STUDIES ON THE ENTOMOGENOUS FUNGUS *Fusarium pallidoroseum* (Cooke) Sacc. ASSOCIATED WITH COWPEA APHID *Aphis craccivora* Koch.

BY

FAIZAL M.H.

THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF SCIENCE IN AGRICULTURE

FACULTY OF AGRICULTURE
KERALA AGRICULTURAL UNIVERSITY

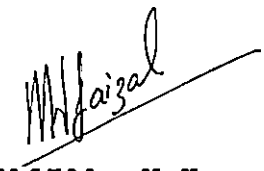
DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM

1992

170381
632.6
FAI/ST
KERALA AGRICULTURAL UNIVERSITY LIBRARY
THRISSUR
680 654

DECLARATION

I hereby declare that this thesis entitled "Studies on the entomogenous fungus Fusarium pallidoroseum (Cooke) Sacc. associated with cowpea 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 to me of any degree, diploma, associateship, fellowship or other similar titles of any other University or Society.



FAIZAL, M.H.

Vellayani

7 - 3 - 1992.

CERTIFICATE

Certified that this thesis entitled "Studies on the entomogenous fungus Fusarium pallidoroseum (Cooke) Sacc. associated with cowpea aphid Aphis craccivora Koch." is a record of research work done independently by Sri.Faizal M.H. under my guidence and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

Susamma Mathai

Dr. Susamma Mathai.
Chairman, Advisory Committee,

Associate Professor,
Department of Agricultural Entomology,
College of Agriculture, Vellayani.

Vellayani,

7-3-1992.

APPROVED BY

Chairman

Dr. Susamma Mathai Susamma Mathai

Members

1. Dr. John Kuriyan

2. Dr. D. Dale

3. Dr. C.K. Peethambaran

Dr. K. S. Pillai
(External Examiner) 04/08/92

ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude and indebtedness to Dr. Susamma Mathai, Associate Professor of Entomology and Chairman of the Advisory Committee for her valuable guidance and encouragement throughout the course of this investigation and in the preparation of this thesis.

I am greatly obliged to the members of the Advisory Committee, Dr. John Kuriyan, Professor and Head of the Department of Entomology, Dr. D.Dale, Professor of Entomoly and Dr. C.K. Peethambaran, Associate Professor of plant Pathology for their valuable suggestions and encouragement.

I am extremely thankful to Dr. N. Mohan Das, Professor, Research co-ordination for the help rendered in the formulation of this project.

I am deeply indebted to Dr. Abraham Jacob, Professor of Entomology, RARS, Pattambi for his pertinent suggestion and valuable help given in the preparation of this thesis.

My thanks are due to Dr. Saifudeen, Associate Professor of Agricultural Chemistry and Soil Science and Dr (Mrs) P. Saraswathy, Professor and Head of the department of Agricultural Statistics and Shri. Ajithkumar, Junior programmer, Department of Statistics for the help.

I take this opportunity to express my sincere thanks to all the members of the Department of Entomology and my friends for their whole hearted cooperation and help rendered during the course of study.

I am also thankful to Prem, Anil and Udayan of the N.C.Computer Service for their help in getting the thesis type-written.

I am deeply indebted to my parents and brother for their help and encouragement during the course of the study.

The awarding of P.G.Fellowship by Kerala Agricultural University is gratefully acknowledged.

Faizal, M.H.

Vellayani

7-3-1992

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

INTRODUCTION

Although broad spectrum pesticides are likely to remain as the major means of crop protection for some more years to come, increased use of biological and other agents will be necessary to overcome problems of pesticide resistance in insects and possible hazards to the environment.

Utilization of micro-organisms such as fungi, bacteria, viruses, protozoa, nematodes and rickettsiae are gaining importance now-a-days as a tool in integrated pest management programmes. These microorganisms are widely distributed in nature and frequently kill large numbers of insects through natural epizootics.

The idea of utilization of fungi for the control of insects was not conceived until after Bail demonstrated in 1861 (quoted by Baird 1956) that a fungus infection could be initiated in insects. From the later part of the ninteenth century onwards a number of attempts were made to control insects by the artificial distribution of pathogenic fungi, there being cases of success as well as failures.

In many cases their utilization merely involves timing their application, so that the insects become diseased earlier than would naturally occur, preventing heavy feeding damage to crops. The method thus has the superiority that it causes the least disturbance to the ecosystem.

Entomogenous fungi could provide inexpensive long lasting control of insect pests in areas of frequent rainfall and high humidity (Dresner, 1949) Kerala, being such an area, offers considerable scope for the use of entomogenous fungi in pest management programmes.

The pea aphid Aphis craccivora Koch is an important pest of cowpea Vigna unguiculata (L.) Walp and many other leguminous crops. It infests young shoots, flowers and tender pods. The loss of plant sap due to their feeding results in stunted growth and delay in initiation of flowering (Nair et al., 1976). Moreover it also acts as a vector of a number of viral diseases such as rosette, mottle, stunt and stripe in various legumes (Porter et al., 1984).

Since the peak incidence of the pea aphid coincides with the active reproductive phase of the crop, application of chemical insecticides may leave toxic residues remaining in/on the produce. It may also lead to the destruction of natural enemies and consequent resurgence of the pest. In this context a feasible approach to combat this pest is to employ a suitable and effective pathogenic agent which can be used either alone or in combination with less hazardous insecticides.

Hareendranath et al. (1987) reported Fusarium pallidoroseum (Cooke) Sacc as a fungal pathogen on pea aphid Aphis craccivora. Preliminary studies have proved the effectiveness of this fungus against the pest (Hareendranath, 1989).

The present project was undertaken to make detailed studies on the fungus F. pallidoroseum in the following lines.

I. Mass culturing of the fungus using cheaper and easily available materials adopting standard techniques.

The materials tried were :

1. Wheat bran
2. Rice bran
3. Wheat bran + straw bits
4. Rice bran + tapioca bits
5. Rice bran + straw bits
6. Straw bits alone.

II. Assessment of the effect of different degrees of sterilization on the growth and sporulation of the fungus adopting different methods of sterilization

a. Degrees of sterilization - full, partial and no.
b. Sterilization techniques - solarization and heat sterilization.

III. Evaluation of different formulations of the fungus in the control of the insect.

IV. Assessment of the effect of some common pesticides on the growth and sporulation of the fungus.

V. Pot culture experiments to evaluate the results of laboratory trials.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Fusarium spp as insect pathogens

The taxonomic position of the genus *Fusarium* according to Alexopoulos and Mims (1983) is as follows:

Division	:	Eumycota
Sub division	:	Deuteromycotina
Class	:	Duteromycetinae
Sub class	:	Hyphomycetidae
Order	:	Moniliales
Family	:	Tuberculariaceae

Mycelium of *Fusarium* is septate and white in colour, hypha elongated and branched. *Fusarium* typically produces two types of conidia viz., long multiseptate crescent shaped macroconidia borne on sporodochia and small spherical or oval microconidia on simple or branched hyphae.

Most species of *Fusarium* are saprophytic or plant parasitic. But a few species have been found to cause insect mycoses.

Reinking (1921) found Fusarium episphaeria f. cocophila as a parasite of scale insects on citrus in Philippines. Teodoro.(1937) reported F. juruanum Hennings on coccids on coconut and F. parasiticum Fautrey on coccids on citrus from Philippines. Morqueer and Nystrakis (1944) found that F. lateritium Champignon normally saprophytic on leaves became parasitic when it entered the galls of Phylloxera vitifolia (Fitch) . Steinhaus (1949) reported F.aleyrodis Petch (white fringe fungus) parasitic on Dialeurodes citri and D. citrifolii.

Steinhaus and Marsh (1962) reported species of Fusarium infecting Monochamus notatus (Drury) and Coccus viridis, F. epishaeria on numerous scale insects, Fusarium sp. on Eurygaster pacifica Lattin, larvae of Chilo zonellus, larvae and pupae of Platynota rostrana Walker and larvae of Carpocapsa pomonella (Linnaeus). In Georgia pea aphid Macrosiphum pisum (Harris) was infected by a species of Fusarium in addition to Entomophthora and Trichothecium (Rachvelishrili, 1965). Gabriel (1968) reported infection by F. episphaeria cocophila on Aonidiella aurantii, Chrysomphalus aonidium (Linnueus) and Coccus viridis. MacLean and Giese (1968) detected Fusarium sp. parasitic on Xyloterinus politus Say. Madelin (1968) found F. citriculatum Montagne regularly associated with some of

the cerambycid beetles. Live cerambycid larvae collected in the field were actively infected with the fungus.

Viswanathan (1972) reported Fusarium oxysporum on Coccus viridis. In field tests it was found to cause 90 per cent mortality within 10 days when the fungal spore suspension was applied on the plants and kept in natural condition. Atwal et al. (1973) reported Chilo partellus (Swinhoe) as a new host of Aspergillus flavus and Fusarium sp. F. larvarum was the most widely spread parasitic species on Adelges piceae in Canada (Smirnoff, 1973). Popov and Illiesu (1975) reported Beauveria, Spicaria and Fusarium as the most important pathogens of Eurygaster integriceps. Sridhar and Krishnaiah (1975) observed F. equisetti parasitic on larvae, pupae and adults of okra petiole maggot Melangromyza hibisci Spencer. Barson (1976) noticed F. solani (Mart.) as a weak pathogen of larval stages of large elm bark beetle Scolytus scolytus. Kalvesh (1976) isolated F. gibbosum var. bullatum, F. javanicum, F. oxysporum, F. oxysporum var. orthoceras, F. sambueinnum var. minus and F. semitectum from forest pests in Kulando, Jacob et al. (1978) observed the occurrence of F. moniliformae var. subglutinans on the grubs, pupae and adults of Epilachnabeetle, Henosepilachna vigintioctopunctata.

Kuruvilla (1978) found that F. oxysporum when applied at 6.25 x 10^6 spores per ml on Nilaparvata lugens caused 100 per cent mortality within three days. The first and second instar nymphs were more susceptible than third, fourth and fifth instar nymphs and adults. Kuruvilla and Jacob (1978) repoted the occurrence of F. oxysporum Schlect. as a pathogen of the rice brown plant hopper, Nilaparvata lugens in Kerala. Lynch and Lewis (1978) reported the parasitization of egg masses of European corn borer Ostrinia nubilalis by F.oxysporum. Nayak and Sreevastava (1978) observed F. oxysporum infecting Melanitis leda ismene. Beevi (1979) studied the efficiency of F.moniliformae var.subglutians in controlling epilachna beetle under caged conditions in the field and showed that the pathogen at 7.5 x 10^5 conida per ml caused 96.67 per cent mortality in seven days. Devanesan et al. (1979) reported infection of Nephotettix virescens by F. equisetti. Pathogenicity tests conducted by spraying a spore suspension prepared from five day old culture of the fungus caused cent per cent mortality of the nymphs and adults after 48 hours of inoculation.

Gopinathan et al. (1982) reported 100 per cent mortality of brinjal mealy bug Coccidohystrix insolita Green in nine days after spraying with F. equisetti. Beevi et al. (1982) found that F. moniliformae was pathogenic to Mylabris

pustulata and Aulacophora sp. under laboratory conditions. Nagalingam and Jayaraj (1986) reported F. semitectum producing fungal epizootic in colonies of Myzus persicae. Devnath (1987) isolated a species of Fusarium tentatively identified as F. coccophilum from the diaspidid Hemberlesia rapex on tea in north eastern India. Hareendranath et al., (1987) reported F. pallidoroseum (Cooke) Saac. as a potent pathogen of cowpea aphid Aphis craccivora. Mathew and Mohamed Ali (1987) isolated F. solani from larvae of the carpenter worm, Cossus cadambae Moore, a pest of teak. Raghavendran et al. (1987) reported that Fusarium subglutinans was pathogenic to sugarcane scale insect Melanaspis glomerata (Green)and noticed 60 per cent mortality of first and second instar nymphs when sprayed at a concentration of 10^6 to 10^8 spores per ml. Ozino et al. (1988) found F. oxysporum and F.tricinctum pathogenic to Sitobion avenae. Hareendranath (1989) reported that F.pallidoroseum at the rate of 7×10^6 spores per ml and 3.5×10^6 spores per ml was as effective as the insecticide quinalphos 0.05 per cent in controlling Aphis craccivora in the field. Bioassy showed that LC 50 of the fungus to cowpea aphid was 3.408×10^6 spores per ml. Martins et al. (1989) recommended Fusarium sp. as effective biological control agent against Orthezia praelonga on coffee in the

dry season in Espirito santo. Villacarolos and Robin (1989) noticed Entomophthora and Fusarium as most frequently recorded species infecting Heteropsylla cubana Crawford.

Symptoms caused by Fusarium spp on insects

Reinking (1921) studied F. episphaeria and F. coccophila infection on citrus scale and found that the fungus appeared red or pinkish with club shaped fruiting bodies on the edge only or more frequently from the entire margin of the scales. Steinhaus (1949) reported that a pinkish spore mass was found on the edge of Dialeurodes citri when infected with F. aleyrodis. Steinhaus and Marsh (1962) reported the symptoms of Fusarium infection on the larvae of Monochamus notatus. Reddish black spots appeared on thorax (later spreading) and white mycelium issued from the cuticle at the infection site. The body tissues became translucent prior to the emergence of flocculent white mycelium. Atwal et al. (1973) reported that larvae of Chilo partellus infected with Fusarium sp. showed a swelling of the body and presence of necrotic interpleurite spots. The hyphal covering on the body gave it a white appearance.

Kuruvilla (1978) reported that, when F. oxysporum infection was noticed on Nilaparvata lugens, the infected insects became sluggish, turned pale and later assumed a

brownish colouration. Death occurred within 24 to 78 hours after inoculation. Majority of insects dropped down after death while some were seen remaining attached to the plants. Towards death, there was a general softening of the body, but later on it got hardened. Growth of the mycelium on the cadavers was observed after 24 to 48 hours of death. The fungus produced both microconidia and macroconidia on the cadavers. Beevi (1979) found that grubs and beetles of Henosepilachna vigintioctopunctata infected with Fusarium moniliformae var. subglutinans exhibited loss of appetite became sluggish and death occurred in 48 to 96 hours. Growth of the fungus over the cadavers appeared 24 hours after death. Studies on the pathogenicity of F. pallidoroseum showed that cowpea aphid Aphis craccivora infected with the fungus turned pale and assumed a brownish black discoloration. Death occurred in 48 to 72 hours after infection and white mycelial growth appeared on the cadavers 24 to 48 hours after death (Hareendranath, 1989).

Safety of entomopathogenic Fusarium spp to crop plants

Kuruvilla (1978) reported Fusarium oxysporum as nonpathogenic to cotton, rice and tomato. Beevi (1982) observed that F. moniliformae var. subglutinans was safe to

cotton, tomato, bittergourd, brinjal and snakegourd. Nagalingam (1983) found that F. semitectum was safe to all instars of mulberry silk worm, adult honeybee, hymenopterous parasitoides and coccinellid predators. It was also found to be safe to plants like chillies, cabbage, brinjal and tobacco. Studies by Hareendranath (1989) on the safety aspects of F. pallidoroseum showed that it was not pathogenic to rice, bhindi, chillies and tomato and the predator Menochilus sexmaculata.

Mass production of entomogenous fungi

Ease and cheapness of production and application are among the principal characteristics of a desirable pathogen (Butcher 1958). Difficulty in producing adequate quantities of pathogens has been a serious impediment towards the development and application of microbial agents in plant protection programmes. There have been many attempts in the past to develop techniques of mass production of entomogenous fungi.

Celino (1930) tried 12 kinds of media for the mass production of Beauveria bassiana. The media tested were water agar, potato dextrose agar, nutrient beef agar,

potato dextrose leaf miner extract agar, cane juice peptone water agar, synthetic agar poato cylinder, steamed rice, corn meal and oat meal agar plus two per cent dextrose. Synthetic agar and corn meal agar gave the fastest growth. Bartlett and Lefebvre (1934) used Forbe's medium without the addition of beef broth. Rice with the addition of peptone (Pascalet 1939) or bran (Mc Coy and Carver, 1941; Dresner, 1949; York, 1958) had also been used successfully for the mass culture of the pathogen.

Strains of B. bassiana produced the highest amount of spores on Sabouraud maltose agar followed by Molish medium, blood agar base, Raulin - Thom and Czapek dox media, potato dextrose and corn meal agar (Hall, 1954). Schaerffenberg (1957) recommended the addition of three per cent peptone to a malt extract medium for a large scale production of spores of the pathogen. Hall and Dunn (1958) recommended Sabouraud dextrose agar medium fortified with cereal for large scale production of Entomophthora exitialis.

Cooked slices of potato tuber inoculated with the spores of Entomophthora aphidis held in petridishes at a constant temperature of 76°F gave a heavy crop of spores ready to harvest after 10 days (Shands et al., 1958).

Martignoni (1964) developed suitable methods of mass production of Beauveria and Metarrhizium using wheat, corn or potato as culture substrates. Pristavko and Goral (1967) proposed three main methods for mass production of B. bassiana.

1. Reproduction on solid nutrient media in sterile condition with natural aeration. This method was accepted for small scale laboratory production.

2. Growth under semi-sterile condition on liquid, pasty and solid nutrient media in flat vessels. This mehod could be satisfactorily used to produce enough materials for laboratory and field tests.

3. Production in liquid media on a shaking machine or industrial type fermentation tanks with artificial aeration.

Bell (1974) found that B.bassiana and M.anisopliae could be grown satisfactorily on a semisolid medium whose base was wheat bran Villacorta (1976) developed a technique for mass culturing of M. anisopliae in granular form. Rice grains were placed in bottles and soaked in water. After covering the top of the bottles, they were autoclaved for 30

minutes at 127^{0}C and were agitated to permit granulation of the rice. Inoculated rice was then added to the autoclaved rice for incubation. The fungus produced in granular form was used to control Spodoptera frugiperda, Diatraea saccharalis and hoppers on pasture grasses. Aquino et al. (1977) used rice grains as the substrate for mass multiplication of M. anisopliae in a different way. Rice grains mixed with water was placed in one litre bottles capped with aluminium foils. A hypodermic syringe was used to inoculate the medium. Two weeks after inoculation the fungus and medium were removed from the bottle, drained in a sieve and placed in plastic sacks. They were then stored at a low temperature until the fungus was required for use.

Easwaramoorthy and Jayaraj (1978) cultured Cephalosporium lecanii infecting coffee green bug Coccus viridis on moist sterile sorghum grains. The fungus sporulated abundantly by three weeks time. Fully sporulated fungal mat along with sorghum grains were taken out, blended with water filtered and used in trials at a concentration of $16x10^6$ spores per ml of spray fluid.

