Fusarium pallidoroseum (Cooke) Sacc AS A BIOCONTROL AGENT FOR THE PEA APHID *Aphis craccivora* Koch

By

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DECLARATION

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

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CERTIFICATE

Certified that this thesis entitled "*Fusarium pallidoroseum* (Cooke) Sacc as a biocontrol agent for the pea aphid *Aphis craccivora* Koch" is a bonafide record of research work done independently by Smt. Sunitha. V.S. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

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INTRODUCTION

1. INTRODUCTION

Pulses form an important ingredient of the daily diet of Indian masses. For the past several decades the production of pulses have increased annually by 11.41 per cent only. The statistics of area and production in the major pulse growing states indicated that even though the area is slightly increased, there was reduction in the production, especially,in the southern states. One major reason for lower production is the drop in production per unit area. Several factors are responsible for the reduction in the yield per unit area. One of the important factors is the incidence of pests and diseases. The black pea aphid, *Aphis craccivora* Koch is a serious pest of cowpea, *vigna unguiculata* and many other pulse crops like black gram, green gram, lablab, ground nut, peas, beans etc. This insect also acts as a vector for viral diseases of legumes like rosette, mottles, stunt and stripe (Porter *et al.*, 1984). Peak aphid infestation occurs during the active reproductive phase of the crop and this results in marked reduction in the yield of the crop.

One of the most widely used methods to control aphids is the use of insecticides. But the wisdom of using chemical insecticide on a massive scale is now being questioned because of environmental and health risks associated with it. Moreover it is becoming increasingly difficult to control insects effectively

with chemicals due to the problem of insecticide resistance and pest resurgence. These problems have led to intensified efforts on developing non-chemical methods of plant protection. One such method is the use of pathogenic microorganisms to check the pest.

Fungal infections in insects are common and widespread and often deciminate insect populations in spectacular epizootics. Fungi are the principal pathogens of sucking insects. Entomogenous fungi could provide effective, inexpensive and long lasting control of insect pests in areas with high rain fall and humidity (Dresner, 1949). Kerala being such an area, offers considerable scope for the use of entomogenous fungi in pest management programmes.

Hareendranath *et al.* (1987) reported *Fusarium pallidoroseum* (Cooke) Sacc as an efficient fungal pathogen on pea aphid, *A. craccivora*. Subsequently, Faizal *et al.* (1992) found that *F. pallidoroseum* at the rate of 3.5×10^6 sporoes per ml was very effective for controlling aphids under laboratory conditions. They also found that spore suspension in water and wettable powder formulation containing diatomaceous earth as inert material were highly effective. However, information on the performance of the fungus under field conditions is lacking. The present investigations were hence taken up with the following objectives:

1. To determine the most effective spore concentration of *F. pallidoroseum* to control field population of aphids.

2. To find out the effect of *F. pallidoroseum* on the various host plants of *Aphis craccivora*.

3. To study the viability of the fungus in water suspension and diatomaceous earth wettable powder under different storage temperatures.

4. To study whether this biological control agent is safe to be used on common vegetables and medicinal plants and

5. To study, whether this fungus has any effect on natural enemies of aphids and productive insects such as honeybees and silkworms.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Entomogenous fungi includes species from the four classes of fungi Phycomycetes, Ascomycetes, Basidiomycetes and Dueteromycetes (Steinhaus 1949). The majority of publications on insect mycoses discuss *Entomophthora*, *Beauveria*, *Metarrhizium*, *Aspergillus*, *Verticillium*, *Hirsutella*, *Culicinomyces*, *Lagenidium* and *Coelomomyces* (Steinhaus, 1964; Roberts and Yendol, 1971, Ferron, 1981; Hall 1981; Mc Coy, 1981; Dean and Wilding 1981).

2.1 Field efficacy of entomogenous fungi

The extent of survival of a biological agent under field conditions is an indication of its efficacy. The biocontrol agent, in order to be effective, must survive for a long period under adverse physical chemical and biological conditions which exist in the field.

Field experiment with *Entomophthora aphidis* showed that conidial suspension of the fungus containing 6 x 10^6 spores/ml killed 76 to 100 per cent *Aphis gossypii*, *Aphis pomi*, *Sitobion avenae* and *Myzus persicae* (Hussey and Tinsley, 1981). Squibbs (1934) reported that the Indian strain of *Cephalosporium lecanii* proved effective for controlling the scale insects of potted plants of coconut. Evalakhova (1938) studied the control of *Ceroplastes sinensis* with *Cephalosporium lecanii*. Effective

control of the pest was obtained when the fungal spores were applied as a dust to infested mandarin orange. Veigas (1939) found that spore suspension of *Verticillium lecanii* sprayed on coffee was effective in reducing the population of *Coccus viridis* in Brazil.

Easwaramoorthy and Jayaraj (1978) reported that the white halo fungus *Cephalosporium lecanii* was highly effective in the control of coffee green bug, *Cocus viridis* under field conditions with two fortnightly applications of $16x10^6$ spores/ml and 73.1 per cent mortality of the insect was noticed after the second application. The fungus was more effective as a high volume spray compared to low volume spray. Khalil *et al.* (1983) studied the effectiveness of *V. lecanii* against the aphid species *Brachycaudus*, *Macrosiphoniella sanbornii* and *M. persicae* on cucumber in glass house and *Macrosiphum avenae* on wheat under modified field conditions and showed that the fungus was highly effective against these aphids at 25±2°C and 100 per cent relative humidity. Control of *M. persicae* and *Macrosiphum avenae* was rapid and reached cent per cent in 25 days, while that of *M. sanbornii* and *Brachycaudus* sp was slower and it took 30 and 35 days respectively to reach 100 per cent control. Voronina *et al.* (1987) reported the effectiveness of resting spores of the fungus *Entomophthora thaxteriana* against field population of pea aphid *Achanthosiphon pisum*. The natural infection was 20-30 per cent when the spore preparation was used. The infection increased to 2-3 fold in 16 days.

Grunberg *et al.* (1988) reported that microbial pesticide based on *V. lecanii* under green house conditions was found to be effective for the biological control of aphids especially *A. gossypii*, *M. persicae*, *Brachycaudus helichrysi*, *M. sanbornii* and *A. fabae*. Li and Kanu (1989) identified thirteen species of Entomophthorales from aphids attacking vegetables and the infection rate on aphids was over 80 per cent in some fields during epidemics of fungi. Sopp *et al.* (1989) found that blastospores of *V. lecanii* was effective in controlling *A. gossypii* affecting *Chrysanthemum* under green house conditions. Field tests in China showed that the entomogenous fungus *Paecilomyces cicadae* isolated from cicadid *Platylomia pieli* had high pathogenicity to the cruciferous pest *Pieris rapae* (Chen *et al.* 1990).

Burchard and Trzebiezky (1990) conducted a field study using *Beauveria brongniartii* against adults of *Melolontha hoppocastani* on oak in forest of South Western Germany. Infection after six weeks was found to average 85 or 67 per cent and there was a subsequent 6 fold decrease in larval density. Badilla and Alves (1991) found out that *Beauveria bassiana* isolates were effective against the sugarcane pest *Sphenophorus levis*. Field tests in Sao Paulo at 4.5×10^{11} conidia/piece of treated sugarcane resulted in 92.3 per cent mortality of the pest. Lima *et al.* (1991) tested *B. bassiana* against *Oedaleus senegalensis* and *Diabolocantops axillaris* under field conditions.

They obtained significant mortality of the insects when *B. bassiana* was sprayed directly on to insects. Bateman *et al.* (1991) successfully used oil based ULV sprays of *Metarrhizium flavoviride* conidia to control adults of *Schistocerca gregaria*. Dorschner *et al.* (1991) found out that the aphid derived isolate of *B. bassiana* was effective against the hop aphid *Phorodon humulii* (Aphididae) in laboratory and under field conditions. Potential for long term control was demonstrated by them when conidial suspensions were applied to aphid infested potted hop plants. Bing *et al.* (1992) observed immediate suppression of *Ostrinia nubilalis* in maize plants under field conditions by foliar application of *B. bassiana*.

Zhang *et al.* (1992) formulated *B. bassiana* as pure powder and wettable powder, both of which were equally effective against *Ostrinia furnacalis*. In field trials it was found that more than 80 per cent control of the pest was achieved when the formulations were used against larvae. Verkleif *et al.* (1992) studied the efficiency of entomopathogenic *Metarrhizium anisopliae* formulated as granulates of dried mycelium, against *Sitona lineatus* in faba bean field. However, they obtained only 33 per cent mycoses in the middle of the season. Maniania (1993) conducted field experiments to evaluate the efficiency of 3 formulations of *B. bassiana* (isolate ICIPE 35) ie., a dry rice grain based inoculum, a soaked dry rice grain based inoculum and an acqueous conidial suspension against larvae of *Chilo*

partellus. The aqueous formulation and dry rice grain based inoculum showed similar performance. The dry rice grain based inoculum which is easy to apply induced the highest fungal infection rates in pest population two weeks after application. Devi (1994) identified the fungus *Nomuraea rileyi* against *Spodoptera litura* on *Ricinus communis* Var VPI. Larval mortality was initilly observed in the field at 9 days after spraying with *N. rileyi* conidia. Even the lowest dose of 2 x 10*11* spores per litre resulted in a significant cumulative larval mortality. Baker *et al*. (1994) applied conidia of *Metarrhizium flavoviride*, isolate F. 1985 on population of *Phaulacridium vittatum* on pasture in Australia and observed that one month after treatment, densities of *P. vittatum* averaged 4.5/m^2 in treated fields.

