (cm)
1	8.7 (17.14)	3.33	7.90 (16.32)	12.22	7.70 (16.10)	14.44	7.00 (15.33)	22.22	7.00 (15.33)	22.22	6.00 (14.17)	33.33	6.00 (14.17)	33.33	8.90 (17.25)	1.11	9.00 (17.45)
2	8.4 (16.84)	5.61	7.50 (15.89)	15.73	7.73 (16.14)	13.14	7.00 (15.33)	21.34	6.83 (15.14)	23.25	5.90 (14.09)	33.70	5.90 (14.09)	33.70	8.76 (17.21)	1.57	8.97 (17.42)
3	7.93 (16.35)	11.88	7.00 (15.33)	22.22	7.00 (15.33)	22.22	6.60 (14.84)	26.66	6.30 (14.52)	30.00	5.16 (13.13)	42.66	4.96 (12.87)	44.89	8.00 (17.21)	11.11	9.00 (17.45)
4	7.50 (15.88)	16.01	6.30 (14.53)	29.45	6.56 (14.84)	26.54	5.87 (14.01)	34.27	5.50 (13.55)	38.41	4.53 (12.28)	49.27	4.09 (11.67)	54.20	7.56 (15.96)	15.34	8.93 (17.38)
5	7.09 (15.88)	21.22	5.56 (13.63)	38.22	6.00 (14.17)	33.33	5.33 (13.34)	40.78	4.80 (12.65)	46.67	3.83 (11.28)	57.44	2.93 (9.86)	67.44	7.50 (15.88)	16.67	9.00 (17.45)
6	6.47 (14.72)	27.55	5.00 (12.91)	44.01	5.83 (13.97)	34.71	4.40 (12.10)	50.73	4.00 (11.53)	55.21	2.97 (9.91)	66.74	2.96 (9.91)	66.85	7.03 (15.37)	21.28	8.93 (17.38)

MAS – Months After Storage
MCD – Mean Colony Diameter
PIOC – Percentage Inhibition Over Control

CD for comparison of treatment combinations = 0.32

Figures in parentheses are values after angular transformation

4.6.2 Effect of carrier materials on virulence of the fungus

4.6.2.1 At room temperature

The mortality rate of aphids sprayed with inoculum formulated using different carrier materials showed marked variations. After one month of storage at room temperature, all the formulations except those of bran, saw dust and peat recorded more than 97 per cent mortality (Table 9). The least mortality percentage of 29.67 was observed when the fungus stored in peat was sprayed on aphids. As the storage period increased from one to six months, reduction in mortality was recorded in various formulations. However when formulations using diatomaceous earth, charcoal and cadaver were stored over a period of 6 months the reduction in mortality rate was less than 14 per cent. While in the case of leaf mold formulation reduction in mortality was nearly 27 per cent. Even though with vermicompost formulation 98 per cent mortality was noticed at 1 MAS, the mortality rate reduced considerably thereafter. At the end of 6 months it gave only 30.4 per cent mortality showing 67 per cent reduction in virulence, over a period of 6 months. Least mortality percentage (12.6) was recorded with peat formulation. Similarly in saw dust also the mortality rate was only 26 per cent at 6 MAS.

4.6.2.2 Under refrigeration

The shelf life of spore formulations improved when they were stored in refrigerator (Table 10) when compared to storage under ambient conditions. Except bran, sawdust and peat based formulations all other formulations resulted in cent per cent mortality of aphids when treated one month after storage. In both

Table 9. Effect of carrier materials on virulence of *F.pallidoroseum* formulations stored at room temperature

MAS	Percentage mortality of aphids								
	Diatomaceous earth	Charcoal powder	Leaf mold	Bran	Saw dust	Peat	Vermicompost	Cadaver	Control
1	98.21 (82.29)	100 (90)	97.64 (81.13)	63.41 (52.75)	52.67 (46.51)	29.67 (32.98)	97.89 (81.62)	100 (90)	0.91 (5.47)
2	96.99 (79.98)	95.17 (77.28)	90.38 (71.91)	55.36 (48.05)	50.00 (44.98)	61.07 (51.38)	94.49 (76.40)	100 (90.00)	0.91 (5.47)
3	88.13 (69.82)	96.45 (79.11)	83.41 (65.93)	50.6 (45.36)	43.17 (41.02)	59.33 (50.35	69.09 (56.20)	99.37 (85.26)	6.31 (14.32)
4	86.64 (68.53)	88.13 (69.82)	78.84 (63.29)	42.43 (4o.63)	31.30 (34.00)	27.16 (31.39)	63.46 (52.78)	95.47 (77.69)	0.22 (2.70)
5	88.82 (70.43)	84.14 (66.50)	75.45 (60.27)	42.37 (40.59)	27.90 (31.87)	13.46 (21.51)	41.84 (40.28)	89.33 (70.91)	1.14 (6.14)
6	86.16 (68.13)	86.98 (68.83)	70.94 (57.36)	41.99 (40.37)	25.95 (30.61)	12.60 (20.78)	30.40 (33.44)	89.68 (71.24)	0.22 (2.71)

MAS – Months After Storage

CD for comparison of treatment combinations = 11.98

Figures in parentheses are values after angular transformation

Table 10. Effect of carrier materials on virulence of *F.pallidoroseum* formulations stored under refrigeration

MAS	Percentage mortality of aphids								
	Diatomaceous earth	Charcoal powder	Leaf mold	Bran	Saw dust	Peat	Vermicompost	Cadaver	Control
1	100 (90.00)	100 (90.00)	100 (90.00)	86.98 (68.82)	75.45 (60.27)	43.30 (41.14)	100 (90.00)	100 (90.00)	1.30 (6.55)
2	100 (90.00)	100 (90.00)	100 (90.00)	88.61 (70.24)	68.37 (55.75)	43.16 (41.05)	93.31 (74.98)	100 (90.00)	2.0 (8.13)
3	100 (90.00)	98.21 (82.28)	94.48 (76.38)	82.09 (64.93)	63.40 (52.75)	52.67 (46.51)	81.37 (64.40)	100 (90.00)	1.30 (6.55)
4	100 (90.00)	98.86 (83.84)	99.33 (85.26)	73.48 (58.98)	56.67 (48.82)	45.32 (42.29)	43.31 (41.14)	100 (90.00)	4.00 (11.53)
5	100 (90.00)	98.86 (83.86)	89.99 (71.53)	60.64 (51.12)	46.65 (43.06)	28.42 (32.20)	36.59 (37.21)	100 (90.00)	5.71 (13.83)
6	100 (90.00)	92.50 (90.00)	73.80 (59.18)	48.77 (44.27)	35.17 (36.36)	21.72 (27.77)	34.43 (35.92)	100 (90.00)	3.10 (10.14)

MAS – Months After Storage
Figures in the parentheses are values after angular transformation.

CD for comparison of treatment combinations = 8.805

diatomaceous earth and cadaver based formulations, even after 6 months there was no inhibition in mortality rate. But in charcoal based formulations a reduction of 7.5 per cent mortality rate was noticed when stored over a period of six months while in leaf mold this reduction was 26 per cent. Even though vermicompost exhibited cent per cent mortality at the end of 1 MAS, its efficacy reduced considerably and at the end of 6 months it could kill only 34.4 per cent of aphids. As observed with formulations stored at ambient conditions, under refrigeration also least effective formulations were those of sawdust and peat. At the end of 6 months the mortality rate of aphids after spraying these formulations were 35.17 and 21.72 per cent respectively. However mortality per cent in the control was only 0.22 to 6.31 per cent. It may be noted that at both the temperatures virulence is better conserved in spores formulated in cadaver or diatomaceous earth followed by charcoal powder and leaf mold (Fig.7).

4.6.3 Effect of packing material on the viability of the fungus

4.6.3.1 At room temperature

When *F.pallidoroseum* spores were stored at room temperature in four different carrier materials namely diatomaceous earth, charcoal powder, semidry cadaver and leaf mold and packed in three different packing materials namely glass vial, polypropylene and aluminium foil, a gradual reduction in growth rate was observed over a period of time (Table 11). However, semidry cadaver formulations retained viability upto 12 months whereas diatomaceous earth, charcoal powder and leaf mold formulations retained viability upto 10 months of storage. There was no significant variation among the growth rate of the fungus

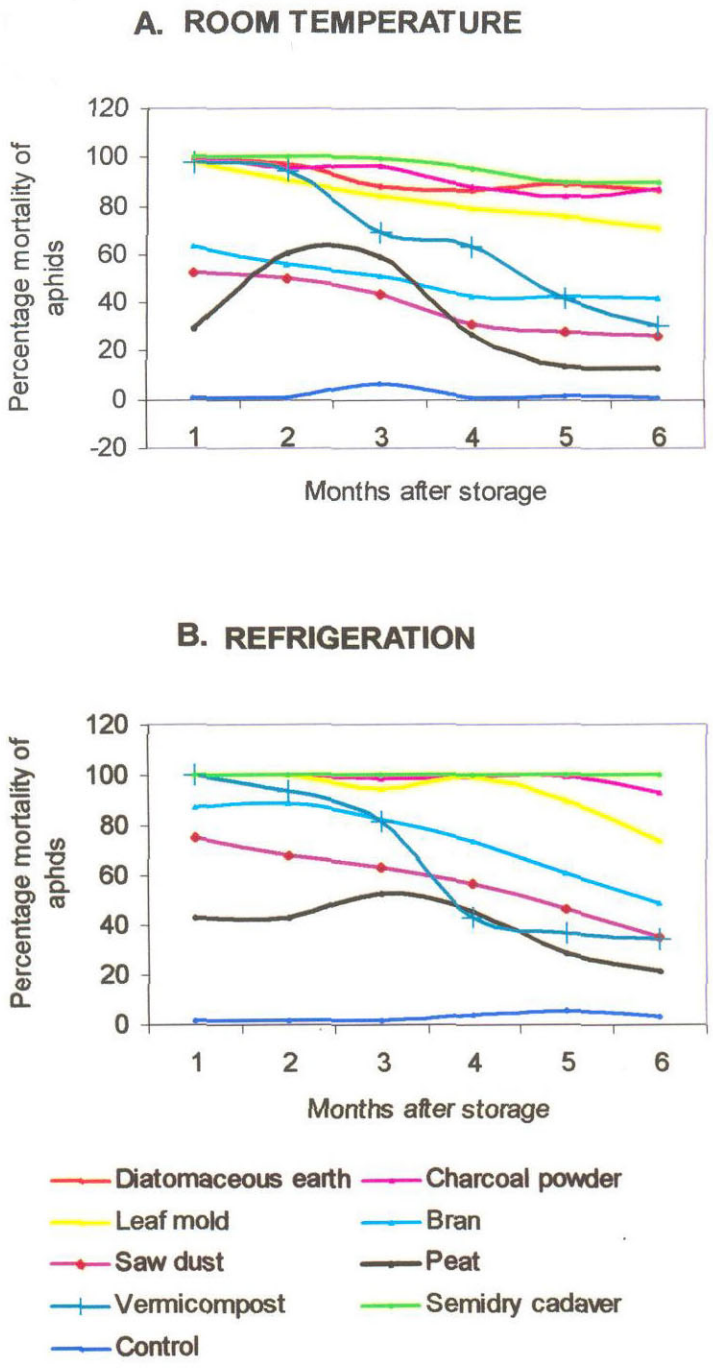


Fig. 7. Effect of carrier materials on virulence of *F.pallidoroseum* spores stored at different temperatures

Table 11. Effect of packing materials on viability of *F.pallidoroseum* formulations stored at room temperature

MAS	Mean colony diameter (cm)											
	Diatomaceous earth			Charcoal powder			Semidry cadaver			Leaf mold		
	G V	P P	A F	G V	P P	A F	G V	P P	A F	G V	P P	A F
1	7.70 (16.10)	7.40 (15.78)	7.23 (15.59)	6.97 (15.30)	6.93 (15.26)	6.73 (15.03)	8.00 (16.42)	8.00 (16.42)	7.87 (16.28)	7.93 (16.35)	7.77 (16.17)	7.50 (15.89)
2	6.97 (15.30)	6.90 (15.22)	6.53 (14.80)	6.57 (14.84)	6.47 (14.73)	6.40 (14.65)	7.60 (16.00)	7.53 (15.92)	7.33 (15.70)	7.00 (15.34)	7.07 (15.41)	6.63 (14.92)
3	6.47 (14.73)	6.17 (14.37)	6.30 (14.53)	5.83 (13.97)	5.57 (13.64)	5.70 (13.81)	7.63 (15.37)	7.10 (15.45)	6.93 (15.26)	6.33 (14.57)	6.10 (14.29)	6.03 (14.21)
4	5.50 (13.56)	5.17 (13.33)	5.40 (13.43)	5.10 (13.05)	5.00 (12.92)	5.07 (13.00)	6.33 (14.57)	6.57 (14.84)	6.03 (14.21)	5.87 (14.01)	5.50 (13.56)	5.27 (13.26)
5	4.10 (11.68)	4.07 (11.63)	4.10 (11.68)	3.03 (10.02)	3.17 (10.24)	3.00 (9.97)	5.83 (13.97)	6.03 (14.21)	5.87 (14.01)	4.90 (12.78)	4.50 (12.24)	4.16 (11.77)
6	2.93 (9.86)	2.97 (9.91)	2.97 (9.91)	2.00 (8.13)	2.00 (8.13)	2.03 (8.19)	5.10 (13.05)	5.00 (12.92)	5.50 (13.56)	4.06 (11.68)	3.50 (10.28)	3.32 (10.49)
7	1.43 (6.87)	1.32 (6.60)	1.30 (6.54)	0.22 (2.70)	0.27 (2.95)	0.72 (4.86)	4.10 (11.68)	4.00 (11.53)	4.17 (11.77)	3.33 (10.51)	2.16 (8.45)	2.16 (8.45)
8	0.43 (3.77)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.06 (1.35)	3.00 (9.97)	2.87 (9.74)	3.03 (10.03)	2.63 (9.33)	1.29 (6.52)	1.38 (6.75)
9	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	1.60 (7.26)	1.79 (7.69)	1.86 (7.85)	1.32 (6.60)	0.22 (2.70)	0.06 (1.35)
10	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.89 (5.42)	1.40 (6.78)	1.50 (7.03)	0.56 (4.30)	0.00 (0)	0.00 (0)
11	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.72 (4.87)	0.78 (5.08)	0.80 (5.12)	0.00 (0)	0.00 (0)	0.00 (0)
12	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.43 (3.75)	0.50 (4.05)	0.40 (3.61)	0.00 (0)	0.00 (0)	0.00 (0)

MAS – Months After Storage
PP – Polypropylene
GV – Glass Vial
AF – Aluminium Foil
CD for comparison of treatment combinations = 0.84
Figures in parentheses are values after angular transformation

with the change in packing material, for a period of six months. Thereafter there was a slight variation in survivability of the formulation depending upon the packing material. Diatomaceous earth formulations retained viability upto 8 months of storage when packed in glass vial, while the survivability was a little bit less (7 months) when stored in polypropylene and aluminium foil containers. In the case of charcoal formulation, aluminium foil packing retained viability up to 8 months while the other two packings lost viability by seven months of storage. With leaf mold formulations aluminium foil could retain viability upto 10 months of storage. In the case of fungal spores stored in semidry cadaver the survivability of the fungus in three different containers did not show any difference till 12 months of storage.

4.6.3.2 Under refrigeration

F.pallidoroseum formulations when stored under refrigeration also showed a gradual reduction in the spore viability over a period of time. However none of the formulations lost viability even at the end of 12 months except aluminium foil packed charcoal formulations (Table12). The spore viability measured in terms of mycelial growth did not vary significantly with change in packing material except in the case of leaf mold formulation which showed variation from ninth month onwards. The mean colony diameter was maximum in the case of glass vial packing during the period of 9 to 12 months after storage followed by polypropylene packing. Even in aluminium foil the survivability could be retained till the end of 12 months.

Table 12. Effect of packing materials on viability of *F.pallidoroseum* formulations stored under refrigeration

MAS	Mean colony diameter (cm)											
	Diatomaceous earth			Charcoal powder			Semidry cadaver			Leaf mold		
	G V	P P	A F	G V	P P	A F	G V	P P	A F	G V	P P	A F
1	8.70 (17.15)	8.50 (16.94)	8.50 (16.94)	7.90 (16.32)	7.87 (16.28)	7.87 (16.28)	8.80 (17.25)	8.87 (17.32)	8.67 (17.11)	7.70 (16.10)	7.80 (16.21)	7.77 (16.17)
2	8.40 (16.84)	8.13 (16.56)	8.23 (16.67)	7.50 (15.89)	7.43 (15.82)	7.33 (15.70)	8.87 (17.22)	8.63 (17.08)	8.50 (16.94)	7.73 (16.14)	7.63 (16.03)	7.53 (15.92)
3	7.93 (16.35)	7.87 (16.28)	7.67 (16.07)	7.00 (15.34)	6.97 (15.30)	6.83 (15.15)	8.00 (16.42)	7.97 (16.39)	8.00 (16.42)	7.00 (15.34)	7.00 (15.34)	6.97 (15.30)
4	7.50 (15.89)	7.47 (15.85)	7.47 (15.85)	6.30 (14.53)	6.20 (14.41)	6.16 (14.37)	7.57 (15.96)	7.60 (16.00)	7.50 (15.89)	6.57 (14.83)	6.50 (14.76)	6.57 (14.83)
5	7.10 (15.45)	7.03 (15.37)	7.00 (15.34)	5.56 (13.63)	5.93 (14.09)	5.80 (13.93)	7.50 (15.89)	7.47 (15.85)	7.23 (15.59)	6.00 (14.17)	6.00 (14.17)	6.07 (14.25)
6	6.47 (14.73)	6.33 (14.57)	6.47 (14.73)	5.00 (12.92)	4.93 (12.83)	4.80 (12.65)	7.03 (15.37)	6.93 (15.26)	6.97 (15.30)	5.83 (13.97)	5.90 (14.05)	6.00 (14.17)
7	6.03 (14.21)	5.90 (14.05)	6.00 (14.17)	4.33 (12.01)	4.47 (12.20)	4.33 (2.01)	6.57 (14.83)	6.50 (14.76)	6.30 (14.53)	5.99 (14.17)	5.66 (13.76)	5.73 (13.85)
8	5.00 (12.92)	5.00 (12.92)	5.03 (12.96)	3.16 (10.24)	3.00 (9.97)	2.93 (9.85)	6.00 (14.17)	6.00 (14.17)	5.97 (14.13)	5.70 (13.81)	5.50 (13.56)	5.50 (13.56)
9	4.70 (12.52)	4.57 (12.33)	4.63 (12.43)	2.53 (9.15)	2.43 (8.97)	2.30 (8.72)	5.70 (13.81)	5.73 (13.85)	5.60 (13.65)	5.33 (13.34)	4.90 (12.78)	4.33 (12.08)
10	4.03 (11.58)	3.93 (11.43)	3.83 (11.29)	1.66 (7.40)	1.60 (7.26)	1.50 (7.03)	5.10 (13.05)	5.07 (13.00)	5.10 (13.05)	4.83 (12.69)	4.00 (11.53)	3.62 (10.97)
11	3.10 (10.13)	2.97 (9.91)	2.87 (9.74)	0.72 (4.86)	0.83 (5.22)	0.86 (5.33)	3.97 (11.48)	4.00 (11.53)	4.03 (11.58)	4.00 (11.53)	3.60 (10.93)	2.87 (9.74)
12	2.73 (9.51)	2.33 (8.77)	2.13 (8.38)	0.18 (2.40)	0.22 (2.70)	00 (O)	2.87 (9.74)	2.48 (9.06)	2.31 (8.74)	2.61 (9.30)	1.73 (7.55)	1.00 (6.80)