For mass production of Fusarium moniliformae var subglutinans sorghum and bajra appeared to be most suitable

as they produced maximum spores with higher virulence. The best combination for all matrials was found to be 30g of material and 25ml of water (Beevi, 1979). Kuruvilla and Jacob (1981a) showed that green gram, wheat or sorghum could be used as substrates for easy mass production of the fungus Fusarium oxysporum infecting rice brown planthopper Nilaparvata lugens.

Nagalingam (1983) reported maize broken grain + black gram husk or red gram husk at 4:1 (w/w) as a suitable media for the mass production of Fusarium semitectum infecting Myzus persicae. Batista et al. (1985) in their studies with liquid media found that the conidia production of B bassiana was 8.8 times as great on bran broth as on rice broth or potato broth. Germination was more than 96 per cent in all three broths. Both conidia production and germination were poor in water alone. Holdom et al. (1986) reported the possibility of using bagasse (Sugercane waste) in a two phase conidial production process of Nomuraea releyi. Although the yields were comparatively low, the cost was very low. Raghavendran et al. (1987) mass cultured Fusarium subglutinans on moist sterile sorghum grains. The fungus sporulated abundantly by three weeks.

Rombach et al. (1988) studied the growth of B. bassiana in liquid medium and subsequent sporulation of dry mycelia. It was concluded that the production of dry mycelia might be a practical method for mass productrion of B. bassiana. Batista et al. (1989) cultured B bassiana and M. anisopliae in two different culture media (rice and soaked bran). The mortalilty rate of banana rhizome weevil Cosmopolites sordidus was between 85 and 97 per cent by both the fungal species on both media. Hareendranath (1989) reported broken maize grain as a suitable mediumfor the mass multiplication of Fusarium pallidoroseum followed by tapioca chips and jack seeds as they produced maximum number of spores.

Effect of pesticides on the growth and development of entomogenous fungi

Hall and Dunn (1959) studied the effect of parathion, malathion, demeton, trithion, DDT, wettable sulphur, dithane, ferbam, Bordeaux mixture (5:5:50) and captan on five fungal pathogens of spotted alfalfa aphid, Therioaphis maculata (Buckt). All the insecticides killed Entomophthora exitialis Hall and Dunn. All but parathion and DDT killed E.virulenta but only trithion prevented the

growth of E. coronata. E.exitialis was the most and E. coronata the least affected by the fungicides. Tests were also made in which resting spores of E. virulenta were soaked in insecticide solution for about six hours before being resuspended in water and sown on agar plates. None of the insecticides killed the spores, though they retarded spore germination to some extent.

Laboratory tests by Dirimanov and Angelova in 1962 with 22 chemical insecticides to determine their effect on the development of B. bassiana showed that fungal growth was not affected by mixing with five per cent DDT dust, but similar treatment with 12 per cent BHC inhibited it. Among the preparations tested in liquids, 0.3 per cent malathion and a mixture of BHC and five per cent pinene prevented development and 0.1 per cent thiometon, 0.2 per cent nicotine sulphate, 0.1 percent parathion or methyl parathion and 0.06 per cent demeton reduced the development substantially. Development was almost normal with 0.1 per cent diazinon, 0.2 per cent DDT, 0.5 per cent toxaphene or parathion and 0.1 per cent dieldrin. Similar resutls were obtained when the insecticides were tested on filter paper.

Evlakhova (1964) found that DDT had a stimulating effect on the growth of B. bassiana and Aspergillus flavus but BHC failed to stimulate growth of either fungus and the gamma isomer showed toxicity. Urs et al. (1967) reported the comparative effect of phosphamidon, parathion, DDT, malathion, endrin and BHC on the development of B. bassiana and M. anisopliae. Phosphamidon was the least harmful followed by endrin, malathion and parathion. Yendol (1968) found that conidial germination of E. conidia was inhibited by the insecticide malathion.

Cadatal and Gabriel (1970) studied the effect of nine insecticides and three fungicides on the development of B. bassiana. M. ansopliae and Entomophthora sp Supracide, carbaryl, endosulfan and endrin exhibited partial to complete inhibition of growth and sporulation at concentrations equal to field recommendations. Fenitrothion, Chlorfenvinphos, lindane, diazinon and DDT were innocuous. The fungicides panogen and granosan L were toxic while Kasumin allowed complete development.

Studies of Uchida (1970) in Japan showed that common fungicides prevented mycelial growth of Aschersonia sp. but insecticides at usual concentration had less adverse effect.

Wilding (1972) observed that Cephalosporium aphidicola was inhibited in vitro by the systemic fungicides benomyl and triarimol but not by dimethrimol.

Fritz (1976) reported the effect of 34 fungicides on the mycelial growth of Basidiobolus ranarum Eidam, Conidiobolus asmodes and Entomophthora virulenta. In general systemic compounds were more injurious than non systemics. Zimmermann (1976) observed that the systemic fungicides calixin and imugan completely inhibited the spore germination of Entomophthora aphidis, E. thaxteriana and E. virulenta. Benomyl and milstem inhibited only the spore germination of E.aphidis Cercobin M showed little toxicity to the fungi.

Easwaramoorthy and Jayaraj (1977) studied the effect of insecticides and fungicides on the growth of coffee green bug fungus Cephalosporium lecanii Zimn. The results showed that dichlorvos reduced the growth of the fungus drastically. Carbaryl, monocrotophos, malathion and endrin also inhibited the growth.Ethyl parathion, BHC and dimethoate were less destructive and acephate, DDT and phosphamidon caused the least inhibition. Boredeaux mixture, dithane M-45 and dithane Z-78 inhibited the growth

completely, sulphur was least inhibitory. Keller (1978) studied the influence of dimilin (Diflubenzuron) on the growth and germination of B. tenella, M. Anisopliae, E. aphidis, E. calicis, E. ignobilis and E. sphaerosperma. The results showed that concentrations of dimilin that were proposed for practical application against insects slightly impaired the growth of B. tenella and M. anopliae but not that of Entomophthora spp. It stimulated the germination of conidia and the formation of secondary conidia in E. aphidis and E. ignobilis. Kuruvilla and Jacob (1981b) found that thiram, dithane M-45 and difoltan completely inhibited the growth of F. oxysporum infecting Nilaparvata lugens. Insecticides like carbaryl and quinalphos showed less inhibitory effect on the growth and sporulation of the fungus.

The growth of the fungus Fusarium semitectum was inhibited by the insecticides heptachlor, monocrotophos, and endosulfan and the fungicides, carbendazim, benomyl and thiram while wettable sulphur and quintozone were innocuous (Nagalingam, 1983).

Yasem De Romero (1986) evaluated the effects of several pesticides currently used in citrus in Argentina on the growth and development of Verticillium lecanii. All

the fungicides tested inhibited growth and conidia production. The inhibitory action of the insecticides were in the order chlorpyrifos < dicofol = tetradifon < Chlorobenzilate. The herbicide glyphosate had no effect on conidia production but reduced mycelial growth by 17 per cent while paraquat reduced both.

Beevi and Jacob (1987) studied the effect of insecticides HCH, quinalphos, malathion, dimethoate and carbaryl and the fungicides, dithane. M-45, captan and thiride on the growth and sporulation of Fusarium moniliformae infecting epilachna beetle Henosepilachna vigintioctopunctata. All the fungicides and HCH inhibited the growth and sporulation of the fungus completely, where as carbaryl and dimethoate had less fungicidal effect. The inhibitory effects of malathion and quinalphos were insignificant.

The effect of the fungicides benomyl and edifenphos and the insecticide carbaryl at 0.1, 1, 10, 100, and 1000 ppm on the entomogenous fungi B. bassiana, Metarhizium anisopliae and Hirsutella citriformis were studied in laboratory. Agoruda et al. (1988). All

combinations of the pesticides inhibited germination of conidia. H . citritormis was more susceptible to benomyl at 10 and 100 ppm.

Sa (1988) studied the effects of tokathion (prothiofos), marshal (carbosulfan), mevinphos, puraquat glyphosate, somp (pendimethalin), previcur (propamocarb), terazole (thiadiazole) and sportak (prochloraz) at dilution of 500, 1000 and 1500 times on the mycelial growth of B. bassiana. Sportak 25 per cent EC was the most toxic followed by previcur. Sportak inhibited fungal growth at all dilution. Paraquat was least toxic.

Saito (1988) studied the effect of 23 fungicides, 42 insecticides and acaricides on the growth of V. lecanii Polyoxin, mepronil, vinclozolin, benomyl, bitertanol and fosetyl were the most toxic fungicides while toxic insecticides and acaricides included fluvalinate, acephate, diflubenzuron, cartap, vamidothion and buprofezin.

Vanninen and Hokkanen (1988) evaluated the effects of four insecticides, five fungicides, four herbicides and a nematicide in vitro on M. anisopliae, B. bassiana, Paecilomyces fumosoroseus and P. farinosus. The compounds

that did not affect the growth or sporulation of any of the fungi tested were insecticides diazinon, pirimicarb and cypermethrin and the nematicide/insecticide oxamyl. Vilas Boas (1988) found that monocrotophos did not inhibit the viability of Beauveria spp in the pathogen - insecticidal mixture. B.bassiana alone caused 27.5 per cent mortality to larvae, but when associated with monorotophos, mortality reached 45.3 per cent.

Combinations of B. bassiana and five insecticide formulations were assayed for compatibility and efficacy for the control of Leptinotarsa decemlineata. In vitro tests with abamectin 0.15 EC, triflumuron 4 flowable, thuringiensin ABG 6162 A (1.5 per cent ai) and carbaryl 50 WP demonstrated no significant inhibition of colony growth of B. bassiana (Anderson et al., 1989).

MATERIALS AND METHODS

MATERIALS AND METHODS

Sterilization of glassware

Petridishes, conical flasks and test tubes were kept for 24 hours in a cleaning-solution containing 60g potassium dichromate and 60ml concentrated sulphuric acid in one litre of water. They were then washed well in tap water, rinsed in distilled water and air dried. All the glassware were then sterilized in a hot air oven at 160°C for two hours. Pipettes and measuring cylinders were autoclaved at 15 psi for 20 minutes.

Preparation of media

Following media were used in these studies.

a. Potato dextrose agar

Peeled potato	-	200 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water	-	1 L

b.Sabouraud dextrose agar

Dextrose	-	40 g
Peptone	-	10 g
Agar	-	20 g
Distilled water	-	1 L

c.Richard's solution

Potassium nitrate	-	10 g
Potassium dihydrogen phosphate	-	5 g
Magnesium sulphate	-	2.5 g
Ferric chloride	-	0.02 g
Sucrose	-	50 g
Distilled water	-	1 L

Rearing of cowpea aphids, Aphis craccivora Koch

Cowpea plants (variety - Kanakamony) were raised in 30 cm diameter earthen pots containing potting mixture of sand, red soil and cowdung in the ratio of 1:1:1. The seeds of Kanakamony were obtained from College of Horticulture, Vellanikara. The crop was raised according to

the recommendations of the Package of Practices of Kerala Agricultural University (1989).

Adult aphids were colonized on 50 to 60 day-old plants by liberating them from an infested branch. The plants were kept watered and free from predators in the insectary. When the plants started drying, the aphids were collected and liberated on fresh plants and thus the culture was maintained.

Maintenance of the culture of Fusarium pallidoroseum (Cooke) Sacc.

The initial culture of the fungus was obtained from that kept in the Insect Pathology Laboratory, College of Agriculture, Vellayani. It was thereafter cultured and maintained on potato dextrose agar or Sabouraud dextrose agar, since they were found to be the most suitable media for the growth of the pathogen.

The virulence of the fungus was maintained by passing it periodically through cowpea aphids and isolating fresh cultures. For this purpose spore suspension of the fungus was prepared by aseptically pouring 5ml of sterile distilled water into heavily sporulating 4 day-old culture

slants. After shaking the tubes the resulting suspension was sprayed on aphid colonies on terminal shoots of cowpea placed in deep petridishes. The death of the aphids was noticed after 24 to 48 hours. Later the dead aphids showing fungal growth were collected and surface sterilized with 0.1 per cent mercuric chloride in 75 per cent ethyl alcohol for one to two minutes, washed in three changes of sterile water and placed either individually or in groups in petridishes containing potato dextrose medium and incubated at room temperature. When the fungal growth was visible, it was subcultured and maintained in slants for further studies.

Mass culturing of the pathogen

In order to find out a suitable medium for mass mutiplication of the fungus trials were conducted with the following materials.

1. Wheat bran.
2. Rice bran
3. Wheat bran + Straw bits
4. Rice bran + Tapioca bits
5. Rice bran + Straw bits
6. Straw bits

Thirty grams each of the above materials (in the case of media having two components, 15g each component was taken.) were taken in separate 250ml conical flasks containing 30ml of distilled water and plugged with cotton. A total of 144 flasks (3 replications each) containing the above materials were prepared for taking observations on growth and sporulation from third day to 10^{th} day after inoculation . Flasks were then autoclaved at 15 psi for 20 minutes. Circular discs of 5mm diameter were cut from outer edge of seven day old cultures of the fungus by means of a sterile cork borer. One disk was transferred to each conical flask containing the material. The flasks were shaken well in order to disperse the inoculum and then incubated at room temperature.

Growth of the fungus was observed by visual comparison. Sporulation was observed as follows: Sterile distilled water (100 ml) was poured into each culture flask. After shaking the flasks for two minutes the resulting suspension was filtered through muslin cloth and collected in an empty sterile 250ml conical flask. Spore concentration in the suspension was estimated using a haemocytometer.

Effect of mass culture substrates on the growth of *F. pallidoroseum*

Powdered materials (200 g each) of wheat bran, rice bran, wheat bran + straw bits, rice bran + tapioca bits, straw bits, rice bran + straw bits were boiled with 500ml of distilled water for 30 minutes. The mixture was strained through a muslin cloth and the volume of filtrate was made up to 1 L with distilled water. After adding agar at two per cent, the media were autoclaved, allowed to cool and asceptically poured into 9cm diameter sterile petridishes. The pertidishes were inoculated with a 5mm mycelial disc of 7 day old fungus. Plain 2.0 per cent agar and PDA served as standards . Three replications were kept for each substrate and all petridishes were incubated at room temperature for 15 days. Diameters of the fungal clonies were measured in opposite directions perpendicular to each other and the mean was calculated.

Effect of different mass culture media on virulence of the pathogen

Mass culture media were prepared and inoculated with the fungus as explained earlier. They were allowed to grow for seven days. Thereafter 100ml of sterile distilled

water was poured into each culture flask. After shaking the flasks for two minutes the resulting suspension was filtered through muslin cloth into empty sterile 250ml conical flasks. The concentration was standardized to 3.5 x 10^6 spores per ml. The test insects were drawn from disease-free culture of pea aphids maintained in the insectary. Wingless adults only were used for the experiment.

The fungal spore suspension (2ml) was applied on to aphids with an atomiser and 15 of them were released on immature pods kept in sterile petridishes. The experiment was replicated thrice. Insect mortality was recorded daily up to the fourth day for each medium. Pea aphids sprayed with only sterile water served as control.

Effect of different degrees of sterilization on the growth and sporulation of the fungus

From the mass culture and pathogenicity studies wheat bran and rice bran were found to be the suitable substrates for the mass multiplication of the fungus F. pallidoroseum and hence for furthur studies these two media only were used.

In order to find out a cheap method of sterilization the following methods were tried :

1. Heat sterilization (full)

The substrates were sterilized at 15 psi for 20 minutes in an autoclave.

2. Heat sterilization (partial)

The substrates taken in conical flasks, plugged with cotton and were placed in boiling water and kept for 30 minutes.

3. Solarization (full)

Substrates taken in conical flasks were kept in solar cooker and kept in sun light for two successive days. The temperature inside the solar cooker was read as 120°C.

4. Solarization (partial)

The substrates taken in conical flasks were kept in open sun light for two successive days. The temperature inside the flask reached upto 45°C

5. No sterilization

The materials (30 g each) were taken in separate 250ml conical flasks, 30ml of distilled water was added to each flask and the contents were mixed properly and plugged with cotton. They were then subjected to the above - said sterilization methods. Five replications were maintained in each case.

The materials subjected to different methods of sterilization were inoculated with a 5 mm diameter culture disc from a seven day old culture of the fungus. The flasks were shaken well in order to disperse the inoculum and then incubated at room temperature. The growth of the fungus was monitored daily by visual observations.

The spore count was taken on the eighth day after inoculation. 100 ml of sterile distilled water was poured into the culture flasks. After shaking the flasks for two minutes the resulting suspension was filtered through muslin cloth. The spore concentration in the suspension was estimated with the aid of a haemocytometer.

Effect of culture filtrate of F. pallidoroseum on Aphis craccivora

100 ml of Richard's solution was taken in seperate 250ml conical flasks and sterilized by autoclaving. Each of the flask was then inoculated with a 5 mm culture disc cut from actively growing seven day old culture of F. pallidoroseum. After incubation for a period of 15 days, the fungal growth was filtered through whatman No.1 filter paper. The filtrates thus obtained were used for the study.

The culture filtrates at 0, 50, 75 and 90 per cent dilutions were prepared by adding required quantity of sterile distilled water. Two ml each of the solution was sprayed on to adult wingless aphids and 15 numbers of them were transfered to immature pods kept in sterile petridishes. Similar applications were made with filtrate from a 15 day old pure media to check weather the toxicity is due to any of its ingredient. Spraying with distilled water alone served as control. Mortality counts were taken 24 hours after treatment. Four replications were kept for each treatment..

Effect of pesticides on the growth and sporulation of the pathogen

a. Insecticides used :

1. Hexachloro cyclohexane (BHC 50 per cent WP at 0.2 per cent)
2. Dimethoate (Rogor 30 per cent at 0.03 per cent)
3. Quinalphos (Ekalux 25 per cent E.C. at 0.05 per cent)
4. Fenthion (Labaycid 80 per cent E.C. at 0.05 per cent)
5. Monocrotophos (Nuvacron 36 per cent E.C. at 0.03 per cent)
6. Phosphamidon (Dimecron 86 per cent E.C.at 0.05 per cent)
7. Mercaptothion (Malathion 50 per cent E.C. at 0.1 per cent)

b. Fungicides used:

1. Captofol (Difolatan 80 per cent WP at 0.2 per cent)
2. Mancozeb (Dithane M 45 at 0.2 per cent)
3. Copper oxychloride (Blue coppr 50 per cent WP at 0.2 per cent)
4. Captan (Captan 75 per cent WP at 0.2 per cent)
5. Zineb (Dithane Z-78 at 0.2 per cent)

Quantities of each pesticide for giving the required concentrations were mixed with 250 ml each of potato dextrose agar medium taken in conical flasks. Flasks

were shaken throughly and the media were poured into sterile petridishes and allowed to solidify. Circular discs (5 mm) were cut from outer edges of seven day old culture by means of sterile cork borer and was placed in the centre of each petridish. Media without pesticide inoculated with the fungus served as control. Each treatment was replicated thrice. Radial growth of the fungus was measured on the 9th day, when growth in the control dish fully covered the media. To assess sporulaion, six discs were cut on the 9th day from different areas of the medium in a dish and each disc was suspended in 100 ml of sterile water taken in a conical flask. After shaking the flasks for two minutes the number of spores in each of the suspension was estimated using a haemocytometer and the average was calculated .