2.2 Fusarium species as insect pathogen

The genus *Fusarium* is plant pathogenic with a saprophytic mode of life. Several works revealed the importance of this pathogen as a biocontrol agent against insect pests. Entomopathogenic species of *Fusarium* were known as early as 1983. Kunchel d Hercularis and Langlois (1891) found *Fusarium acridiorum* (Trabut) to infect desert locust *Shistocerca gregaria* (Forski), A detailed list of insects infected by different species of *Fusarium* is given below:

Fusarium species as insect pathogen

Fungus	Insect	Crop	Reference	Remarks
Fusarium sp	*Monochamus notatus.*		Steinhaus and Marsh 1962	
Fusarium sp				
Fusarium sp	*Coccus viridis*			
Fusarium sp	*Eurygaster pacifica*			
Fusarium sp	*Chilozcrethus* sp			
Fusarium sp	*Platynota rostrana*			
Fusarium sp	*Carpocapsa pomonella*			
Fusarium sp	*Macrosiphum pisum*	Pea	Rachvelishrile 1965	
Fusarium sp	*Xyloterinus politus*		Mac Lean and Giese 1968	
Fusarium sp	*Eurygaster integriceps*		Popov and Illiesu 1975	
Fusarium sp	*Orthezia praelonga*	Coffee	Martins *et al.* 1989	
Fusarium sp	*Orthezia praelonga*	Tea	Ye and Wang *et al.* 1991	
F. aleyrodid	*Dialeurodes citri* and *D. citrifolia*	Citrus	Steinhaus 1949	
F. citriculatum	Cerambycid beetle		Madelin 1968	
F. cocophylla	Scale	Citrus	Teodero 1937	

F. cocophylla	Scale	Citrus	Reinking 1921
F. episphaeria	Scale		Steinhaus and Marsh 1962
F. episphaeria cocophila	*Aonidiella aurantii* *Chrysomphalus acridium* *Coccus viridis*	Coconut	Gabriel 1968
F. equiseti	*Melanagromyza hibisci* Spencer	Okra	Sridhar and Krishnaiah 1975
F. equiseti	*Nephotettix virescens*	Rice	Devanesan *et al.* 1979
F. equiseti	*Coccidohystrix insolita* green	Brinjal	Gopinathan *et al.* 1982
F. gibbosum var *bullatum*	Forest pest	Forest trees	Kalvesh 1976
F. juruanum hennings	Coccids	Coconut	Reinking 1921
F. javanicum	Forest pest	Forest trees	Calvesh 1976
F. larvarum	*Adelger piceae*		Smirnoff 1973
F. moniliformae var *subglutinans*	*Henosepilachna vigintioctopunctata*	Vegetables	Jacob *et al.* 1978
F. moniliformae var *subglutinans*	*Epilachna* beetles	Vegetables	Beevi 1979
F. moniliformae	*Mylabris pustulata* *Aulacophora* sp		Beevi 1982

F. oxysporum	*Coccus viridis*		Viswanathan 1972	
F. oxsporum	Forest pest	Forest trees	Kalvesh 1976	
F. oxysporum	*Nilaparvata lugens*	Rice	Kuruvilla 1978	6.5×10^6 spores
F. oxysporum	*Sitobium avenae*	Wheat	Ozino *et al.* 1988	
F. oxysporum var. orthoceras	Forest pest	Forest trees	Kalvesh 1976	
F. pallidoroseum	*A. craccivora*	Pea	Hareendranath *et al.* 1987	7×10^6 spores/ml and 3.5×10^6 spores/ml
F. pallidoroseum	*A. craccivora*	Pea	Faizal 1992	3.5×10^6 spores/ml
F. sambuainnii var. *minus*	Forest pest	Forest trees	Kalvesh 1976	
F. semitectum	Forest pest	Forest trees	Kalvesh 1976	
F. semitectum	*Myzus persicae*	Pea	Nagalingam and Jayaraj 1986	
F. solani	*Scolytus scolytus*	elm bark	Barson 1976	
F. solani	*Coccus cadambae*	Teak	Mathew and Mohamed Ali 1987	
F. subglutinans	*Melanaspis glomerata*	Sugarcane	Ozino *et al.* 1988	10^6-10^8 spores/ml
F. bicinctum	*Sitobium avenae*	Wheat	Ozino *et al.* 1988	

2.3 Safety of *Fusarium* spp. to crop plants

Any insect pathogenic fungus before its large scale application must be tested against a wide range of crop plants to find out whether they are infected by the fungal pathogen or not.

Fusarium oxysporum a biocontrol agent of *Nilaparvata lugens* was proved to be nonpathogenic to cotton and tomato (Kuruvilla. 1978). Beevi (1982) observed *F. moniliformae* var *subglutinans* to be nonpathogenic to cotton, tomato, bittergourd, brinjal and snake gourd. *F. semitectum* was safe to chillies, cabbage, brinjal and tobacco (Nagalingam, 1986). Studies of Hareendranath *et al.* (1989) clearly showed the nonpathogenic nature of *F. pallidoroseum* on rice, bhindi, chillies and tomato.

2.4 Safety of *Fusarium* spp to beneficial insects and natural enemies

F. semitectum a fungal pathogen of *Myzus persicae* was safe to all instars of mulberry silkworm, adult honey bees, hymenopterous parasitoides and coccinellid predators (Nagalingam 1986). Hareendranath (1989) reported *F. pallidoroseum* to be nonpathogenic to *Menochilus sexmaculata*, an efficient predator of pea aphid.

2.5 Mass production of entomopathogenic fungi

Mass production of insect pathogens had been a serious impediment in the development and application of microbial

pesticides in plant protection programmes. There has been many attempts in the past to develop techniques for the mass production of entomogenous fungi.

Pascalet (1939) reported the use of rice with the addition of peptone for the mass multiplication of the fungus *Beauveria bassiana*, while bran was used as the medium Mc Coy and Carver 1941 and York (1958). Shands *et al*. (1958) used cooked potato slices for the mass multiplication of spores of *Entomophthora aphidis*. Martignoni (1964) reported that *B. bassiana* and *Metarrhizium* could be multiplied using wheat, cane or potato as food source while Bell (1974) obtained good growth using wheat bran medium. Villacorta (1976) and Aquino *et al*. (1977) mass multiplied *M. anisopliae* in soaked sterilised rice grain.

The fungus *Cephalosporium lecanii* infecting coffee green bug *Coccus viridis* was cultured on moist sterile sorghum grains. The sporulated mat was agitated with water, filtered and used at a concentration of 16×10^6 spores/ml (Easwaramoorthy and Jayaraj (1978) Beevi *et al*. (1979) reported sorghum and bajra to be the most suitable food source for mass production of *F. moniliformae*. Kuruvilla (1981) observed green gram, wheat or sorghum as substitute for easy mass production of the fungus *F. oxysporum*., infecting rice brown plant hopper *Nilaparvata lugens*.

F. semitectum infecting *Myzus persicae* was multiplied in a medium containing broken maize grain and black or red gram husk (Nagalingam, 1986). Batista *et al.* (1985) observed better conidia production by *B. bassiana* in bran broth compared to rice and potato broth. Holdom *et al.* (1986) found sugarcane waste (bagasse) to be a good medium for conidial production of *Nomuraea releyi*. Abundant sporulation of *Fusarium subglutinans* was observed by Raghavendra *et al.* (1987) when the fungus was mass multiplied in sorghum grain.

Susamma Mathai *et al.* (1988) observed that *F. pallidoroseum* gave maximum spore count when wheat bran or rice bran plus tapioca bits were used as substrates followed by wheat bran plus straw bits, rice bran and tapioca bits. Growth of the fungus was very poor on vegetable waste and on tapioca stem peelings. Batista *et al.* (1989) cultured *B. bassiana* and *M. anisopliae* in two different culture media viz. rice and soaked bran. Hareendranath (1989) reported broken maize grain as a suitable medium for the mass multiplication of *F. pallidoroseum* followed by tapioca chips and jack seeds as they produced maximum number of spores, but according to Faizal *et al.* (1992) rice bran and wheat bran were the best. Erkilic (1992) reported two per cent malt sugar to be the best medium for mass production of spores of *V. lecanii*, the fungal pathogen of peach aphid *M. persicae*. Devi (1994) could mass produce *Nomuraea rileyi* in crushed sorghum containing one per cent yeast extract.

2.6 Formulation of mycoinsecticides

Formulation is the processing of a pesticidal compound by any method which will improve its properties of storage, handling, application, effectiveness and safety. Formulation of mycoinsecticide is equally important as that of a chemical pesticide.

The possibility of rearing entomophthorous fungi sapro as well as biotropically has enabled 2 different preparations to be obtained from *Entomophthora thaxteriana* for the purpose of aphid control (Voronina *et al*. 1987). These are Mikoafidin based on resting spores and suitable for the development of primary epizootic foci, and Mykoafidin T based on toxins suitable for use as a biological insecticide. Mikoapfidin increased infection 2 to 3 fold 16 days after treatment of plants in the stages of flowering and mass pod formation against a back ground of high population of pea aphid and 20-30 per cent natural infection. Mikoafidin T was effective against many species of aphids, while others were resistant. Those most susceptible were the melon aphid (*A. gossypii*), the green bug, (*Schizaphis graminum*) and the bean aphid (*A. pomi*) against which 0.05 per cent emulsion preparation gave 80-100 per cent mortality after 8h.