MAS – Months After Storage
PP – Polypropylene
GV – Glass Vial
AF – Aluminium Foil
CD for comparison of treatment combinations = 0.62
Figures in parentheses are values after angular transformation

4.6.4 Effect of packing material on the virulence of the fungus

4.6.4.1 At room temperature

In order to find out the efficacy of the formulated material stored in different containers at room temperature, samples were drawn at monthly intervals for a period of 12 months and they were sprayed on aphids. The mortality was recorded at 24 h interval (Table 13). The mortality rate at the end of one month ranged from 96.45 (charcoal formulation in polypropylene) to 100 and they did not differ significantly among different treatments. When samples drawn from two month old material was sprayed on aphids, cent percent mortality was observed only with semidry cadaver stored in glass vial. All other formulations resulted in a mortality percentage ranging from 76.13 (charcoal formulation in polypropylene) to 98.86 (semidry cadaver formulation in aluminium foil). During the third month more than 99 per cent mortality was recorded when sample of semidry cadaver stored in glass vial was used. In all other formulations mortality ranged from 76.75 to 96.45. More than 40 per cent reduction in mortality rate was noticed between four and five month old samples of charcoal based formulations. Mortality rate at the end of four months ranged from 85.52 (polypropylene packed charcoal) to 98.13 (glass vial packed charcoal formulation) while at the end of five months it ranged from 41.93 (polypropylene packing) to 44.61 (glass vial packing). However such a sudden reduction in mortality rate was not observed among other formulations. Mortality rate of charcoal based formulation stored in glass vial was almost half the rate of semidry cadaver (89.34) and diatomaceous earth formulations (88.82) stored in the same container. A similar trend was noticed during the sixth month also. During this period mortality rate with semidry cadaver ranged from

Table 13. Effect of packing materials on virulence of *F.pallidoroseum* formulations stored at room temperature

MAS	Cumulative per cent mortality of aphids											
	Diatomaceous earth			Charcoal powder			Semidry cadaver			Leaf mold		
	G V	P P	A F	G V	P P	A F	G V	P P	A F	G V	P P	A F
1	98.21 (82.29)	98.86 (83.85)	100 (90.00)	100 (90.00)	96.45 (79.11)	98.86 (83.85)	100 (90.00)	99.56 (86.14)	98.86 (83.85)	97.64 (81.14)	96.87 (79.78)	100 (90.00)
2	97.00 (79.99)	88.88 (70.49)	87.92 (69.83)	95.18 (77.280	76.13 (60.73)	86.03 (68.02)	100 (90.00)	95.48 (77.69)	98.86 (83.85)	96.45 (79.11)	96.45 (79.11)	95.02 (77.08)
3	88.13 (69.82)	86.99 (68.83)	81.85 (64.75)	96.45 (79.11)	86.26 (68.22)	80.00 (63.41)	99.33 (85.26)	87.76 (69.50)	95.48 (77.69)	83.41 (65.93)	76.75 (61.15)	78.06 (62.05)
4	86.64 (68.53)	83.64 (66.12)	80.71 (63.92)	98.13 (69.82)	85.52 (67.61)	87.92 (69.63)	95.48 (77.69)	67.43 (55.18)	81.37 (64.40)	79.84 (63.29)	77.38 (61.58)	77.53 (61.68)
5	88.82 (70.43)	77.43 (61.61)	73.80 (59.19)	44.61 (41.89)	41.93 (40.34)	43.16 (41.05)	89.34 (70.91)	65.42 (53.96)	84.91 (67.11)	75.45 (60.27)	60.86 (51.25)	72.87 (58.58)
6	86.16 (68.14)	73.80 (59.19)	78.27 (62.19)	43.31 (41.14)	40.66 (39.60)	40.44 (39.48)	89.69 (71.24)	58.05 (49.61)	83.41 (65.93)	70.94 (57.36)	63.37 (52.73)	64.72 (53.54)
7	83.64 (66.12)	77.01 (61.32)	70.50 (57.27)	41.88 (39.99)	41.22 (39.93)	45.90 (42.68)	79.89 (62.97)	55.35 (48.05)	80.43 (67.54)	56.62 (48.44)	55.50 (48.14)	65.14 (53.79)
8	64.72 (53.54)	68.85 (56.05)	69.44 (56.42)	45.98 (42.68)	37.90 (37.98)	39.95 (39.91)	65.39 (53.94)	53.38 (46.92)	50.00 (47.98)	59.82 (50.82)	36.65 (37.24)	38.59 (38.39)
9	58.74 (50.01)	59.34 (50.36)	58.31 (49.76)	39.32 (38.82)	38.55 (38.37)	39.99 (39.21)	66.03 (54.33)	51.33 (45.75)	55.39 (48.07)	39.29 (38.80)	39.86 (39.13)	41.29 (39.97)
10	47.95 (46.35)	54.69 (47.67)	56.00 (48.43)	31.31 (34.01)	26.52 (30.98)	40.57 (39.55)	63.35 (52.72)	44.63 (41.90)	50.00 (44.98)	39.99 (39.21)	39.33 (38.82)	37.95 (38.01)
11	39.22 (38.76)	41.95 (40.35)	43.97 (41.52)	39.32 (38.82)	33.20 (35.17)	32.61 (34.81)	51.33 (45.75)	46.66 (43.07)	43.99 (41.53)	37.25 (37.60)	35.81 (36.74)	33.88 (35.58)
12	28.42 ( )	31.95 (34.00)	28.89 (32.50)	34.51 (35.96)	33.12 (35.12)	33.91 (35.60)	58.07 (49.63)	47.33 (43.45)	43.29 (41.12)	39.96 (38.92)	37.76 (38.90)	28.86 (32.48)

MAS – Months After Storage
PP – Polypropylene
GV – Glass Vial
AF – Aluminium Foil
CD for comparison of treatment combinations = 9.60
Figures in parentheses are values after angular transformations

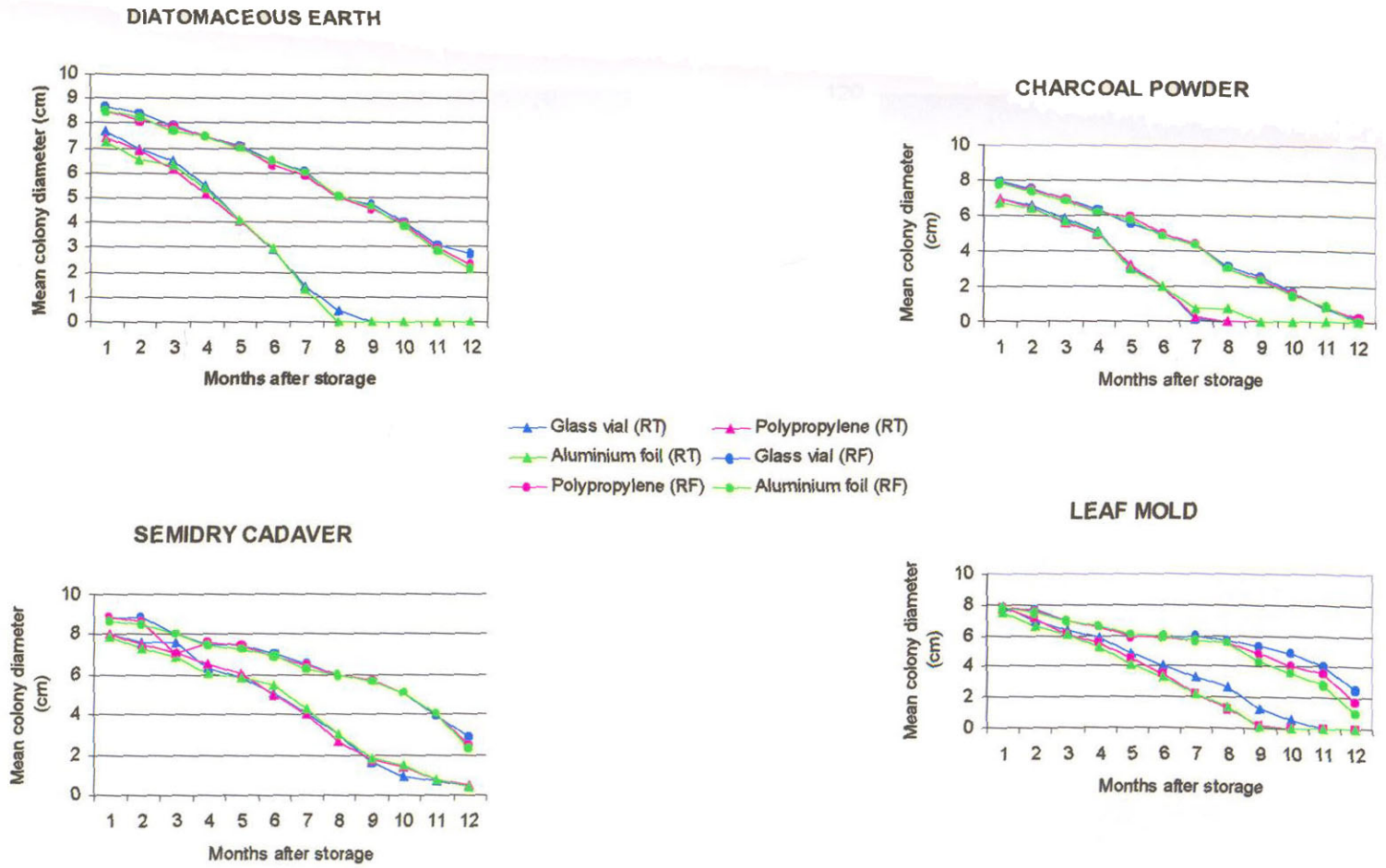


Fig. 8. Effect of packing materials on viability of *F.pallidoroseum* spores stored at different temperatures

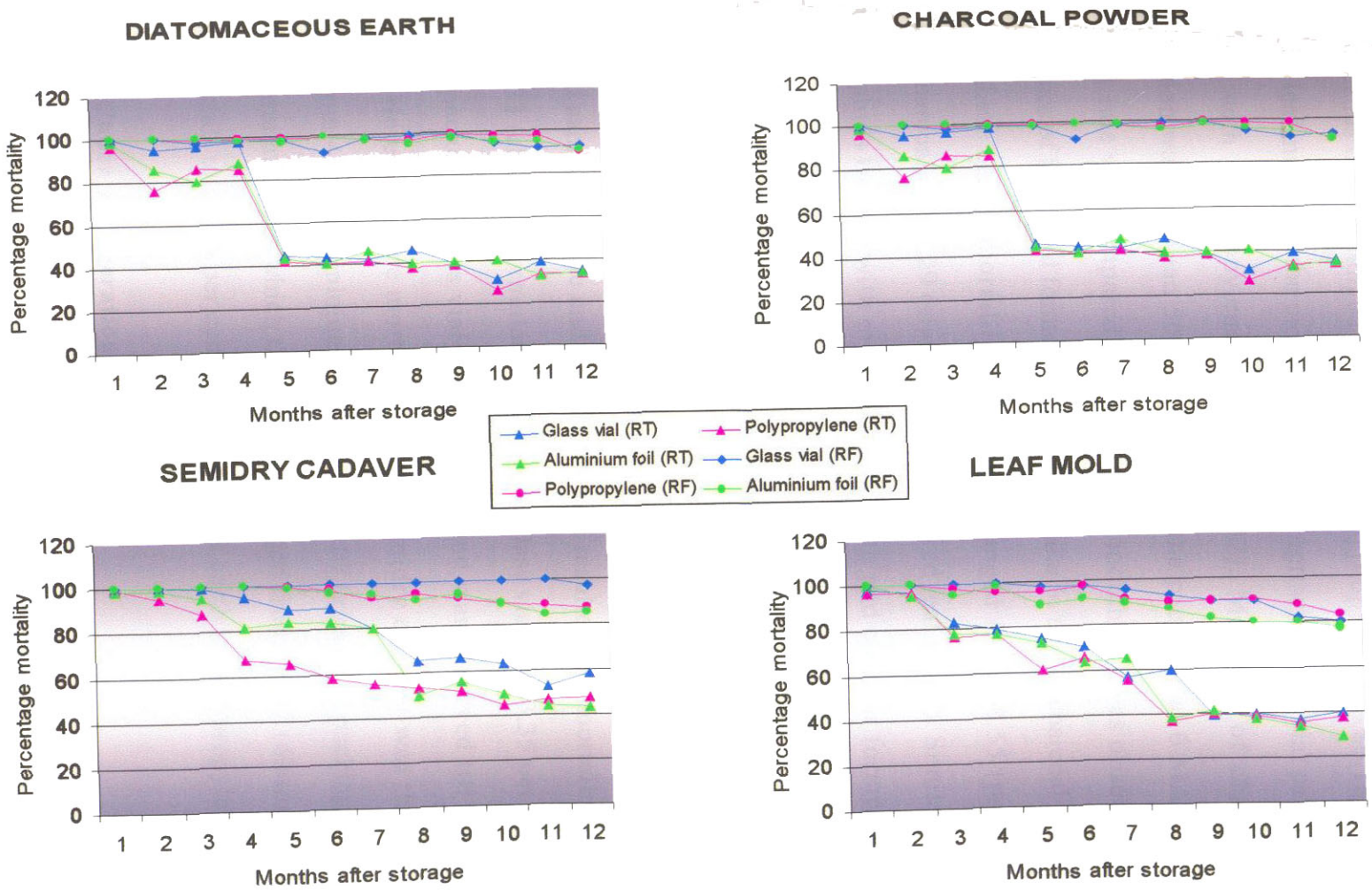


Fig. 9. Effect of packing materials on virulence of *F.pallidoroseum* spores stored at different temperatures

58.05 (polypropylene packing) to 89.69 (glass vial packing) while with diatomaceous earth it ranged from 73.80 (polypropylene) to 86.16 (glass vial) and with leaf mold also the mortality rate was high with glass vial packing (70.94) and low with polypropylene packing (63.37). Mortality of charcoal based formulation was less than 50 per cent in all the packings at 6 MAS. When the mortality rate was compared at the end of 9 months it was observed that the performance of leaf mold formulations were similar to that of charcoal formulations. In both these cases mortality percentage ranged from 38.55 (polypropylene packed charcoal) to 41.29 (aluminium foil packed leaf mold). Similarly mortality rate of aphids treated with diatomaceous earth and cadaver formulations were equal and ranged from 59.33(polypropylene packed semidry cadaver) to 66.03 (glass vial packed cadaver formulation).

Eventhough the fungus failed to grow in diatomaceous earth, charcoal and leaf mold samples, at the end of 10 months it resulted in a mortality rate ranging from 31.33 (charcoal formulation packed in glass vial) to 63.35 per cent (semidry cadaver packed in glass vial). A similar trend was observed till the end of 12 months. Here again the mortality rate of semidry cadaver was the highest and ranged from 43.99 (aluminium packing) to 58.07 (glass vial packing) compared to other formulations in which the morality ranged from 28.42 (diatomaceous earth in glass vial) to 41.95 per cent (diatomaceous earth in polypropylene).

4.6.4.2 Under refrigeration

F.pallidoroseum formulations when stored in different packing materials and kept under refrigeration, it was found that all the formulations resulted in cent

per cent mortality till the end of two months of storage (Table 14). Thereafter there was slight reduction in the mortality rate in charcoal and leaf mold formulations. But this reduction in mortality observed was not significant till the end of seven months except in the case of aluminium foil packed leaf mold formulation (90 per cent). After eight months of storage only diatomaceous earth and semidry cadaver formulations resulted in cent per cent mortality whereas with others the mortality percentage ranged from 86 (aluminium foil packed leaf mold) to 90 per cent (polypropylene packed diatomaceous earth and charcoal formulations). Even at the end of 12 months of storage under refrigeration more than 75 per cent mortality was observed with all the formulations, whereas when kept at room temperature there was only 28 to 58 per cent mortality. Here also rate of loss in virulence was found to be less when compared to that of viability (comparison of Fig.8 and 9). Significant variation was not noticed in the mortality rate due to change in packing material, even though the highest mortality rates recorded were with glass vial packings.

The results revealed that packing materials used in this experiment did not make any significant variation in the spore qualities as they were equally effective based on the viability and virulence of the formulations stored in them.

4.7 Compatibility of *F.pallidoroseum* with botanicals and quinalphos

4.7.1 Effect of botanicals and quinalphos on the mycelial growth of the fungus

The effect of botanicals and quinalphos on growth of the fungus was studied on PDA using Poisoned Food Technique. *F.pallidoroseum* completely covered a 9 cm petridish within 9 days after inoculation in the control, where the fungus was grown on PDA (Table15). The growth of the fungus in media poisoned with 5 per

Table 14 Effect of packing materials on virulence of *F.pallidoroseum* formulations stored under refrigeration

MAS	Cumulative per cent mortality of aphids											
	Diatomaceous earth			Charcoal powder			Semidry cadaver			Leaf mold		
	G V	P P	A F	G V	P P	A F	G V	P P	A F	G V	P P	A F
1	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
2	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
3	100 (90.00)	100 (90.00)	99.78 (87.28)	98.21 (82.29)	99.33 (85.26)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	98.21 (82.29)	95.48 (77.69)
4	100 (90.00)	100 (90.00)	99.78 (87.28)	98.86 (83.85)	99.56 (86.14)	98.70 (83.42)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	96.00 (78.43)	98.86 (83.85)
5	100 (90.00)	100 (90.00)	100 (90.00)	98.86 (83.85)	99.11 (84.56)	98.33 (82.54)	100 (90.00)	99.10 (84.51)	98.86 (83.85)	98.21 (82.29)	96.00 (78.43)	90.00 (71.54)
6	100 (90.00)	100 (90.00)	99.78 (87.28)	92.51 (74.08)	99.10 (84.51)	99.33 (85.26)	100 (90.00)	97.00 (79.99)	96.45 (79.11)	98.21 (82.29)	97.78 (81.41)	92.28 (73.83)
7	100 (90.00)	100 (90.00)	100 (90.00)	98.86 (83.85)	97.78 (81.41)	97.78 (81.41)	100 (90.00)	93.30 (74.97)	95.17 (77.27)	96.00 (78.43)	91.44 (72.95)	90.00 (71.54)
8	100 (90.00)	100 (90.00)	98.70 (83.42)	99.10 (84.51)	97.31 (80.53)	95.56 (77.81)	100 (90.00)	95.48 (77.69)	92.28 (73.83)	93.30 (74.97)	90.00 (71.54)	86.50 (68.42)
9	100 (90.00)	99.56 (86.14)	90.86 (83.85)	99.10 (84.51)	99.56 (86.14)	98.21 (82.29)	100 (90.00)	92.28 (73.83)	94.28 (76.13)	90.00 (71.54)	90.00 (71.54)	82.09 (64.94)
10	100 (90.00)	97.78 (81.41)	95.65 (77.94)	95.48 (77.69)	98.86 (83.85)	96.00 (78.43)	100 (90.00)	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	90.69 (72.20)	80.00 (63.41)
11	95.48 (77.69)	92.08 (73.62)	90.00 (71.54)	92.28 (73.84)	97.64 (81.12)	95.48 (77.69)	99.78 (87.28)	88.73 (70.36)	84.81 (67.04)	82.09 (64.94)	88.05 (69.75)	80.10 (63.48)
12	94.28 (76.13)	92.28 (73.83)	90.00 (71.54)	92.89 (74.50)	90.00 (71.54)	90.69 (72.20)	97.41 (80.70)	87.40 (69.18)	84.45 (66.75)	80.00 (63.41)	82.74 (65.43)	77.53 (61.68)