Evaluation of different formulations of the fungus in the control of Aphis craccivora

Spore suspension of F. pallidoroseum was prepared from mass culture media. The spore suspensions were then concentrated by centrifuging at 5000 rpm for 15 minutes. While centrifuging, the temperature was kept constant at 10^{o}c. The supernatant solution was drained off and the spore count in the sediment was estimated and then mixed with different inert materials as follows.

1. Dust formulation using diatomaceous earth as inert material

The concentrated spores were mixed with diatomaceous earth powder. It was standardized to contain 3.5×10^6 spores per gram of formulation.

2. Wettable powder formulation using diatomaceous earth

It was prepared by mixing the dust formulation with equal quantity of water containing 0.1 per cent teepol (wetting agent).

3. Wettable powder formulation using talc

Prepared as above, the substrate being talc in place of diatomaceous earth.

4. Wettable powder formulation using talc

Prepared as in the case of WP formulation using diatomaceous earth.

Test insects were drawn from disease-free culture of A. craccivora maintained in the insectary. Adult wingless aphids only were used for the experiment (15 aphids were used in each replication). Two grams of dust formulation

/4ml of WP formulation of the fungal spores were applied to aphids using a cloth bag/atomiser and the same were released on immature pods kept in sterile petridishes. The experiment was replicated four times. Insect mortality was recorded up to the fourth day after treatment . Aphids sprayed with only sterile water served as control.

Control of A. craccivora with F. pallidoroseum spore formulations - Pot culture experiment

A pot culture experiment was conducted to test the efficacy of F.pallidoroseum spore formulations against A. craccivora under field conditions. The experiment was replicated four times. The treatments were as follows :

T_1 - Dust formulation (diatomaceous earth)

T_2 - Dust formulation (talc)

T_3 - Wettable powder formulation (diatomaceous earth)

T_4 - Wettable powder formulation (talc)

T_5 - Spore suspension in water

T_6 - Culture filtrate of F. pallidoroseum grown in Richard's solution, at 50 per cent dilution

T_7 - Quinalphos 0.05 per cent spray

T_8 - Control (sprayed with distilled water alone)

Cowpea plants were raised in 30 cm diameter pots as mentioned earlier. Aphids were relased on 30 days-old plants. Sucessful development of aphid colonies was noticed in 30 days after release.

Spore formulations of the fungus were prepared as mentioned earlier. Application was done in the evening. Dust formulations were applied at the rate of 30 g/plant using a muslin cloth bag. Wettable powder formulations were applied at the rate of 60 g/plant using an atomiser. 30ml of spore suspension containing 3.5 x 10^6 spores/ml and 30ml of culture filtrate at 50 per cent dilution were applied using an atomiser.

Pretreatment counts of aphids were taken by following the sampling technique evolved by Banks (1954). Aphid infestation was grouped into various classes as follows:

Class	Description
Zero (o)	Where there was no aphid present
Very light (v)	Where there was one aphid to a small colony of some scattered individulals confined to young leaves.
Light (L)	Where there were scattered aphid colonies present on the stem and leaves and not confined to crown and upper leaves.
Medium (M)	Aphids present in large numbers, not inrecognizable colonies but diffused and infesting a large proportion of the stem and leaves.
Heavy (H)	Aphids present in very large numbers, very dense, infesting all leaves and stem,the stem usually black with aphids.

Number of aphids for each class was found out by counting 10 samples from each class and taking average of it. The samples were picked up from the field carefully with out causing any disturbance to aphid colonies and kept sealed in individual labelled bottles containing 95 per cent ethanol and counting done later. Before counting each sample, aphids were dislodged from the leaves and stem by slow agitation in the alcohol with the use of a camel hair brush. From the samples containing the dislodged aphids, a small portion was taken with an ordinary aspirator and transferred to a petridish. A graph paper was pasted at the

bottom of the petridish. When all aphids settled in the petridish excess alcohol was carefully drained with the aspirator so that the aphids did not move while counting. Counting was done under a stereo-microscope. All the aphids in each sample were counted. 10 samples were counted from each class and the average of each class was worked out as follows.

Class	Number of aphids per sample										Mean no. of aphids per class
	1	2	3	4	5	6	7	8	9	10	
V	9	11	4	6	1	3	5	8	8	2	6.7
L	75	38	43	102	57	43	64	82	91	76	67.1
M	213	187	112	161	130	243	121	180	205	210	176.2
H	420	582	311	355	570	447	491	515	428	432	455.1

Post-treatment counts of aphids were taken four days after treatment. For this purpose the live insects that remained after treatment were dislodged into petridishes containing 95 per cent ethanol. Counting was done as mentioned earlier

Assessment of spore viability of *F. pallidoroseum* in the formulations

The fungal spore formulations were prepared as mentioned earlier. The viability of spores in the formulation was tested after 1, 4, 7, and 14 days of preparation.

Adult wingless aphids (15 in number) were treated with formulations as mentioned earlier. The insect mortality was recorded four days after treatment. The experiment was replicated thrice.

RESULTS

RESULTS

Mycosis of *Aphis craccivora* caused by *Fusarium pallidoroseum*

Symptomatology

The infected insects became sluggish and turned pale, later on developing a brownish black discolouration. Death occurred within 24 to 72 hours of inoculation. The body of the dead insects became hard and seen adhering to the plant covered with white mycelial growth of the fungus (Plate 1). Growth of the mycelium over the cadaver was observed after 24 to 48 hours of death (Plate 2a and 2b). The conidia of the fungus (zero to seven septate) could be observed under a compound microscope (Plate 3)

Mass culturing of *F. pallidoroseum*

The growth of the fungus on different mass culture substrates are shown in Plate 4a and 4b . Growth characteristics and sporulation of the fungus in different substrates are given in Table 1 and 2 respectively.

Pooled analysis of data showed that the error variances are heterogenous. But interaction was absent.

Plate 1. Cowpea aphid killed by *Fusarium pallidoroseum* remaining attached to the plants

Plate 1.

Plate 2a & 2b. *Fusarium pallidoroseum* growing on the cadavers of cowpea aphid

2a winged aphid

2b wingless aphid

Plate 2a.

Plate 2b.

Plate 3. Conidia of *Fusarium pallidoroseum*

Plate 3.

Plate 4a & 4b. Growth of *Fusarium pallidoroseum* on different mass culture substrates.

1. Wheat bran
2. Rice bran
3. Wheat bran + straw bits
4. Rice bran + tapioca bits.
5. Rice bran + straw bits
6. Straw bits.

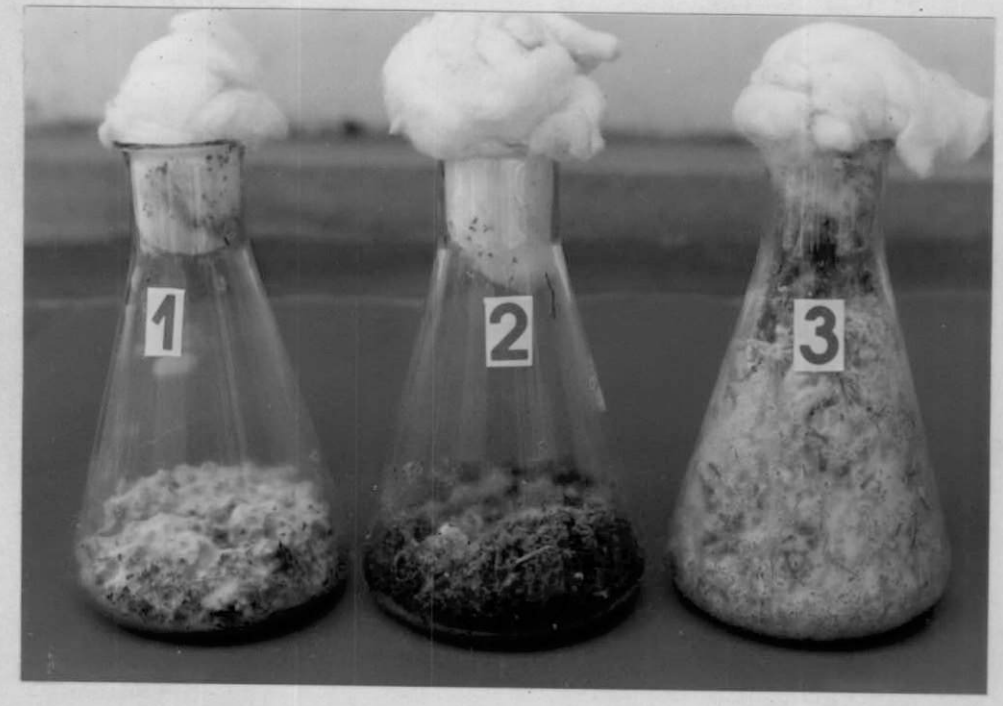


Plate 4a.

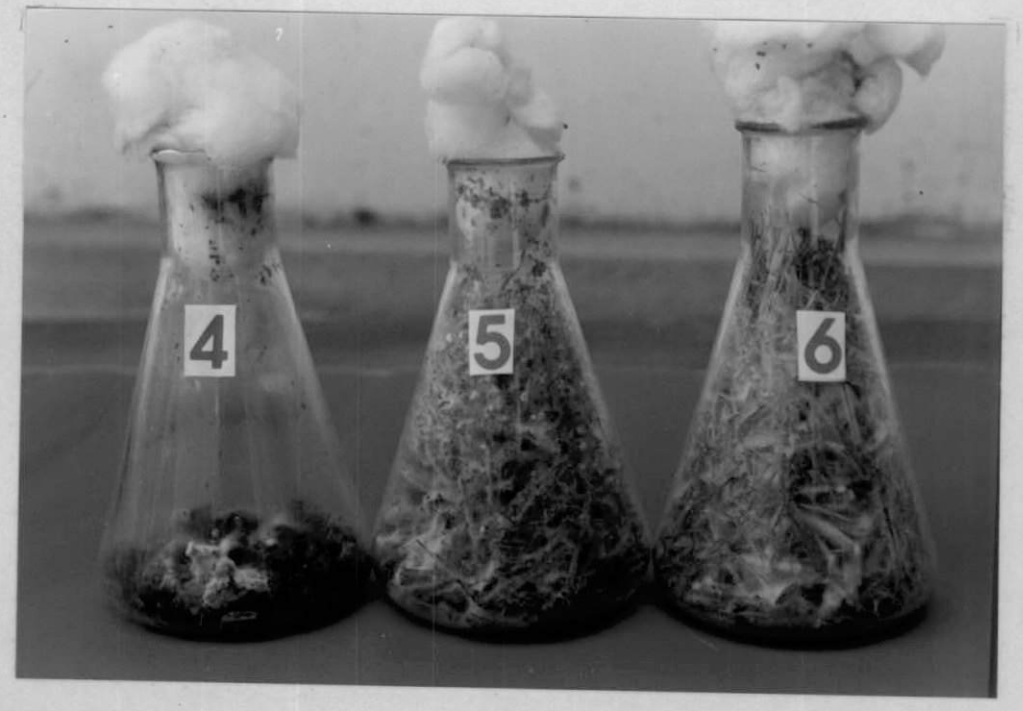


Plate 4b.

Table 1. Growth characteristics of *Fusarium pallidoroseum* on different mass culture media

Sl No.	Media	Growth Characteristics
1.	Wheat bran	White cushiony mycelium which covered the entire surface of the media. The growth of the fungus was very fast .
2.	Rice bran	Diffused mycelial growth was noticed. Growth was slow in the early days but later picked up.
3.	Wheat bran + straw bits	Dense white mycelium was noticed ramifying the entire media.
4.	Rice bran + tapioca bits	Mycelial growth was thin.
5.	Rice bran + straw bits	White fluffy mycelium scattered in the media.
6.	Straw bits	The growth was slow but later white mycellium covered the entire media.

Table 2. Sporulation of *Fusarium pallidoroseum* in different mass culture substrates

(Figures in parenthesis are values after logarithmic transformation)

Media	Days after inoculation							
	3	4	5	6	7	8	9	10
	Mean table x 10^6 spores per ml							
Wheat bran	2.226	2.368	3.447	3.860	5.329	6.193	6.526	6.437
	(0.347)	(0.374)	(0.537)	(0.587)	(0.727)	(0.791)	(0.815)	(0.809)
Rice bran	1.671	2.177	3.144	4.155	5.667	6.454	6.345	6.497
	(0.223)	(0.338)	(0.498)	(0.619)	(0.753)	(0.810)	(0.802)	(0.813)
Wheat bran + straw bits	0.826	1.107	2.841	3.300	4.684	5.593	5.306	5.567
	(-0.083)	(0.044)	(0.454)	(0.519)	(0.671)	(0.748)	(0.725)	(0.746)
Rice bran + tapioca bits	0.462	0.366	1.085	1.951	2.444	3.137	2.678	2.897
	(-0.336)	(-0.436)	(0.036)	(0.290)	(0.388)	(0.497)	(0.428)	(0.462)
Rice bran + straw bits	1.064	1.414	2.023	3.502	4.771	5.783	5.613	5.673
	(0.027)	(0.150)	(0.306)	(0.544)	(0.679)	(0.762)	(0.749)	(0.753)
Straw bits	0.986	1.604	3.123	2.893	4.074	5.409	5.519	5.723
	(-0.006)	(0.205)	(0.495)	(0.461)	(0.610)	(0.733)	(0.742)	(0.758)
C D Values	0.345	0.451	0.179	0.152	0.078	0.117	0.107	0.098

Hence data left un-pooled. Significant difference was observed on the sporulation of the fungus grown on different mass culture media.

On the 3rd day after inoculation treatments differed significantly . Wheat bran recorded maximum sporulation followed by rice bran. Rice bran + tapioca bits recorded the least sporulation. A similar trend was noticed on 4th and 5th day after inoculation.

From 6th to 10th day after inoculation rice bran and wheat bran showed maximum sporulation. Wheat bran + straw bits, rice bran + strawbits and strawbits alone also showed improved sporulation at these intervals. In all substrates tried there was a steady increase in sporulation with age of the culture upto 8th day after inoculation. Thereafter the sporulation remained more or less constant or even decreased (Fig.1). The sporulation in all the mass culture substrates except rice bran + tapioca bits were found to be on par on 8th, 9th and 10th day after inoculation.

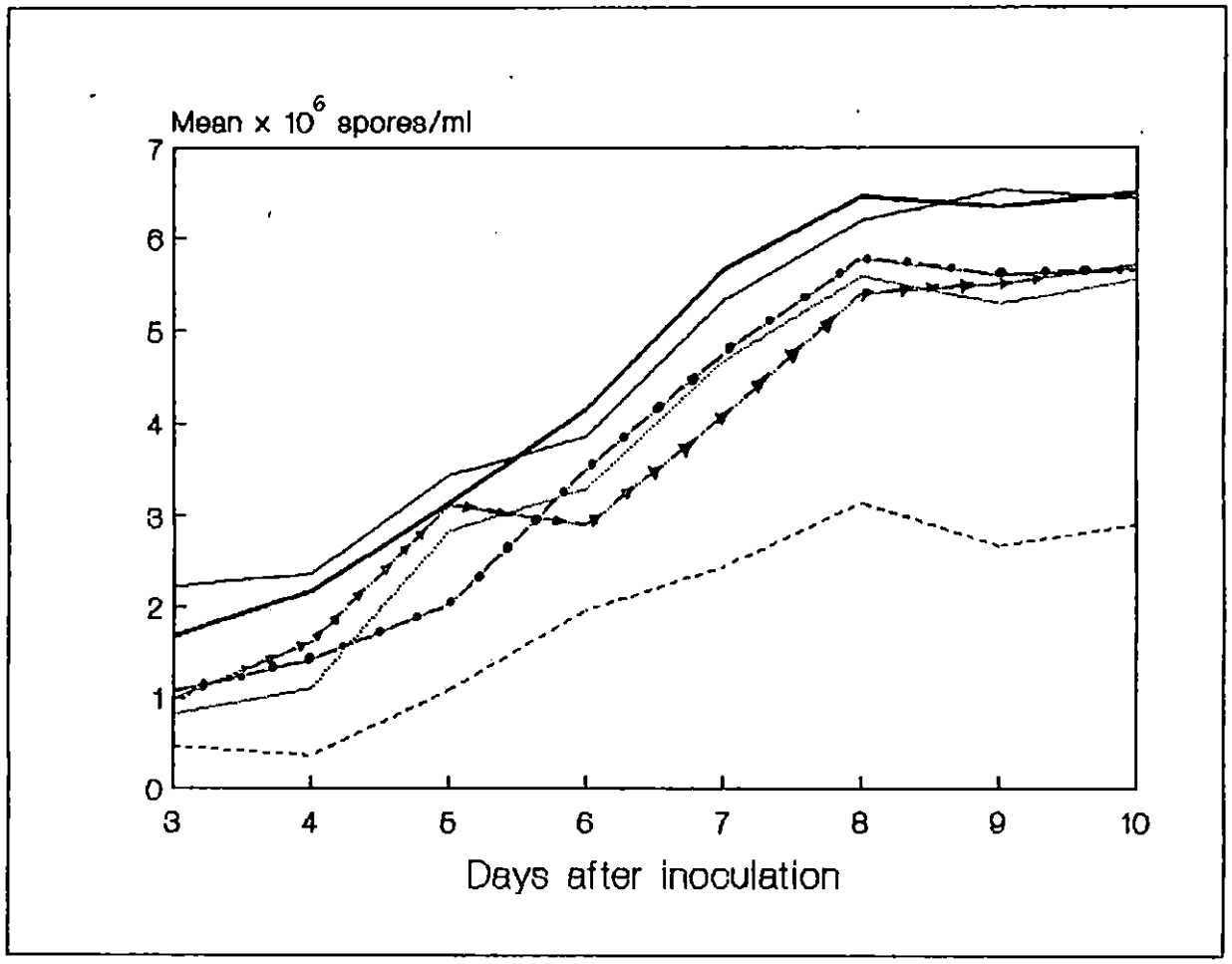


Fig. 1 Sporulation of *Fusarium pallidoroseum* on different mass culture media from third to tenth day after inoculation

Wheat bran

Rice bran

Wheat bran + straw bits

Rice bran + tapioca bits

Rice bran + straw bits

Straw bits

Growth of F. pallidoroseum on the agar extracts of mass culture media.

The mean mycelial colony dimeter of F. pallidoroseum on agar extracts of mass culture substrates are given in Table 3. Treatments differed statistically at one per cent level. Maximum colony diameter was observed in potato dextrose agar medium (90.00 mm) followed by wheat bran agar (88.33 mm), rice bran + straw bit agar (87.65 mm), and wheat bran + straw bits agar (82.31 mm), rice bran agar (77.65 mm) and straw bits agar (74.00 mm) in the decreasing order. The least growth was observed in plain agar (33.87mm). The mycelial growth on PDA was on par with wheat bran agar which differed statistically from the other treatments.