Chudare (1988) studied the effect of conidial spores and spore suspension in water prepared from the cultures of *E. thaxteriana*, *E. virulenta*, and *Basidiobolus* sp. These fungal

formulations effectively controlled *A. fabae*, *A. pomi*, *Macrosiphum rosae*, *Hyalopterus pruni* and *Anuraphis subterranea*.

The blastospore suspension in water from the fungus *V. lecanii* was prepared by Sopp *et al*. (1989). This formulation when sprayed by an ultra low volume, electrostatically charged rotary atomiser at a concentration of 2.5×10^{13} spores under green house condition gave complete control of chrysanthemum aphids. The oil based formulation of *V. lecanii* is a very effective mycoinsecticide against *M. persicae* Erkilic (1991) studied the efficiency of spore suspension in water of *V. lecanii* in controlling green peach aphids. Stirmanova (1994) reported a commercial formulation of *V. lecanii* available as verticillin against white fly.

Burchard *et al*. (1990) reported 85 per cent control of *Melolontha hippocastani*, a pest of oak, by spraying blastospore suspension of *Beauveria brongniarti* at a concentration of 2×10^{14} spores/ha. Zoebisih and Stimae (1990) conducted preference test in the laboratory using adults of *Solenopsis invicta* which were offered baits of pieces of maize, wheat or vermiculite alone or mixed with soya oil and the *B. bassiana* spores at 15-30 per cent. This preparation gave only 33.9 per cent accumulated mortality after 7 days. While submerged culture fermentation is usually regarded as the most economical and convenient method for mass production of biopesticides by

industry, Auld (1992) described various methods of liquid and solid state culture for fungi. Formulation of fungal spores or mycelia as dry powder is well established. He also reported the use of oils as spray supplements to be a promising means of overcoming dew or high humidity requirements for many fungi. Badilla *et al*. (1991) reported spore suspension of *Beauveia sp* containing 4.5 x 10^{11} conidia/piece of treated sugarcane resulted in 92.3 per cent mortality of sugarcane weevil *Sphenophorus levis*. *B. bassiana* formulations in mineral oil water emulsion, when sprayed directly on grass hopper *Oedaleus senegalensis* and *Diabolocatantops axillaris* caused complete destruction of the pest (Lima *et al*. 1991). Dorschner (1991) observed that the conidial suspensions of *B. bassiana* in water at a concentration of 10^{8} conidia/ml caused destruction of hop aphid *Phorodon humuli* in 3-4 days.

Shimazu *et al*. (1992) devised a novel method for applicaton of *B. bassiana* for the control of *Minochamus alternatus* the host of pine wood Nematode *Bursaphelenchus xylophilus* by implanting wheat bran pellets with *B. bassiana* in tree trunks. For this the fungus was cultured on wheat bran pellets and implanted under the bark of *Pinus* sp. Zhang (1992) reported 95 per cent mortality of larvae of *Ostrinia fumacata* by using wettable powder formulation of *B. bassiana* containing 50x10^{9} spores/g. Wright and Chandier (1992) developed a formulation of *B. bassiana* bait containing cotton products, grand lure (boll weevil pheromone) a sticker and ultraviolet protectant

(Nufilm (7) to control the boll weevil *Anthonomus grandis*. Maniania (1993) reported formulations of *B. bassiana* containing dry rice grain based inoculum and a soaked dry rice grain based inoculum for the control of larvae of *Chilo partellus*. He also reported that the aqueous conidial suspension of *B. bassiana* is very effective in controlling *C. partellus*. Chiue (1993) observed that *B. bassiana* cultured on wine derivatives mixed with sand to form granules containing $2x10^8$ conidia/g was effective in controlling *Ostrinia furnacalis*. *B. bassiana* prepared as powder was used against fire ant *Solenopsis invicta* by Oi *et al*. (1994). He found that infection by conidial powder in late autumn and early summer resulted in peak infection of 60 and 52 per cent. Puterka *et al*. (1994) reported that the conidia of *B. bassiana*, *Paecilomyces fumosoroseus* and *Verticillium lecanii* suspended in water were very effective in controlling pear psylla, *Cacopsylla pyricola*.

Bateman (1991) reported the oil based ULV sprays containing conidia of *Metarrhizium flavoviridae* readily killed adults of *Schistocerca gregaria* in field at 73 to 80 per cent relative humidity. Prior *et al*. (1992) formulated the conidia of *M. flavoviridae* in oil diluents suitable for controlled droplet application using rotary atomiser. This preparation was tried against locust *S. gregaria*. They also found that foliar applications of a granular formulation of maize grits containing conidia is effective for the control of *O. nubilalis*. This

entomopathogenic fungus could be formulated in granulates of dried mycelium against pea weevil *Sitona lineatus*.

Chen *et al*. (1990) showed that the spore suspension in water prepared from entomogenous fungus *Paecilomyces cicadae* at 2.4 - 4.6 x 10^7 spores/ml when applied at egg stage of *Pieris rapae* gave 90 per cent control of newly hatched larvae. Li *et al*. (1993) reported fungi *Zoophthora radicans* and *Erynia neoaphidis* could be formulated as dried mycelial preparations.

Devi (1994) found that *Nomuraea rileyi* spores suspended in water at a concentration of 10^{11} spores/litre was very effective in controlling first instar larvae of *Spodoptera litura* on *Caster*. Faizal *et al*. (1994) reported that spore suspension of *F. pallidoroseum* in water was superior to wettable powder formulation with diatomaceous earth as inert material causing equal mortality in 4 days. Dust formulation of spores using talc was the least effective.

2.7 Storage of the fungal biocides

There are a number of species of fungi which are capable of infecting insects. In spite of their potential as agents of insect control, use of fungi based biopesticides on commercial scale is limited. This is primarily due to the problems of large scale production, poor stability and lack of effective delivery system.

Chen *et al.* (1990) observed that the entomogenous fungus *Paecilomyces cicadae* isolated from the cicadid, *Platylomia pieli* showed high pathogenicity to crucifer pest *P. rapae*. The pathogenicity of the fungus was maintained after storage for one year at room temperature. *B. bassiana* formulated as wettable powder when stored for about 8 months at 10-20°C gave a spore germination rate of more than 85 per cent (Zhang *et al.* 1992).

Tverdigukov *et al.* (1993) formulated *B.bassiana* commercially as Boverin for large scale use and long term storage. This was effective in controlling green house white fly, thrips, colorado potato beetle and codling moth of apple. Studies conducted by Li *et al.* (1993) found that *Erynia radicans* and *E. neoaphidis* could be stored at 4°C for several weeks, but were largely destroyed by freezing or milling.

Faizal *et al.* (1994) formulated the fungus *F. pallidoroseum* as wettable powder with diatomaceous earth and water suspension. Water suspension of spores caused 100 per cent mortality of aphids after 3 days of treatment and wettable powder formulation of spores containing diatomaceous earth caused a mortality of 90.25 per cent. Steinhaus (1994) studied the effect of environment on the formulation, survival and germination of primary conidia and capilliconidia of *Neozygites fresenii* and found that semidry cadavers of *A gossypii* could be stored at -15°C. The discharge of conidia from cadavers began

approximately one hour after rehydration. The capilliconidia of this remained infective for 14 days at 75 per cent or 100 per cent RH at 20°C and 25°C. Stirmanova (1994) prepared commercial forms of Boverin from *B. bassiana* and verticillin from *Verticillium sp* with improved shelf life for field use.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Multiplication of cowpea aphid - *Aphis craccivora*

Cowpea plants were raised in 30 cm diameter earthen pots containing sand, soil and cowdung in the ratio of 1:1:1. Cowpea seeds of variety Malika were obtained from Instructional farm, College of Agriculture, Vellayani. The crop was raised following the package of practices recommendations of the Kerala Agricultural University (Anonymous 1993).

One month after planting aphids were inoculated on the tender shoots. Normally they got colonized in 40-50 days. When the plants started drying the aphids were transferred on to fresh plants and thus the culture was maintained.

3.2 Sterilization of glassware

Petridishes, conical flasks and test tubes were kept for 24 h in a cleaning solution containing 60 kg potassium dichromate and 60 ml concentrated sulphuric acid in one litre of water. They were then washed well in tap water, rinsed in distilled water and air dried. They were sterilized in a hot air oven at 160°C for two hours. Pipettes and measuring cylinders were autoclaved at 1.05 kg/m^2 for 20 minutes.

3.3 Preparation of media

Potato Dextrose Agar (PDA) was used for growing *Fusarium pallidoroseum*.

Peeled potato	-	200 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water	-	1 li.

Peeled potato was cut into small bits and cooked in 500 ml water and the extract filtered. To this extract molten agar containing dextrose was added and mixed well and made up to one litre. This was poured into 250 ml conical flasks and closed with cotton plug. The flasks were then autoclaved at 1.05 kg/m^2 for 20 minutes.