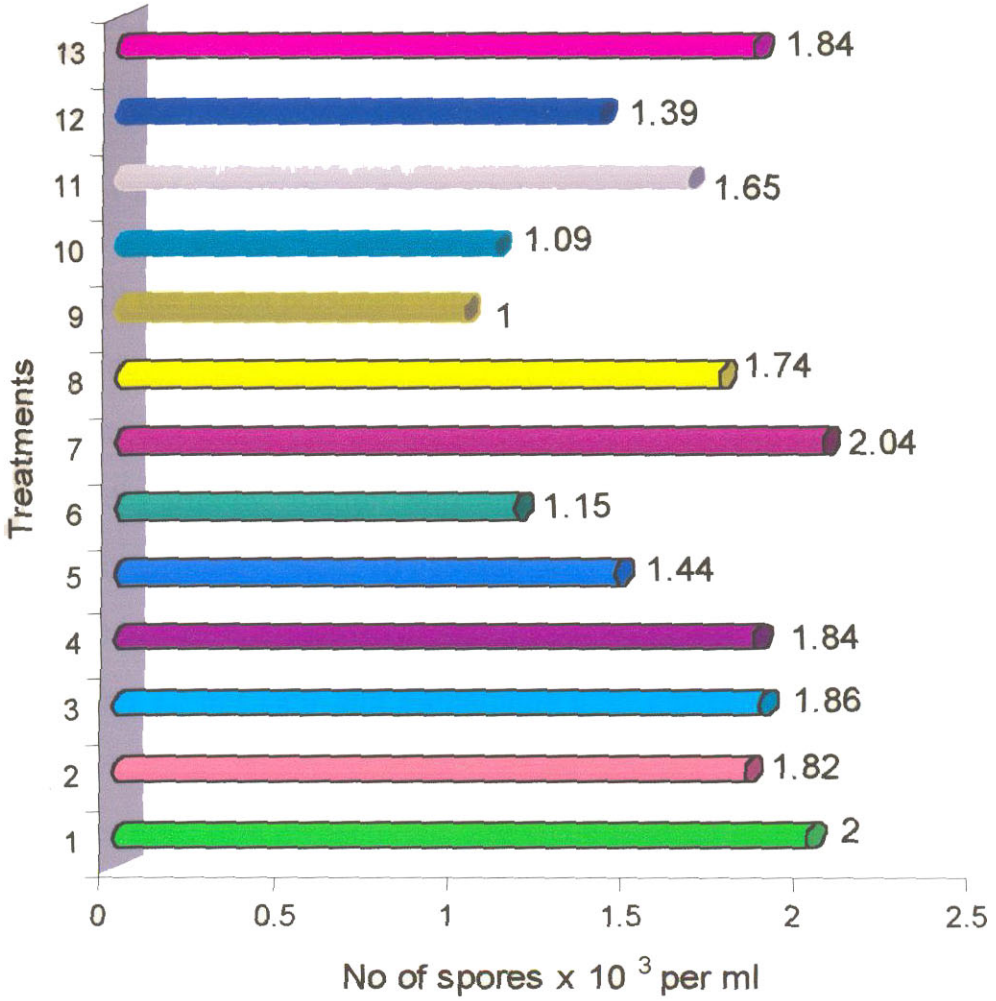
MAS – Months After Storage
PP – Polypropylene
GV – Glass Vial
AF – Aluminium Foil
CD for comparison of treatment combinations = 8.03
Figures in parentheses are values after angular transformations

Table 15. Mean diameter of fungal growth on PDA poisoned with botanicals and quinalphos.

Sl No.	Treatments*	Mean colony diameter (cm) at days after inoculation							
		2	3	4	5	6	7	8	9
1	5% BLE	2.02 (1.42)	3.02 (1.73)	4.02 (1.97)	5.02 (2.24)	6.02 (2.45)	7.02 (2.64)	8.02 (2.83)	9.00 (3.00)
2	10% BLE	1.99 (1.41)	2.90 (1.70)	3.90 (1.97)	5.00 (2.23)	5.90 (2.42)	6.80 (2.60)	7.80 (2.79)	8.80 (2.96)
3	5% HLE	1.96 (1.40)	2.89 (1.70)	3.80 (1.49)	4.81 (2.19)	5.90 (2.42)	6.90 (2.62)	8.00 (2.82)	8.96 (2.99)
4	10% HLE	2.00 (1.41)	3.00 (1.73)	4.00 (2.00)	5.00 (2.23)	5.90 (2.42)	6.80 (2.60)	7.80 (2.79)	8.80 (2.96)
5	5% TLE	1.93 (1.38)	2.80 (1.67)	3.80 (1.94)	4.80 (2.19)	5.80 (2.40)	6.50 (2.50)	7.67 (2.76)	8.67 (2.94)
6	10% TLE	1.95 (1.39)	2.75 (1.65)	3.75 (1.93)	4.75 (2.17)	5.75 (2.39)	6.50 (2.50)	7.50 (2.73)	8.50 (2.91)
7	5% NLE	2.04 (1.42)	3.00 (1.73)	4.00 (2.00)	5.00 (2.23)	5.80 (2.40)	6.80 (2.60)	7.80 (2.79)	8.80 (2.96)
8	10% NLE	1.90 (1.37)	2.90 (1.70)	4.00 (2.00)	4.75 (2.17)	5.65 (2.37)	6.65 (2.57)	7.75 (2.78)	8.80 (2.96)
9	10% NSO	1.96 (1.40)	2.95 (1.71)	3.96 (1.98)	4.95 (2.22)	5.80 (2.40)	6.80 (2.60)	7.80 (2.79)	8.80 (2.96)
10	10% MSO	1.99 (1.41)	2.99 (1.72)	4.00 (2.00)	5.00 (2.23)	6.00 (2.44)	7.00 (2.64)	8.00 (2.82)	8.96 (2.99)
11	S S(10%)	1.95 (1.39)	2.95 (1.71)	3.95 (1.98)	4.95 (2.22)	6.95 (2.63)	7.75 (2.78)	8.85 (2.97)	8.85 (2.97)
12	Q (0.05%)	1.95 (1.39)	2.59 (1.60)	3.90 (1.98)	4.90 (2.21)	5.80 (2.40)	6.80 (2.60)	7.80 (2.79)	8.80 (2.96)
13	Control	2.00 (1.41)	3.10 (1.76)	4.00 (2.00)	5.00 (2.23)	6.00 (2.44)	7.20 (2.68)	8.10 (2.84)	9.00 (3.00)

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

* BLE – Bougainvillea leaf extract
HLE – Hyptis leaf extract
TLE – Thevetia leaf extract
NLE – Neem leaf extract
NSO – Neem seed oil emulsion
MSO – Marotti seed oil emulsion
SS - Soap solution
Q - Quinalphos



- 5% Bougainvillea leaf extract
- 10% Bougainvillea leaf extract
- 5% Hyptis leaf extract
- 10% Hyptis leaf extract
- 5% Thvetia leaf extract
- 10% Thvetia leaf extract
- 5% Neem leaf extract
- 10% Neem leaf extract
- 10% Neem seed oil emulsion
- 10% Marotti seed oil emulsion
- 10% Soap solution
- 0.05% quinalphos
- Control

Fig. 10. Sporulation of *F.pallidoroseum* on PDA poisoned with different botanical insecticides and quinalphos

cent leaf extract of bougainvillea behaved similar to control. In all other treatments the growth of the fungus ranged from 8.67 cm to 8.98 cm indicating that none of the botanical insecticides nor quinalphos inhibited the growth of the fungus (Plate 12).

4.7.2 Effect of botanicals and quinalphos on the sporulation of the fungus

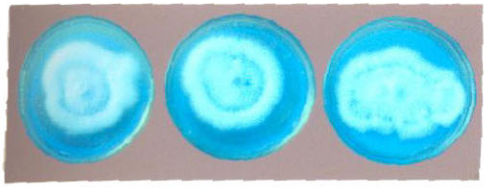
In all the media poisoned with either botanicals or insecticide the fungus failed to produce macroconidia. Hence the population difference of conidia among the different treatments was compared based on spore load of microconidia on the seventh day after inoculation. Maximum inhibition of conidia was observed in treatments incorporated with 10 per cent seed oil emulsions of neem ($1.0x10^3$ spores ml^{-1}) and marotti ($1.09x10^3$ spores ml^{-1}) (Fig.10). Thevetia leaf extracts, both at 5 and 10 per cent and quinalphos 0.05 per cent exhibited moderate inhibition when compared to control, their spore load being $1.44x10^3$, $1.15x10^3$, $1.39x10^3$ per milli litre respectively. The spore load estimated from 10 per cent leaf extracts of hyptis, bougainvillea and neem were on par with that of control, while 5 per cent leaf extracts of neem, hyptis and bougainvillea were found to enhance sporulation significantly ($2.04x10^3$, $2.0x10^3$, $1.82x10^3$ spores ml^{-1}).

4.8 Assessment of time and method of application of *F.pallidoroseum* formulations under field conditions

4.8.1 On population control of *A. craccivora*

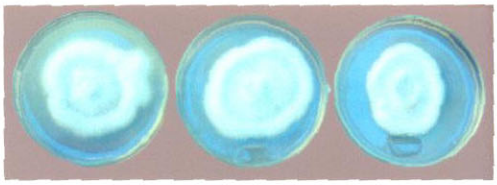
4.8.1.1 Prophylactic treatment

Effect of prophylactic application of the biopesticide *F.pallidoroseum* was studied by applying the fungal spores at three weeks after planting (WAP), when

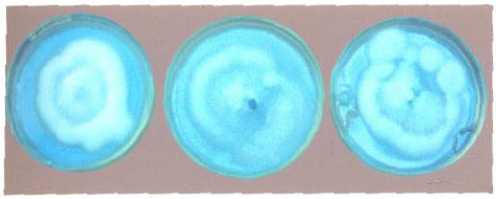


Control BLE 5% BLE 10%

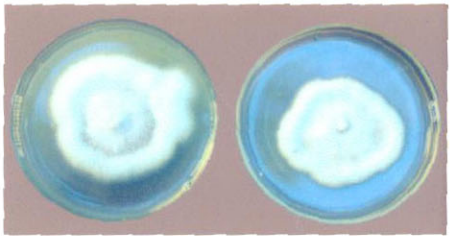
Control HLE 5% HLE 10%



Control NLE 5% NLE 10%



Control NSO 10% MSO 10%



Control Q (0.05)

Plate 12. Growth of *F. pallidoroseum* on PDA - poisoned with different botanical insecticides and quinalphos

the plants were free from aphid infestation. Results of the study clearly indicated that there is no significant difference in aphid population when the spores were applied either by spraying or broadcasting. Similarly significant difference was not observed even when different carrier materials were used for preparing the biopesticide (Table 16).

Observations on the population fluctuation of aphids revealed that both in prophylactic as well as control plots infestation of aphids occurred only when plants reached 4 WAP. A population build up was observed thereafter during the successive stages and it reached a maximum of 274.4 aphids per plant (population in terminal 15 cm twig) at 7 WAP in prophylactic treatment and 353.43 at 8 WAP in the case of control. Thereafter a population decline was noted during ninth week in both the cases and it was absolutely nil during the tenth WAP. From eleventh week onwards when the crop reached it's late reproductive phase there was only negligible incidence of the pest. The mean aphid count per plant per 15 cm terminal twig was only 2.56.

4.8.1.2 Curative treatment

Effect of application of the biopesticide after incidence of aphid infestation was studied by applying it at 6 WAP by spraying as well as broadcasting. Analysis of variance revealed that there was no significant difference in aphid population when the formulated spores were sprayed or broadcasted or when different materials were used as carriers (Table 17).

Table 16. Effect of carrier material and method of application of *F. pallidoroseum* formulations on population buildup of *A.craccivora* (Prophylactic treatment)

WAP	Control	Mean aphid population per 15 cm terminal twig								
		Diatomaceous earth		Charcoal powder		Semi dry cadaver		Leaf mold		Treatment Mean
		Spraying	Broadcasting	Spraying	Broadcasting	Spraying	Broadcasting	Spraying	Broadcasting	
4	12.74	12.33 (2.50)	14.33 (2.69)	20.42 (2.97)	16.38 (2.71)	12.26 (1.90)	20.42 (2.97)	24.50 (3.10)	20.42 (2.97)	17.60
5	75.97	69.64 (3.82)	60.02 (3.93)	105.63 (4.22)	113.28 (4.50)	24.50 (3.10)	69.60 (4.19)	92.86 (4.40)	104.30 (3.99)	123.58
6	212.76	194.97 (5.05)	137.03 (4.75)	220.91 (5.24)	234.65 (5.26)	172.13 (5.12)	148.87 (4.96)	188.98 (5.06)	214.87 (5.10)	200.30
7	223.93	283.72 (5.57)	214.94 (5.29)	283.72 (5.61)	210.62 (4.78)	319.72 (5.75)	267.68 (5.26)	378.46 (5.94)	236.72 (5.68)	274.40
8	353.49	165.81 (4.99)	116.12 (4.70)	117.39 (4.69)	117.39 (4.52)	155.75 (5.03)	51.80 (3.78)	152.95 (4.97)	90.15 (4.50)	120.90
9	109.67	70.97 (4.06)	32.63 (3.47)	20.42 (2.96)	24.46 (3.10)	75.05 (4.20)	20.45 (2.88)	38.19 (3.30)	20.42 (2.77)	37.80
10	00	00	00	00	00	00	00	00	00	00
11	6.90	00	4.11	00	00	00	00	4.11	12.33	2.56
12	9.71	00	4.11	00	00	00	00	4.11	12.33	2.56

CD for comparison of treatment combinations = 0.876
Figures in parentheses are values after logarathmic transformation
WAP – Weeks After Planting

Table 17. Effect of carrier material and method of application of *F. pallidoroseum* formulations on population buildup of *A.craccivora* (Curative treatment)

WAP	Control	Mean aphid population per 15 cm terminal twig								
		Diatomaceous earth		Charcoal powder		Semi dry cadaver		Leaf mold		Treatment Mean
		Spraying	Broadcasting	Spraying	Broadcasting	Spraying	Broadcasting	Spraying	Broadcasting	
4	12.74	4.11 (0.84)	12.26 (1.90)	8.22 (1.67)	8.22 (1.67)	16.38 (2.74)	8.22 (1.06)	12.33 (2.51)	16.31 (2.13)	10.75
5	75.97	38.13 (3.52)	24.50 (3.52)	60.02 (3.93)	38.11 (3.59)	60.02 (4.06)	38.11 (3.59)	57.37 (3.97)	41.00 (2.71)	44.65
6	212.76	132.71 (4.92)	132.71 (4.92)	201.41 (5.26)	211.73 (5.33)	401.8 (5.71)	335.68 (5.74)	214.21 (5.36)	134.31 (4.56)	280.56
7	223.93	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	353.49	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	109.67	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	6.90	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.11	0.53
12	9.71	4.11	8.22	0.0	0.0	.0.0	0.0	4.11	4.11	2.16

CD for comparison of treatment combinations = 0.490
Figures in parentheses are values after logarathmic transformation

Population of aphids in the field was first recorded during the fourth week after planting (10.75 per 15 cm of terminal twig per plant) which gradually increased to a maximum of 226.35 at the time of application of the biopesticide. After three to five days of treatment there was complete destruction of aphids. This situation continued upto the end of 12WAP. However very mild reinfestation was recorded during the eleventh WAP (0.52 per 15 cm of terminal twig per plant) and 12 WAP (2.56 per 15 cm of terminal twig per plant) when the crop started drying.

A comparison of weekly mean population build up of aphids (Fig.11) in the control plot and in the plots subjected to prophylactic and curative treatment with *F.pallidoroseum* clearly showed that cent per cent control of pest is possible by application of spore formulation at 6 WAP, when there is aphid infestation and that application of the biopesticide before pest incidence is not at all effective in controlling the pest. Crop stage has a direct effect on population build up of the pest. Peak infestation always coincided with the reproductive stage of the crop.

4.8.2 On the average yield of cowpea

A comparison on the average yield obtained from plots subjected to prophylactic and curative treatment of *F.pallidoroseum* when applied using different carrier materials and methods clearly revealed that there is significant difference in the average yield obtained from prophylactic and curative application (Table 18). The average yield obtained from prophylactic treatment ranged from 748.33 to 848.33 g per plant while that from curative application ranged from 958.33 to 1051.67 g per plant. In the control plot, yield recorded was 790 to 845.80 g per

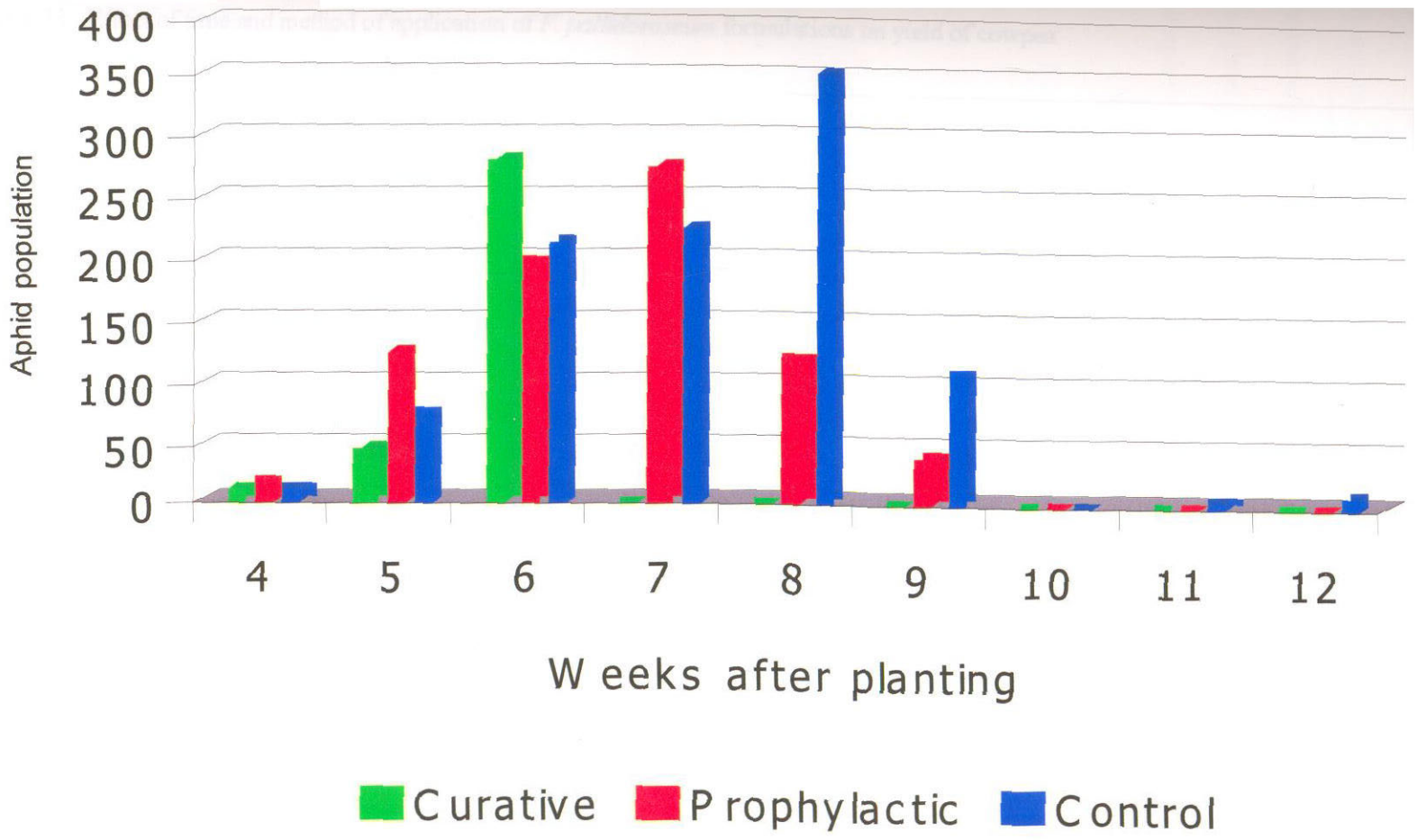


Fig. 11. Effect of prophylactic and curative control of *A. craccivora* with *F.pallidoroseum* formulations under field conditions

Table 18. Effect of time and method of application of *F. pallidoroseum* formulations on yield of cowpea

Sl No	Carrier material	Application method	Average yield per plant (g)				
			Prophylactic (3 WAP)		Curative (6 WAP)		Control
			Yield	PDC	Yield	PDC	Yield
1	Diatomaceous earth	Spraying	848.33	7.30	1051.67	33.12	790.00
		Broadcasting	848.33	-5.60	1020.00	27.02	803.00
2	Charcoal powder	Spraying	785.00	-2.40	1001.67	24.43	805.00
		Broadcasting	776.67	-8.10	1018.33	20.47	845.80
3	Semidry cadaver	Spraying	808.33	1.60	1006.67	22.51	821.67
		Broadcasting	840.00	1.60	1023.33	23.78	826.67
4	Leaf mold	Spraying	806.00	0.40	1038.33	28.14	810.00
		Broadcasting	748.33	-8.30	958.33	17.36	816.67

CD for treatment combinations = 75.23

PDC – Percentage Deviation over Control

plant which was not significantly different from that obtained from prophylactic treatment. The change in carrier material or application method (spraying / broadcasting) did not make any significant difference in the average yield per plant, but timely application of the biopesticide which resulted in cent per cent destruction of aphids increased the yield correspondingly by 17- 33 per cent.