Virulence of F. pallidoroseum grown on different mass culture media.

Results presented in Table 4 show that 100 per cent mortality was obtained in three days with spores harvested from wheat bran and rice bran media.

Table 3. Growth of *Fusarium pallidoroseum* on agar extracts of mass culture media.

Sl. No.	Media	Mean mycelial colony diameter (mm)
1.	Wheat bran agar	88.33 (9.398)
2.	Rice bran agar	77.65 (8.812)
3.	Wheat bran + straw bits agar	82.31 (9.073)
4.	Rice bran + tapioca bits agar	43.30 (6.580)
5.	Rice bran + Straw bits agar	87.65 (9.362)
6.	Straw bits agar	74.00 (8.602)
7.	Plain agar	33.87 (5.820)
8.	Potato dextrose agar	90.00 (9.487)
C.D. Value $\overline{o}$		0.352

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

Table 4. Cumulative per cent mortality of Aphis craccivora treated with Fusarium pallidoroseum spores harvested from different mass culture media

Media	Cumulative per cent mortality* at intervals (days)			
	1	2	3	4
Wheat bran	60.06 (50.78)	87.92 (69.63)	100.00 (90.00)	100.00 (90.00)
Rice bran	53.35 (46.90)	82.34 (65.12)	100.00 (90.00)	100.00 (90.00)
Wheat bran + straw bits	33.22 (35.18)	58.05 (49.61)	89.11 (70.70)	100.00 (90.00)
Rice bran + tapioca bits	21.68 (27.74)	37.57 (37.79)	70.11 (56.83)	83.29 (65.84)
Rice bran + straw bits	31.06 (33.86)	48.84 (44.32)	85.36 (67.48)	99.25 (85.00)
Straw bits	21.84 (27.85)	42.21 (40.50)	53.35 (46.90)	83.60 (66.09)
Control	0 (0)	1.54 (7.14)	4.39 (12.10)	14.60 (22.45)
SE Values	2.512	4.161	4.256	3.888
C D Value	7.621	12.623	12.909	11.794

* Mean of 3 replications of 15 insects each (Figures in parentheses are values after angular transformation)

Analysis of variance showed significant difference between treatments at one per cent level for all four days. One, two and three days after inoculation a cumulative per cent mortality of 60.06, 87.92 and 100 respectively were obtained with spores harvested from wheat bran and 53.35, 82.34 and 100 per cent mortality were noticed with spores harvested from rice bran. Further, the per cent mortality after 24 and 48hrs in both the treatments were not significantly different from each other . On the 4th day, treatment with spores harvested from wheat bran + straw bit also showed 100 per cent mortality, which was on par with spores harvested from rice bran + straw bits, in which mortality of 99.25 per cent was recorded. These four treatments statistically differed from the other treatments. Of all the treatments excepting control spores harvested from rice bran + tapioca bits recorded the least mortality, except on the 1st day.

Effects of different degrees of sterilization on the growth and sporulation of the fungus.

Different degrees of sterilization were tried only on two mass culture media namely, wheat bran and rice bran as they were found to be superior to others.

170381

The growth of F. pallidoroseum on these media subjected to different degrees of sterilization are shown in Plate 7 and 8.

Those media which were exposed to sunlight for two days for sterilization and those without sterilization got fully contaminated and no data could be collected from them (Plate 5 and 6). Sporulation of the fungus on the other treatments were recorded and analysed statistically.

Table 5 shows the mean number of spores / ml, 8 days after inoculation, in the different treatments.

No interaction was observed between media and degrees of sterilization showing that media and degrees of sterilization remained independent.

Analysis of variance showed significant difference between the two media at 5 per cent level. Wheat bran media with a mean sporulation of 4.526×10^6 spores/ml was found superior to rice bran medium with a mean sporulation of 3.739×10^6 spores/ml.

KERALA AGRICULTURAL UNIVERSITY LIBRARY
THRISSUR
680 654

Plate 5. Growth of Fusarium pallidoroseum in mass culture of - substrates subjected to ~~full~~ Partial solarization.

1. Rice bran
2. Wheat bran

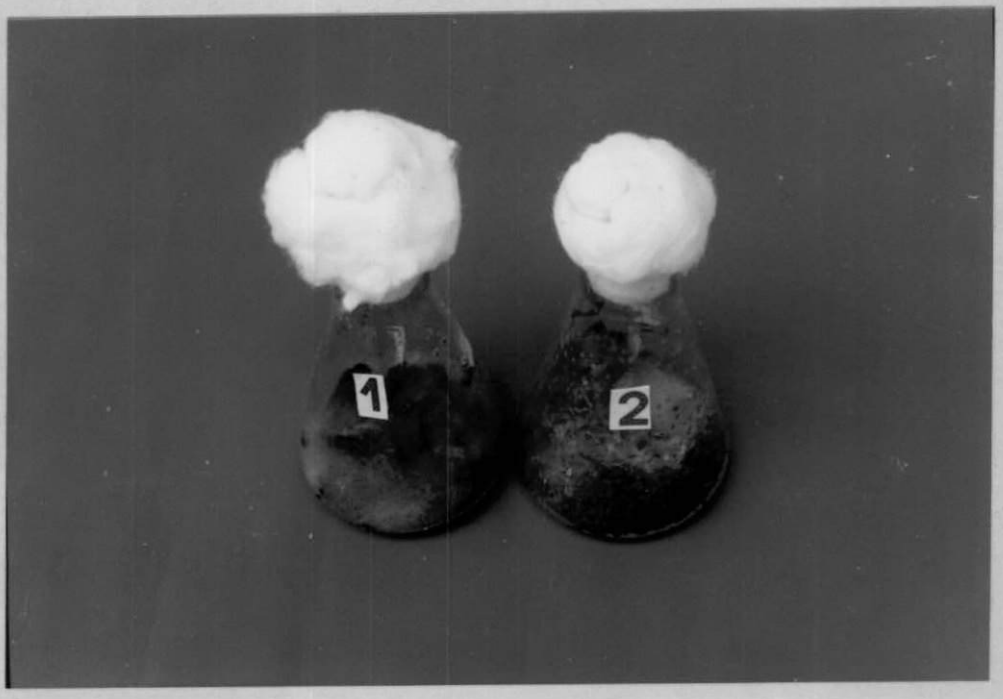


Plate 5.

Plates 6. Growth of *Fusarium pallidoroseum* in mass culture substrates which received no sterilization.

1. Rice bran
2. Wheat bran

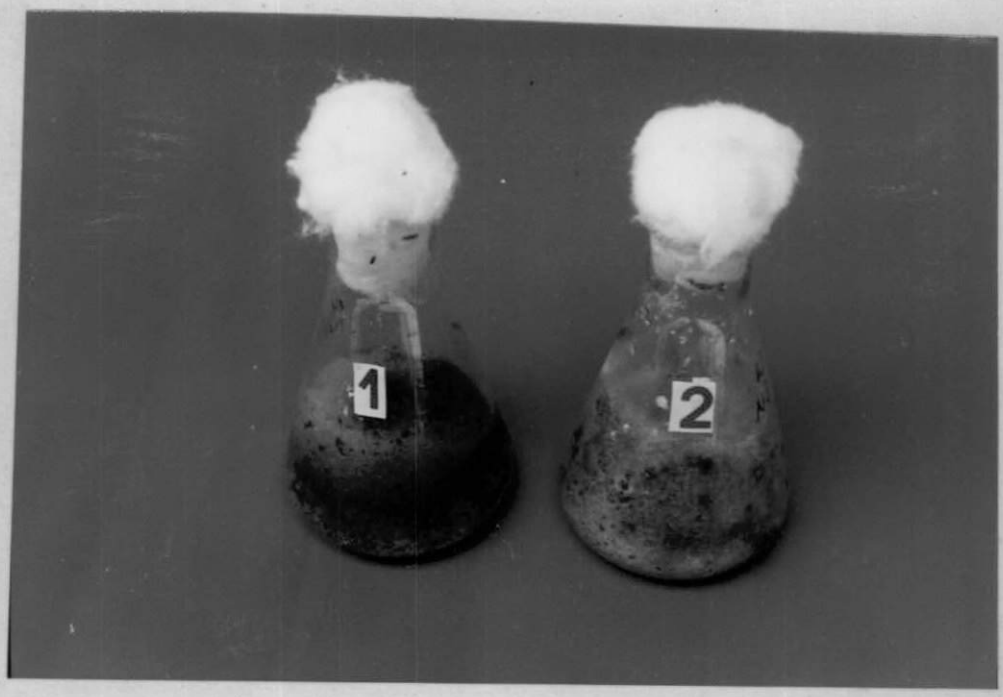


Plate 6.

Plate 7. Growth of Fusarium pallidoroseum in wheat bran subjected to different degrees of sterilization

1. Heat sterilization (full)
2. Heat sterilization (partial)
3. No sterilization
4. Solarization (full)
5. Solarization (partial)

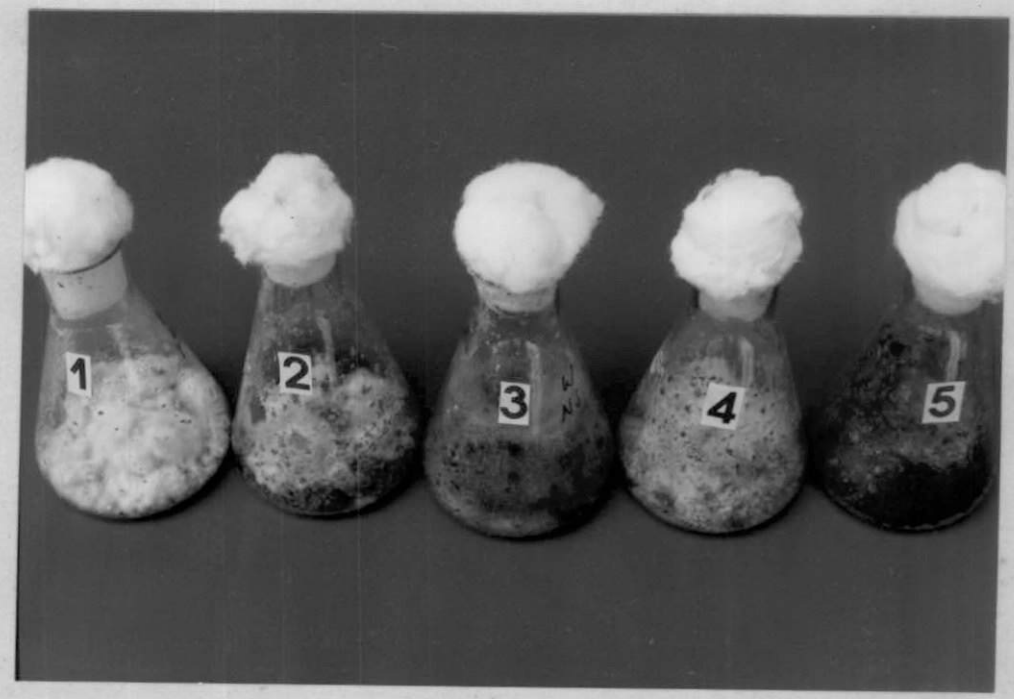


Plate 7.

Plate 8. Growth of *Fusarium pallidoroseum* in rice bran subjected to different degrees of sterilization

1. Heat sterilization (full)
2. Heat sterilization (partial)
3. No sterilization
4. Solarization (full)
5. Solarization (partial)

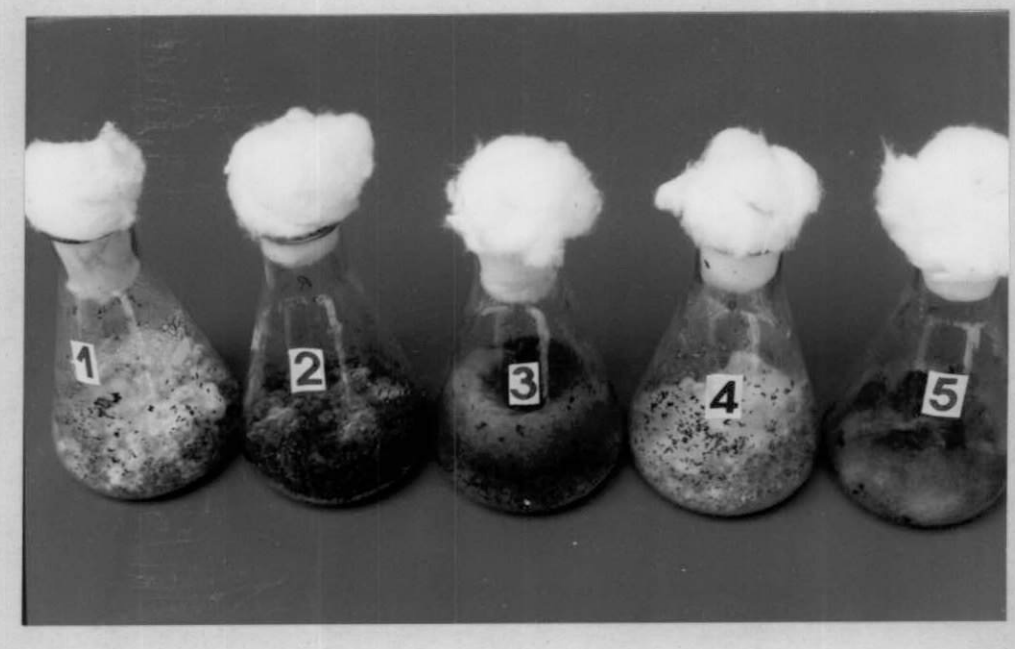


Plate 8.

Table 5. Sporulation of Fusarium pallidoroseum in mass culture substrates subjected to different degrees of sterilization. (Mean table x 10^4 spores/ml).

Media	Degree of sterilization			Mean
	Heat Sterilization (full)	Heat sterilization (partial)	Solarization (full)	
Wheat bran	6.979 (2.642)	1.964 (1.402)	4.635 (2.153)	4.526 (2.065)
rice bran	6.798 (2.607)	1.222 (1.059)	3.296 (1.816)	3.739 (1.827)
Mean	6.889 (2.625)	1.543 (1.230)	3.966 (1.984)	

C D Value for comparison between media - 0.332

C D Value for comparison of degrees of sterilization - 0.235

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

* Those media which received partial solarization and those without sterilization got fully contaminated indicating their unsuitability and were avoided from the experiment.

Statistically significant differences were observed between differnt degrees of sterilization. Full heat sterilization method with mean sporulation of 6.889 x 10^6 spores/ml was found significantly superior to all other degrees of sterilization followed by solarization (full) which recorded a mean sporulation of 3.966 x 10^6 spores/ml. Partial heat sterilization recorded the least sporulation (1.543 x 10^6). The results are illustrated in fig. 2.

Effect of culture filtrates of F. pallidoroseum on A. craccivora

Table 6 shows the mean per cent mortality of aphids sprayed with different dilutions of culture filtrate. Analysis of variance table (Appendix 1) showed significant difference at 1 per cent level between treatments.

Culture filtrate without dilution was found to cause the highest mortlity of 99.57 per cent, followed by culture filtrate at 50 per cent dilution with a mortality rate of 89.9 per cent. Richard's solution alone recorded the least mortality of 13.0 per cent while the aphids sprayed with distilled water failed to get killed.

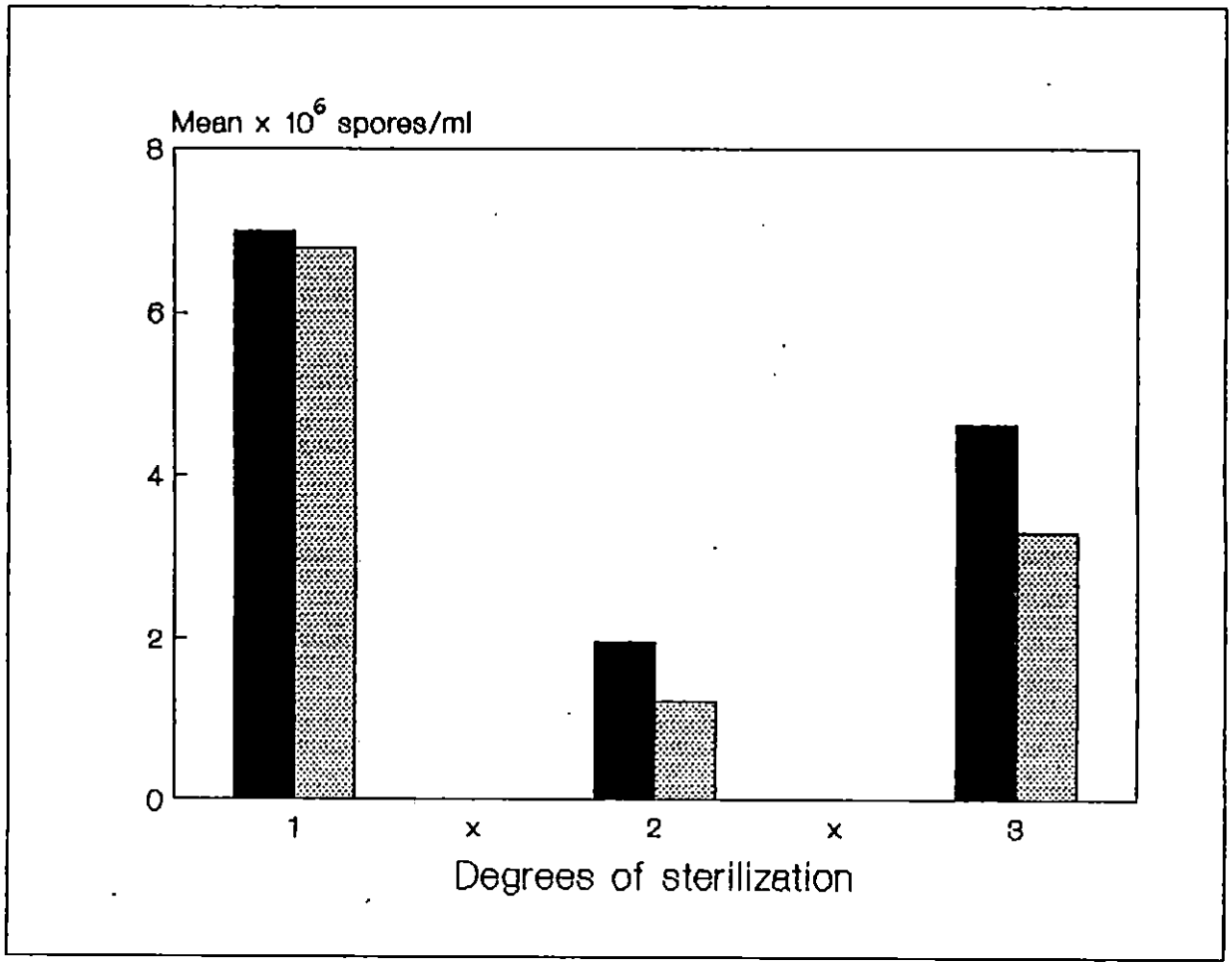


Fig. 2 Sporulation of *Fusarium pallidoroseum* in mass culture substrates subjected to different degrees of sterilization

Wheat bran

Rice bran

1 Heat sterilization (full)

2 Heat sterilization (partial)

3 Solarization (full)

Table 6.Mean per cent mortality of pea aphids treated with different dilutions of culture filtrate of *Fusarium pallidoroseum*

Treatments	Mean Percent mortality of aphids.
Culture filtrate without dilution	99.57 (86.25)
Culture filtrate at 50 per cent dilution	89.9 (70.49)
Culture filtrate at 75 per cent dilution	48.10 (43.94)
Culture filtrate at 90 per cent dilution	29.10 (32.86)
Culture media	13.00 (21.17)
C D Value	11.122

* Mean of 4 replications of 15 insects each

* In control no mortality could be observed

(Figures in parenthesis are values after angular transformation)

Effect of pesticides on the growth and sporulation of F. pallidoroseum

Table 7 presents the data on the radial growth and sporulation of the fungus in media mixed with different pesticides at their field doses and figures 3,4 and 5 illustrate the results.