3.4 Isolation and maintenance of fungal culture

F. pallidoroseum (Cook) Sacc culture used for the study was obtained from the Insect Pathology laboratory of the College of Agriculture, Vellayani and was maintained on potato dextrose agar medium. The virulence of the fungus was maintained by passing it periodically through cowpea aphids and then isolating the fungus afresh. The spore suspension for inoculating the aphids was prepared by aseptically pouring 5 ml of sterile distilled water into a heavily sporulating 7 day old culture grown in PDA slants. After shaking the tubes for a few min

the suspension containing the spores was taken out and sprayed using an atomizer on aphid colonies maintained on twigs of cowpea. The lower cut end of the twigs was kept dipped in small bottles containing water to prevent drying up of the twigs. Mortality rate of aphids were noted after 24 to 48 h. Fungal growth on the aphids was observed within 2-3 days after death of the insect. They were collected and surface sterilised with 0.1 per cent mercuric chloride for one minute, washed in 3 changes of sterile water and placed on petridishes containing PDA and incubated at room temperature for 3 to 4 days. When the fungal growth was visible, the indentity of the fungus was established by microscopic examination. The mycelial tips were then subcultured and maintained in slants for further studies.

3.5 Mass culturing of pathogen in the laboratory

For mass multiplication of the fungus fresh insect free rice bran was used, as it was observed as the cheapest and most suitable substrate for large scale multiplication (Faizal 1992).

Rice bran medium was prepared by autoclaving slightly moistened rice bran (30 g) kept in 250 ml conical flasks. Inoculation of the medium was done by using a pure culture of *F. pallidoroseum* maintained in PDA slants. The flasks were shaken well for a few minutes to mix the inoculum with rice bran and incubated at room temperature. White fluffy growth of the fungus

was observed within 24-28 h, after inoculation. This was kept for 7 days for maximum growth and sporulation. For preparing spore suspension 100 ml of tap water was mixed thoroughly with a 7 day old culture of the fungus. A suspension so obtained was filtered through a muslin cloth and the spore load of solution was estimated using haemocytometer.

3.6 Formulation of the fungal spores

a) Water suspension

Spore suspension of *F. pallidoroseum* was prepared from the mass culture media and concentrated by centrifuging the spore suspension at 5000 rpm for 15 min in a refrigerated centrifuge. The temperature inside the centrifuge was maintained at 10°C. Supernatent fluid obtained after centrifugation was drained off and the spore count in the sediment was estimated using haemocytometer. The sediment was then diluted with water to get the following spore count per ml. The spore load was fixed based on the work of Hareendranath (1989).

1. 7×10^6 spores/ml
2. 3.5×10^6 spores/ml
3. 1.75×10^6 spores/ml
4. 0.875×10^6 spores/ml

b) Wettable powder

The diatomaceous earth wettable powder of the spores of *F. pallidoroseum* was prepared by adding 20 g diatomaceous earth(60µm) to the spore concentrate of 7 x 10^7 spores/ml required to give 1000 ml spray fluid. To this required quantities of water was added to give the above mentioned dilutions.

3.7 Efficacy of different formulations and doses of *F. pallidoroseum* spores against pea aphid under field conditions

A field experiment was conducted to find out the efficacy of *F. pallidoroseum* spore formulation at different concentrations against pea aphid *A. craccivora*. The cowpea variety Malika obtained from Instructional farm, College of Agriculture, Vellayani was used as the host plant.

The crop was raised in 3 cents of land in the Instructional farm, following the package of practices recommendations of the Kerala Agricultural University (1993). The experiment was laid out in randomised block design with 10 treatments with 3 replications. The treatments were as follows.

Treatment No.	Concentration of spore suspension in the spray fluid
T_1 (Spore suspension in water)	7×10^6 spores/ml
T_2 (Spore suspension in water)	3.5×10^6 spores/ml
T_3 (Spore suspension in water)	1.75×10^6 spores/ml
T_4 (Spore suspension in water)	0.875×10^6 spores/ml
T_5 (Wettable powder formulation)	7×10^6 spores/ml
T_6 (Wettable powder formulation)	3.5×10^6 spores/ml
T_7 (Wettable powder formulation)	1.75×10^6 spores/ml
T_8 (Wettable powder formulation)	0.875×10^6 spores/ml
T_9 Absolute control	Water spray
T_{10} Insecticidal control	Quinalphos 0.05% spray

The experiment was conducted in two seasons viz. April to July 1994 and September to December 1994. Peak population density of aphid in cowpea was noticed 7-8 weeks after planting. Pretreatment counts of aphids were taken and the different formulations were sprayed during the evening. To provide adequate humidity in the environment, the plants were irrigated just before spraying. Teepol was added to the formulations as adhesive at the rate of 1 ml/litre. Spraying was done using hand sprayer. To avoid spray drift contamination, cotton cloth screens were used. After spraying observations on the number of aphids were taken at 4 days interval, till the final harvest

(32 days). The percentage mortality was calculated by using the formula.

$$\frac{\text{Pre treatment count - Post treatment count}}{\text{Pre-treatment count}} \times 100$$

3.8 Assessment of population density of *A. craccivora*

The pre treatment counts of aphids were taken following the sampling technique illustrated by Banks (1952). The aphid population was grouped into 5 classes.

Class	Description
1. Zero (N)	Where there was no aphid present
2. Very light (VL)	Where there were one aphid to a small colony of some scattered individuals confined to young leaves.
3. Light (L)	Where there were scattered aphid colonies present on the stem and leaves and not confined to crown and upper leaves.
4. Medium (M)	Aphid present in large numbers not in recognizable colonies but difused and infesting a large portion of the stem and leaves.
5. Heavy (H)	Very large number, very dense, infesting all leaves and stem. Stem usually black with aphids.

Mean number of aphids in each class was estimated by counting ten samples using standard random number technique. The samples were picked up from the field carefully with out causing any disturbance to aphid colonies and kept in alcohol. Aphids were dislodged by slow agitation. A small portion was transferred to a petridish using an aspirator. A graph paper was placed at the bottom of the petridish. When all the aphids were seen settled excess alcohol was carefully drained off and 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.

Average number of aphids/class

SEASON I - April-July

Class	Number of aphids/sample										Mean number of aphids class
	1	2	3	4	5	6	7	8	9	10	
VL	14	11	7	9	3	6	12	4	8	1	7.5
L	35	42	33	56	64	44	46	38	53	64	47.5
M	108	141	137	150	114	111	138	129	144	158	133.0
H	324	381	362	333	358	364	346	371	313	328	348.0

SEASON II - September-December

VL	11	7	14	2	5	3	11	6	9	13	6.2
L	56	44	91	72	89	93	65	33	67	52	66.2
M	191	246	150	152	231	174	202	192	163	220	191.5
H	365	414	505	543	465	477	468	590	440	528	478.6

Aphid counting was done by this method

3.9 Pot culture studies

Pot culture studies were conducted to find out the efficacy of *F. pallidoroseum* in controlling *Aphis craccivora* attacking different host plants. The best treatment selected from the two field experiments viz. the spore suspension having 7 x 10^6 spores/m concentration was used for pot culture studies. The following crops were used for the study.

1. Grain cowpea (*Vigna unguiculata*) (Variety, Charodi from Department of Plant Breeding, College of Agriculture, Vellayani).
2. Green gram (*Vigna radiata*) (Variety CO_5, from Agriculture College and Research Institute, Madurai)
3. Balck gram (*Vigna mungo*) (Variety CO_4 from Agriculture College and Research Institute, Madurai)
4. Cluster bean (*Cyamopsis tetragonoloba*) (Local variety from Instructional farm, College of Agriculture, Vellayani).

Treatments - 5 including one control

Replications - 6

Pots (30 cm^2 diameter) were filled with potting mixture containing sand, top soil and cowdung in the ratio 1:1:1. Seeds were sown and watered daily. Aphids got colonized by about six weeks after seeding. Spraying was done 45 days after seeding and observations were taken at 2, 3 and 4 days interval.

3.10 Effect of storage temperature on viability and infectivity of spores

The viability of spores stored at refrigerated conditions and at room temperature (22.0 (min) to 32.6°C (max) were estimated at monthly intervals for a period of 10 months. The following formulations were used for the study.

1. 7×10^6 spores/ml water suspension at room temperature
2. 7×10^6 spores/ml water suspension at 4°C.
3. 7×10^6 spores/ml Diatomaceous earth wettable powder at room temperature (spore count obtained after diluting the diatomaceous earth WP with water at the time of observation.
4. 7×10^6 spores/ml diatomaceous earth at 4°C mixed with spores and spore count obtained after diluting the diatamaceous earth WP with water at the time of observation.

The treatments were replicated 6 times. At monthly intervals, 1 ml spore suspension and 1 g of diatomaceous earth were taken and sprayed on to the adult wingless aphids. Adequate quantities of water was added in diatomaceous earth formulation so as to get the required spore count. It was then sprayed on to the adult wingless aphids (20 in number) kept on tender pods in sterile petridishes. The mortality of aphids was recorded 4 days after spraying. Simultaneously, a small drop of the spore suspension was inoculated on PDA slants. Seven days after

inoculation spore count was estimated using haemocytometer. The effect of storage under different conditions on the growth of the fungus was studied by recording the growth rate of the fungus on PDA. For this a drop of the spore suspension was placed at the centre of PDA in 9 cm petridish and the growth of the fungus was recorded at the end of 7 days.

3.11 Pathogenicity of *F. pallidoroseum* to different crop plants

Pathogenicity of the fungus to the following crop plants were tested by leaf and soil inoculation methods. Pathogenicity of the fungus in the crop plants was recorded for a period of three months. The following plants were used for the study.