4.9 Biosafety evaluation of *F.pallidoroseum*

4.9.1 Safety test of the fungus to crop plants and other nontarget organisms

4.9.1.1 Crop plants

Certain strains of *F.pallidoroseum* were reported to be pathogenic to crop plants such as amaranthus, pineapple, drumstick, mulberry, banana, passion fruit and sesamum. To find out whether the strain used for the management of *A.craccivora* is pathogenic to the above plants, pathogenicity test was conducted by soil and leaf inoculation of the spore suspension at a concentration of $7x10^6$ spores ml^{-1}. Results of the study indicate that none of the plants reported to be the host of *F.pallidoroseum* are infected by the strain used for the present investigation either when soil or leaf inoculated.

4.9.1.2 Natural enemies

In order to find out whether the strain of *F.pallidoroseum* used for the study is pathogenic to the commonly observed natural enemies of *A.craccivora* like coccinellids, *Menochiles sexmaculata, Coccinella septumpunctata,* and *Micraspis crocea*; syrphids, *Ischiodon scutellare, Ischiodon* sp., hemerobid;

spiders, *Lycosa pseudoannulata, Tetragnatha maxillosa* and *Oxyopus* sp., dragonflies and preying mantids, an experiment was laid out by artificially inoculating these insects with *F. pallidoroseum* spores at a concentration of 7 $x10^6$ spores ml^{-1} under laboratory conditions. The study revealed that the fungus is not pathogenic to the tested natural enemies. All the treated grubs of coccinellids, maggots of syrphids and larvae of haemerobid kept under observation pupated normally and emerged as healthy adults as in the case of control group of insects. The treated adult coccinellids laid eggs which emerged normally. The spiders, dragonflies and preying mantis did not develop any symptoms of mycosis.

4.9.1.3 Productive insects and pollinators

None of the treated pollinators *Bombus sp, Camponotus sp, Syrphus sp and Xylocopa* sp and stingless bee *Trigona iridipennis* developed any symptoms of mycosis.

4.9.1.4 Cross infectivity of *F. pallidoroseum* to other insect pests and mites

None of the treated lepidopteran, coleopteran, hemipteran or orthopteran pests or the mites (mentioned in para 3.11.4) developed disease symptoms when inoculated with $7x10^6$ spores ml^{-1} of *F. pallidoroseum.*

4.9.1.5 Cross infectivity to other aphids

Nine species of aphids collected from different crop plants sprayed with spore suspension at a concentration of $7x10^6$ spores ml^{-1} were kept under

observation for development of disease symptoms (Table 19). Of these, two speceis namely *Myzus persicae* and *Aphis gossypii* developed symptoms of mycosis. In both these cases Koch's postulates could be established for pathogenicity.

Table 19. Cross infectivity of *F. pallidoroseum* to other aphid species

Sl No	Aphid species	Host plant	Symptom
1	*Aphis gossypii*	Coccinia, Bringal	Positive
2	*Aphis malvae*	Hibiscus	Negative
3	*Aphis spiraecola*	Eupatorium	„
4	*Hysteroneura setariae*	Guinea grass	„
5	*Myzus persicae*	Sorghum	Positive
6	*Pentalonia nigronervosa*	Banana	Negative
7	*Toxoptera odinae*	Cashew	„
8	Unidentified species	Tulsi, Pepper	„

4.9.2 Safety evaluation of *F.pallidoroseum* on rabbits

All the rabbits treated with the spore suspension of the fungus at a concentration of $7x10^7$ spores ml^{-1} remained alert throughout the experimental period and did not show any clinical signs of diseases.

The mean body temperature (Table 20 & 21) recorded at weekly intervals remained within the physiological limits both in acute and chronic toxicity studies. It was 39.00 ± 0.09^0C in acute toxicity group and 38.94 ± 0.15^0C in chronic toxicity

Table 20. Body temperature of rabbits treated with *F. pallidoroseum* through different routes - Acute toxicity studies

Treatments	Mean body temperature					
	Hours after treatment					
	1/4	1	2	3	4	24
Oral F.p	38.86	39.00	39.00	38.93	39.13	38.93
Oral D.w	39.00	39.00	39.00	39.00	39.00	38.93
Dermal F.p	39.26	39.00	39.06	39.06	38.93	38.93
Dermal D.w	39.06	39.00	39.00	38.93	38.93	38.80
Subcutaneous F.p	39.13	39.00	39.06	39.00	39.00	38.93
Subcutaneous S.n.s..	39.00	39.06	39.00	39.00	39.13	38.86
Intranasal F.p	39.00	39.00	39.00	39.00	39.00	38.93
Intranasal D.w	38.86	39.06	39.00	39.00	39.00	38.93
Intravenous F.p	39.00	39.2	39.2	39.00	38.93	38.93
Intravenous S.n.s	39.13	39.26	39.13	38.93	39.00	38.86

CD : not significant

F.p - *F. pallidoroseum*

D.w - Distilled water

S.n.s -Sterile normal saline

Mean = 39.00

S.E = 0.09

Normal body temperature =38.7-39°C

Table 21. Body temperature of rabbits treated with *F.pallidoroseum* through different routes - Chronic toxicity studies

Treatments	Mean body temperature at weekly intervals (°C)							
	Weeks after treatment							
	1	2	3	4	5	6	7	8
Oral F.p	38.77	38.96	38.83	38.96	38.83	38.93	39.00	39.00
Oral D.w	38.96	38.86	38.90	38.80	39.06	38.83	39.00	38.93
Dermal F.p	38.86	39.03	39.03	38.93	39.06	38.86	38.83	39.06
Dermal D.w	38.83	38.86	38.96	38.93	38.96	38.93	39.03	39.06
Subcutaneous F.p	39.00	39.00	39.03	39.03	38.96	38.80	38.86	39.00
Subcutaneous S.n.s	38.83	38.83	38.83	38.93	38.86	38.93	39.00	39.00
Intranasal F.p	38.93	39.03	38.93	39.03	39.03	39.03	39.00	38.93
Intranasal D.w	38.93	39.03	38.93	38.93	38.76	38.86	38.96	38.60
Intravenous F.p	38.56	38.83	38.86	38.93	38.96	39.00	38.80	38.80
Intravenous S.n.s	38.90	38.96	39.00	38.96	38.96	38.96	38.96	38.83

CD : Not significant

F.p - *F. pallidoroseum*

D.w - Distilled water

S.n.s - Sterile normal saline

Mean = 38.94

SE = 0.15

Normal value = 3 8.7-39°C

group. The mean respiratory rate (Table 22 & 23) and mean pulse rate (Table 24 & 25) were also within physiological limits. The respiratory rate was 46.45±2.67 in acute toxicity group and 44.53±1.23 in chronic toxicity group. The pulse rate recorded was 130.07±2.99 in acute toxicity group and 123.00±4.02 in chronic toxicity group. However, the respiratory rate and pulse rate vary significantly in animals tested for acute toxicity. These variations were observed in dermal, subcutaneous, intranasal and intravenous roots of administration.

Haematology examination ruled out the possibility of any subclinical disease in chronic toxicity group of animals (Table 26). Haemoglobin content (9.66±0.4 $g^{-1}mm^3$) total WBC (8.29±1.02x$10^3/mm^3$), percentage of blood components viz. packed cell volume (38.79±1.77), neutrophils (34.73±5.59), lymphocytes (69.13±1.92), monocytes (5.13±0.94), eosinophils (2.33±0.54) and basophils (0.39±0.34) recorded from the blood samples drawn from chronic toxicity group of animals were within the normal range in all the routes of administration.

Mean body weight of rabbits did not show significant variation among the experimental and control groups or within the various experimental groups (Table 27). At the termination of the experimental period the mean body weight ranged from 2017 g to 2167 g in experimental group and 2090 g to 2153 g in control group. It was found that all the animals belonging to the experimental as well as their respective control groups followed a modified exponential growth pattern and in all the cases the growth rate ('b') tends to one (Table 28).

Post mortem examination did not show any consistent pathology in any of the experimental groups (Plate 13) although there were occasional congestion with or without mild haemorrhage in the mucosa, peritonium, liver, adrenal and kidney.

Table 22. Respiratory rate of rabbits treated with *F.pallidoroseum* through different routes - Acute toxicity studies

Treatments	Respiratory rate at hours after treatment					
	1/4	1	2	3	4	24
Oral F.p	44.33	44.66	44.66	45.66	46.00	46.33
Oral D.w	44.66	45.33	45.66	45.33	45.00	45.66
Dermal F.p	51.66	47.66	46.00	45.33	45.00	45.33
Dermal D.w	52.33	49.00	45.66	45.33	45.66	45.33
Subcutaneous F.p	52.33	48.66	44.66	45.00	45.33	44.33
Subcutaneous S.n.s.	52.33	49.33	45.33	45.00	45.66	43.66
Intranasal F.p	52.00	48.33	44.33	44.66	45.66	43.33
Intranasal D.w	51.66	47.33	45.00	44.66	46.00	45.00
Intravenous F.p	53.66	45.33	44.33	44.66	45.33	45.00
Intravenous S.n.s	53.33	49.33	45.00	44.66	45.33	45.33

F.p - *F. pallidoroseum*

D.w - Distilled water

S.n.s -Sterile normal saline

CD = 2.303

Normal rate = 45 -55

Table 23. Respiratory rate of rabbits treated with *F.pallidoroseum* through different routes - Chronic toxicity studies

Treatments	Respiratory rate at weeks after treatment							
	1	2	3	4	5	6	7	8
Oral F.p	44.00	42.33	43.00	44.00	44.33	42.66	43.00	42.00
Oral D.w	43.33	42.00	44.00	42.33	43.33	44.00	44.33	42.00
Dermal F.p	48.00	43.00	44.00	43.33	44.00	43.00	44.66	42.66
Dermal D.w	46.00	43.00	44.00	42.66	43.66	43.66	43.66	43.66
Subcutaneous F.p	44.33	45.33	44.00	45.33	44.33	45.33	46.00	46.00
Subcutaneous S.n.s	46.00	44.33	45.00	45.66	45.00	44.00	45.33	46.00
Intranasal F.p	45.00	45.33	46.00	46.33	45.33	44.66	44.66	46.00
Intranasal D.w	44.33	44.66	45.66	46.33	46.33	45.00	45.00	45.66
Intravenous F.p	44.33	44.66	45.66	44.66	46.00	45.66	44.66	46.00
Intravenous S.n.s	44.33	45.00	45.33	44.33	43.66	46.00	45.33	45.66

CD : Not significant

F.p- *F. pallidoroseum*

D.w- Distilled water

S.n.s -Sterile normal saline

Mean = 44.53

S.E = 1.23

Normal rate = 45-55

Table 24 Pulse rate of rabbits treated with *F.pallidoroseum* through different routes - Acute toxicity studies.

Treatments	Pulse rate at hours after treatment					
	¼	1	2	3	4	24
Oral F.p	125.00	124.33	125.00	125.00	125.00	124.66
Oral D.w	131.00	127.66	127.66	125.00	125.00	124.66
Dermal F.p	134.66	130.66	130.33	130.00	129.66	129.00
Dermal D.w	137.00	132.33	133.33	132.33	132.66	131.66
Subcutaneous F.p	137.00	133.33	131.33	131.33	130.66	129.66
Subcutaneous S.n.s	133.00	130.66	130.00	129.39	129.33	129.33
Intranasal F.p	133.00	131.00	130.33	129.66	129.66	129.66
Intranasal D.w	133.00	130.00	130.00	129.66	129.66	129.33
Intravenous F.p	134.66	133.00	132.33	129.66	129.33	129.33
Intravenous S.n.s	133.66	131.33	130.00	129.33	129.33	129.66

F.p - *F. pallidoroseum*

D.w - Distilled water

S.n.s -Sterile normal saline

CD = 1.94

Normal rate = 120-130

Table 25. Pulse rate of rabbits treated with *F.pallidoroseum* through different routes - Chronic toxicity studies

Treatments	Pulse rate at weeks after treatment							
	1	2	3	4	5	6	7	8
Oral F.p	119.66	121.00	120.00	120.33	120.00	120.00	119.66	119.66
Oral D.w	118.33	119.00	118.00	118.00	117.66	117.66	117.33	116.66
Dermal F.p	125.33	124.66	125.00	125.00	125.66	125.00	124.33	125.00
Dermal D.w	129.66	128.33	130.00	129.66	129.33	130.00	129.66	129.66
Sub cutaneous F.p	129.66	129.00	128.66	129.00	129.66	130.00	130.00	129.66
Subcutaneous S.n.s	118.66	119.00	118.00	120.33	118.33	118.33	118.33	119.00
Intranasal F.p	124.33	123.66	123.33	125.00	124.33	123.33	123.33	124.00
Intranasal D.w	125.33	124.33	123.66	125.33	125.00	123.33	124.33	125.00
Intravenous F.p	120.33	120.00	120.00	121.66	121.66	120.00	120.00	120.00
Intravenous S.n.s	126.00	125.33	126.66	127.33	126.66	125.00	125.00	125.00

CD: Not significant

F.p - *F. pallidoroseum*

D.w - Distilled water

S.n.s -Sterile normal saline

Mean = 123.50

S.E = 4.02

Normal rate = 125-130

Table 26 Blood parameters of rabbits treated with *F. pallidoroseum* through different routes - Chronic toxicity studies

Treatments	Haemoglobin (g/mm^3)	W B C ($10^3/mm^3$)	P C V (%)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
Oral F.p	9.20	6.66	38.33	25.33	69.66	6.00	2.66	0.67
Oral D.w	9.13	7.66	40.66	29.33	67.33	5.33	3.33	0.00
Dermal F.p	9.63	7.66	38.66	30.00	70.33	5.33	2.33	0.00
Dermal D.w	9.56	8.33	35.33	33.00	65.33	5.66	2.33	0.67
Subcutaneous F.p	9.70	8.66	37.66	39.33	71.00	6.33	1.33	0.00
Subcutaneous S.n.s	9.57	8.33	37.33	37.66	69.00	5.66	1.66	0.33
Intranasal F.p	9.77	9.66	39.33	31.33	68.33	5.33	2.00	0.33
Intranasal D.w	9.30	8.66	39.00	41.00	71.00	4.33	2.33	1.00
Intravenous F.p	10.30	10.00	41.33	38.33	71.33	4.00	2.33	0.67
Intravenous S.n.s	10.50	7.33	40.33	41.66	68.00	3.33	2.00	0.33
Mean	9.66	8.29	38.79	34.73	69.13	5.13	2.23	0.39
S E	0.44	1.02	1.77	5.59	1.92	0.94	0.54	0.34
Normal values	8-15	6-12	30-50	17-52	42-80	5-8	0-3	0-5

CD : Not significant

Table 27. Body weight of rabbits treated with *F.pallidoroseum* through different routes - Chronic toxicity studies.

Treatments	Mean body weight at weekly intervals (g)							
	1	2	3	4	5	6	7	8
Oral F.p	760	873	1070	1243	1483	1700	1897	2070
Oral D.w	772	963	1173	1362	1550	1750	1947	2125
Dermal F.p	727	893	1076	1227	1437	1627	1817	2070
Dermal D.w	763	953	1142	1305	1513	1707	1900	2153
Subcutaneous F.p	716	883	1047	1297	1483	1637	1820	2017
Subcutaneous S.n.s	750	920	1115	1320	1523	1733	1920	2093
Intranasal F.p	710	867	1057	1263	1463	1667	1857	2167
Intranasal D.w	738	928	1115	1323	1540	1725	1927	2120
Intravenous F.p	720	873	1087	1283	1560	1773	1960	2160
Intravenous S.n.s	743	937	1137	1338	1555	1725	1916	2090

CD : Not significant

F.p - *F. pallidoroseum*

D.w - Distilled water

S.n.s -Sterile normal saline

Table 28. Growth modeling of rabbits treated with *F. pallidoroseum*and distilled water through different routes of administration

Treatment	Growth modeling - $y = k + ab^x (x + 0\text{-}7)$	χ^2
Oral F.p	$-614.26 + 1374.26 \times 1.1056^x$	8.19
Oral D.w	$70183 + {}^-69411.76 \times 0.9971^x$	0.34
Dermal F.p	$-2051.11 + 2777.77 \times 1.05^x$	1.99
Dermal D.w	$-4763 + 5527.24 \times 1.031^x$	1.68
Subcutaneous F.p	$6616.96 + -5900 \times 0.966^x$	5.58
Subcutaneous S.n.s	$-10211.53 + 10961 \times 1.017^x$	1.60
Intranasal F.p	$6975.13 + 7685.13 \times 1.023^x$	9.61
Intranasal D.w	$-18132.63 + 18870.96 \times 1.01^x$	1.78
Intravenous F.p	$-2081.24 + 2801.24 \times 1.063^x$	6.33
Intravenous S.n.s	$21119.45 + {}^-20376.12 \times 0.99^x$	1.38

$\chi^2 5 = 11.00$

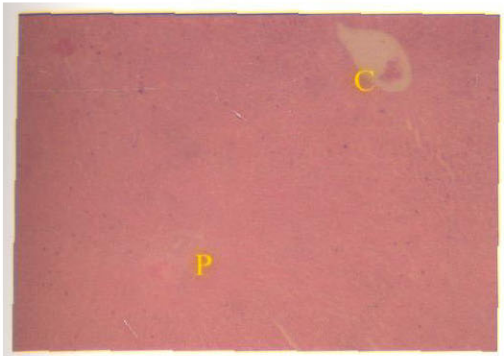


Plate 14

Liver showing normal morphology (original magnification 10x). The portal tract (P) and central vein (C) are visible

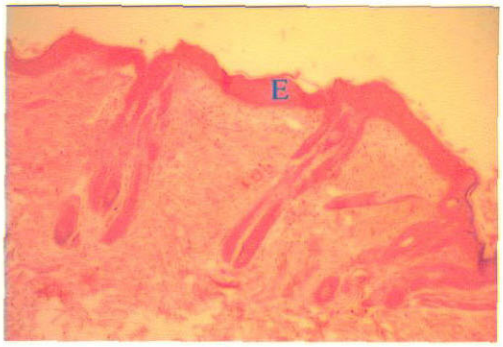


Plate 15

Skin showing normal morphology (original magnification 4x). The epidermis (E) and some hair follicles are clearly seen here

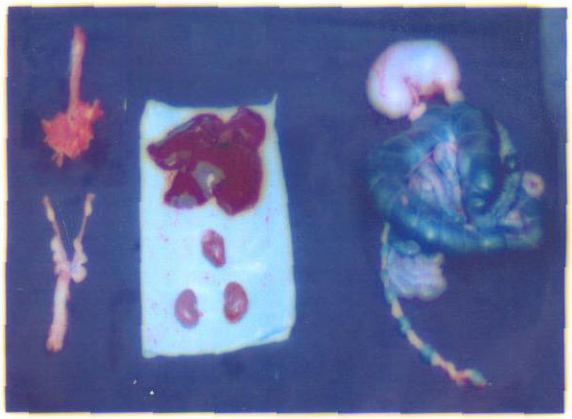


Plate 13

Internal organs of rabbit treated with *F. pallidoroseum* spores

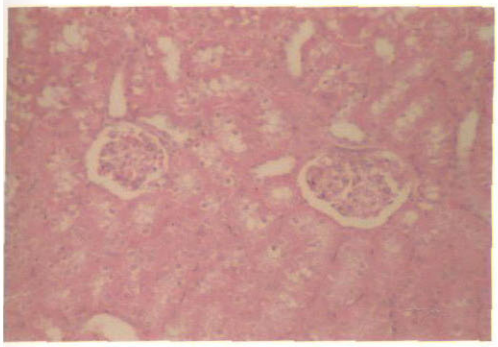


Plate 16

A section of kidney (original magnification 10x) showing normal glomeruli and tubules with mild oedema and hydropic degeneration

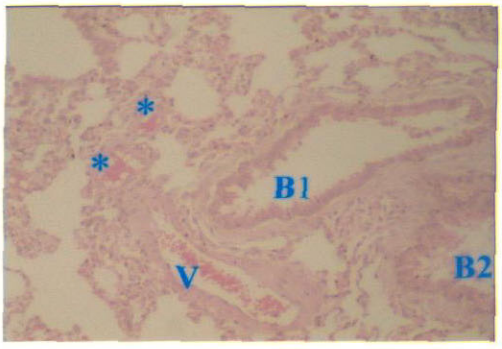


Plate 17

Lung (original magnification 10x): Some of the large broncheoles had normal appearance (B1) whereas others had degenerated epithelium (B2). A congested blood vessels (V) and some oedematous alveoli (*) are also seen in this photograph

Table 29. Relative weight of internal organs to mean body weight of rabbits treated with *F. pallidoroseum* through different routes - Chronic toxicity studies.