It is seen that there was no growth in the media with HCH indicating that HCH was highly toxic to the fungus. There was fungal growth in the combinations with all the other insecticides but the radial growth attained in 10 days showed variation (Plate 9a and 9b). Quinalphos was found to be most harmful and recorded a growth reduction of 72.5 per cent followed by fenthion with a growth inhibition of 70.56 per cent. Phosphamidon and dimethoate were less inhibitory, the per cent reduction in the growth being 32.50 and 31.94 respectively. Mercaptothion and monocrotophos were found to be least inhibitory with only 19.16 and 11.39 per cent reduction respectively.

There was considerable inhibition of sporulation also in all the combinations with insecticides. Mercaptothion and quinalphos caused maximum reduction in sporulation, i.e 54.81 and 54.07 per cent respectively.

Table 7. Effect of pesticides on the growth and sporulation of Fusarium pallidoroseum

Pesticides	Concentration (per cent a.i.)	Diametre of colony (in mm) on 10th day	per cent inhibition over control	sporulation on 10th day (spores/ml)	per cent increase (+) or decrease (-) of spores over control
Monocrotophos (Nuvacron)	0.05	79.75	11.39	2.2×10^6	-45.68
Mercaptothion (Malathion)	0.01	72.75	19.16	1.83×10^6	-54.81
Dimethoate (Rogor)	0.03	61.25	31.94	2.60×10^6	-35.80
Phosphamidon (Dimecron)	0.05	60.75	32.50	2.2×10^6	-45.68
Fenthion (Labaycid)	0.05	26.5	70.56	3.0×10^6	-25.93
Quinalphos (Ekalux)	0.03	24.75	72.50	1.86×10^6	-54.07
HCH (BHC)	0.02	No growth	100.0	—	—
Copper oxychloride (Blue copper)	0.2	22	75.56	—	—
Mancozeb (Dithane M-45)	0.2	19.75	78.06	—	—
Captafol (Difolatan)	0.2	12	86.67	—	—
Captan (Captan)	0.2	No growth	—	—	—
Zineb (Dithane Z-78)	0.2	No growth	—	—	—
Control		90.0		4.05×10^6	

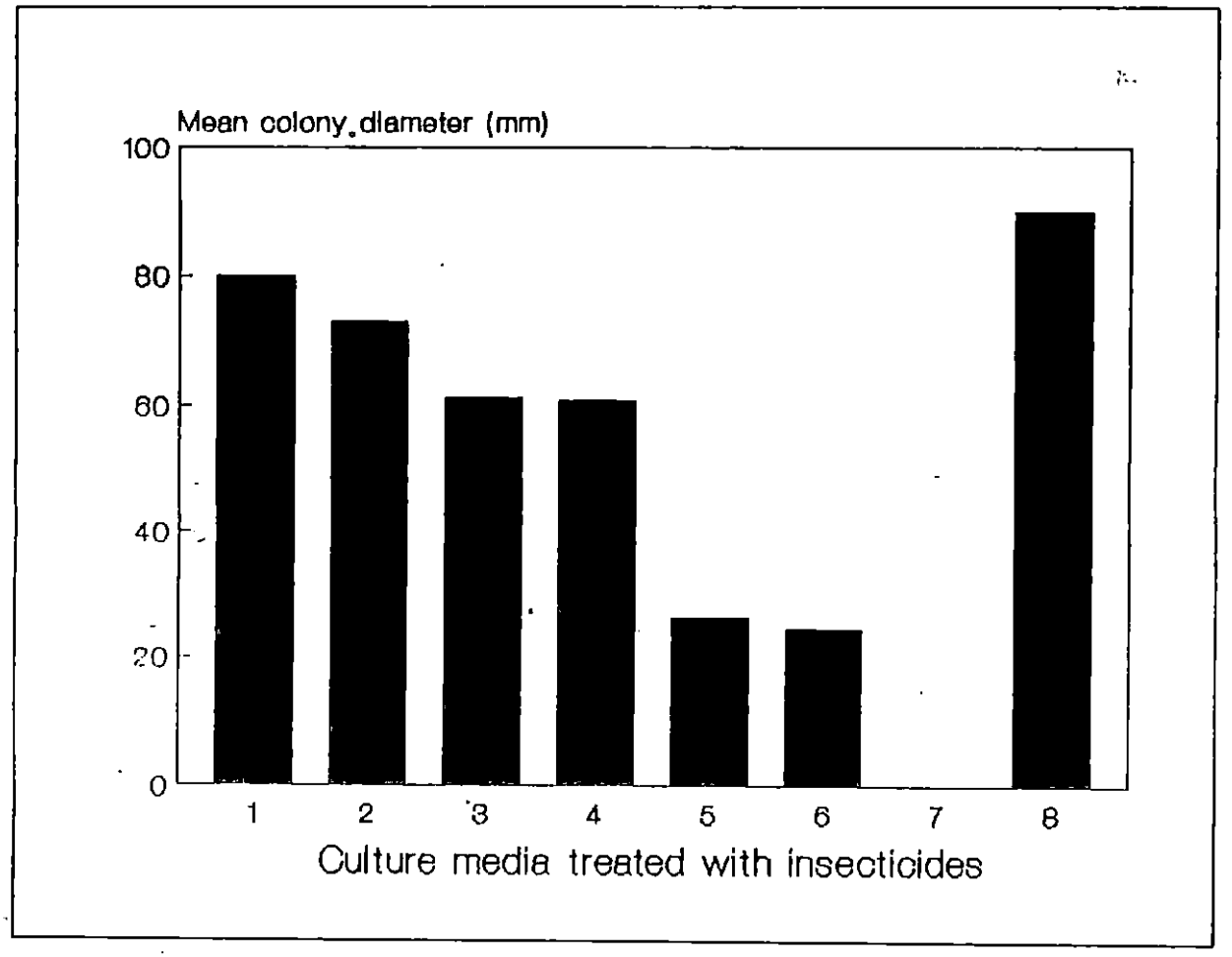


Fig. 3 Effect of insecticides on the growth of *Fusarium pallidoroseum*

1 Monocrotophos

2 Mercaptothion

3 Dimethoate

4 Phosphamidon

5 Fenthion

6 Quinalphos

7 HCH

8 Control

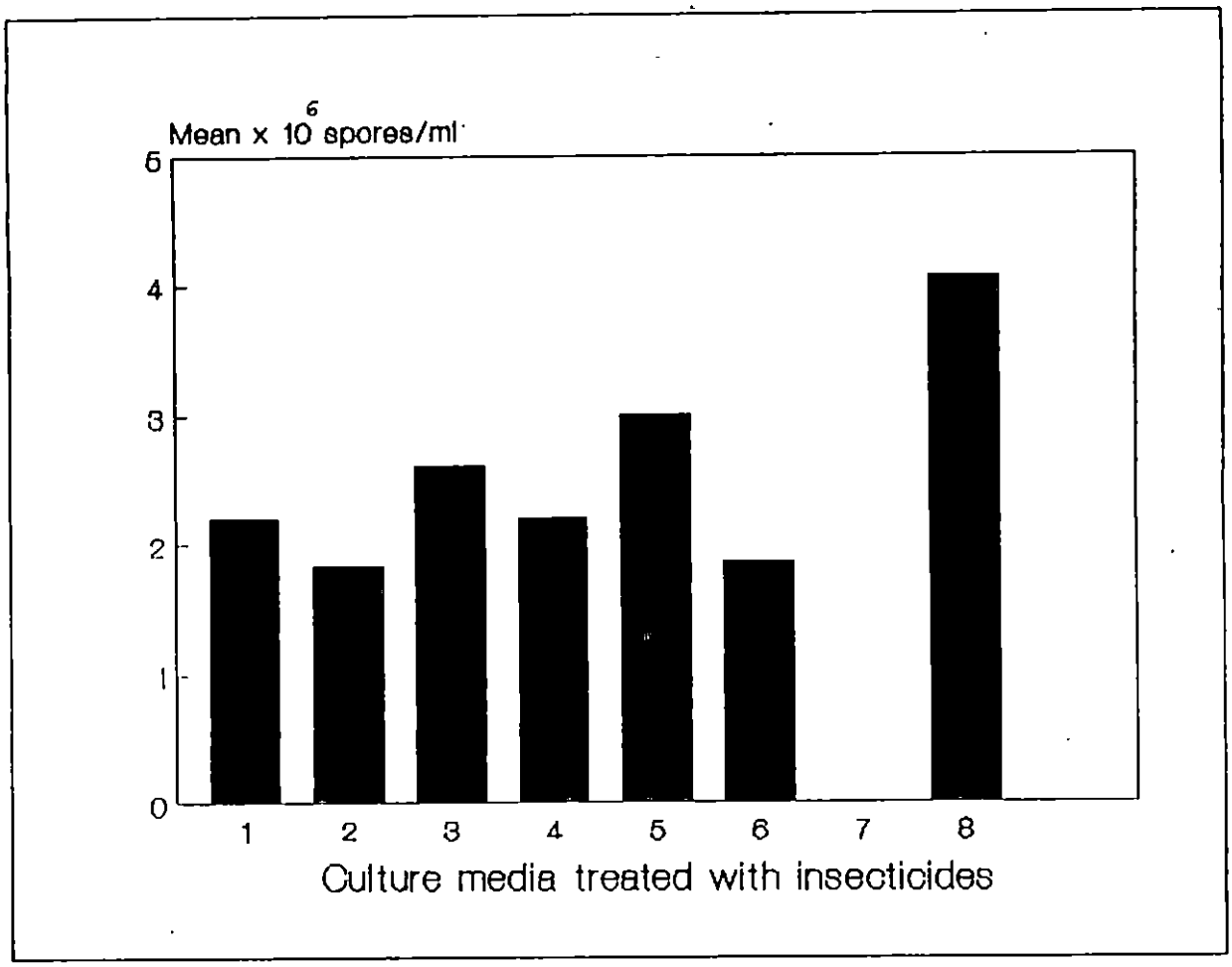


Fig. 4 Effect of insecticides on the sporulation of *Fusarium pallidoroseum*

1 Monocrotophos

2 Mercaptothion

3 Dimethoate

4 Phosphamidon

5 Fenthion

6 Quinalphos

7 HCH

8 Control

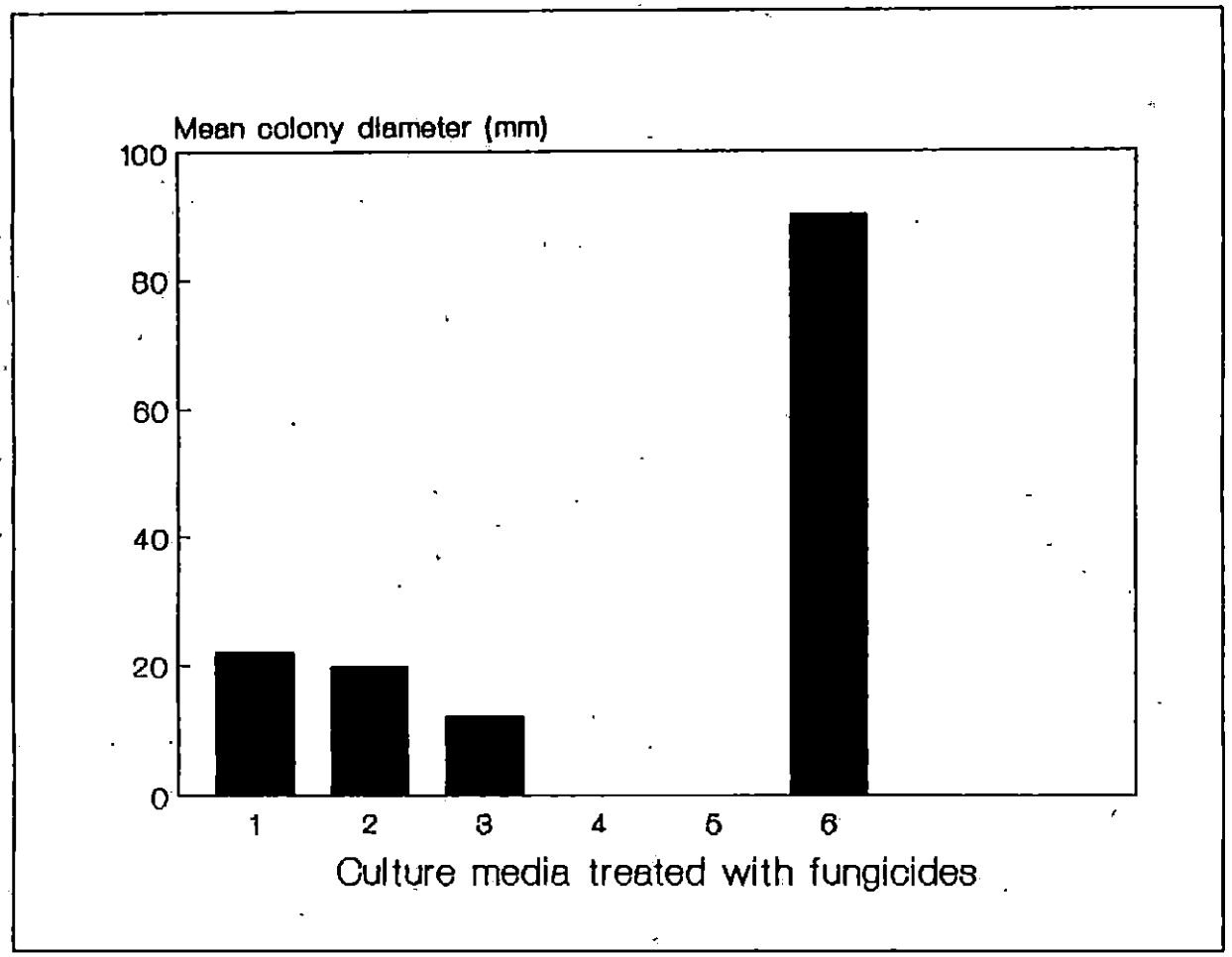


Fig. 5 Effect of fungicides on the growth of *Fusarium pallidoroseum*

1	Copper oxychloride
2	Mancozeb
3	Captofol
4	Captan
5	Zineb
6	Control

Plate 9a & 9b. Growth of *Fusarium pallidoroseum* on insecticide treated potato dextrose agar medium

1. HCH
2. Quinalphos
3. Fenthion
4. Phosphamidon
5. Dimethoate
6. Mercaptothion
7. Monocrotophos.
8. Control.

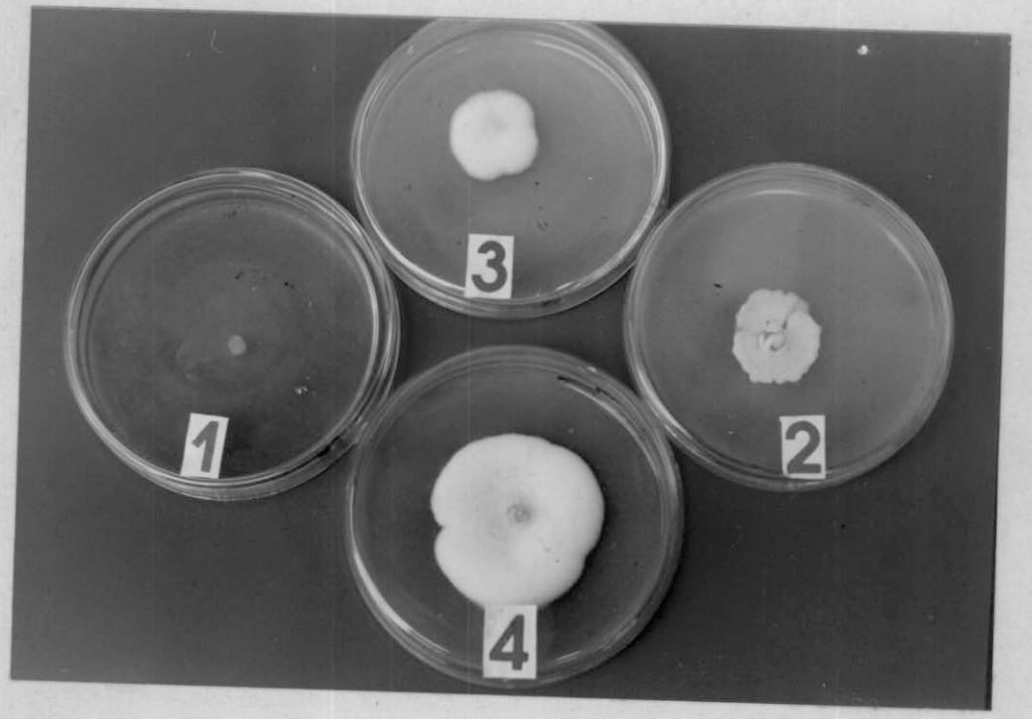


Plate 9a.