Vegetables:

Okra	-	*Abelmoschus esculentus*
Brinjal	-	*Solanum melongena*
Amaranthus	-	*Amaranthus* sp
Tomato	-	*Lycopersicum esculentum*
Chillies	-	*Capsicum* sp
Snake gourd	-	*Trichosanthes anguina*
Bitter gourd	-	*Momordica charantia*

Medicinal plants:

Adathoda	-	*Adathoda vasaka*
Ocimum	-	*Ocimum sanctum*
Notchi	-	*Vitex nigundo*

i) Soil inoculation

The planting was done on earthern pots 30 cm diameter filled with potting mixture. The top 10 cm of the soil in earthern pots was mixed with 250 ml of spore suspension (7×10^6/ml) of *F. pallidoroseum* and the seeds/cuttings of the test plants were planted immediately ater inoculation. Soil with out the fungal inoculant served as the control. All the package of practices recommendations were conducted.

ii) Leaf inoculation

Healthy leaves of the test plants were used for leaf inoculation. Spore suspension of the fungus was appplied on the leaf surface after making pin prick injury. A moist cotton wool was placed on the inoculated area and leaves were covered with polythene bags to supply high humidity. Uninoculated plants served as the control. Three leaves per plant were inoculated and there were three replications for each crop.

3.12 Pathogenicity of *F. pallidoroseum* on the natural enemeies of *A. craccivora*

The pathogenicity of *F. pallidoroseum* was tested on the following natural enemies of *A. craccivora*.

1. *Coccinella septumpunctata* - Lady bird beetle (Coccinellidae)
2. *Menochilus sexmaculaus* - Lady bird beetle (Coccinellidae)
3. *Scymnus* sp. - Lady bird beetle (Coccinellidae)

Second instar grubs of coccinellids reared in the laboratory were used for the experiment ten grubs of each species were taken in a petridish and sprayed with spore suspension containing 7 x 10^6 spores per ml. using an atomiser. After spraying each treated grub was reared individually in specimen tubes, till adulthood and provided with fresh aphid cultures daily and grubs were observed for occurrence of fungal infection.

3.13 Pathogenicity of *F. pallidoroseum* on beneficial insects

1. Honey bees (*Apis mellifera*)

A total of 15 adult worker bees were sprayed with the spore formulation containing 7 x 10^6 spores/ml. After spraying the bees were kept in separate specimen tubes and cotton wool soaked in sugar syrup was provided occasionally as food. The bees were observed for a period of 2 weeks. The dead bees were surface sterilized and inoculated in PDA slants.

Silkworm (*Bombyx mori*)

Second and fourth instar larvae were used for this experiment. Twenty larvae each of second and fourth instars were sprayed with the spore suspension and kept in separate petridishes having four larvae each and provided with chopped mulberry leaves as food. They were observed till adult emergence for fungal infection.

3.14 Pathogenicity of *F. pallidoroseum* to other species of aphids

Three species of aphids viz. *Toxoptera aurantii*, *A. gossypii* and *A. malvae* were used for the study. Aphids colonized on tender pods of cowpea were sprayed with the spore suspension prepared from fresh cultures containing $7x10^6$ spores/ml and the twigs were kept in small bottles containing water. They were observed for about ten days.

3.15 Pathogenicity of a strain of *F. pallidoroseum* isolated from *Eichhornia crassipes* to aphids

Spore suspension prepared from a culture tube of *F. pallidoroseum* isolated from water hyacinth was sprayed on to the aphids colonised on tender twigs of cowpea, the lower end of which was kept in water. The aphids were watched daily for the occurrence of any disease. Naturally dead ones were surface sterilized and incubated on culture tubes.

The water hyacinth plants were inoculated with the spore suspension of *F. pallidoroseum* (Cooke) Sacc under laboratory conditions to find out whether this fungus is pathogenic to this weed plant. Pin pricks were made on the leaves and the spots were covered with cotton wool soaked in spore suspension. The leaves were then covered with polythene bags to prevent drying of the spots. They were observed daily for a period of 10 days.

RESULTS

4. RESULTS

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

4.1 Symptomatology

The affected aphids turned pale and showed mild tremor, later developed a brownish black discolouration, death occurred within 24-72h of inoculation. The cadaver was hard and mummified and was seen adhering to the plant covered with white mycelial growth of the fungus. Growth of the mycelium over the cadaver was observed after 24-48 h of death (Plate 1). The fungus produced small spherical or oval microconidia and large sickle shaped multiseptate macroconidia (Plate 2). Mycelium of fusarium is septate and white in colour, and the hyphae elongated and branched (Plate 3).

4.2 Effect of different formulations and doses of *F. pallidoroseum* on the percentage reduction of aphids, at different intervals

Influence of different formulations and doses of *F. pallidoroseum* on *A. craccivora* during April-July (Season I) is given in Table-1 (Appendix-1). Water suspension of the fungus at

Plate 1 *Fusarium pallidoroseum* growing on the cadavers of cowpea aphid.

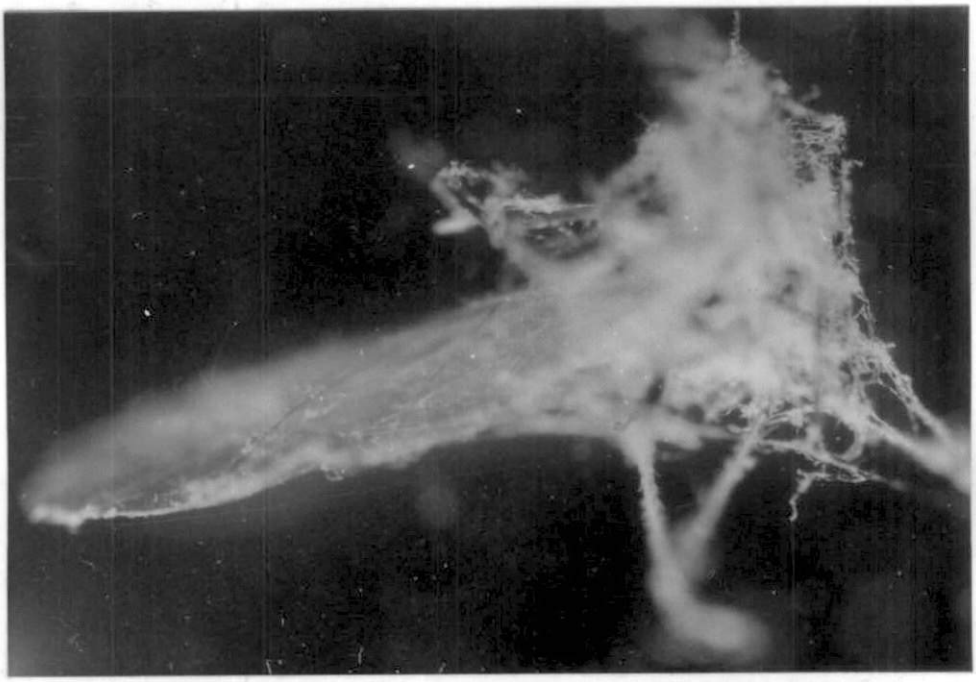


PLATE - I

Plate 2 Conidia of *Fusarium pallidoroseum*.

Plate 3 Mycelium of *Fusarium pallidoroseum*.

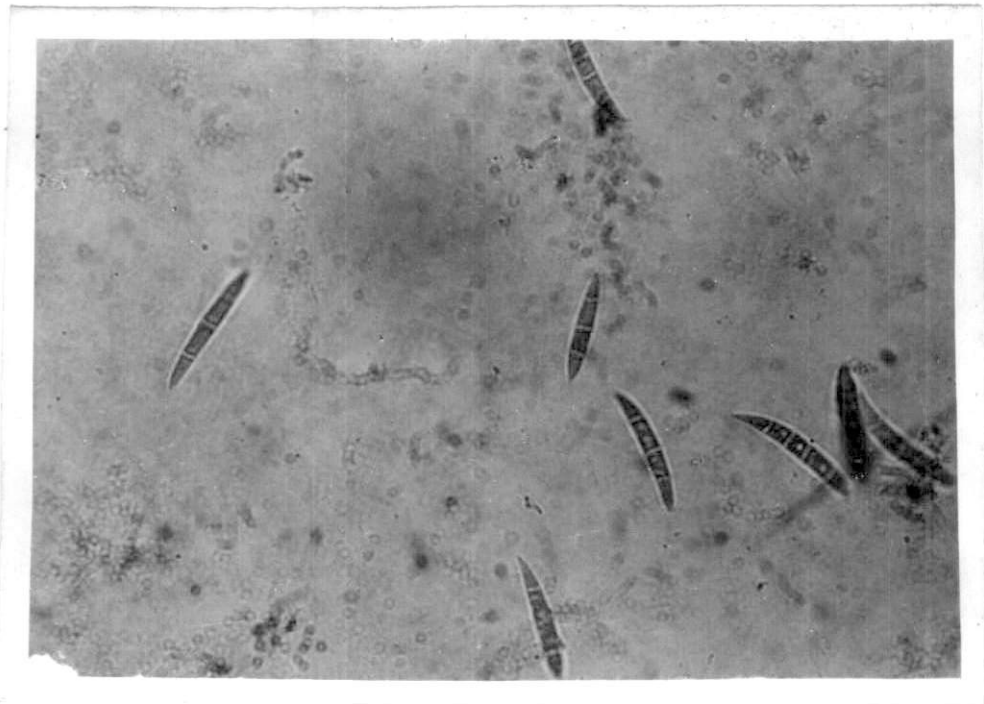


PLATE - 2

PLATE - 3

Plate 4 *Aphis craccivora* killed by *Fusarium pallidoroseum* remaining attached to vegetable cowpea.