Treatments	Heart	Lung	Liver	Lt. kidney	Rt. Kidney
Oral F.p	0.0029	0.0049	0.034	0.0028	0.0028
Oral D.w	0.0029	0.0048	0.034	0.0029	0.0028
Dermal F.p	0.0029	0.0045	0.033	0.0028	0.0028
Dermal D.w	0.0028	0.0047	0.033	0.0027	0.0027
Subcutaneous F.p	0.0028	0.0045	0.033	0.0026	0.0026
Subcutaneous S.n.s	0.0030	0.0048	0.034	0.0030	0.0029
Intranasal F.p	0.0028	0.0047	0.033	0.0030	0.0028
Intranasal D.w	0.0029	0.0048	0.033	0.0030	0.0027
Intravenous F.p	0.0028	0.0048	0.033	0.0030	0.0027
Intravenous D.w	0.0029	0.0048	0.033	0.0030	0.0028
Mean	0.0028	0.0047	0.033	0.0028	0.0028
S E	0.005	0.013	0.055	0.009	0.009
CD	-	-	-	0.0002	0.0002

F.p - *F. pallidoroseum*

D.w - Distilled water

S.n.s - Sterile normal saline

The relative weights of heart, lungs and liver were similar in experimental and control groups (Table 29). The mean weight of kidney in the experimental group treated subcutaneously was less when compared to respective control group. However these organs had normal histology and hence reckoned as normal.

Histological observations substantiated the gross findings. Although some lesions were noticed, they were not restricted to the organs and tissues of animals belonging to any experimental group. The deviation in morphology, if any, recorded were reversible in nature. These could very well occur as a part of physiological response and therefore had no pathological significance. Some examples of these histological appearance are shown in Plates (14 to 17).

4.10 Toxin produced by *F.pallidoroseum*

4.10.1 Isolation of toxin

The toxins from the culture filtrates of seven day old cultures of *F.pallidoroseum* were concentrated and a paper chromatogram was developed in butanol : formic acid : water (75:15:10 v/v) solvent system. The paper was dried for 14 to 16 h under a hood and sprayed with bromophenol blue. This produced an yellow colour to the spot and was the indication of presence of fusaric acid (Plate 18).

4.10.2 Purification of toxin

One litre of the culture filtrate subjected to the procedure for purification of fusaric acid (mentioned in para 3.12.2) yielded 112.5 mg of fusaric acid. It was again spotted on a chromatographic paper to confirm the rf value.

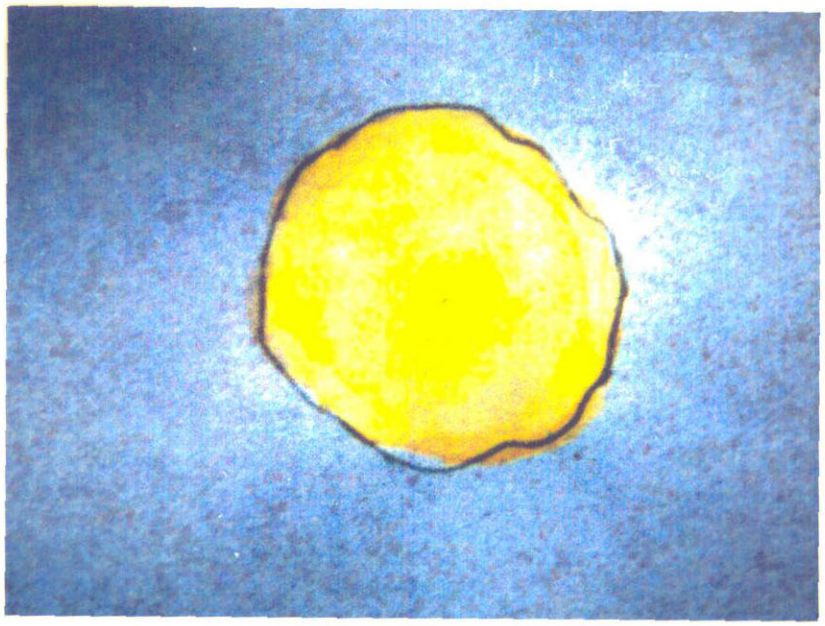


Plate 18. Detection of fusaric acid on chromatographic paper

4.10.3 Effect of fusaric acid

4.10.3.1 On *A.craccivora*

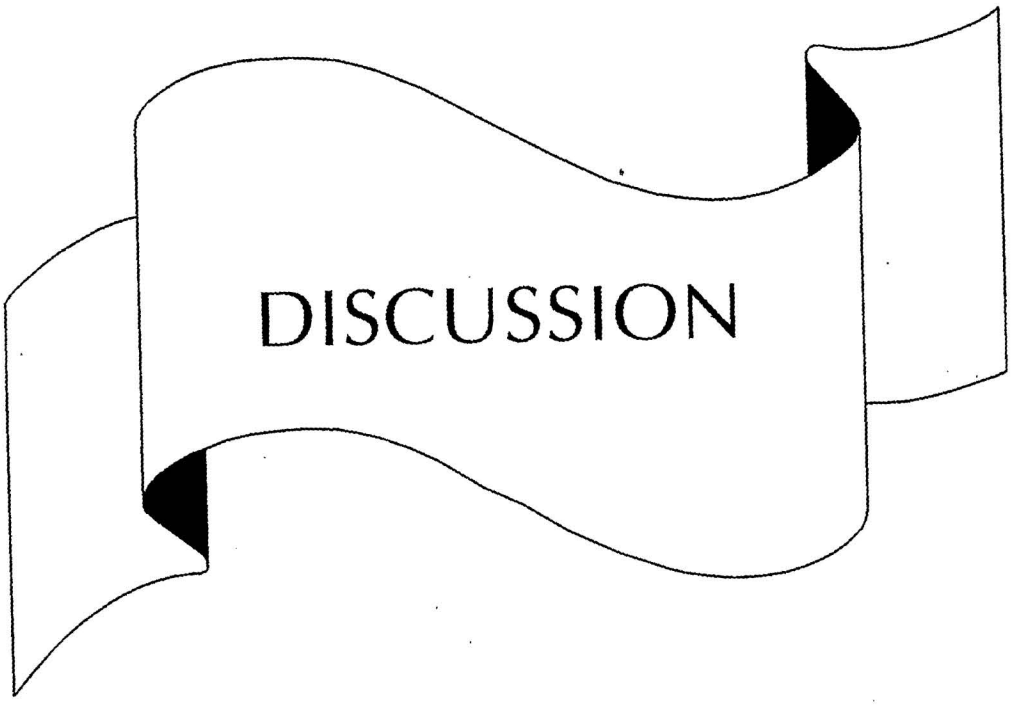
Effect of application of fusaric acid on mortality of aphids was assessed by spraying fusaric acid at four different concentrations (1000 ppm, 500 ppm, 100 ppm and 10 ppm). Mortality was recorded at 12 h interval (Table 30). It was observed that 1000 ppm solution of fusaric acid resulted in cent per cent mortality after 72 h of treatment, while only 46.60 per cent was recorded with 500 ppm solution. At lower concentration (100 &10 ppm) aphids were not killed even after 72 h. One ppm acetone solution resulted in 28 per cent mortality. When the fungus spore was administered it took only 60 h to cause cent per cent death of aphids.

Table 30 Per cent mortality of aphids treated with fusaric acid

Concentration of fusaric acid (ppm)	Hours after treatment				
	24	36	48	60	72
1000	12.33	41.30	54.60	78.66	100
500	Nil	Nil	Nil	46.66	46.66
100	-do-	-do-	-do-	Nil	Nil
10	-do-	-do-	-do-	-do-	-do-
1 ppm acetone	-do-	-do-	-do-	28.00	28.00
Fungal spore @ $7x10^6ml^{-1}$	-do-	9.0	23.30	100	-

4.10.3.2 On coccinellid predators of *A.craccivora*

None of the adults, pupae or first, second, third and fourth instars of *Menochiles sexmaculata, Coccinella septumpunctata* and *Micraspis crocea* topically injected with 1000, 500, 100 and 10 ppm solutions of fusaric acid show any abnormalities or mortality till the end of 72 h after treatment. All the treated stages continued and completed their life cycle as in the case of control group. Treated eggs of coccinellids also emerged normally.


DISCUSSION

5. DISCUSSION

Pea aphid *Aphis craccivora* Koch. a pest of regular occurrence in cowpea and other pulse crops is found associated with their vegetative and reproductive phase causing heavy yield loss. Extent of natural control on pest population is not adequate at peak infestation period. The use of insecticides for the control of persistent pests in a short duration crop like pulses is beset with danger of pesticide residue and attendant toxic hazards. The phenomenon of resurgence has also been a serious problem. An ecologically sound approach with minimum use of insecticide is necessary in this context to combat this pest.

Entomogenous fungi are recognized as potent biocontrol agents for pest control. Kerala with its high humid condition, suited for the survival and multiplication of the fungi renders them as an important tool in Integrated Pest Management programme. *Fusarium pallidoroseum* (Cooke) Sacc. was observed causing heavy mortality of aphid *A.craccivora* (Hareendranath, 1987). Preliminary studies conducted at College of Agriculture, Vellayani proved its efficacy under field conditions (Faizal, 1992 and Sunitha, 1997). A good candidate pathogen for use in microbial control should be amenable to economically viable mass production techniques, formulation using cheaper and easily available carriers and should have at least a shelf life of 4 to 12 months. For its effective utilization in the field, time and method of application should be standardized. Above all it should be

compatible with other pest management tactics and should be specific and safe to nontarget organisms including man.

5.1 Development of mass production and formulation techniques for *F. pallidoroseum*

Development of a pathogen as a biopesticide requires basic research on its morphological characters. Morphological characters of the entomopathogen *F.pallidoroseum* which was used for the present investigation were studied in detail to find out whether this pathogen is the same as that recorded as a plant pathogen. The study clearly indicated that the plant pathogenic strain of *F.pallidoroseum* reported by Booth and Sutton (1984) and Santhi (1994) were different from this entomopathogenic strain. The morphological characters of this strain were similar to that recorded by Hareendranath (1987) working with *A.craccivora*. A comparison of the measurement of macro and micro conidia of the plant pathogenic strain and the entomopathogenic strain showed that the macro conidia of plant pathogenic strain was longer in size and had more number of septa.

The spore size of *F.pallidoroseum* showed variation when it was collected from the infested aphids and culture medium (Potato Dextrose Agar – PDA). The size of macroconidia was reduced by threefold when the fungus was grown on PDA, compared to that collected from the naturally infested aphids. However marked variations were not observed in breadth of the spores from aphid and PDA. The reduction in spore size observed in artificial medium may be due to the fact that the fungus is subjected to nutritional stress when compared to its natural

host. This phenomenon of the fungus have been observed in many other plant pathogenic fungi like *Phomopsis nymphaceae* and *Phyllosticta sp.* pathogenic to aquatic weeds (Nagaraj and Ponnappa,1970). Further, the mycelial characters of the fungus also showed variations due to continuous subculturing. When the fungus was isolated on PDA from the infected aphid, the colony was fluffy and pale white in colour. With continuous subculturing, mycelium of the fungus turned pure white.

All fungi grow upon some nutritional substratum in their natural environment which are ideally suited for its natural supply of nutrients. However the artificial substrate on which microorganisms are grown in the laboratory consists of soluble constituents which are easily available and substantially different from natural substratum which is generally made up of insoluble constituents. Under such artificial conditions fungal growth gets diversified in form and colour (Nelson *et al.*, 1983). According to them, cultures of the same fungus may look different when grown on different culture medium or when the fungus is subcultured several times on same medium. Tribe (1987) opined that sometimes media rich in sugar such as PDA and malt agar induce pigment production and thus *Fusarium spp.* blossom out in their full diversity and colour. The more and more white and cottony appearance of the fungal cultures observed with repeated culturing on PDA as observed in the study may be attributed to this fact.

Another peculiarity observed with *F.pallidoroseum* culture was that repeated passage through PDA, reduced the number of macroconidia produced. Moreover virulence got reduced with every subculturing, revealing the fact that

virulence is directly dependant on the number of macroconidia produced. The conidial virulence remained constant in first and second subculture (100 per cent mortality of aphids) and gradually decreased thereafter, to loose nearly half of its activity by third subculturing. This indicated that subculturing should be kept to a minimum and isolates should never be subcultured more than three times before use in mass production and that routine maintenance of isolates for mass production should include regular passage of the pathogen through its host. It is possible that some nonobservable physiological or biochemical changes could and did occur during these serial passages. Many entomopathogenic fungi like *Nomuraea rileyi, Beauveria bassiana* and *Verticillium lecanii* are known to attenuate during successive subculturing resulting not only in loss of virulence but also variation in conidiation characteristics. It is generally believed that the virulence of entomopathogens increases with successive passages through a susceptible host and conversely decreases following successive passages on artificial medium (Steinhaus, 1949; Schaerffenberg, 1964; Muller Kogler, 1967; Hall, 1981; Ignoffo *et al.,* 1982; Hajek *et al.,* 1990; Nina *et al.,* 1998).

In any microbial control programme, production of sufficient quantities of good quality inoculum becomes essential to its success. *F.pallidoroseum* grew and sporulated well on leaves of cowpea and pongamia, rice bran and oil seed cakes. The suitability of plant leaves for mass multiplication of entomopathogenic fungi was reported by earlier workers also. Sudharma *et al.* (1999) got good growth of *F.pallidoroseum* on clerodendron leaves while Lopes *et al.* (1998) observed leaves of *Phoenix dactylifera* and *P.canariensis* as ideal medium for growth of *M. anisopliae, B.bassiana*, and *Paecilomyces farinosus.*

Use of oil cakes for mass production of beneficial fungi were reported to be successful by Sharma and Trivedi (1987) who could obtain rapid growth of *P.lilacinus* on oil seed cakes of gingelly, cotton, linseed, mustard and groundnut. Mani and Anandan (1989) could also harvest spores of *P.lilacinus* from oil cakes.

Ability of bran to support growth and sporulation of entomopathogenic fungi has already been reported by earlier workers. Wheat bran or rice bran was found to be an ideal substrate for mass production of *F.pallidoroseum* (Faizal and Mathai,1996). Bran was reported to be an ideal substrate for mass production of various entomopathogens like *M.anisopliae* and *B.bassiana* (York, 1954; Bell, 1974; Hussey and Tinsley, 1981; Anitha, 2000).

The suitability of leaves and oil cakes which are cheapest form of organic nitrogen and protein and that of bran which is rich in carbohydrates, proteins and vitamins is attributed to the availability of basic nutrients in the balanced form (Burges and Hussey, 1971).

Poor growth and sporulation of *F.pallidoroseum* was observed in sawdust, coir pith and soil. Inferiority of coir pith for mass multiplication of *F.pallidoroseum* infective to *Eichornia crassipes* was reported earlier by Santhi (1994).

Cow dung and neem cake were found to suppress the growth of *F.pallidoroseum*. Suppression of *F.pallidoroseum* by neem cake may be due to the presence of inhibitory alkaloids azadirachtin (A to K) at high concentration. Inhibition of various plant pathogenic fungi by neem has been reported by many

workers (Narayana Bhatt and Sivaprakasam, 1994; Senthilnathan and Narasimhan, 1994; Ganapathy and Narayanasamy, 1994).

Optimisation of a liquid medium to promote rapid growth of a given isolate is one of the simpler steps in the development of mass production procedure. Among various liquid substrates tried, coconut water culture yielded maximum biomass and spore yield when compared to boiled rice water or water obtained by washing raw rice. This finding is in accordance with that of Manisekarame and Letchoumanane (1996) who tested coconut water, par boiled rice water and raw rice gruel for mass production of *F.pallidoroseum* infecting rice leaf roller. They could obtain maximum biomass from coconut water and reported that rice based media were not suitable for the fungus. Suitability of coconut water for mass production of entomopathogenic fungi was also reported by Dangar *et al.* (1991) (*M.anisopliae*) and Ibrahim and Low (1993) (*B.bassiana* and *P.lilacinus*). Thus it may be concluded with the support of many findings that coconut water (composition – TSS-5.4%, SS%-30, minerals-0.5% (K, Ca, Mg, P, Cu), protein-0.1%, fat-0.1%) wasted from copra making industry may successfully be utilized for easy and economic multiplication of entomopathogenic fungi.

Different responses of the fungus in biomass and conidial production on different media may be related to the different nutritional composition and carbon to nitrogen ratio of various media (Hildebrand and Mc Cain, 1978; Churchill,1982; Zangh and Watson, 1997; Nina, *et al.*, 1998). Only if carbon and nitrogen source is in the form that can be metabolised by the fungus, it can grow and sporulate well.

Plain water lacks essential nutrients required for growth and sporulation of *F. pallidoroseum*. Further as reported by Burges and Hussey (1971) it may also contain harmful amount of certain trace elements.

From the results it is evident that a wide range of solid and liquid media are available for mass production of fungi for biological control. Choice of the substrate depends upon a number of factors including local availability, cost and isolate preference. In general, for maximum sporulation and growth, apart from the nutrient status of the medium, surface area to volume ratio is also essential (Jenkins *et al.,* 1988). The individual substrate particle in the medium should remain separate after hydration and sterilization. An ideal substrate should not only contain particles of ideal size but also maintain structural integrity during preparation for production process (Bradely *et al.*, 1992).