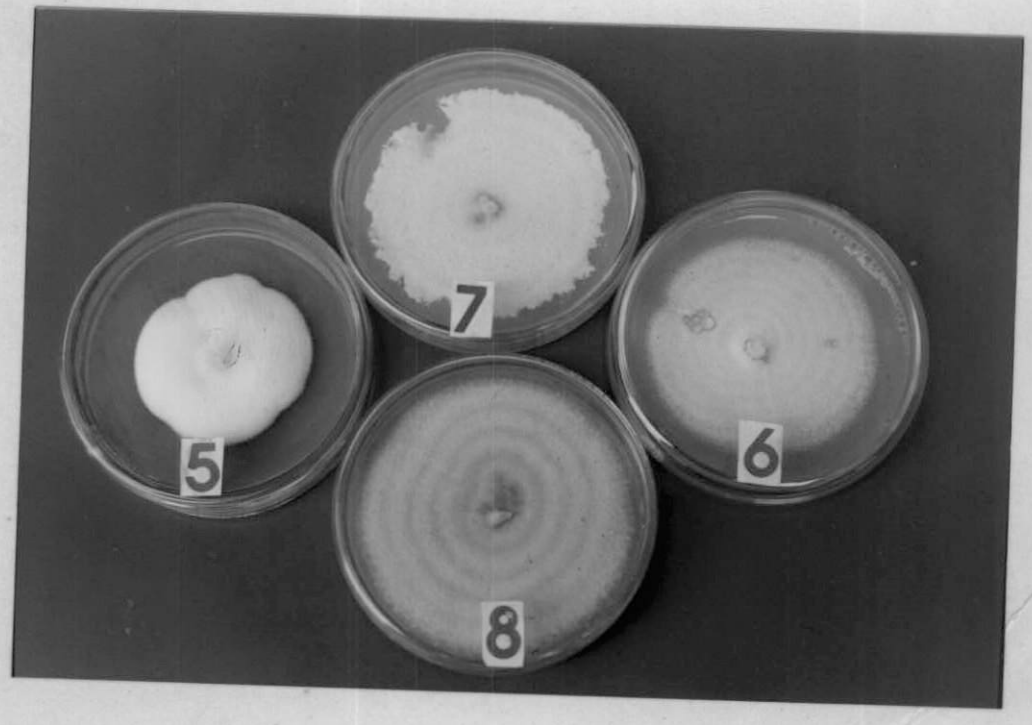


Plate 9b.

Monocrotophos and phosphamidon recorded a per cent inhibition of 45.68. Dimethoate and fenthion had comparatively less inhibitory effect with a reduction of 35.80 and 25.93 per cent respectively.

Among the fungicides captan and zineb were most inhibitory permitting no growth (Plate 10). Captafol, mancozeb and copper oxychloride recorded 86.67, 78.06 and 75.56 per cent inhibition respectively over the control. Sporulation was completely inhibited in all the fungicidal treatments.

Efficacy of different spore formulations of F. pallidoroseum in controlling the aphid A. craccivora under laboratory conditions.

Analysis of variance table (Appendix I) showed that the treatments differed significantly at 1 per cent level on all four days after treatment.

On the first day after treatment spore suspension in water caused 63.49 per cent mortality which was found statistically superior to other treatments(Table 8). Diatomaceous earth WP, formulation of spores caused a

Plate 10. Growth of *Fusarium pallidoroseum* on fungicide treated potato dextrose agar medium.

1. Captan
2. Zineb
3. Captafol
4. Mancozeb
5. Copper oxychloride
6. Control

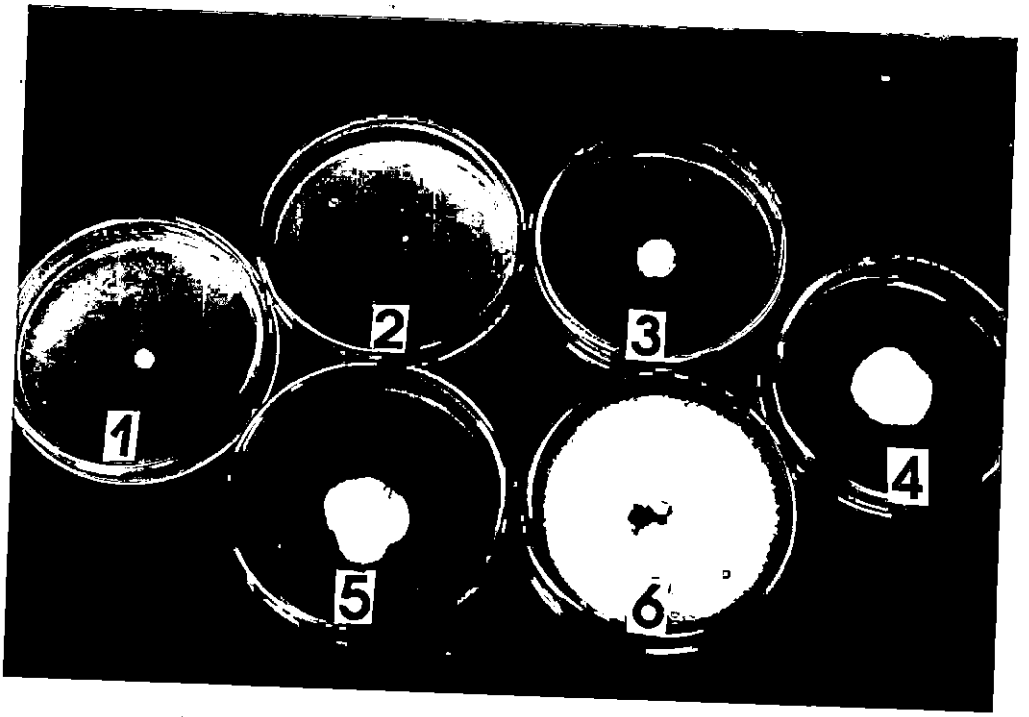


Plate 10.

mortality of 50.00 per cent which was on par with talc WP which recorded a mortality of 41.54 per cent which was on par with diatomaceous earth dust which caused a mortality of 33.25 per cent. Dust formulation containing talc as the inert material caused least mortality (28.15per cent) excepting the control.

Two days after treatment spore suspension in water caused a cumulative per cent mortality of 95.07 which was significantly higher than that in all other treatments. Other treatments followed the same trend as that of one day after treatment.

On the 3rd day after treatment spores suspension in water caused a cumulative mortality of 100 per cent which differed significantly from all other treatments. WP formulation of spores containing diatomaceous earth caused a mortality of 90.25 per cent which was found significantly higher than that in other treatments. Talc WP, diatomaceous earth dust and talc dust were on par with cumulative mortality percentages of 76.88. 72.30. 70.27 respectively. In control a mortality of 1.68 per cent was recorded.

Table 8.Cumulative per cent mortaity of Aphis craccivora treated with different spore formulations of Fusarium pallidoroseum under laboratory conditions

Spore formulations	Cumulative per cent mortality* at intervals (days)			
	1	2	3	4
Dust (diatamaceous earth)	33.25 (35.20)	53.35 (46.90)	72.30 (58.22)	95.07 (77.14)
Dust (talc)	28.15 (32.03)	44.97 (42.10)	70.27 (56.94)	88.88 (70.49)
Wettable powder (Diatomaceous earth)	50.00 (44.98)	66.75 (54.76)	90.25 (71.78)	99.58 (86.25)
Wettable powder (talc)	41.54 (40.11)	56.74 (48.85)	76.88 (61.23)	97.51 (80.89)
Spore suspension in water	63.49 (52.91)	95.07 (77.14)	100.00 (90.00)	100.00 (90.00)
Control (distilled water)	0 (0)	0 (0)	1.68 (7.44)	6.21 (14.42)
C D Value	6.46	7.23	8.46	11.98

* Mean of 4 replications of 15 insects each

(Figures in parentheses are values after angular transformation)

On the 4th day after treatment cumulative per cent mortality with spore suspension in water was on par with diatomaceous earth WP with 100 and 99.58 per cent respectively.

Efficacy of different formulations of F. pallidoroseum against A. craccivora on cowpea grown in pots

Table 9 shows the final mean population of aphids, adjusted with the initial population (Analysis of co-variance) after the application of different treatments.

Analysis of variance table (Appendix I) showed significant difference between treatments at 1 per cent level.

Quinalphos 0.05per cent, spore suspension in water and diatomaceous earth WP formulation of spores were found equally effective and superior to all other treatments in controlling the aphids with a mean final population of 5.5, 8.94 and 19.11 respectively. Among the formulations tried spore dust with talc was the least effective. However, it was better than the control in which a final population of 360 aphids was recorded.

Table 9. Efficacy of different formulations of *Fusarium pallidoroseum* against *Aphis craccivora* on cowpea grown in pots

Treatments	Final mean population of aphids after application of treatments adjusted with the initial population.
1. Spore dust (diatomaceous earth)	50.17 (7.15)
2. Spore dust (talc)	71.54 (8.52)
3. Spore wettable powder (diatomaceous earth)	19.11 (4.48)
4. Spore wettable powder (talc)	55.40 (7.51)
5. Spore Suspension in water	8.94 (3.15)
6. Culture filtrate (at 50 per cent dilution)	50.37 (7.17)
7. Ekalux 0.05 per cent	5.50 (2.55)
8. Control	360.00 (19.00)

C D value for comparison of treatments = 3.354

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

Viability of formulation of F. pallidoroseum at different intervals after preparation.

Cumulative per cent mortality of aphids treated with diferent formulations of F. pallidoroseum spores, at different intervals after preparation is shown in table 10 and illustrated in figure 6.

Analysis of variance showed no significant interaction between formulations and intervals after preparations in causing mortality. But the formulation and period after preparation was found to differ significntly at 1per cent level.

Diatomaceous earth WP formulation was found significantly superior to other treatments with a mean percentage mortality of 66.57 per cent Diatomaceous earth dust formulation recorded a mean mortality per centage of 58.29 and was on par with talc dust and talc WP formulation which caused a mean mortality of 53.55 and 54.61 per cent respectively.

Table 10. Cumulative per cent mortality* of Aphis craccivora treated with different formulations of Fusarium pallidoroseum at different intervals after preparations

Days after preparation	Formulations				Mean
	Spore dust (diatomaceous earth)	Spore dust (talc)	Spore WP (diatomaceous earth)	Spore WP (talc)	
2	73.84 (59.21)	70.74 (57.23)	87.43 (69.21)	72.30 (58.22)	76.08 (60.97)
4	69.20 (56.27)	70.40 (57.02)	85.69 (67.75)	73.02 (58.68)	74.57 (59.93)
7	56.88 (48.93)	44.92 (42.07)	63.44 (52.78)	46.60 (43.03)	52.96 (46.70)
14	33.25 (35.20)	28.15 (32.03)	29.72 (33.02)	26.53 (30.99)	29.41 (32.81)
Mean	58.29 (49.90)	53.55 (47.09)	66.57 (55.69)	54.61 (47.73)	

C D Value for comparison of periods after formulation - 5.304

C D Value for comparison between different formulation - 5.304

* Mean of 4 replications of 15 insects each

(Figures in parentheses are values after angular transformation)

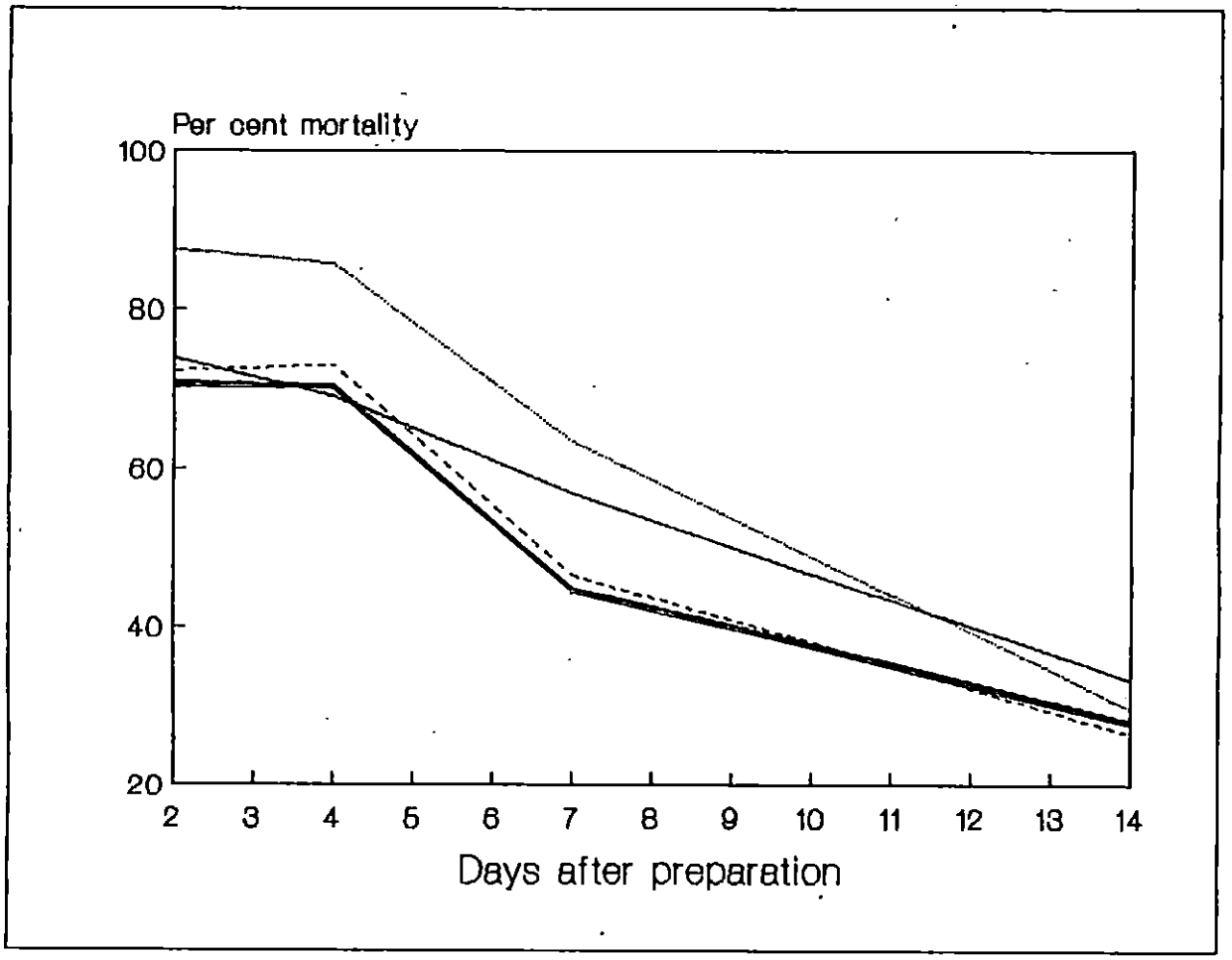


Fig. 6 Viability of formulations of *Fusarium pallidoroseum* at different intervals after preparation

Spore dust (diatomaceous earth)

Spore dust (talc)

Spore WP (diatomaceous earth)

Spore WP (talc)

Viability of spores 2 and 4 days after preparation was found superior to that at other intervals with a mean mortality per cent of 76.08 and 74.51 respectively. The least mortality rate of 29.41 per cent was noticed 14 days after formulation.

DISCUSSION

DISCUSSION

Entomogenous fungi are recognized as potent bio-control agents for pest control. Kerala with its high humid conditions suited for the survival and multiplication of the fungi renders them as an important tool in the integrated pest management programmes . Fusarium pallidoroseum (Cooke) Sacc. was observed causing heavy mortality of cowpea aphids, Aphis craccivora Koch. (Hareendranath et al .,1989). A good candidate pathogen for use in microbial control of insect pests should be amenable for easy mass production at competitive cost. Easier and convenient methods of application and compatibility with commonly used pesticides are other attributes enhancing the utility of a pathogen in field control programmes. The present studies have been taken up to gather data on the above lines to judge the suitability of F. pallidoroseum as a biocontrol agent for field application against cowpea aphids.

It was found that the growth of F. pallidoroseum was quick and complete on wheat bran than on the other substrates. It was followed by wheat bran + straw bits. On rice bran and straw the growth was slow in the early stages but later covered the entire media. Rice bran + straw bits and rice bran + tapioca bits proved inferior .

In all the substrates tried, there was a steady increase in sporulation with age of the culture up to 8 days after inoculation. Thereafter the sporulation remained more or less constant or even decreased.

Wheat bran and rice bran showed maximum sporulation from 3rd to 10th day after inoculation. The sporulation in all the mass culture substrates except rice bran + tapioca bits were found to be on par on 8th, 9th and 10th day after inoculation. Hussey and Tinsely (1981) reported wheat bran as a suitable substrate for the mass production of Beauveria bassiana. Henis et al. (1978) reported wheat bran as a suitable medium for the mass culturing of the antagonistic fungi, Trichoderma harzianum. Batista et al. (1989) cultured B. bassiana and Metarrhizium. anisopliae in soaked bran. Gokulapalan (1989) noticed wheat bran and rice bran as superior substrates for culturing Trichoderma sp. used for the control of sheath blight of rice. Hareendranath (1989) reported maize grain as a suitable mass culture substrate for F. pallidoroseum. Thus the observations of the present study that wheat bran and rice bran are suitable substrates for growing the biocontrol fungihas been supported by many earlier works.

The results of the present study indicate that the growth and sporulation of the fungus was closely related with its nutrition. Detailed physiological studies are needed to find the suitable nutritive media in order to have a good harvest of spores.

Growing the fungus on agar extracts of the mass culture substrates would give some indication on the nutrient status of the materials. In the present experiment F. pallidoroseum was grown on agar extracts of the mass culture substrates with potato dextrose agar and pure agar as standards. Mycelial growth was maximum on PDA whereas it was the least on pure agar. Among the substrates wheat bran showed maximum growth indicating that wheat bran is a preferred medium for the growth of the fungus . Mean mycelial diameter in rice bran agar was 77.65mm whereas that in rice bran + straw bits agar was 87.65mm. Straw bits agar produced a mean mycelial colony of 74.00mm diameter. Wheat bran + straw bits agar also produced fairly good growth of 82.31mm. Rice bran + tapioca bits agar produced very poor growth. These results indicate that straw bits have some factor responsible for the growth of mycelium while tapicoa bits probably may contain certain principle inhibitory to the sporulation of the fungus. In the previous experiment

also it was found mixing of straw bits with either wheat bran or rice bran gave fairly good sporulation. However further biochemical studies are required to bring out importance of each of these materials.

Spores harvested from different substrates showed significant variation in their virulence. The cumulative per cent mortality caused by spores harvested from wheat bran and rice bran were the highest, there being no significant difference between them. The spores harvested from the other substrates caused less mortality of aphids in the initial stages indicating a low virulence. It is generally believed that the virulence of a fungal pathogen is influenced by the media in which it is grown (Madelin,1963). Voukassovitch (1925) found that spores of Spicaria farinosa from a peptone medium killed silk worm, where as those from potato dextrose agar did not. Schaerffenberg (1957) observed that to conserve the virulence of the pathogenic fungi particularly B. bassiana it was essential to culture them in proteinaceous media. This procedure was also adopted by Wallengren and Johanson (1929) with M. anisopliae. Beevi (1979) noticed higher virulence of F. moliliformae var. subglutinans when cultured in Richard's medium than when it is cultured in Coon's

medium or potato dextrose agar. Kuruvilla and Jacob (1981 a) reported higher virulence of F. oxysporum cultured on green gram and wheat than those grown on sorghum, ragi and redgram. Hareendranath (1989) found that the spores of F. pallidoroseum harvested from Sabouraud dextrose agar was significantly superior in virulence than from other media.