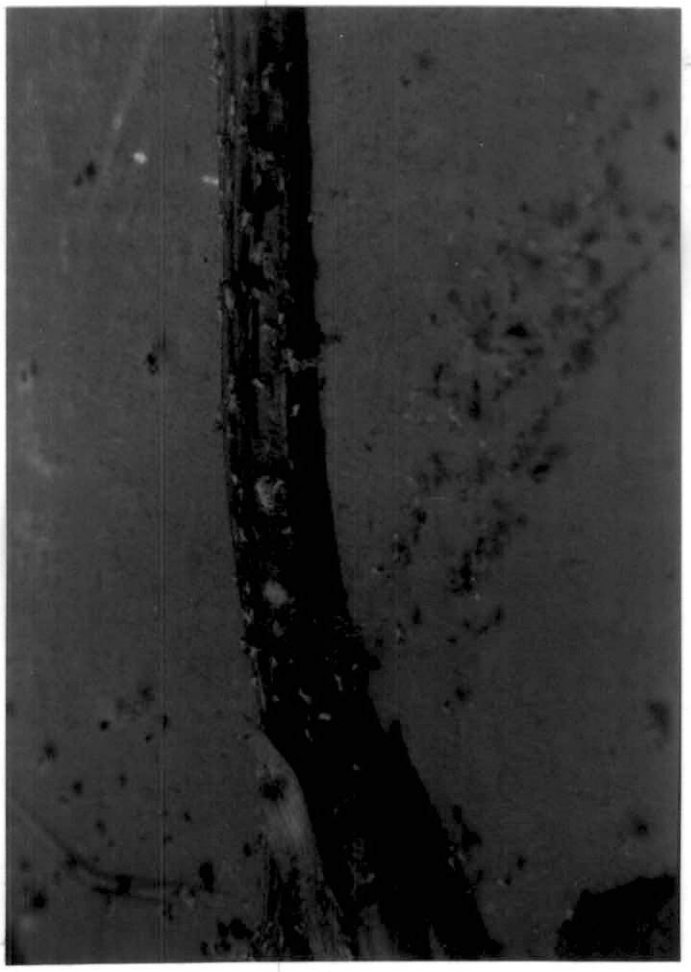


PLATE 4

the highest concentration (T_1) reduced the aphid population by 94.14 per cent at the end of 4 days after treatment and 98.4 per cent at the end of 8 days and 100 per cent mortality of the aphids was noticed after 12 days. But as the spore concentration decreased from $7x10^6$ to $35x10^6$ the period taken for cent per cent death increased from 12 days in treatment T_1 ($7x10^6$ spores/ml water suspension) to 24 days in T_2 ($3.5x10^6$ spores/ml). In treatment T_2 only 67.47 per cent reduction in aphid population was noticed at the end of 4 days. A further decrease in spore concentration to $1.75x10^6$ delayed its inhibitory effect on the aphid population and at the end of 4 days there was a marginal increase in the population of aphids (1.63%). Even at the end of 8 days, only 21.49 per cent of aphids were killed as a result of treatment; However, the death rate of the aphids gradually increased and cent per cent aphids were killed on 32nd day after treatment. A similar trend was noticed in treatment number 4, where the concentration was only $0.875x10^6$. In this treatment, an increased population count of aphids, over pretreatment count. was observed up to 8 days after treatment (79.74%). Even at the end of 32 DAT *F. pallidoroseum* at this concentration, could inhibit only 91.58 per cent of population of aphids. When wettable powder formulation using diatomaceous earth as carrier was used in the field, the result obtained was almost similar to that observed when a water suspension of the fungus was used. When the highest concentration of spores in diatomaceous earth

(7 x 10^6 spores/ml) was used, 98 per cent of aphids were killed at the end of 4 days and 100 per cent in 16 days. At a lower concentration of $3.5x10^6$ spore load the inhibition was only 39.42 and 99.6 per cent at the end of 4 days and 20 days respectively. None of the aphids survived after 20 days. A similar trend was noticed with a spore concentration of 1.75 x 10^6. However, in this case cent per cent mortality of aphids was recorded only in 28 days after treatment. A further reduction in spore load $0.875x10^6$, increased the aphid population upto 8 DAT. But from 12th day onwards (75.37%) the population of aphids gradually reduced and at the end of 32 DAT 97.06 per cent of aphids were infected.

When water was sprayed on aphid infected plants a reduction of 98.47 per cent was noticed at 4 DAT. However the population of aphids gradually increased and at the end of 20 DAT, the population recorded was 54.64 per cent more than that noticed before treatment. During subsequent days also, the population recorded was more than all the treatments receiving fungal inoculum. Even at the end of 32 days, 20.67 per cent of the aphids were noticed on the water sprayed plants. The pattern of population fluctuation of aphids on plants sprayed with quinalphos was different from that of all other treatments. Here a cent per cent mortality of the aphids was observed 4 days after treatment. However the aphid multiplied gradually and at

Table 1 Effect of different formulations and doses of *F. pallidoroseum* on the percentage reduction of aphids at different intervals (April to July) Season I (Mean of 3 replications)

Treatments	Percentage reduction of aphids as compared to pretreatment							
	4 DAT	8 DAT	12 DAT	16 DAT	20 DAT	24 DAT	28 DAT	32 DAT
T_1 7×10^6 spores/ml water suspension	94.14	98.40	100.00	100.00	100.00	100.00	100.00	100.00
T_2 3.5×10^6 spores/ml water suspension	67.47	91.40	94.48	87.22	99.73	100.00	100.00	100.00
T_3 1.75×10^6 spores/ml water suspension	-101.63*	21.49	88.90	83.96	85.49	96.17	99.73	100.00
T_4 0.875×10^6 spores/ml water suspension	-121.21*	-179.74*	71.83	84.23	50.29	75.24	82.58	91.58
T_5 7×10^6 spores/ml spore suspension with diatomaceous earth	98.15	98.15	99.70	100.00	100.00	100.00	100.00	100.00
T_6 3.5×10^6 spores/ml spore suspension with diatomaceous earth	39.42	76.29	99.60	98.52	99.60	100.00	100.00	100.00
T_7 1.75×10^6 spores/ml spore suspension with diatomaceous earth	44.23	65.92	98.67	97.25	94.49	99.46	100.00	100.00
T_8 0.875×10^6 spores/ml spore suspension with diatomaceous earth	-100.0013*	-116.44*	75.37	82.19	78.61	86.61	90.43	97.06
T_9 Water spray	98.47	82.43	66.61	6.92	-154.64*	64.51	60.74	79.33
T_{10} Quinalphos 0.05%	100.00	99.12	32.96	-189.51*	-191.43*	42.99	39.99	68.80

DAT - days after treatment

* -ve sign indicate increase in the number of aphids than pretreatment

Fig.1 Effect of different doses of spores in water suspension of F.pallidoroseum on mortality of aphids.(Season I)