Apart from the sporulating ability, spore type of *F.pallidoroseum* also vary based on media on which it is grown. Leafy substrates, bran, oil cakes and coconut water produced more macroconidia which caused in cent per cent mortality of aphids while coir pith, sawdust, soil and rice based liquid media produced only microconidia with less mycelial growth. The spore suspensions prepared from these media produced only less than 50 per cent cumulative mortality of aphids. There was a direct relationship between the macroconidia count and time taken for kill. As the proportion of macroconidia decreased, the time taken for kill of the target pest increased. Cent per cent mortality was observed within 2 to 5 days when aphids were inoculated with spore suspensions predominant in macroconidia while the cumulative per cent mortality recorded was only 50 per cent even at the end

of seven days when inoculated with those predominant in microconidia. In the cadaver of aphids mostly macroconida were observed. This indicates that the fungus produces macroconida when the nutritional status of the substrate is satisfied. Under such conditions the fungus acts as a better pathogen. The better infectivity of the fungus grown on leafy substrates, bran and oil cakes may be due to this factor. Exact reason for the increased virulence of macroconidia is not known. But it may be speculated that, macroconidia being multi septate in nature has got several germinating points which possibly improves attachment of spores to the host and better mycelial coverage in the haemocoel of the insect in shortest possible time, compared to that of microconida. Many workers have reported that the virulence of fungal culture depends on concentration of spores produced (Hall and Papierok,1982; Liu *et al.*, 1989; Putreka *et al.*, 1994; Jones *et al.*, 1996; Yoon *et al.*, 1999). But variation in virulence of the macro and micro type of conidia has not been reported so far.

From the foregoing results it is obvious that medium has an essential influence on the type of spore formed and the concentration of infective spores determines the infectivity and time taken for infection. Madelin (1968) stated that the virulence of a fungal pathogen is influenced by the media in which it is grown. While working with *F.pallidoroseum* pathogenic to *A.craccivora* Hareendranath (1989) observed that the spores harvested from Sabourd's Dextrose Agar was superior than any other media. Similar effects were also reported by earlier workers while working with different entomopathogenic fungi (Voukassovitch, 1925; Beevi, 1979; Kuruvilla and Jacob, 1981; Cliquet and Scheffer, 1997).

In order to improve the properties of storage, handling, application and effectiveness of an entomopathogen it should be formulated. Ferron (1981) considered short shelf life of the formulation is one of the major factors which limits the use of microbial pesticides. Goettel and Robberts (1992) suggested that only through improved formulation that enhanced shelf life, persistence, efficacy and field targeting of fungi for insect control can be achieved. Estimates of the required storage time varied from 3 to 6 weeks to 12 to 24 months as reported by Moore and Prior (1993).

Performance of the formulated biocide during six months of storage was studied as a base line against which its further shelf life was assessed. In this study eight different dry formulations using diatomaceous earth, fine charcoal powder, leaf mold, bran, sawdust, peat, vermicompost, and semidry cadaver were stored at room temperature as well as under refrigeration. There was a decrease in effectiveness of spores when the formulated mycoinsecticide was stored at room temperature.

Viability of spores was markedly reduced within 5 to 6 months of storage at room temperature. However, at the end of six months, semidry cadaver retained 57 per cent viability whereas diatomaceous earth and sawdust formulations retained 33 to 39 per cent viability. It was only 25 to 28 per cent in charcoal, leaf mold and bran formulations. Peat and vermicompost formulations lost viability four and five months after storage respectively.

Storage under refrigeration was found to improve the viability of spores. As in the case of storage at room temperature, under refrigeration also maximum

viability was observed with spores stored in cadaver which could retain 79 per cent viability even after six months. The viability of diatomaceous earth and leaf mold formulations in particular was doubled when compared to storage under ambient conditions. When the viability of spores stored in peat and vermicompost was totally suppressed under room temperature by four months storage, there was 50 per cent retention of spore viability when stored in a refrigerator for the same period.

Viability of spores present in the formulated material depends upon physical, chemical and biotic factors of the carrier. One of the important factors responsible for viability of spores is the pH of the carrier material. In general the germinating ability of entomopathogenic fungus decreased with decrease in pH of the carrier material (Groden and Lockwood,1991). The best carrier material for retention of viability as found in this study was cadaver followed by diatomaceous earth, saw dust, charcoal and leaf mold. Of these cadaver had an alkaline pH while the others have neutral to slightly alkaline pH. Survival of conidia in peat, which is highly acid in nature was minimum. Least survival of fungal conidia in peat was also reported by Balaraman (1980) and Villacorta (1976). This clearly indicates that the carrier material selected for long time storage of *F.pallidoroseum* should be slightly alkaline, neutral or slightly acidic in nature.

In nature, *Fusarium* survive dry periods as mycelium or conidia either in encased insect cadavers or as aerial conidia. Dormancy of conidia is exogenous and not constitutive. So the key to prolonging their survival while in storage is to stop germination and, as with the preservation of mycelium, to reduce metabolism as

ıuch as possible (Burges, 1998). This is most easily done either by drying or by eeping it under low temperature. In the present study also higher survival was bserved under these conditions. Drying of conidia in the carrier material is usually ıdden but drying of the fungus in insect cadaver is relatively slow. This slow rying of conidia in a conducive medium (cadaver) might have enhanced the survival bility of the fungus. Reason for the better survival of conidia under refrigerated ondition may be that drying of spores under low temperature takes place at a ower rate compared to the ambient. Similar observations have been reported by ell *et al.* (1998), working with the entomogenous fungus *Zoophthora radicans.*

Faizal (1992) observed that *F. pallidoroseum* formulated in water, iatomaceous earth and talc could retain 75 per cent viability only up to four days nd thereafter the viability was significantly reduced. He observed that diatomaceous arth formulation is superior to water or talc formulations. While studying the effect f temperature on viability of *F. pallidoroseum* spores, Sunitha (1997) found that ıere was a decrease in effectiveness when stored at room temperature and that efrigeration could retain atleast one third of the initial sporulation and colony growth f spores in water and diatomaceous earth formulations.

Zhang *et al.* (1992) obtained 85 per cent germination of *Beauveria assiana* when its wettable powder formulation was stored for eight months under efrigeration. Aregger (1992) observed a reduction in spore viability of *B. brongniartii* with increase in storage time when it was stored at 23°C for 24 ıonths when compared to storage at 2°C.

It is necessary to evolve a formulation in which viability and virulence of the fungus could be simultaneously conserved during storage. When *F.pallidoroseum* formulations were stored under room temperature and its virulence evaluated in terms of mortality of *A. craccivora*, it was observed that virulence of the spores could be well retained in cadaver, charcoal and diatomaceous earth resulting in 86 to 89 per cent mortality of aphids followed by leaf mold which resulted in 70 per cent mortality even at the end of six months. Virulence of bran, sawdust, vermicompost and peat formulations got significantly reduced during storage. Storage under refrigeration was found to retain 100 per cent virulence of the spores in cadaver and diatomaceous earth formulations even after six months, followed by charcoal (92 per cent) and leaf mold formulations (74 per cent).

These studies indicate that for a storage period of six months, cadaver is the best carrier material to retain spore viability under ambient conditions and under refrigeration diatomaceous earth, saw dust, charcoal and leaf mold were also found to be good carriers. The carrier materials found best for retention of virulence was cadaver, diatomaceous earth, charcoal and leaf mold. Therefore to store *F. pallidoroseum* spores without loss of viability as well as virulence, semidry cadaver is the best carrier since it can be stored even under ambient conditions. For storage in a refrigerator diatomaceous earth, charcoal powder and leaf mold can also be used as ideal carriers. The improved action of diatomaceous earth and charcoal formulations may be due to the additive effect of the carrier materials acting as "physical poisons". They could break the water barrier of insect cuticle thereby increasing transpiration across the cuticle resulting in dehydration and death (Mewis and Ulrichs, 1999).

Even though loss of pathogenicity is a common problem with biocides, there are several reports on retention of viability of mycoinsecticides, and in many reports storage under low temperature is found to give better shelf life. Sunitha (1997) reported that *F. pallidoroseum* spores suspended in water and stored at room temperature for six months resulted in 62 per cent mortality and at refrigeration it resulted in 88 per cent mortality. Diatomaceous earth formulations stored for the same period resulted in 75 per cent mortality and when kept in refrigerator it resulted in 98 per cent mortality. However Chen *et al.* (1990) reported the retention of pathogenicity of *Paecilomyces cicadae* for one year even when stored at room temperature. Li *et al.* (1993) observed that *Erynia neoaphidis* and *E. radicans* could be stored at 4°C for several weeks, but its virulence was lost when deep freezed. But Steinkraus and Slaymaker (1994) reported the conidial storage of *Neozygites fraseni* in cadaver of *Aphis gossypii* under deep freezed condition (-15°C) without loss of virulence.

In the present study it was observed that certain stored formulations which lacked viability when sprayed on aphids resulted in mortality especially in the case of peat and vermicompost (13 to 42 per cent mortality). One of the factors responsible for this situation may be 'fungistasis'. The term 'fungistasis' describe the phenomenon whereby viable propagules not under the influence of endogenous or constitutive dormancy, fail to germinate sometimes even when conditions of temperature and moisture favourable for germination are given. This phenomenon have also been reported in *Fusarium* sp. by Watson and Ford (1972) and Sunitha (1997). Failure of the formulated spores to germinate in artificial media after 4 to 6 months of storage may be due to this phenomenon. While, mortality of aphids

treated with the same spore suspension may be due to the release from fungistasis by factors of biotic origin produced by the fungus (Singh, 1984). Another possible reason for mortality of aphids treated with spore formulations which lack spore germination may be due to the presence of toxins produced by the fungus. Sunitha (1997) has also reported such a phenomenon with *F.pallidoroseum* formulations in water and diatomaceous earth.

Observations on spore viability of the four best formulations using cadaver, diatomaceous earth, charcoal powder and leaf mold each packed in glass vial, polypropylene and aluminium foil revealed that both at room temperature as well as under refrigeration glass vial is the suitable container to retain spore viability when compared to the other two. When kept at room temperature, eventhough there was no significant difference in the colony diameter among differently packed materials upto six months, thereafter glass vial was found to extend the spore viability by one month for diatomaceous earth and leaf mold formulations. But for the charcoal formulation kept at room temperature, aluminium foil packing was found to be good. However packing material was not found to be a factor determining the viability of spores stored in semidry cadaver. When the biocide packings were kept in a refrigerator the effect of packing material on spore viability was not reflected till the end of the experimental period (12 months) except in the case of leaf mold formulation in which mean colony diameter vary significantly and was higher in glass vial packing followed by polypropylene and aluminium foil.

Observations on percentage mortality of aphids treated with different biocide packing kept at room temperature revealed that there was a gradual

reduction in mortality over 12 months storage. When nearly 100 per cent mortality was observed at one month after storage with all the packings, it ranged from 28 to 58 per cent at the end of one year. Comparison of percentage mortality among the different biocide packings, the packing material did not show any significant difference in most of the samples drawn.

Contradictory observations were made by different workers on the efficacy of different containers for storing mycoinsecticides. Jin *et al.* (1993) suggested that suitable packaging materials for conidial storage of *Beauveria bassiana* is polyethylene- aluminium foil-polyethelene laminate which is permeable to gases. They suggested that the packing material should be heat sealable, autoclavable and impermeable to water but permeable to oxygen, while Moore (1996) pointed out the importance of an air tight container in the maintenance of spore viability.

5.2 Application technology of *F.pallidoroseum*

In the present agricultural scenario emphasis is being given for IPM and also for reduced pesticide use. Several botanicals have been found to show insecticidal properties. In order to find out whether the botanicals which are effective against aphids have any inhibitory action against *F.pallidoroseum* compatibility test of six different botanicals were conducted using poisoned food technique. Results of the study showed that the botanicals did not inhibit the mycelial growth while there was significant variation in the sporulating ability of the fungus in different treatments. The nutritional status and the inhibitory principle

produced in different botanicals vary. Variations in these might have influenced the sporulating ability of *F.pallidoroseum*. The finding that even if certain media can support mycelial growth they cannot support sporulation is in agreement with that observed by Cochrane (1958) who showed that more nutrients are required for sporulation than that needed for mycelial growth.

Seed oil emulsions of neem and marotti at 10 per cent concentration were found to be highly inhibitory to the sporulating ability of *F.pallidoroseum*. Leaf extracts of bougainvillea, hyptis and neem at five and ten per cent were not at all inhibitory to the sporulation of the fungus. On the other hand, it enhanced sporulation when used at lower concentration (five per cent) which is the recommended field dose for aphid control. Any poisonous substance when used at very low concentrations are beneficial to living organisms. Thus, when neem cake containing high concentration of azadirachtin was used for mass production it was found to suppress *F.pallidoroseum*, but neem leaves which contain only low concentration of this alkaloid were found beneficial. Even here, neem leaf extract at five per cent was found to be more beneficial than 10 per cent extract. Parmar and Devakumar (1993) stated that neem at lower concentrations act as a growth regulator while at higher concentrations act as an inhibitor. Another possible reason for the noninhibitory action of neem leaf extract even at higher concentration may be that an active substance with antifungal activity may be viable only at optimum concentration (Alpana and Singh, 1997). When leaf extract of neem was found to be noninhibitory, seed oil emulsion was found inhibitory to the fungus. This may be due to the lower concentration of alkaloids in leaves when compared to seeds. It is also possible that other tissue debris present in leaf extracts interfere with the inhibitory action.

It is clear from these observations that botanical leaf extracts at lower concentrations can be mixed with *F.pallidoroseum,* the biocontrol agent of *A.craccivora* to enhance its management efficacy. Hence, instead of preparing the spore suspension in water it will be advantageous if lower concentrations of leaf extracts are used. Sudharma and Hebsy (2000) reported that *F.pallidoroseum* with very low concentration (two per cent) of neem oil did not reduce the efficacy of the fungus. Babu *et al.* (2000) also reported that efficacy of a biocontrol agent would be increased by mixing it with botanicals. *B.bassiana* at sublethal doses when mixed with neem seed kernel extracts caused significantly high mortality of *Spodoptera litura* when compared to the commonly used insecticide.

Integration of any fungal isolate with an insecticide requires a thorough knowledge of compatibility between these two agents particularly with reference to growth and sporulation. Quinalphos, one of the insecticides commonly used for aphid control when used at recommended dose (0.05 per cent) showed only partial inhibition on sporulation of the fungus and no inhibition to the mycelial growth indicating that, factors affecting germination and growth may be different from those affecting sporulation in presence of insecticides. Faizal (1992) observed that the entomopathogenic *F.pallidoroseum* was highly inhibited by HCH while quinalphos, fenthion and phosphamidon showed comparatively less inhibition.

Studies on compatibility of *F.pallidoroseum* with botanicals and quinalphos clearly indicated that in IPM strategy against aphids this fungus could successfully be integrated. Under conditions when the use of an insecticide becomes necessary to tackle a pest complex eventhough there could be a reduction in

sporulating ability of the fungus, it is not completely wiped out from the field. Hence the mycelial growth remaining on the treated plant surface or host cadaver may produce spores on newly infesting aphids once the efficacy of quinalphos decreases, thereby it may again become effective as a mycoinsecticide.

Field studies conducted using diatomaceous earth, charcoal powder, semidry cadaver and leaf mold as carrier materials revealed that there was no difference in the efficacy of these formulations in controlling aphids. Similar observations were recorded earlier by Faizal (1992) and Sunitha (1997). They found that *F. pallidoroseum* formulations using diatomaceous earth and water as carrier material made no difference in the extent of mortality of *A.craccivora* under field conditions.

F.pallidoroseum formulations when sprayed or broadcasted on plants at a concentration of $7x10^6$ spores ml^{-1} resulted in cent percent control of the pest within 3 to 5 days after treatment. But Faizal (1992) observed that spraying was found to be effective than dusting while using *F. pallidoroseum* against *A.craccivora* and that spray formulations using diatomaceous earth or talc resulted in maximum mortality when compared to dust formulations using these carriers. He opined that spraying is better than dusting since spray fluid can meet the requirement of humidity for mycosis. In the present experiment mortality percentage recorded was not significantly different in both spraying and broadcasting. This might be due to the provision of enough humidity for mycosis, because just before dusting, the plants were irrigated as well as wetted facilitating better adhesion of dust particles to the plant surface. Thus *F. pallidoroseum* spores when sprayed

›r broadcasted behaved in a similar manner resulting in same extent of control ›f *A. craccivora*. Wilding(1981) suggested that even wetting of host plant can ›e a determining factor in the initiation and development of mycosis. Apart from his as suggested by Moore *et al.*(1996) water available on the aphid surface night be sufficient for germination of conidia and subsequent infection.

Regarding pest control using a living organism the most important factor o be optimised is its time of application in the field. In the present field experiment t was observed that prophylactic application of a mycoinsecticide *F.pallidoroseum*) against *A. craccivora* did not reduce the pest infestation. nstead, the population increased and reached maximum, coinciding with the pod ›earing stage of the crop as in the case of untreated crop. This reveals that ›urvival of spores in the field is less in the absence of host insect or, the population ›f viable and virulent spores which might have survived in the absence of aphids was not enough to initiate an epizootic to check the pest population.

In the absence of host insect, time taken by the fungus to multiply and ›ause infection in the fresh infestation of aphids may be prolonged resulting in ›educed or no control of aphids. If the target pest was a soil insect, prophylactic ›pplication of an entomopathogen would have been effective since it will pick up infection while burrowing into the soil. Moreover soil can well protect the pathogen ›pores from uv rays of the sun (Burges,1998).

Field experiments pointed out that application of the biopesticide *F.pallidoroseum* should be timed with the presence of host insect in the field and that prophylactic treatment was not effective. When *F. pallidoroseum* at a

concentration of 7x 10^6spores ml^{-1} was applied after aphid incidence (curative control measure) it was found that irrespective of the carrier material or method of application there was cent per cent control of the pest within three to four days after treatment. Reinfestation of aphids was not seen for the succeding weeks eventhough a very mild population was noticed at the termination of experimental period (12 weeks after planting- 4 weeks after treatment) when the plants started drying up. It was 0.51 aphids in 15 cm terminal twig per plant as against 9.71 aphids in the control plot. Low intensity of reinfestation in the curative plot may be due to the infection of fresh aphids by the spores harboured in the cadaver of the earlier treated insects. Further it becomes clear that the fungus does not prefer plant surface for long term survival. Sunitha (1997) reported an absence of *A.craccivora* till four weeks after treatment with *F. pallidoroseum.*

The time taken for cent per cent mortality under field conditions as observed from this experiment was only three to four days, while Sunitha (1997) reported that it took about 12 days for cent per cent destruction of the pest. Reduced time requirement for the present strain to cause cent per cent mortality may be due to the increased virulence resulted from repeated passage through the host insect, which agrees with the demonstration of Fargues (1981) that virulence is increased after first or second passage which happens because of quicker adaptation of the strain.

Yield loss assessment is an important factor in pest management programme. Any pest management programme can be said to be successful only if the yield obtained is increased due to the particular management practice

adopted. An increase in yield of 17 to 33 per cent was recorded in plants treated with *F. pallidoroseum*. Whatever be the method of application or carrier material used, time of application of the biopesticide should be only after pest incidence. Similar results on increase in yield was reported earlier by Sunitha(1997). She also observed a proportionate decrease in yield with decrease in concentration of spore in the spray fluid.

Popularity of any plant protection measure depends not only on the control obtained but also on the benefit derived from its use. Cost benefit ratio determines the economic viability of any plant protection measure.

According to package of practices recommendations of the Kerala Agricultural University (1996), three repeated sprayings of quinolphos (0.05%) are to be given at fortnightly intervals for a single crop of cowpea for the management of pea aphid. For protecting one cent area of cowpea from aphids, 18 ml Ekalux (100 ml ekalux costs Rs 48) is required for a season. The cost of the insecticide alone is Rs 8.64 (Rs 9/-) and the cost of three spraying approximately equals to Rs 15/-. Thus the total cost involved for spraying an area of one cent of cowpea works out to be Rs 24/-.