The foregoing results indicate that for mass culture of F. pallidoroseum wheat bran and rice bran are the best among the substrates as regards growth, sporulation and virulence. However, further studies with other cheap materials may provide a more suitable mass culture media. Leaves which have insecticidal properties like those of neem can be tried.

The common sterilization method is by autoclaving which needs costly equipments and better technical knowledge. Hence it may not be a feasible technique for an ordinary farmer. So, cheaper and easily practicable low cost methods of sterilization are needed in order to popularize this technique. The present studies on growth and sporulation of F. pallidoroseum on mass culture substrates subjected to different methods of sterilization revealed the unsuitability of no sterilization and partial solarization. The growth of other saprophytic fungi

on these media completely inhibited the growth of F. pallidoroseum. Growth and sporulation of the fungus could be observed on substrates subjected to full solarization and partial heat sterilization but differed significantly from those subjected to full heat sterilization. Full solarization had given fairly good sporulation though it was inferior to full heat sterilization. Sporulation could also be observed in partial heat sterilization. Considering the cheapness and practicability, these methods can be utilized for mass production of the pathogen for large scale application in the field. Very few studies have been made in this line by earlier workers. Pristavko and Goral (1967) studied the growth of B. bassiana under semi-sterile condition on liquid, pasty and solid nutrient media and suggested this as a suitable method to produce enough materials for laboratory and field tests. Hussey and Tinsley (1981) reported that in China B. bassiana was mass cultured on materials like wheat bran, rice powder, compost humus and ground corn stalks which were steamed but not autoclaved before use.

Many entomopathogenic fungi overcome their hosts after a limited growth in the haemocoeal indicating the presence of toxins which are presumed to cause

host mortality. Culture filtrate (without dilution) of F. pallidoroseum when sprayed on A. craccivora showed 99.57 per cent mortality. A 50 per cent diluted solution was also effective (89.7 per cent mortality). This may be due to the presence of some toxic substances, produced by the fungus during its growth. Culture filtrate of Aspergillus flavus isolated from a moribund mosquito larvae, contained two chloroform extractable compounds toxic to Culex larva by addition to their habitat water (Toscano and Reever, 1973). Filtrate of M. anisopliae cultures were found toxic to Oryctus rhinoceros (Vey and Quiot, 1975). Two strains of Fusarium solani, one pathogenic to bark beetle Scolytus scolytus and another to lobster, Homarus americanus produced in liquid media substances toxic to adult bower flies (Claydon et al., 1977). Mori and Takaishi (1989) isolated 'monocerin' an insecticidal constituent from entomogenous fungi F. larvarum .

Detailed studies on the constituents present in the culture filtrates are needed to identify the exact toxic chemicals responisble for killing the aphide. Identification of strains with early production of rapidly acting toxin will afford better crop protection.

Insecticides and fungicides are regularly applied on the crop for the control of pests and diseases. Such pesticide treatments have been reported to reduce growth, sporulation and germination of spores of several entomogenous fungi (Hall and Dunn, 1917; Yendol, 1968; Wilding, 1972). Hence the effect of some of the pesticides commonly used for pest control in cowpea were studied in the present investigation.

These studies showed that HCH completely inhibited the growth of F. pallidoroseum. This was followed by quinalphos, fenthion and phosphamidon . Mercaptothion and monocrotophos were found to be least inhibitory for the growth of the fungus. Among fungicides captan and zineb completely inhibited the growth. Captafol, mancozeb and copper oxychloride also caused considerable inhibition. Fungicides have been reported to reduce germination and growth of entomopathogenic fungi considerably (Hall and Dunn, 1959; Yendol, 1968; Kuruvilla and Jacob, 1981b; Beevi and Jacob, 1987) . This is not true for insecticides (Benz 1971) Dirimanov and Angelova (1962) reported that BHC 12 per cent inhibited growth of B. bassiana. Urs et al.(1967) found that BHC was the most toxic to B. bassiana and M. anisopliae. Easwaramoorthy and Jayaraj (1977) also

observed that it caused the least inhibition of B. bassiana and Cephalosporium lecani . Beevi and Jacob (1987) noticed complete inhibition of growth of F. moniliformae var. subglutinans by HCH . In the present study quinalphos and fenthion caused considerable inhibition of growth. Easwaramoorthy and Jayaraj (1977) observed that ekalux (quinalphos) was harmful to Cephalosporium lecani . But Kuruvilla and Jacob (1981a) reported it to be less harmful to F. oxysporum . Beevi and Jacob (1987) also noticed less inhibitory action for ekalux against F. moniliformae var.subglutinans. Mercaptothion and monocrotophos was found to be less harmful to F. pallidoroseum in the present study. Easwaramoorthy and Jayaraj (1977) obtained the same result with Cephalosporium lecani . Urs et al. (1967) reported malathion (mercaptothion) to be less inhibitory for the growth of B. bassiana and M.anisopliae Beevi and Jacob (1987) found malathion as a compatible insecticide with F. moniliformae var. subglutinans.

Observation on the sporulation of F. pallidoroseum in media mixed with pesticides showed that in general all the insecticides inhibited sporulation. However, the reduction in sporulation was not proportionate with that of the vegetative growth. Similar observations were made by Kuruvilla and Jacob (1981b) in the case of F. oxysporum but

Beevi and Jacob (1987) reported that the reduction in sporulation was proportionate to that of vegetative growth in F. moniliformae var. subglutinans. Sporulation was more when compared to vegetative growth in media containing fenthion and dimethoate . The reverse was true in the case of mercaptothion . Quinalphos inhibited both growth and sporulation .

In all the fungicide treated media, sporulation was inhibited completely. The present observations coupled with the previous reports, indicate that the compatibility of various insecticides with different species of entomogenous fungi did not show any uniform pattern.

For easy and convenient handling the pathogen should be formulated in a convenient form. Dust and wettable powder formulations of F. pallidoroseum spores were hence prepared using diatomaceous earth and talc as inert materials and its effectiveness tested against cowpea aphid under laboratory conditions. None of the formulations was able to cause a quick insect mortality to the extent that caused by spore suspension in water. This indicates that the procedure of formulation decreased the virulence of the pathogen. On the 4^{th} day after treatment wettable powder

formulation using diatomaceous earth as inert material caused mortality of 97.51 per cent and was found to be on par with mortality caused by spore suspension in water. In all the other cases spore suspension in water was found to be significantly superior followed by WP (diatomaceous earth), WP (talc), and dust (diatomaceous earth). Dust formulation (talc) was found to be the least effective. These results indicate the superiority of diatomaceous earth to talc as an inert material. Backman Rodriguez- Kabana (1975) reported diatomaceous earth as a suitable inert material for the formulation of mycofungicide with Trichoderma harzianum used against Sclerotium rolfsii.

Wettable powder formulations of fungal spores were found to be more effective than dusts. This may be because of the requirement of humidity for insect mycosis. Humidity and even wetting of the host plant were considered to be the determining factors in the mycosis caused by entomophthorales (Wilding, 1981). Dust formulation using talc recorded the least mortality of aphids in the present study. Sachithanandam et al. (1987) reported that NPV formulations using talc as inert material were inferior to the unformulated virus. He also observed wettable powder formulation to be more effective than the dust.

Pot culture studies on the effect of spore formulations of F. pallidoroseum showed that the fungal spore suspension in water and wettable powder formulation of spores containing diatomaceous earth as inert material were equally effective in controlling the aphids. These treatments were found to be as effective as insecticidal treatment with quinalphos 0.05 per cent in bringing down the aphid population . Hareendranath (1989) reported that the application of spore suspension of F. pallidoroseum in water to be equally effective as quinalphos 0.05 per cent in controlling the aphids. The field efficacy of entomogenous fungi has been reported by many workers. Kuruvilla and Jacob (1979) reported that F. oxysporum applied at the rate of 6.025×10^6 spores per ml on brown plant hopper, Nilaparvata lugens caused 100 per cent mortality. Easwaramoorthy et al. (1978) reported that Verticillium lecani caused 95.6 per cent mortality of coffee green bug Coccus viridis .

Studies on the viability of spores of F. pallidoroseum in different formulations showed a decrease in its effectiveness due to storage . It would be seen that the [illegible] spores retained about 75 per cent viability till 4 days of storage and thereafter a significant decrease

was noticed in the virulence in all the formulations. Ferron (1981) considered short storage life of formulation as one of the factors which limits the use of microbial pesticides.

Further studies are needed to obtain a formulation in which the viability and virulence of the fungus could be simultaneousley retained for a longer period. Addition of nutrient materials like starch cornmeal etc. to the formulation may be advantageous to the fungus. Mycelial formulation using alginate pellets can also be tried to evolve a better formulation in which the viabillity and virulence of the fungus can be kept for a longer period.

Aphis craccivora is a serious pest of cowpea causing heavy economic loss to farmers. They usually resort to application of chemical pesticides to combat this noxious pest. At least 3 application of insecticides are required for a single season. For spraying 400 m^2 of cowpea crop, on an average 40 ml of ekalux (quinalphos 40 ml) is required. This is equivalent to 120 ml for a season. So cost totals up around Rs.36/-.

Present studies indicate that spraying of F. pallidoroseum at 3.5 x 10^6 spores/ml concentration is as effective as spraying of 0.05 per cent quinalphos. It is also found that 200 ml of fungal spore suspension at the required concentration can be obtained from 30 g of an 8 day-old culture of the fungus in wheat bran. A single application is sufficient for a season since the pathogen persists in the environment long. The spore suspension of 20 liters required for 400 m^2 can be cultured in 3 kg of wheat bran, the cost of which is only Rs. 5/-. The present studies show that the fungus can be cultured in substrates subjected to full solarization and partial heat sterilization. So the farmer can adopt this microbial control if the culture and the technical know-how is provided. The maximum total cost comes to only Rs.10/- for a cowpea crop grown in 400 m^2 area including cost of culture and sterilization where as Rs 35/- is required if chemical control is adopted . Moreover, chemical pesticides may cause destruction of natural enimies and results in consequent resurgence of the pest and also leaves toxic residues in/on the produce.

Nagalingam (1983) reported F. semitectum (old name of F. pallidoroseum (Booth & Sutton, 1984)) was safe to all instars of silk worm, adult honeybee, hymenopterous

parasitoides and coccinellid predators. It was also found to be safe to plants like chillies, cabbage, brinjal and tobacco. Hareendranath (1989) found F. pallidoroseum to be safe to rice, bhindi, chillies, tomato and predator Menochilus sexmaculata. Thus the present studies coupled with previous reports indicate the suitability and feasibility of using F. pallidoroseum as a bio-control agent of cowpea aphid A. craccivora.

SUMMARY

SUMMARY

Detailed investigations on the entomogenous fungus, Fusarium pallidoroseum (Cooke) Sacc. infecting cowpea aphid, Aphis craccivora Koch. were carried out. The studies covered symptomatology, mass culturing of the pathogen on cheaper substrates, effect of degrees of sterilization on culture substrates on the growth and sporulation of the pathogen, toxin production, if any, by the fungus, effect of pesticides on the growth and sporulation, preparation of spore formulation, efficiency of these formulations in controlling the aphids under laboratory and field conditions and the viability of spores in the formulations.

Death of infected insects occurred in 24 to 72 hours, after which the body became hard. Growth of the mycelium over the cadaver appeared 24 to 48 hours after death. The dead insects were seen adhering to the plant covered with white mycelial growth of the fungus.

For mass production of F. pallidoroseum wheat bran and rice bran were found to be comparatively suitable substrates as they produced maximum number of spores with

higher virulence. Maximum sporulation was noticed 8 days after inoculation, after which it appeared to be more or less constant or decreased. Growth of the pathogen was the highest in wheat bran agar followed by rice bran + straw bits agar, wheat bran agar, straw bits agar, rice bran agar and straw bits agar in the descending order.

Studies on the growth and sporulation of the fungus in mass culture substrates subjected to different sterilization methods showed the superiority of full heat sterilization over other methods. Fairly good growth and sporulation of the fungus was also observed in substrates subjected to full solarization . Cheapness and practicability render this method highly suitable for large scale multiplication of the fungus for field use.

Culture filtrates of F. pallidoroseum grown on Richard's media when sprayed on to aphid caused 99.57 per cent mortality. The filtrate diluted with equal amount of water was also found effective in controlling the aphids.

Effect of some pesticides on the growth and sporulation of the fungus was assessed by growing the fungus on media mixed with them. It was found that HCH completely inhibited the fungal growth. It was followed by quinalphos,

fenthion and phosphamidon, the percentage inhibition being 72.5, 70.56 and 32.5. Monocrotophos and mercaptothion were found to be the least inhibitory. All the insecticides inhibited sporulation to varying extent, mercaptothion ~~alathion~~ causing maximum inhibition of 54.81 per cent followed by quinalphos ~~nekalux~~ 54.07 per cent. Fenthion was the least inhibitory to sporulation. Among fungicides captan and zineb inhibited the growth completely whereas copper oxychloride, mancozeb and captofol allowed some growth which did not produce any spores.

Out of the different spore formulations tried none was able to cause insect mortality to the extent of that caused by spore suspension in water in the early period. Wettable powder formulation with diatomaceous earth as inert material caused equal mortality in 4 days as spore suspension in water. Dust formulation of spores (talc) was the least effective.

Pot culture studies showed that application of spore suspension in water and wettable powder formulation of spores with diatomaceous earth as inert material was as effective as insecticide, quinalphos 0.05 per cent in controlling the pest.

Studies on the viability of spores in the formulation showed a decreasing trend with increase in storage period and it retained substantial virulence up to four days of storage.

REFERENCE

REFERENCES

Aguda, R.M., Rombach, M.C. and Robert, D.W. (1988). Effect of pesticides on germination and growth of three fungi of rice insects. International Rice Research Newsletter. 13 (6) : 39-40.

Alexopoulos, C.J. and Mims, C.W. (1983). Introductory Mycology. Wiley Eastern Limited, New Delhi. pp 567-568.

Anderson, T.E., Hafek, A.E., Roberts D.W., Presler, H.K. and Robertson,J.L. (1989). Colorado potato beetle Coleoptera : Chrysomelidae. Effects of combination of B. bassiana with insecticides.J. econ. Ent. 82 (1):83-89.

*

Aquino, M.DE L.N., Vital, A.F.,Calalcanti, V.L.B. and Nascimento, M.G. (1977). Culture of Metarrhizium anisopliae (Metch) Sorokin in polypropylene sacks (preliminary note) Boletin Techico da Comissao Executiva de Defesa Fitssanitaria da Lavoura Canavieira de pernambaco 5 : 11.

Atwal, A.S., Singh, B. and Battu, G.S. (1973). Chilo partellus (Swinhoe) a new host of Aspergillus flavus Link. and Fusarium sp. Curr. Sci. 42 : 585.

Backman, P.A. and Rodriguz-Kabana, R. (1975). A system for the growth and delivary of biological control agent to the soil. Phytopathology 65 : 819-821.

Baird, R.B. (1956). Use of fungus disease in biological control of insects. Proceedings of 10th International Congress of Entomology. 4 : 489-692.

Banks, C.J.(1954). A method of estimating populations and counting large numbers of Aphis fabae Scop. Bull. Entomol. Res. 45 :751-756.

Barson, G. (1976). Fusarium solani, a week pathogen of the larval stages of the large elm beetle Scolytus scolytus (Coleoptra : Scolytidae).J.Invertebrate pathol. 27: 307-309.

Bartlett, K.A. and Lefebvre, C.L. (1934). Field experiment with Beauveria bassiana (Bals.) Vuill, a fungus attacking the European corn borer. J. econ. Ent. 27 : 1147-1157.

Batista Filho, A., Camargo, L.M.P.C.A., Myazaki, I., Cruz, B.P.B. and Olivera, D.A. (1989). [Biological control of the banana root borer (Cosmopolites sordidus Germar, 1824) by entomogenous fungi in the laboratory]. Biologico. 53 (1-6) : 1-6.

Batista Filho, A., Cruz, B.P.B., Camargo, L.M.P., C.DE. A. and Oliveria, D.A., (1985). [Growth of Beauveria sp, isolated from cotton weevil (Anthonomus grandis. Boheman) in natural liquid media]. Biologico. 51 (3) : 17-21.

Beevi, N.S. (1979). Studies on entomogenous fungi associated with lady bird beetle Henosepilachna vigintioctopunctata (Fabr.) (Coccinellidae:Coleoptra). Master of Science in Agriculture thesis. Kerala Agricultural University.

Beevi, N.S. (1982). Succeptibility of different pests and plants to infections of Fusarium moniliformae var. subglutinans. Entomon 7 : 235-236.

Beevi, N.S. and Jacob, A. (1987). Effect of pesticides on the growth and sporilation of Fusarium monniliformae Var. subglutinans infecting Epilachna beetle Henosepilachna vigintioctopunctata (Fabr.) on bitter gourd. National symposium on integrated pest control-Progress and perspectives,October 15-17, 1987 Proceedings-November 1988. pp 267-269.

Bell, J.V. (1974). Mycoses. In "Insect Diseases" (E.A. Cantwell, ed.). 1 : 185-236. Marcel Dekker, New York.

Benz, G. (1971). Synergism of Micro-organisms and Chemical Insecticides. In "Microbial control of insects and mites" (H.D. Burges and N.W. Hussey. eds.). pp 327-353. Academic Press, New York.

Booth, C. and Sutton, B.C. (1984). Fusarium pallidoroseum the correct name for F. semitectum Auct. Trans. Brit. mycol. Soc. 83 : 202-204.

Butcher, G.E. (1958). General summary and review of utilization of disease to control insects. Proc. 10th Internatl. Congr. Ent. (Montreal 1956). 4 : 695-701.

Cadatal, T.D. and Gabriel, B.P. (1970). Effect of chemical pesticides on the development of fungi pathogenic to some rice insects. Philipp. Ent. 1 : 379-395.

Celino, M.E. (1930). A fungus disease of the coconut leaf miner (Promecotheca cumingi Baly.) Philipp. Agric. 19 (4) : 253.

Claydon, N., Grove, J.F. and Pople, M. (1977). Insecticidal secondary metabolic products from the entomogenous fungus F. solani. J. Invertebr. pathol. 30 : 216-223.

Devenasan, S., Jacob, A., Kuruvilla, S. and Mathai, S. (1979). Infection of Nephotettix virescens (stal.) (Cicadellidae : Hemiptera) by Fusarium equiseti. (Corda) Sacc. Entomon 4 : 304-305.

Devnath, S. (1987). Fusarium coccophilum (Desm) Wr & Rg. an interesting entomogenous fungus recorded on Hemiberlesia rapex (Comstock) a scale insect pest of tea. Two and a Bud. 34 29-30.