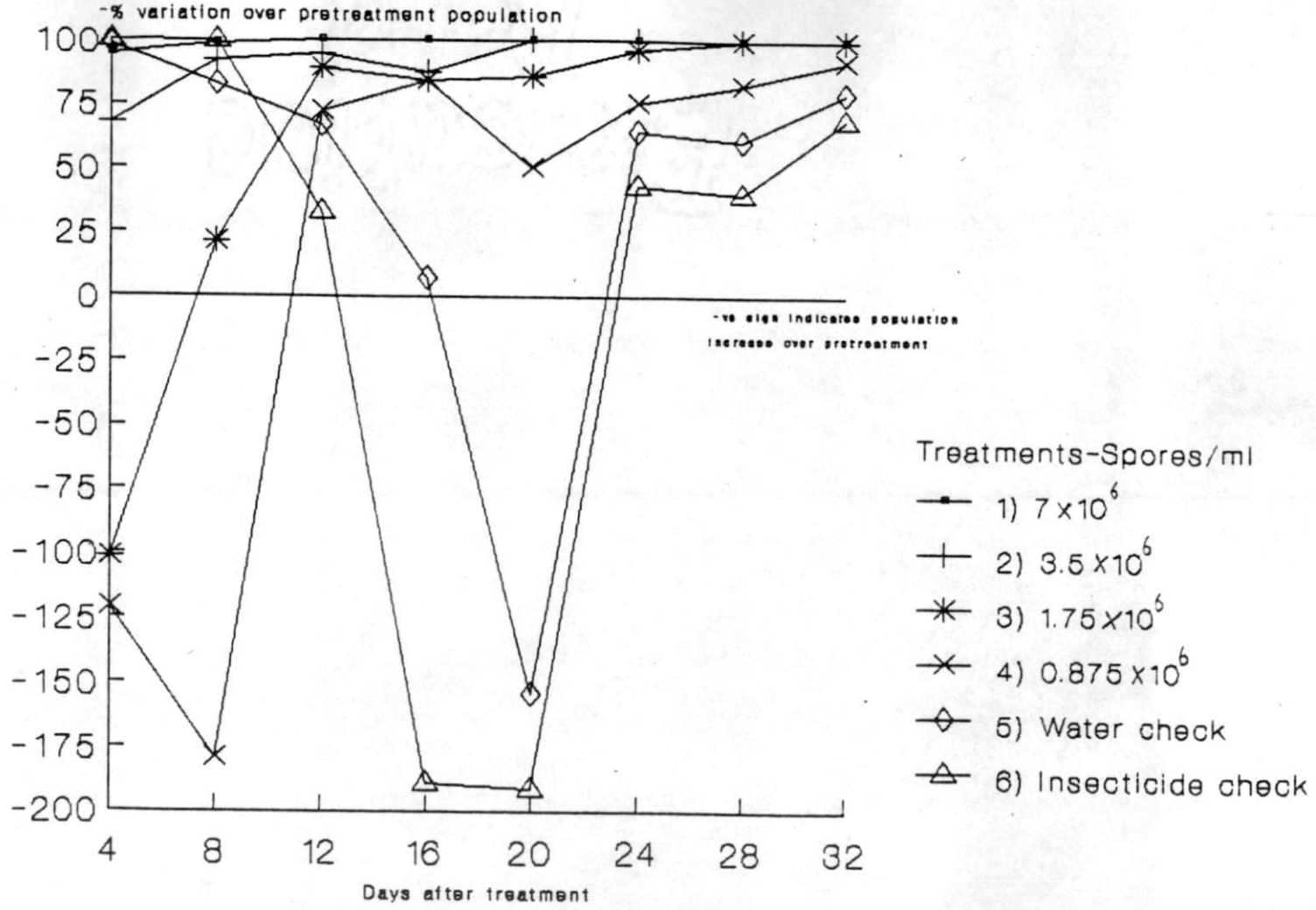
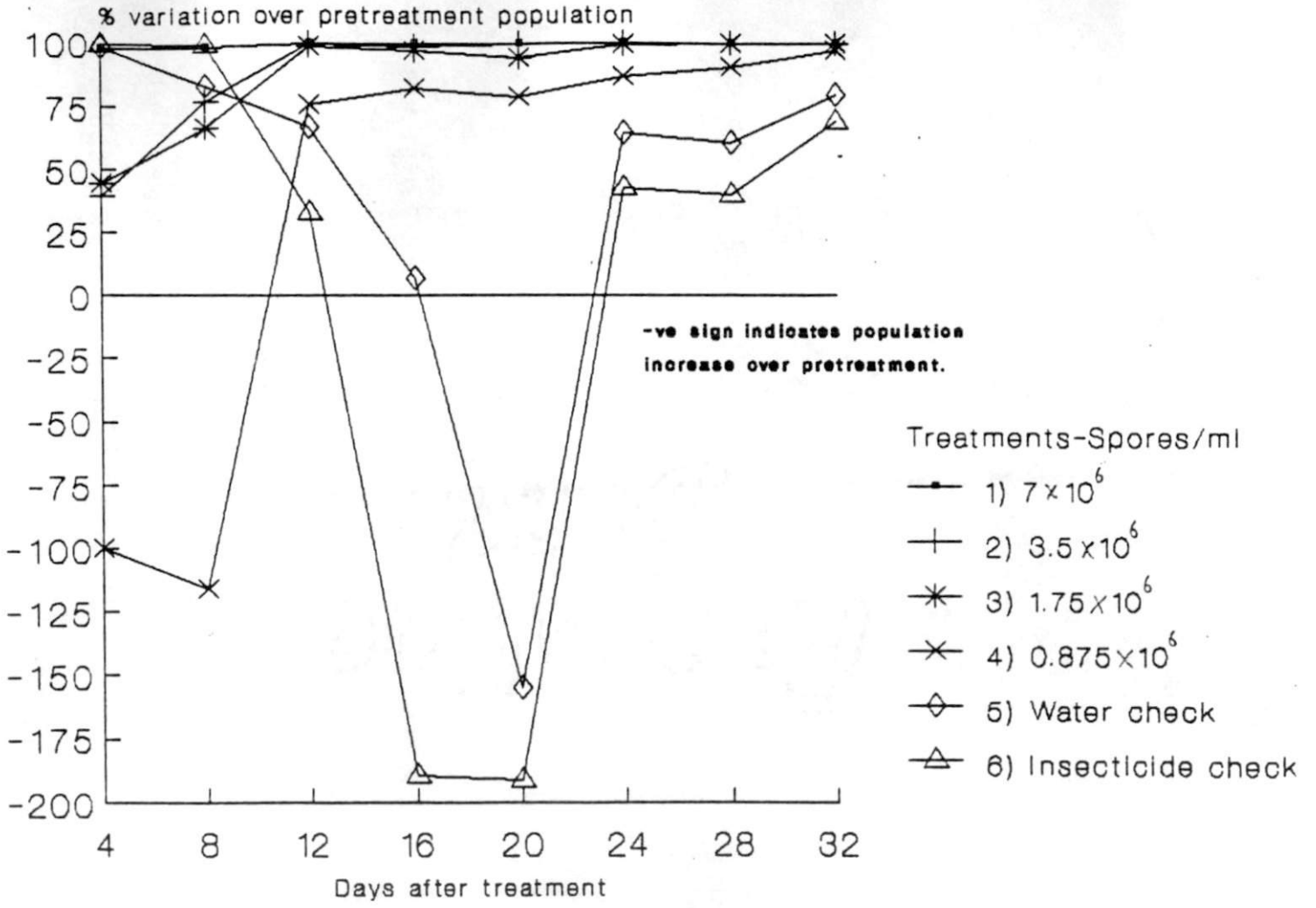


Fig.2 Effect of different doses of wettable formulations of F.pallidoroseum on mortality of aphids.(Season I).



the end of 16 DAT 89 per cent more aphids were recorded compared to the pretreatment figures. The population further increased and by the end of 20 days it was 91.43 per cent more than the original value. Even at the end of 32 DAT, there was only 68.8 per cent reduction in the population of aphids which was even less than the reduction noticed in the plants received water spray.

During the second season (September to December) the highest concentration of spores in water reduced the aphid population by 91.22 and 95.59 percentages respectively on 4^{th} and 8^{th} DAT. There after cent per cent control was observed and there was no further build up of aphids. As was observed in the first season during this season also a reduction in the spore concentration from $7x10^6$ spores/ml (T_1) to $3.5x10^6$ spores/ml (T_2), the time taken to reach cent per cent mortality increased (12 to 16 days). In this case the percentage control obtained from 4^{th} to 12 DAT were 80.91, 88.59 and 99.76 percentages respectively. As the spore concentration was decreased to 1.75 x 10^6 spores/ml there was a delay in the inhibition of aphids and even at the end of 32 DAT the percentage reduction was only 92.51. However more than 90 per cent inhibition of the aphids was obtained from 20 DAT. Almost a similar trend with a reduced aphid inhibition was noticed in treatment with the least spore count ($0.875x10^6$ spores/ml). In this treatment more than 75 per cent of the aphids were alive at

4 DAT and even at the end of 32 days, 30 per cent of the aphids were alive.

The inhibitory effect of *F. pallidoroseum* when formulated as wettable powder using diatomaceous earth was in general not as effective as that of water suspension at higher concentrations (T_5 and T_6) ($7x10^6$ and $3.5x10^6$ spores/ml), but the general trend of inhibition was similar to that of water suspension. At the highest concentration T_5 ($7x10^6$ spores/ml wp) even though the inhibition was only 77.48 per cent compared to 91.22 in water suspension, during subsequent periods the inhibitory nature of this treatment was very effective and cent per cent inhibition was noticed from 12 DAT as in the case of water suspension. At a spore concentration of 3.5×10^6, even though more than 90 per cent inhibition was noticed from, 8th day after treatment, at the end of 32 DAT 2.42 per cent of the aphids survived on the plant compared to cent per cent inhibition from 16th DAT in the corresponding spore concentration in water suspension. At a concentration of $1.75x10^6$ spores/ml, the inhibition at the end of 4 DAT was 68.96, compared to 51.54 in water suspension. A similar increased inhibition was noticed in this treatment, compared to that of water spray and at the end of 32 DAT only 0.41 per cent aphids survived compared to 7.49 per cent in water suspension. Almost a similar trend was recorded at a lower concentration of $0.875x10^6$ spores/ml. In this treatment, the inhibition was 38.54, during 4th DAT and there was a gradual

Table 2 Effect of different formulations and doses on the percentage reduction of aphids at different intervals September to December. Season II (Mean of 3 replications)

Treatments	Percentage reduction of aphids as compared to pretreatment							
	4 DAT	8 DAT	12 DAT	16 DAT	20 DAT	24 DAT	28 DAT	32 DAT
T_1 7×10^6 spores/ml water suspension	91.22	95.59	100.00	100.00	100.00	100.00	100.00	100.00
T_2 3.5×10^6 spores/ml water suspension	80.91	88.59	99.76	100.00	100.00	100.00	100.00	100.00
T_3 1.75×10^6 spores/ml water suspension	51.54	61.98	85.33	88.60	94.24	94.26	93.94	92.51
T_4 0.875×10^6 spores/ml water suspension	24.03	32.59	50.68	49.98	52.72	55.88	55.73	70.04
T_5 7×10^6 spores/ml spore suspension with diatomaceous earth	77.48	99.62	100.00	100.00	100.00	100.00	100.00	100.00
T_6 3.5×10^6 spores/ml spore suspension with diatomaceous earth	82.20	98.65	99.55	97.58	97.58	93.00	93.00	97.58
T_7 1.75×10^6 spores/ml spore suspension with diatomaceous earth	68.96	85.10	94.13	88.37	90.53	95.02	97.40	99.59
T_8 0.875×10^6 spores/ml spore suspension with diatomaceous earth	38.54	52.02	46.42	80.01	85.59	74.12	83.69	95.45
T_9 Water spray	89.87	77.03	43.63	57.45	47.47	27.43	9.68	51.30
T_{10} Quinalphos 0.05%	97.71	96.80	89.59	75.41	30.27	-109.36*	-132.74*	-116.83*