Instead of three sprays of ekalux only one spray of *F.pallidoroseum* at a concentration of $7x10^6$ spore ml^{-1} is required. From a seven day old 900 g rice bran culture of the fungus spore suspension of $7x10^6$ spore ml^{-1} can be prepared which is enough for one spray. For spraying one cent of cowpea, cost of rice bran including sterilization etc. works out to be Rs 15/-. Application charge for

one cent land approximately equals Rs 5/-. Thus the cost of control of aphids using *F.pallidoroseum* works out to be Rs 20 only, compared to Rs 24/- when insecticide was used.

5.3 Biosafety evaluation of *F.pallidoroseum*

Due to the importance of protecting the natural ecosystem which constitutes crop plants, natural enemies and beneficial organisms, the possible action of entomopathogens in an agroecosystem and its effect on nontarget organisms must be considered. To confirm the nonpathogenic nature of *F. pallidoroseum* while inoculating various nontarget organisms, the inoculum level was increased from normal standardized field dose against aphids by ten times ($7x10^7 spores^{-1}$ ml). The results revealed that *F. pallidoroseum* is safe to crop plants like amaranthus, banana, drumstick, mulberry, passion fruit, pineapple and sesamum while there are reports of pathogenicity by another strain (plant pathogenic) of this fungus on the above tested plants. The fact that present isolate is nonpathogenic to crop plants which have been reported to be the host of the same fungus shows that the isolate which is being used in this study is a different strain. Such strain variation is a common phenomenon with many microorganisms.

Pathogenicity tests conducted on natural enemies of insect pests viz. coccinellid predators like *Menochiles sexmaculata*, *Coccinella septempunctata* and *Micraspis crocea;* syrphid, *Ischiodon scutellare, Ischiodon* sp., haemerobid, spiders like *Pardosa pseudoannulata, Tetragnatha maxillosa, Oxyopus* sp., preying mantis and dragon fly proved that *F.pallidoroseum* is safe to all stages of

all these natural enemies tested. Similar observations with respect to safety of this fungus to natural enemies were reported by Nagalingam (1983) Hareendranath (1989) and Sunitha (1997).

F.pallidoroseum was also found to be safe to the productive insects like stingless bee, *Trigona iridipennis* and helpful insects *Syrphus* sp., *Bombus* sp., *Xylopa* sp. and *Camponotus compressus* when treated at a concentration of $7x10^6$spores ml^{-1}. Safety of this entomopathogen to honey bees, *Apis indica* and silkworm, *Bombyx mori* was reported earlier by Nagalingam (1983) and Sunitha (1997).

High host specificity of entomopathogens is a problem while dealing with a pest complex. But at the same time, if the host range is too wide the pathogen would be equally unsuitable. Specificity with some isolates is confined to orders of insects as with the isolates of *Metarhizium* and *Beauveria*. *F. pallidoroseum* strain isolated from *A.craccivora* used in the present study was earlier found to be highly host specific. During this course of investigation *Aphis gossypii* and *Myzus persicae* were found to take up infection by *F. pallidoroseum.* But none of the treated lepidopteran, coleopteran, orthopteran or other hemipteran pests (mentioned in para 3.11.4) took infection. Inability of this fungus to produce penetrant germ tubes for the infection process on non hosts could be due to the presence of specific inhibitors or absence of proper stimuli required for germ tube development which follows host invasion (Boucias and Latge, 1987).

Manisegarame and Letchoumanane (1996) reported an epizootic of *F.pallidoroseum* on rice leaf roller *Cnaphalocrocis medinalis* in the Karaickal region of Tamil Nadu. The strain used in the present study when inoculated on the

ıme insect was not found to develop infection even on the earlier instars. Such a ːcognition of difference in susceptibility of the host insect may be attributed to ıe gradation in specificity of strains from different geographical regions. Ignoffo ıd Gracia (1981) while working with the entomopathogen *Nomuraea rileyi* noted ıat caterpillars of *Trichoplusia ni* are susceptible to strains from different ːographical regions (Missouri, Missisippi and Brazil), but that a biotype of the ıngus from Florida is 7 to 17 times less infective than others. Therefore to enable ːlection of suitable isolates of enomopathogens suitable screening tests should be ɔught. Development of a chemotaxonomic data bank of entomopathogenic fungi ·ill enable the location of specific isolates.

The level of hazard involved in the exploitation of an entomopathogen as iocontrol agent in agriculture needs to be assessed before its large scale production ıd release. The entomopathgen *F.pallidoroseum* used in the present study when valuated for its toxicity to rabbits revealed that the spore suspension at a ɔncentration which is ten times the expected atmospheric contamination did not ause any disease or mortality in rabbits tested for acute or chronic toxicity ːudies.

Spore suspension administered orally, dermally, subcutaneously, ıtranasally or intravenously did not make any significant difference in mean body ːmperature, respiratory rate or pulse rate in the case of animals subjected to hronic toxicity studies. These parameters recorded at weekly intervals fall within ıe physiological limits reported by Sastry (1985). However the respiratory and ·ulse rates vary significantly in animals tested for acute toxicity in the case of

dermal, subcutaneous, intranasal and intravenous treatments. But variations were also noticed in their respective control groups indicating that these variations which occurred within 24h of treatment was solely due to the experimental procedures.

The finding that *F.pallidorosuem* spores measuring 9.0± 2.19 x 2.19mm did not cause any respiratory problem is supported by the finding of Austwick (1980) that only the spores which are lesser than three mm in diameter would cause allergenic or respiratory problems as these are resulted by sensitation through lungs. With increased size (>3mm) the spores will not get lodged in the alveoli and consequently these do not pose a serious risk as in the case of *Metarhizium* spores.

Haematology examination ruled out the possibility of any subclinical disease. The blood parameters like haemoglobin content, total WBC, and the percentage of blood components like packed cell volume, neutrophils, lymphocytes, monocytes, eosinophils and basophils fall within the normal range reported by Sastry (1985). The mean body weight of rabbits did not show any significant variation among the experimental and control groups. The relative weights of heart, lungs and liver were similar in experimental and control groups. Mean weight of kidney in the experimental group of animals treated subcutaneously was less when compared to its respective control group. However on histopathlogical examination it was reckoned as normal.

To date, no artificial infection has been caused in mammals by entomopathogenic fungi which are potential biocontrol agents. Studies conducted by Shadduck *et al.* (1982) showed that contamination of rabbits, rats and mice

by *M. anisopliae* conidia by inhalation, intraperitonial injection or ingestion cause neither disease nor morbidity. Similar results were obtained by Ignoffo (1981) with *Nomuraea rileyi;* Wilding (1981) with *Entomophthora virulenta*; Mc Coy (1990) with *Hirsutella thompsonii*; Hall(1981) with *Verticillium lecanii*; Umphlett and Mc Gray (1981) using *Lagendium gigantium*; Elkadi *et al.*(1983) with *M. anisopliae* and Jeevanand and Kannan (1995) with *M. flavoviridae*.

5.4 Role of toxin in pathogenicity of *F.pallidoroseum*

Toxicity of certain entomopathogens like *Beauveria* spp. and *Paecilomyces* spp. causing respiratory problems has been reported by Austwick (1980) and Sakaguchi (1983). Certain entomopathogens like *Aspergillus flavus* and *A. parasiticus* eventhough very effective against insect pests have not been selected as biocontrol agents due to the toxic and carcinogenic properties of its toxins. Toxicity of plant pathogenic *Fusarium spp.* have been reported by Lucia *et al.* (1984). They observed that *F. poae* applied on the depilated area of the flank of rabbits showed cellular degenerative process. Rats fed with the same isolate developed cerebral haemmorhage. Intratracheal inoculation of *F.oxysporum* microspores in rabbits induced necrotic and non cellular lesions with hyphal growth. So far no entomopathogenic *Fusarium* has been reported to cause diseases in mammals. One of the possible reasons for this is the non preference of entomopathogens to a temperature between 33°C and 37°C, which is the body temperature of most of the mammals (Roberts and Campbell,1977). The present investigation on biosafety to higher animals confirmed that the fungus is absolutely safe to them.

Some fungal genera do occur in nature primarily as insect pathogens and there is some evidence that their pathogenicity to insect host may sometimes be mycochemically mediated. The fungal genera *Penicillium, Aspergillus* and *Fusarium* have been extensively investigated for metabolic production. Many entomopathogenic fungi overcome hosts after a limited growth in the haemocoel indicating the presence of toxins which are presumed to cause host mortality. Faizal (1992) reported 99.57 per cent mortality of *Aphis craccivora* when treated with culture filtrate of *F. pallidoroseum* which he opined to be due to toxins. Attempts made for isolation of toxic substance from *F. pallidoroseum*, during the course of investigation led to the detection of fusaric acid from the fungal culture filtrate. It was found that one litre of culture filtrate could yield upto 112.5 mg of this toxin. Mahadevan and Sridhar (1974) could quantify only 80 to 100 mg of fusaric acid from one litre of *F. oxysporum* culture filtrate. Higher yield of the toxin may be due to the higher content of fusaric acid in this species.

Studies conducted for evaluating the effect of fusaric acid on *A. craccivora* revealed that the toxin at a concentration of 1000 ppm caused cent per cent mortality of aphids 72 h after treatment and with 500 ppm it was only 46.6 per cent at the end of 72 h. With 10 ppm and 100 ppm there was no mortality at all indicating that only at very high concentrations, fusaric acid can cause death of aphids. When the fungal spores resulted in 100 per cent kill of aphids within 60 h, fusaric acid at 1000 ppm took 72 h, revealing slow action of the mycochemical compared to the pathogen. But Faizal (1992) reported cent per cent kill of *A.craccivora* with undiluted culture filtrate of *F. pallidoroseum* within 24 h. This indicates the possibility of more than one mycochemical in *F. pallidoroseum* cultures.

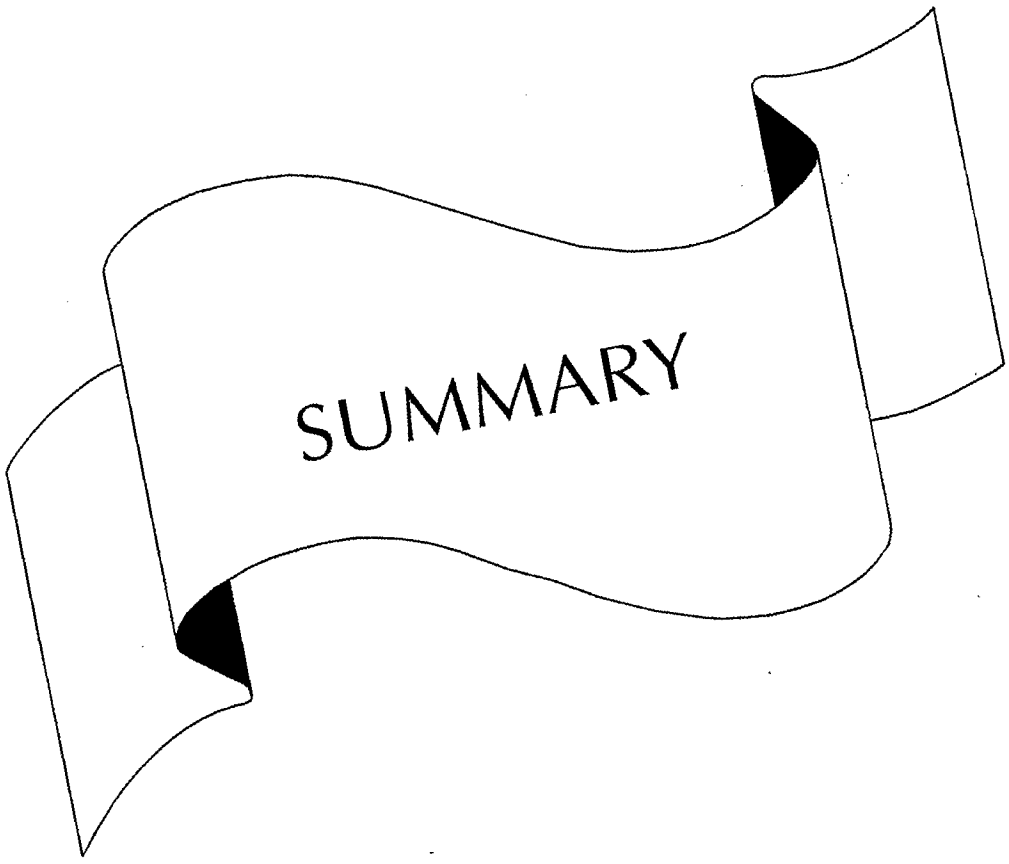
Susha (1997) also detected the presence of fusaric acid in the culture filtrate of *F.pallidoroseum* pathogenic to the aquatic weed *Eichornia crassipes*. Fusaric acid has also been detected from eight other species of *Fusarium* by Bacon *et al.* (1996). Claydon *et al.*(1997) found *F.solani* produced naphthazarin pigments fusarbin, anhydrofusarbin, and javanicin which were toxic to adult blowflies *Calliphora erythrocephala*. Other important groups of mycotoxins are trichothecenes (Rodricks *et al.*, 1977) zearaloenones (Mirocha *et al.*, 1977) and beuvericin (Gupta *et al.*, 1991).

Mycotoxin production has also been well documented in genera *Beauveria* and *Metarhizium*. Mycochemicals so far identified from *Beauveria spp.* include beauvericin (Hamill *et al.*, 1969), beauverolides H and I (Elsworth and Grove, 1977), bassionolide (Suzuki *et al.*, 1977) and those from *Metarhizium spp.* include destruxins A and B (Kodaira,1961), swainsonine (Hino *et al.*, 1985) and 16 other isolates of destruxins (Jegorov *et al.*, 1992). Fungal toxins have also been identified from entomopathogenic genera like *Entomophthora, Aspergillus, Nomuraea, Verticillium, Paecilomyces, Cordyceps* and *Iceria.*

The role of fungal toxins in pathogenisis of insects is controversial. When Gillespie and Claydon (1989) suggested more clarification in this aspect, Evans (1989) attributed the death of host insect infected by fungi to toxins. Moore and Prior (1993) opined that toxins may interfere with some aspects of host defense, but many pathogenic fungi do not produce toxins.

It may be concluded that *F. pallidoroseum* causes death of *A. craccivora* both by invasion of host tissue by spore germination and multiplication in the

haemocoel as well as by production of toxins. The scope of using the toxin isolated in the present study directly for the control of *A.craccivora* is very limited as evident from the dose response of the mycochemical. However, further investigation is needed to study the mode of action of toxins, the range of toxins present in each species and pest control potential of the mycotoxins.


SUMMARY

6. SUMMARY

Investigations were carried out on the entomopathogen *Fusarium* ›*allidoroseum* (Cooke) Sacc. at the College of Agriculture, Vellayani. The study ıimed at exploring the possibility of using this entomopathogenic fungus as an ›ffective biopesticide for an ecologically sound pest management programme for he major pest of cowpea and other pulse crops, *Aphis craccivora.* The areas of ·esearch include symptomatology and morphology of the fungus , identification of ess expensive natural substrates for mass production , standardisation of suitable ›arrier materials for formulation and storage of spores, and evaluation of storage emperature and packing material with a view to improve shelf life, compatibility ›f this biopesticide with other pest control methods. Its specificity and safety to ıon target organisms like crop plants, natural enemies, beneficial insects and higher ınimals were also studied. The time and method of application of the biopesticide n the field was standardized and the toxin present in the fungus was detected, ›urified and effect tested on *A. craccivora* and its natural enemies.

Mortality of the aphid infected by *F.pallidoroseum* occured within two ›o four days after infection and growth of the mycelia over the cadaver appeared ·our to five days after infection. The fungus produced two types of conidia, the ıacroconidia and microconidia, of which macroconidia were found to be more ınfective. Subculturing was found to reduce the virulence of conidia, which was ›ompletely lost after seventh serial passage.

Leafy substrates, oil seed cakes and bran were found to be the ideal solid substrates and coconut water, the ideal liquid substrate for large scale production of the fungus as they supported maximum biomass and infective conidia.

Spore suspensions prepared from these cultures when sprayed on healthy aphids resulted in cent per cent mortality within 4 to 6 days depending on the concentration of macroconidia present in the suspension which in turn depends on the culture media. Spore suspensions prepared from rice based liquid media were less virulent and those from sawdust, soil, coir pith were least virulent as they contained mostly microconidia. Neem cake, cowdung and plain water did not support the growth and sporulation of *F. pallidoroseum*.

Spores harvested from *F.pallidoroseum* cultures could be well stored at room temperature in semidry cadaver of *A.craccivora* with retention of more than 50 per cent viability even after six months, while carrier materials like diatomaceous earth, charcoal powder, leaf mold and saw dust could maintain the same extent of viability at the same temperature only till the end of four months of storage. Viability of spores stored in bran, peat and vermicompost were greatly reduced within 2-3 months of storage at room temperature.

Storage under refrigeration was found to be better for maintenance of viability of spores. Under such condition it was found that semidry cadaver, diatomaceous earth, leaf mold and fine charcoal retained more than 50 per cent viability even at the end of six months of storage. Among these cadaver was the

best (79 per cent viability) followed by diatomaceous earth, leaf mold and charcoal powder with a viability of 73, 65 and 56 per cent respectively.

Virulence of spores stored at room temperature could be fairly maintained even though loss of viability was noticed over a period of time. After six months of storage the spore suspension prepared from cadaver, diatomaceous earth and charcoal formulations resulted in 86 to 90 per cent mortality of aphids followed by leaf mold formulation which caused 71 per cent mortality. Reducing the storage temperature was found to improve the retention of infectivity of spores. Even after six months, spore suspensions prepared from cadaver and diatomaceous earth formulations resulted in cent per cent destruction of aphids followed by that of charcoal powder (92 per cent) and leaf mold (74 per cent) mortality.

A gradual reduction in viability of the fungus was observed over a period of time when the formulations were packed in three different packing materials namely glass vial, polypropylene and aluminium foil kept at room temperature. However semidry cadaver formulations retained viability upto 12 months of storage, irrespective of material used for packing. Diatomaceous earth, charcoal and leaf mold formulations did not reflect any significant change in viability of spore based on packing material till the end of six months. Thereafter the survivability was found to be extended by one month for glass vial packing of diatomaceous earth and leaf mold formulations and aluminium foil packing of charcoal formulation. Shelf life of the spores stored in polypropylene and aluminium foil lasted for six months while that in glass vial lasted for seven months. Packing material was not found to influence the viability till nine months except in the case of leaf mold

formulation when stored under refrigeration. After nine months of storage glass vial packing showed better retention followed by polypropylene.

Assessment of virulence of spore formulations in different packing materials revealed that glass vial packing improved the infectivity of spores compared to aluminium foil and polypropylene packing. Mortality rate of aphids was greatly increased when sprayed with formulations stored under refrigeration. It ranged from 28 to 58 per cent with formulations stored at room temperature, the range was 72 to 92 per cent with formulations stored at lower temperature for a period of 12 months. Here also glass vial packing was found to improve the infectivity of spores.

Compatability studies with six different botanical insecticides commonly used for pest control in cowpea showed that none of the botanicals inhibited the mycelial growth of the fungus while it's sporulation was inhibited significantly by 10 per cent seed oil emulsion of neem and marotti. Ten per cent leaf extracts of neem, hyptis and bougainvillea leaves were not at all inhibitory. Instead these leaf extracts enhanced sporulation when used at a lower concentration (5 per cent).

The commonly used insecticide for aphid control namely quinalphos at 0.05 per cent concentration moderately inhibited the sporulating ability, eventhough it did not cause any significant inhibition on mycelial growth of the fungus.