*

Dirimanov, M. and Angelova, R. (1962). The effect of insecticides on the development of the fungus Beauveria bassiana (Bals.) Vuill. Rast. Zasht. 10 : 63-67.

*

Dresner, E. (1949). Culture and use of entomogenous fungi for the control of the insect pests. Inst. pfl. Res. 15:319-335.

Easwaramoorthy, S. and Jayaraj, S. (1977). Effect of certain insecticides and fungicides on the growth of the coffee green bug fungus Cephalosporium lecanii. Zimm. Madras Agric. J. 64 : 243-246.

Easwaramoorthy, S. and Jayaraj, S. (1978). Effectivness of the White Halo Fungus Cephalosporium lecanii against field population of coffee green bug Coccus viridis. J. Invertebrate pathol. 32 (1) : 88-96.

*

Evalakhova, A.A. (1964). The effect of DDT and BHC on the growth and virulence of entomopathogenic fungi. Trudy Vser. Inst. Zashch. Rast. 21 (1) : 95-100.

Ferron, P. (1981). Pest control by the fungi Beauveria and Metarrhizium. In "Microbial control of pests and plant diseases" (H.D. Burges, ed.) Academic press, New York.

Fritz, R. (1976). The action of some fungicides on the mycelial growth of three species of Entomophthorales. Entomophaga. 21 : 234-249.

Gabriel, B.P. (1968). Entomogenous micro organisms in the Philippines, New and past records. Philipp. Ent. 1 :97-130.

Gokulapalan, C. (1989). Effect of plant, protection chemicals on foliar pathogen and phylloplane mycoflora of rice. Ph.D thesis, Kerala Agricultural University. pp 134.

Gopinathan, P.B., Beevi, N.S. and Nair, M.R.G.K. (1982). occurence of Fusarium equiseti (Corda) Sacc as a fungal pathogen of bringal mealy bug Coccidohystrix insolita. (Green) Entomon 7 : 120.

Hall, I.M. (1954). Studies on micro organisms pathogenic to the sod web worm. Hilgaradia. 22: 535-565.

Hall, I.M. and Dunn, P.H. (1958). Artificial dissemination of entomophthorous fungi pathogenic to the spotted alfalfa aphid in California. J. econ. Ent. 51 341-344.

Hall, I.M. and Dunn, P.H. (1959). The effect of certain insecticides and fungicides on fungi pathogenic to the spotted alfalfa aphid. J. econ.Ent. 52:28-29.

Hareendranath,V. (1989). Control of Aphis craccivora Koch. with fungal pathogens and their impact on the natural enemies of the pest. Master of science thesis . Kerala Agricultural University.

Hareendranath,V., Vasudevan Nair,K.P.and Suma Paulose (1987). Fusarium pallidoroseum (Cooke) Sacc. as a fungal pathogen of Aphis craccvori Koch. Enotmon: 12 : 392-394.

Henis, Y., Gaffer, A. and Baker,R.(1978). Integrated control of Rhizoctonia solani damping off of radish: Effect of successive plantings , PCNP and Trichoderma harzianum on pathogen and desease. Phytopathology. 68 : 900-907.

Holdom,D.G., Klashorst,G., Van De (1986). Inexpensive culture media and methods for Nomuraea releyi. J. Invertebr. pathol. 48: (2) :

Hussey, N.W. and Tinsley, J.W. (1981). Improvement of insect pathology in the Peoples Republic of China. In " Microbial control of pests and plant diseases 1970 - 1980." (H.D. Burges, ed.). Academic press, New York. pp.785-796.

Jacob, A., Kuruvilla,S., Philip,B.P. and Asari P.A.R. (1978). Fusarium moniliformae var.subglutinans Wollenw and Reink. pathogenic to the spotted beetle, Epilachna vigintioctopunctata F. Agri.Res.J. Kerala. 16:262-263.

*

Kalvesh,T.K.(1976). Entomophilus fungi protective to forest belt pest of Kalandinskay steppe. Izv.SIB. OTD. AKAD. NAUK SSR SER.BIOL.NAUK.2 : 85-86.

Kerala Agricultural University (1989). Package of Practices Recommendations.

*

Keller, S.(1978). Investigations on the influence of Dimilin (Diflubenzuron) on the growth and germination of the conidia of some fungi pathogenic to insects. Anzeiges fur Schudlangs kunde, Pflanzenschutz. Umweltschutz 51 (6) : 81-83.

Kuruvilla,S.(1978). Studies on entomogenous fungi of brown plant hopper Nilaparvata lugens Stal (Delphacidae: Hemiptera) Master of science thesis Kerala Agricultural University.

Kuruvilla, S. and Jacob,A. (1978). Fusarium oxysporum Schlect as an entomogenous fungus on Nilaparvata lugens Stal. Abstract of Papers, symp.on Rice Research and Development. KAU 41

Kuruvilla,S. and Jacob,A. (1979). comparative suceptibility of nymphs and adults of Nilaparvata lugens Stal. to Fusarium oxysporum Schelt and it's use in microbial control. Agri.Res. J.Kerala 17 : 287-288.

Kuruvilla,S. and Jacob,A. (1981a). Mass culturing of Fusarium oxysporum schlt an entomogenous fungus of brown plant hopper Nilaparvata lugens Stal Agri. Res. J. Kerala 19 (1) : 66-68.

Kuruvilla,S. and Jacob,A. (1981b). Effect of pesticides on the growth and sporulation of Fusarium oxysporum Schlt pathogenic to brown plant hopper Nilaparvata lugens Stal. Agri. Res. J. Kerala. 19 (2) : 102-104.

Lynch, R.E. and Lewis,L.C. (1978). Fungi associated with eggs and first instar larvae of the European corn borer J.Invertebr. pathol. 32 : 6-11.

Mac Lean, D.B. and Giese,R.L. (1968). Fungi associated with Xyloterinus politus (Say) (Coleoptra : Scolytidae) J.invertebr. pathol. 10 (2) : 185-189.

Madelin, M.F.(1963). Diseases caused by Hyphomycetous fungi. In "Insect pathology an Avanced Treatise" (E.A. Steinhaus, ed.), vol. 2, pp 233-271.Academic Press New York.

Madelin, M.F. (1968). Fungal parasites of insects Annu. Rev. Entomol. 11 : 423-448.

Martignoni, M.E. (1964). Mass production of insect pathogens. In " Bioligical control of insects pest and weeds" (P.De Bach, ed.) pp - 579-609. Reinhold, New York.

Martins , D.S. , Paulin, A.E. and Galvao, M.M. (1989). Incidence of Orthezia praelonga Douglas 1893 in coffee in Esprito tanto. Articulcao Pesquisa -Extensao 9: 1-18.

Mathew, G. and Mohammed Ali, M.I. (1987). Microbial pathogens causing mortality in the carpenter worm, Cossus cadambae Moore. (Lepidoptera : cossidae) a pest of teak (Tectonia grandis Lin f.) in Kerala (India) Journal of Tropical Forestary. 3 : 349-351.

Mccoy,E.E. and Carver, C.W. (1941). A method for obtaining spores of the fungus Beauveria bassiana (Bals) Vuill in quantity. Jour. New York Ent. Soc. 49 : 205-210.

Mori, K. and Takaish, H. (1989). Synthsis of monocerin, an anti fungal, insecticidal and phytotoxic leptaketide metabolite of Exserohilum. monocerus. Tetrahedron. 45 :1639-1946.

*

Morqueer, R., and Nystrakis,N.F. (1944). Role des Fusariees entomophytes comme destructeurs d'insectes. Bull Soci. Hist. Nat. Toulouse. 79 : 281-318.

Nagalingam,B. (1983). Studies on the ecology and bio control agents of Myzus persicae (Sulzer) Ph D thesis . Tamil Nadu Agric. Univ. coimbatore.

Nagalingam,B. and Jayaraj, S. (1986). First record of Fusarium semitectum Berk and Rav as an entomophagous fungus. Current Science. 55 (7) : 377-388.

Nair, K.K., Ananthakrishnan, T.N. and David David, B.V. (1976). General and Applied Entomology. Tata Mc Graw Hill publishing co. Ltd, Delhi. pp 203-204.

Nayak, P and Srivastava, R.P. (1978). Occurrence of a new fungal disease on green horned caterpillar of rice. Curr. Sci. 47: 380-381.

Ozino, O.I., Arzone, A.and Alma, A.(1988). Entomogenous fungi of Sitiobion avenae (F) in piedmont cereal crops. Redia 71 : 173-183.

Pascalet, P. (1939). Lalutta biolongique contra Staphanoderes hampei un scolyte du cafeicr au camerou. Revue de bot appl. et. agric. tropic.19 : 753-764.

*

Popov,C. and Illiesu, H. (1975). The parasitic microflora of Eurygaster integriceps in the diapause period Probleme de protectia plantelor. 3 : 125-136.

Porter, D.M., Smith,D.H. and Rodriguez - Kabana,R. (1984). compendium of peanut diseases St. Pauls, Minnesota. pp.18-226.

Pristavko, W.P. and Goral, V.M. (1967). The mass production of Beauveria bassiana. In " Insect pathology and microbial control " (P.A., Vander Loan, ed.) pp. 118-119. North Holland publ. Co. Amsterdam.

*

Rachvelishrili,E.V. (1965). Some data on the injurious insect fauna of peas in Georgia Trudy Inst. Zashch Rast. Thilisi. 17 : 21-25.

Raghavendran, R., Easwaramoorthy, S. and David,H.(1987). Pathogenicity of Fusarium subglutinans to sugarcane scale insect Melanspis glomerata (Green) J. Biol. control. 1 : 118-121.

Reinking, C.A. (1921). Citrus disease of the Philippines, Southern China,Indochina and Stam.Philipp. Agric. 9:121-180.

Rombach, M.C., Aguda, R.M. and Roberts, D.W. (1988). Production of Beauveria bassiana (Deuteromycotina : Hyphomycetes) in different liquid media and subsequent conidiation of dry mycelium Entomophaga. 33 (3) :

*

Sa, C.Y. (1988). [The effect of certain pesticides on Beauveria bassiana] Chinese Journal of Entomology. 8 (2) : 157-160.

Sachithanandam, S., Rabinbra, R.J. and Jayaraj. (1989). Pot culture studies on the efficacy of NPV formulations against the tobacco cut worm Spodoptera litura (F.) larvae on ground nut. J. Biol. Control. 3 (1) : 44-46.

Saito, T. (1988). [control of Aphis gossypii in green houses by a mico insecticidal prepearation of verticillium lecanii and the effect of chemicals on the fungus]. Japanese Journal of Applied Entomology and Zoology. 32 (2) : 224-227.

*

Schaerffenberg, B. (1957). Beauveria bassiana (Vuills) Link also parasit. des kurtoffelkafers (Leptinotarsa decimlineata). Anz. Schadling skunde. 30 : 69-74.

Shands, W.A., Thompson, G., Simpson, G.W. and Wave, H.E. (1958). Preliminary studies on Entomogenous fungi for the control of potato infesting aphids in Maine J. econ. Ent. 51 (2) : 184-186.

Smirnoff, W.A. (1973). A fungus disease of Adelges piceae and their possible use for the control of this species. Report of forest research laboratory, Dept. Fisheries and Forestry Quebec, Canada.

Sridhar, T.S. and Krishnaiah, K. (1975) Fusarium equiseti (Ida) Sacc a fungus infecting okra petiole maggot (Melanagromyza hibisci spencer). curr. Sci. 44 : 447.

Steinhaus, E.A. (1949). Principles of Insect Pathology McGraw Hill, New York. pp. 757.

Steinhaus, E.A. and Marsh, G.A. (1962). Report of diagnoses of diseased insects 1951-1961. Hilgardia 33 : 490.

Teodoro, N.G. (1937) Ann enumeration of Philippine fungi. Dept. Agric. and comm. Tech. Bull. 4 : 585.

Toscano, N.N. and Reever, E.L. (1973). J. Invertebr. pathol. 22 : 55-59.

*

Uchida, M.. (1970). Studies on the use of parasitic fungus Aschersonia sp for controlling citrus white fly Dialeurodes citri. Bull. Kang. 18 : 66-74.

Urs, N.V.R., Govinda, H.G. and Shivashankarashastry, K.S. (1967). The effect of certain insecticides on the entomogenous fungi Beauveria bassiana and Metarrhizium anisopliae. J. Invertebrate pathol. 9 : 398-403.

*

Vanninen, I. and Hokkanen, H. (1988). Effect of pesticides on four species of entomopathogenic fungi in vitro Annales Agriculturae Fenniae 27 (4) : 345-353.

*

Vey, A. and Quiot, J.M. (1975) C.V. Lebel Seanc. Acad. Sci. Paris ser. D. 280 : 931-934.

Vilas Boar, A.M. and Alves, S.B. (1988) Pathogenicity of Beauveria spp and its effect associated with monocrotophos on castnia licus (Drury 1770) [Lepidoptera: Castniidae] Anais da Sociedade Entomologica do Brasil 17 (2) : 305-332.

Villacarolos, L.T. and Robin, R.P. (1989). Entomogenous fungi infecting Hetropsylla cabana Crawford (Homoptera : Psyllidae) in Leyte, Philippines. Tropical pest management 35 : 120-122.

*

Villacorta, A. (1976). Technique for the mass culturing of the entomopathogenic fungus Metarrhizium anisopliae Metch, in granular form. Anais da sociedade Entomologiica do Brazil 5 : 102-104.

Viswanathan, P.R.K. (1972). A Fusarium disease of Coccus viridis. J. coffee Res. 2 : 25-27.

*

Voukassovitch, P. (1925) Contribution a'l'e'tude d'un Champignon entomophyte Spicaria farinosa (Fries) var. verticilloides Fron. Ann. epiphyt. 11 ; 73-106.

*

Wallengren, H. and Johanson, R. (1929). On the infection of Pyrausta nubilalis Hb. by Metarrhizium anisopliae (Metsch) Sor. Sci. Repts. Intern. Corn. borer Invest. 2 : 131-145.

Wilding, N. (1972). The effect of systemic fungicides on the aphid pathogen cephalosporium cephidicola. plant pathol. 4 : 137-139.

Wilding, N. (1981). Pest control by entomophthorales. In "Microbial control of pests and plant diseases 1970-1980" [H.D, Burges ed.] pp. 539-554. Academic Pres, New York.

*

Yasem De Romero. M.G. (1986). Effect of some agricultural chemicals on the entomopathogenic fungus Verticillium lecanii (Zimn) Viegas. Rivista de Investigaion, centro de Investigaciones Pura la Ragulaci-on de poblaciones de organismos nocivos Argentina. 4 (4-1) : 56-62.

Yendol, W.G. (1968). Factors affecting germination of Entomophthora conidia. J. Invertibr. pathol. 10: 116-121.

*

York, G.T. (1958). Field tests with the fungus Beauveria sp. for the control of the European corn borer. Iowa State Coll. Jour. Sci. 33 : 123-129.

*

Zimmermann, G. (1976). Laboratory investigations on the effect of systemic fungicides on infection and production of conidia by an aphid infecting Entomophthora (Zygomycetes) in cereal aphids. Zectschrift far pflanzen krankheisten und pflanzenschutz. 85 : 513-524.

(* Originals not seen)

APPENDICES

APPENDIX I

Abstract of anova and ancova (M S values)

Abstract of anova for Table 2

Source	D F	M S Values (days after inoculation)							
		3	4	5	6	7	8	9	10
Treatment	5	0.1716*	0.2611*	0.1086**	0.0416**	0.0523**	0.0395**	0.061**	0.0517**
Error	12	0.0378	0.0643	0.0101	0.0073	0.0019	0.0044	0.0036	0.0030

Abstract of anova for Table 3

Source	D F	M S values
Treatments	7	5.8870**
Error	16	4.1405

Abstract of anova for Table 4

Source	D F	M S values (days after treatment)			
		1	2	3	4
Treatments	6	823.70**	1269.62**	2209.43**	1832.30**
Error	14	18.90	51.95	54.33	45.35

Abstract of anova for Table 5.

Source	D F	M S values
Treatment	5	
A (Substrates)	1	0.4253*
B (Degree of sterilization)	2	4.8704**
AB	2	0.0777
Error	24	0.0646

Abstract of anova for Table 6.

Source	D F	M S values
Treatments	4	2891.55**
Error	15	54.48

Abstract of anova for table 8

Source	D F	M S values (days after treatment)			
		1	2	3	4
Treatments	5	1338.22**.	2544.07**	3024.62**	3138.05**
Error	18	18.91	23.70	32.45	65.01

Abstract of ancova for Table 9 (final population adjusted with the initial population)

Source	D F	M S values
Treatments (adj.)	7	106.248**
Error	23	3.570

Abstract of anova for Table 10

Source	D F	M S values
Treatments	15	630.50**
A (days)	3	2801.25**
B (formulation)	3	245.07**
AB	9	35.40
Error	48	50.99

** Significant at 1 % level

* Significant at 5 % level

STUDIES ON THE ENTOMOGENOUS FUNGUS *Fusarium pallidoroseum* (Cooke) Sacc. ASSOCIATED WITH COWPEA APHID *Aphis craccivora* Koch.

BY

FAIZAL M.H.

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF SCIENCE IN AGRICULTURE

FACULTY OF AGRICULTURE
KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM

1992

ABSTRACT

Studies were conducted on the entomogenous fungus Fusarium pallidoroseum (Cooke) Sacc. infecting cowpea aphid Aphis craccivora Koch.

The infected aphids turned pale and assumed brownish black discolouration. Death occured in 24 to 72 hours after infection and mycelial growth appeared on the cadavers 24 to 48 hours after death.

For mass production of the fungus, wheat bran and rice bran appeared to be comparatively suitable as they recorded maximum growth, sporulation and virulence of the pathogen. Maximum sporulation was noticed 8 days after inoculation.

Culture substrates subjected to full heat sterilization produced maximum growth and sporulation of the fungus followed by those subjected to full solarization .

A mortality per cent of 99.57 was observed when aphids were sprayed with culture filtrate of F. pallidoroseum grown in Richard's medium indicating the presence of toxins.

Studies on the effect of pesticides on the growth of the fungus showed that mercaptothion and monocrotophos were the least inhibitory. Fenthion showed the least inhibition of sporulation. HCH, captan and zineb completely inhibited the growth. Sporulation was completely inhibited in all the fungicidal treatments.

Under laboratory conditions spore suspension of the fungus in water was found to be superior to the spore formulations tried. Among the formulations wettable powder with diatomaceous earth as inert material was found to cause more mortality followed by wettable powder with talc as inert material. Dust using talc as inert material was found to be the least effective.

Spore suspension in water, wettable powder formulation of fungal spores with diatomaceous earth as inert material and quinalphos 0.05 per cent were found to be equally effective in bringing down the population of aphids under field conditions.

The virulence of spores of F. pallidoroseum in formulation was found to decrease with increase in storage period and it retained substantial virulence up to four days of storage.