Field experiments with *F.pallidoroseum* spores revealed that neither the carrier material used viz. diatomaceous earth, fine charcoal powder, semidry cadaver and leaf mold nor the method of application viz. spraying or broadcasting has a significant role in the extent of control of *A.craccivora*. But the time of

application is a critical factor, which determines the efficiency of spores in controlling the pest. Cent per cent control of aphids could be obtained by application of spores at six weeks after planting (WAP) when there was pest infestation on the plants. Prophylactic application of the spore suspension was found to be ineffective. Reinfestation of aphids did not occur in the plots treated with the spore suspensin at six WAP till four weeks after treatment. Thereafter even though very mild infestation was noticed it was 10 times lesser than that observed in plots subjected to prophylactic treatment as well as to control plots.

Management of aphids had a significant effect on the yield of cowpea. The average yield obtained from plots treated with *F.pallidoroseum* as curative control measure showed 17 to 33 percent increase when compared to obtained from control plots or the plots treated prophylactically.

F.pallidoroseum pathogenic to *A.craccivora* is not pathogenic to crop plants like *Amaranthus sp , Moringa olifera, Morus albus, Musa paradasiaca, Passiflora edulis*, and *Sesamum indicum* which have been reported to be diseased by another strain of *F.pallidoroseum* (plant pathogenic)

The fungus was found not pathogenic to the natural enemies of *A. craccivora* namely the coccinellid predators like *Menochiles sexmaculta, Coccinella septumpunctata* and *Micraspis crocea*; syrphids, *Ischiodon scutellare, Ischidon* sp., hemerobid, preying mantis and dragon flies and spiders like *Lycosa pseudoannulata, Tetragnatha maxillosa* and *Oxyopus* sp.

The fungus was found to be safe to beneficial insects like stingless bee *Trigona iridipennis* and pollinators like *Syrphus* sp., *Bombus* sp., *Xylocopa* sp. and *Camponotus* sp.

Cross infectivity studies of the entomopathogen revealed that out of the nine aphid species tested *Aphis gossypii*, and *Myzus persicae* took infection. But none of the treated lepidopteran, coleopteran, hemipteran or orthopteran pests of crop plants took infection when treated at a concentration of 7×10^6spores ml.

Safety test conducted on New Zealand White rabbits proved that the fungal spore even at a concentration that is ten times the expected atmospheric contamination did not cause any acute or chronic toxicity symptoms when treated orally, dermally, subcutaneously intranasally or intravenously. The animals remained alert throughout the experimental period and did not show any clinical signs of diseases. The mean body temperature, respiratory rate, and pulse rate were within the physiological limits. Haematology examination ruled out the possibility of any subclinical disease. The mean body weight and the relative weights of internal organs were similar in experimental and control groups of animals. Post mortem examination did not show any cosistant pathology.

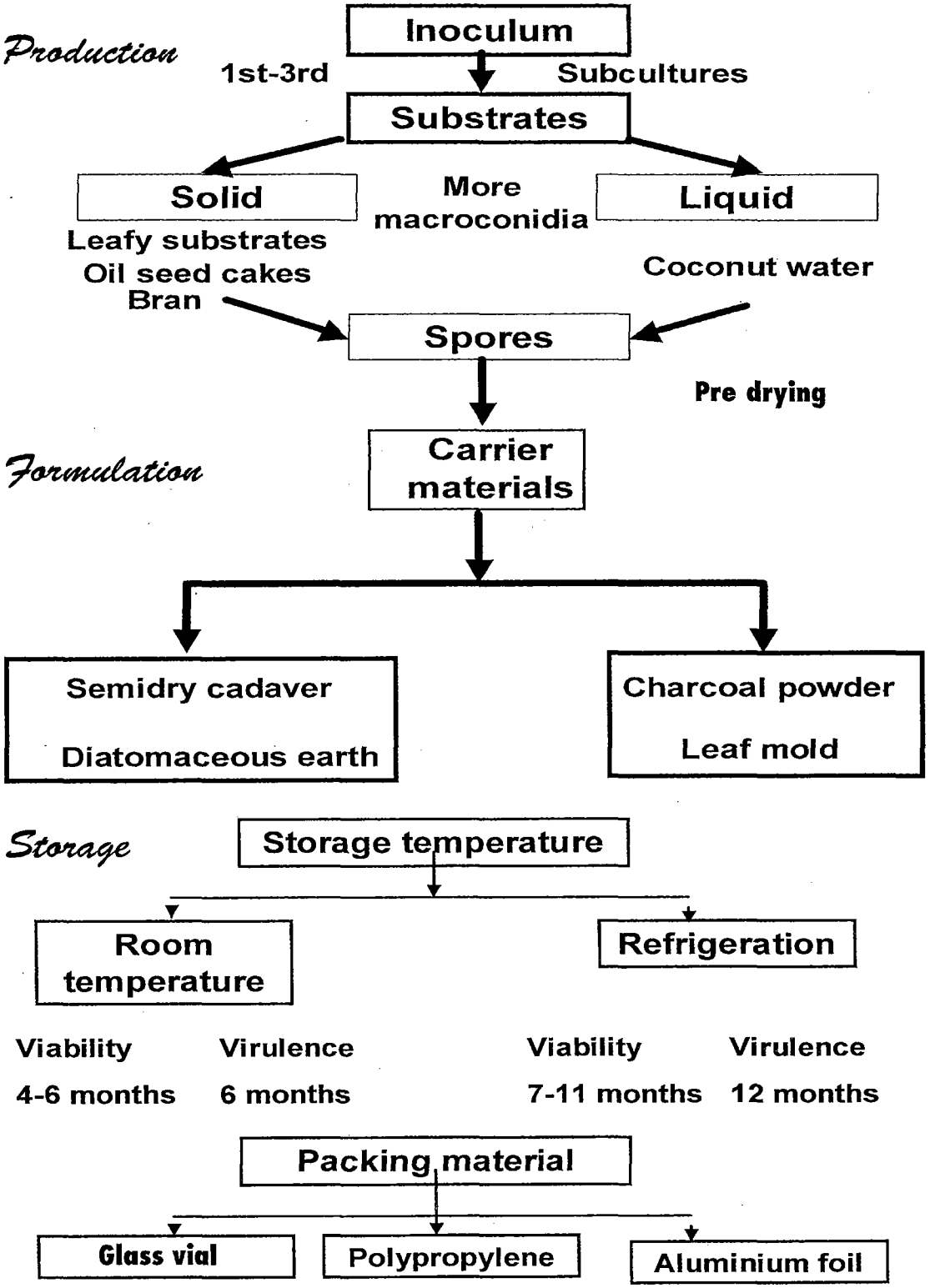
The tissues on histopathological examination were reckoned as normal. Seven day old culture filtrates of *F.pallidoroseum* contained 112.5 mg^{-1}litre of fusaric acid. The toxin when sprayed on *A.craccivora* at 10, 100, 500 and 1000 ppm, the mortality rate observed was 47 and 100 respectively at the end of 72 h after treatment compared to 60 h when treated with *F.pallidoroseum* spores.

F.pallidoroseum is thus proved as an ideal candidate for the management of pea aphid *A. craccivora.* Its utilization in IPM leads to an economically viable and ecofriendly approach in pest management.

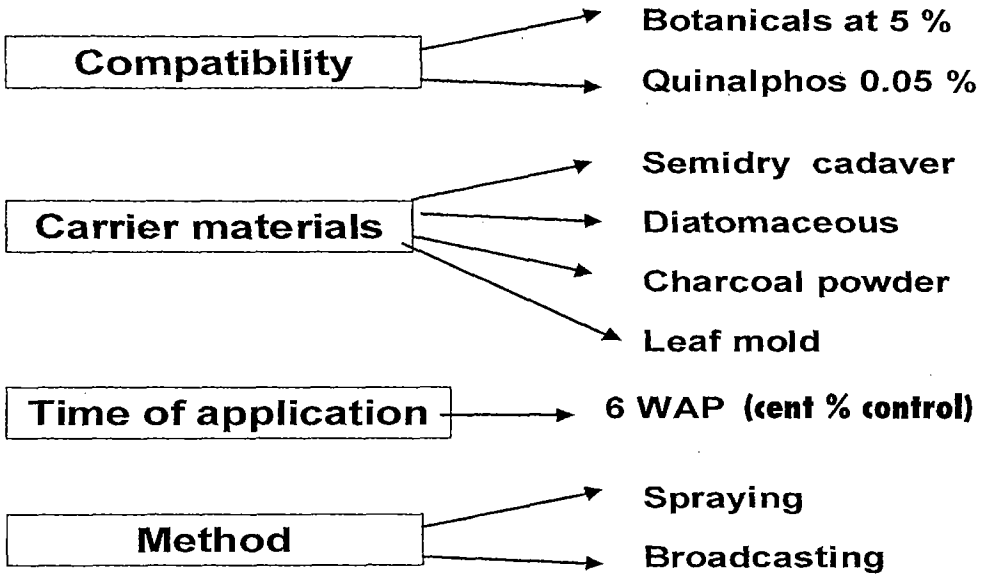
To take better advantage of this pathogen, further investigations may be directed to standardise the C:N ratio of the mass production substrates in order to optimise the production of infective conidia. Study on mode of action of the toxins and range of toxins present in the species, may increase the scope of pest control potential of the mycochemicals produced by the fungus. Attempts may be made to distinguish the genotype at subspecific level for strain characterisation of the fungus and to improve its efficiency ~~of~~ by manipulation of fungal genome.

RESEARCH HIGHLIGHTS

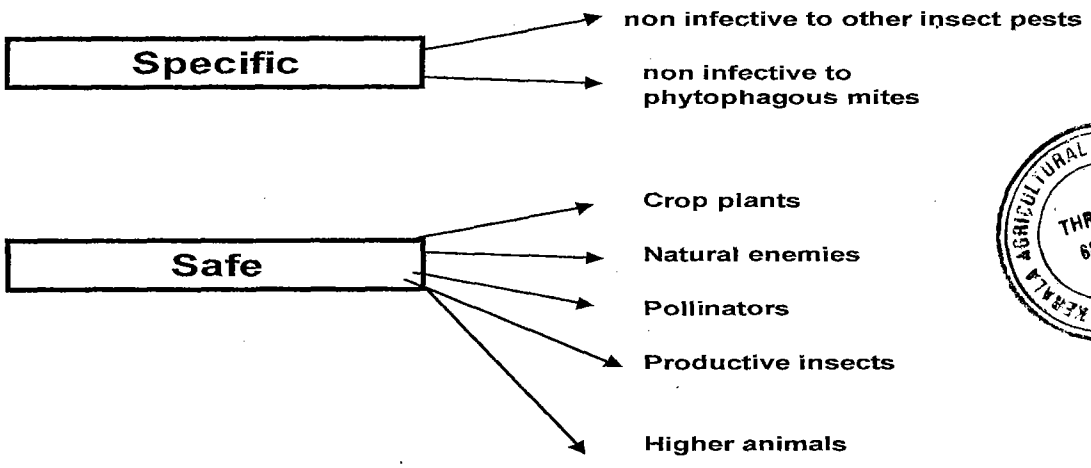
I. Development of mass production, formulation and storage technique



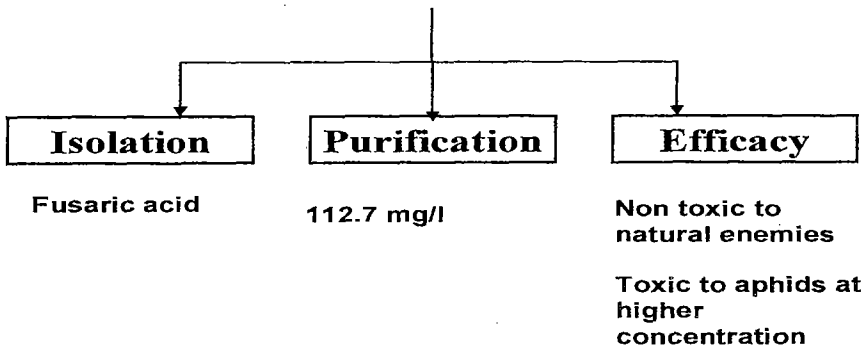
II. Application techniques

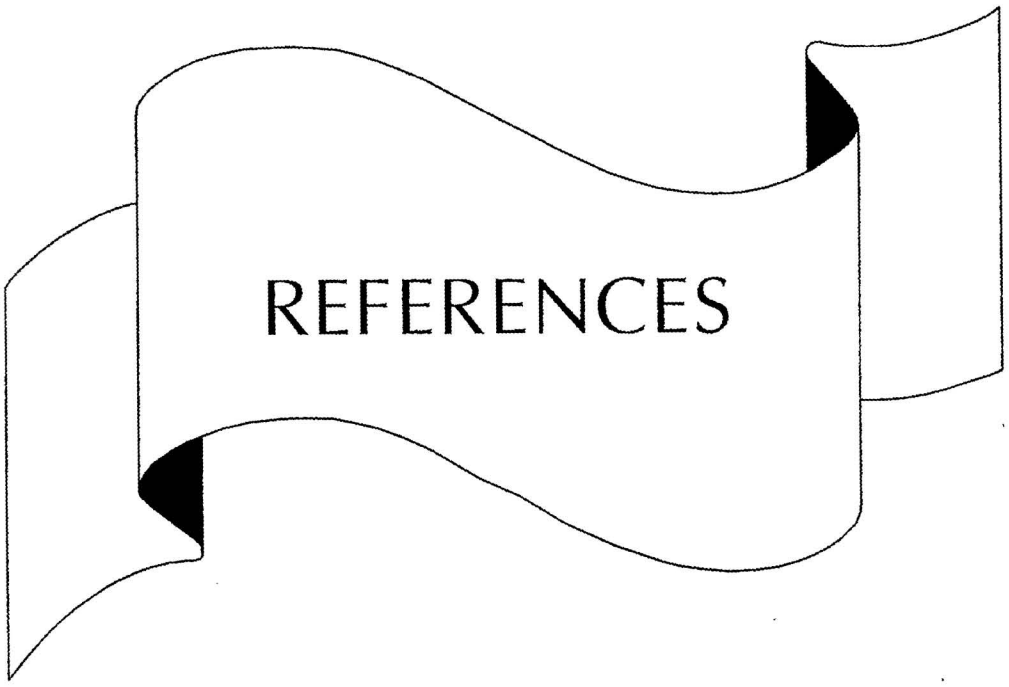


III. Specificity and biosafety



IV. Toxin produced by *F. pallidoroseum*

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PRODUCTION AND EVALUATION OF THE FUNGUS *Fusarium pallidoroseum* (Cooke) Sacc. AS A BIOPESTICIDE AGAINST PEA APHID *Aphis craccivora* Koch.

By

REJI RANI O. P.

ABSTRACT OF THE THESIS
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DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
COLLEGE OF AGRICULTURE, VELLAYANI
THIRUVANANTHAPURAM - 695 522

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ABSTRACT

Investigations on "Production and evaluation of the fungus *Fusarium pallidoroseum* (Cooke) Sacc. as a biopesticide against the pea aphid *Aphis craccivora* Koch." were carried out at the College of Agriculture, Vellayani. *A. craccivora* infected by *F. pallidoroseum* naturally or artificially, died within two days. The fungus on repeated subculturing showed variation in morphology. Number of macroconidia got reduced and the mycelia turned more white with every subculture which resulted in a gradual reduction of virulence. Virulence was completely suppressed by seventh subculture.

Evaluation of different naturally available substrates for mass production of *F.pallidoroseum* revealed that among the solid substrates tried, leafy substrates, bran and oil seed cakes supported maximum biomass and macroconidia. Among the liquid substrates tested mature coconut water was found to support maximum biomass and macroconidia. Increased virulence of spore suspensions prepared from these media was found to be due to the abundance of macroconida in them. Aphids were killed faster when spore suspensions containing more macroconidia were sprayed.

Assessment of shelf life of the fungus for a period of twelve months revealed that for storing spores of *F.pallidoroseum* semidry cadaver of the host

insect (*A.craccivora*), diatomaceous earth, fine charcoal powder and leaf mold were found to be the best carrier material with substantial retention of virulence than viability when compared to other materials tested viz. bran, saw dust, vermicompost and peat.

Storage under refrigeration was found to double the shelf life of the fungus compared to storage under room temperature. Spores retained 24 to 58 per cent virulence after 12 months storage at room temperature, while it retained 77 to 97 per cent virulence when kept in a refrigerator. Viability of the fungus was also found to be doubled and virulence well conserved till the end of twelve months (experimental period) when stored in a refrigerator.

Material for packaging the spore formulations did not make any significant difference till the end of six months of storage. Thereafter glass vial was found to extend the shelf life of *F.pallidoroseum* spores by one month compared to polypropylene and aluminium foil when the spore packings were kept at room temperature while under refrigeration the effect of packing materials were not reflected even after 12 months of storage.

Studies conducted to evaluate the compatibility of *F.pallidoroseum* with commonly used botanicals revealed that mycelial growth of the fungus was not adversely affected by none of the botanicals while seed oil emulsions of neem and marotti highly inhibited the sporulating ability of the fungus. Leaf extracts of thevetia was moderately inhibitory while that of neem, bougainvillea and hyptis were found to enhance spourulation. Commonly used insecticide quinalphos at 0.05 per cent concentration did not suppress growth and sporulation of the fungus completely.

Field experiments conducted to evaluate the time and method of application of the fungus to control *A.craccivora* using different spore formulations revealed that carrier materials like diatomaceous earth, charcoal powder, semi dry cadaver and leaf mold were effective in controlling aphids. *F.pallidoroseum* spores applied by spraying as well as braodcasting were equally effective when applied at six weeks after planting as a curative control measure which could exert cent per cent control of the pest . Prophylactic application of the fungal spores on cowpea plants prior to aphid infestation was not at all effective in controlling the pest. Negligible reinfestation in *Fusarium* treated plants reflected the capability of the fungal spores to survive in the cadaver of the host insect. Management of pea aphid using *F.pallidoroseum* was found to significantly increase the yeild of cowpea by 17 to 37 per cent.

F. pallidoroseum was found to be nonpathoenic to the crop plants like *Amaranthus* sp., *Ananas comosus, Moringa olifera, Morus albus, Musa paradasiaca, Passiflora edulis,* and *Sesamum indicum* which have been reported to be diseased by another strain of *F.pallidoroseum* (plant pathogenic).

F.pallidoroseum was found to be noninfectious to the common predators of *A. craccivora* viz. the coccinellids, *Menochiles sexmaculata*, *Coccinella septumpunctata*, and *Micraspis crocea*; syrphids, *Ischiodon scutellare* and *Ischiodon* sp., hemerobid; dragon flies; spiders like *Paradosa pseudoannulata, Tetragnatha maxillosa* and *Oxyopus sp.* Besides it was found to be safe to the stingless bee *Trigona iridipennis* and the common pollinators like *Bombus* sp., *Camponotus* sp., *Syrphus* sp. and *Xylocopa* sp.

The fungal spores at a concentration of 7 x 10^7spores^{-1}ml was found to be nonpathogenic to New Zealand White rabbits. The spores administered orally, dermally, subcutaneously, intranasally and intravenously did not produce any symptoms of acute or chronic toxicity.

The toxin fusaric acid was isolated from seven day old Czapek's liquid culture of the fungus. The toxin yield was at the rate of 112.7 mg litre^{-1}. It caused death of *A. craccivora* at 500 and 1000 ppm with a mortality rate of 46 and 100 per cent respectively at the end of 72 h after treatment. The speed of action of fusaric acid was found to be slow when compared to the entomopathogen *F.pallidoroseum*. The mortality of aphids treated with *F. pallidoroseum* might be not only due to the properties of fusaric acid but also due to the action of some other toxins as well as the biological activity of the fungus.