DAT - days after treatment
* -ve sign indicate increase in the number of aphids than pretreatment

Fig.3 Effect of different doses of spores in water suspension of F.palllidoroseum on mortality of aphids.(Season II)

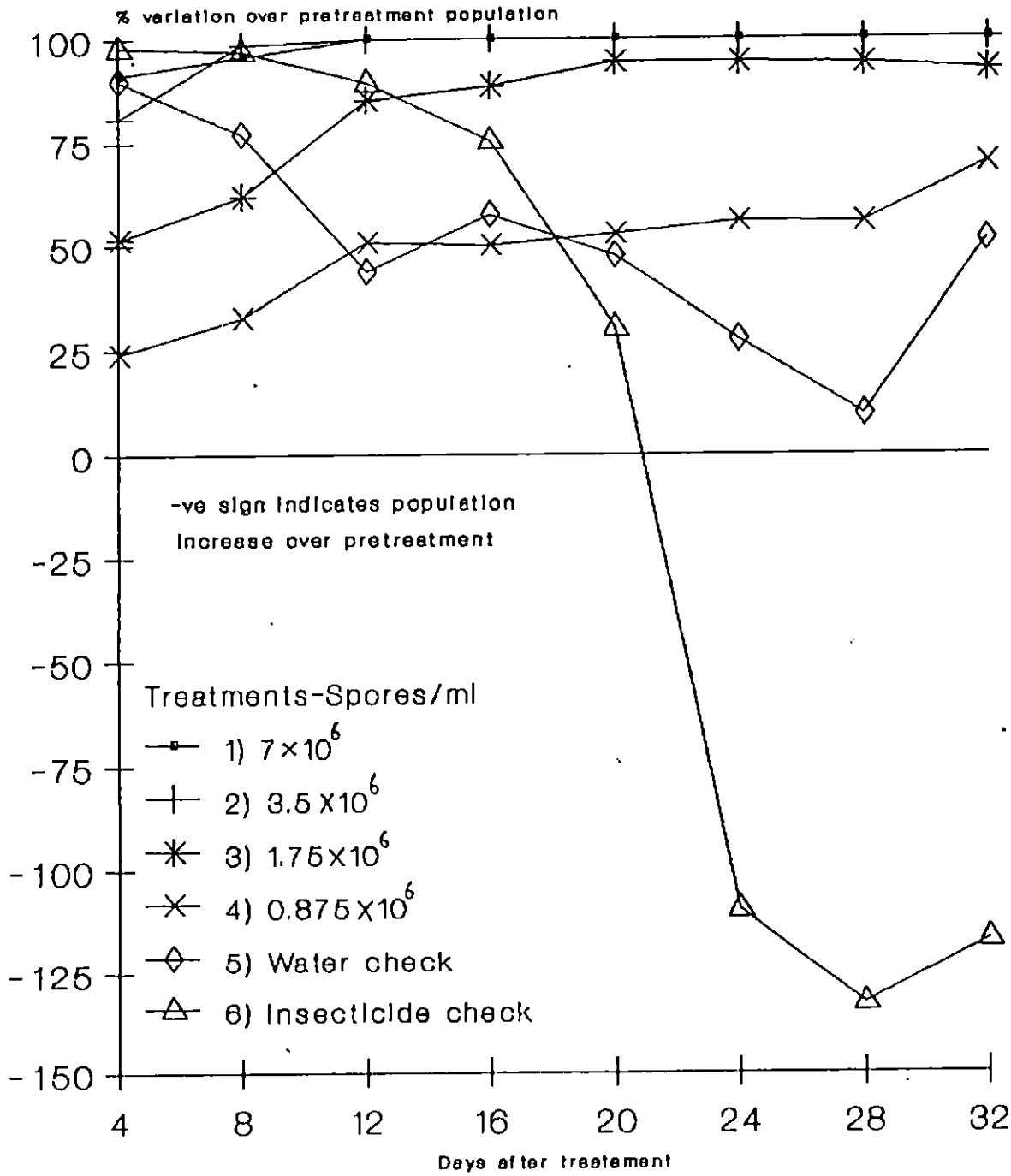


Fig. 4 Effect of different doses of wettable powder formulation of F.pallidoroseum on mortality of aphids. (Season II)

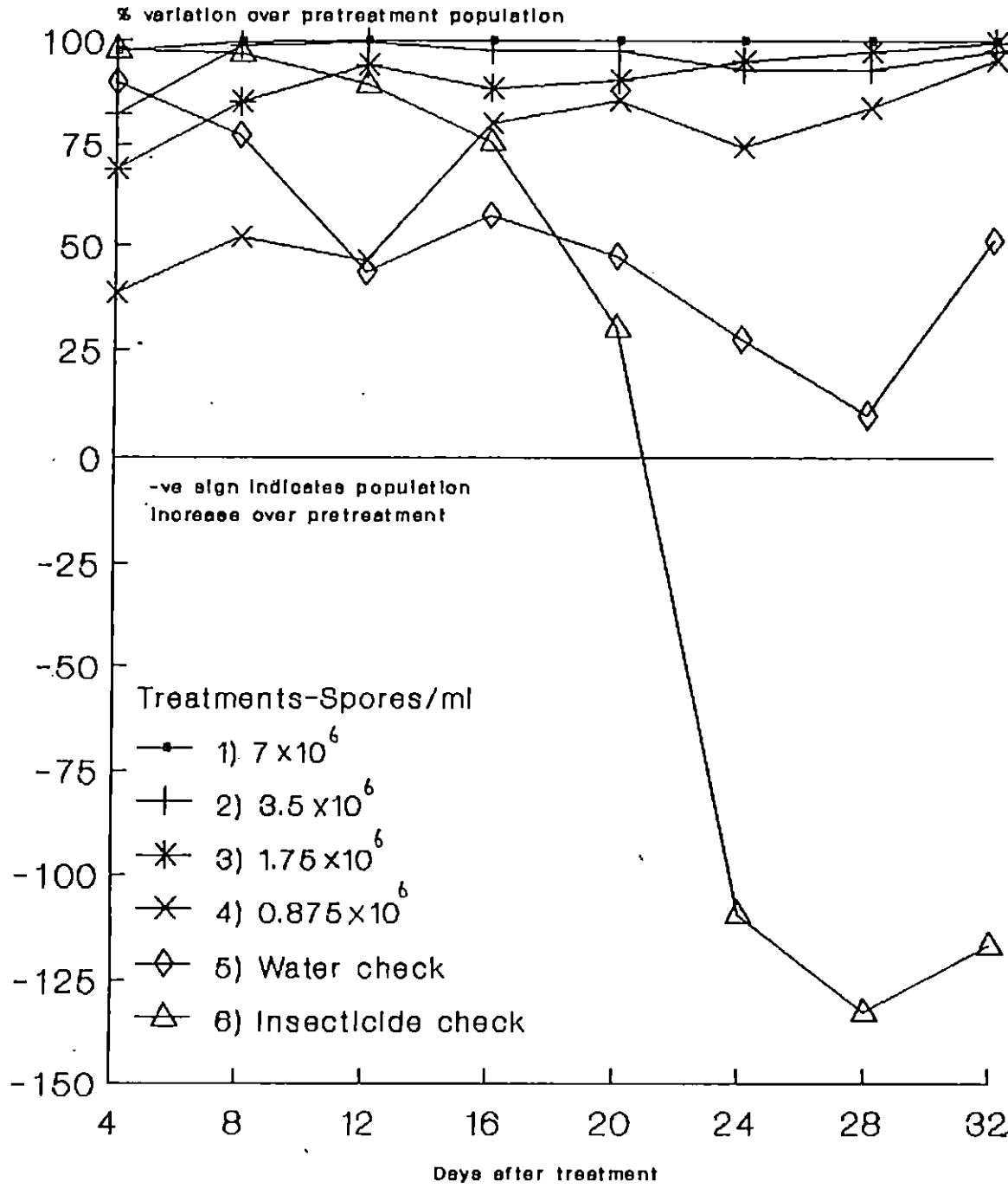


Table 3 Effect of different formulations and doses on the percentage reduction of aphids at different intervals (Pooled mean of two seasons)

Treatments	4 DAT	8 DAT	12 DAT	16 DAT	20 DAT	24 DAT	28 DAT	32 DAT
T_1 7×10^6 spores/ml water suspension	92.68	96.995	100.00	100.00	100.00	100.00	100.00	100.00
T_2 3.5×10^6 spores/ml water suspension	74.19	89.90	97.12	93.61	99.87	100.00	100.00	100.00
T_3 1.75×10^6 spores/ml water suspension	76.59	41.74	87.12	86.28	89.87	95.22	96.84	96.26
T_4 0.875×10^6 spores/ml water suspension	48.59	73.28	61.26	67.11	51.51	65.56	69.16	80.81
T_5 7×10^6 spores/ml spore suspension with diatomaceous earth	87.82	98.89	99.85	100.00	100.00	100.00	100.00	100.00
T_6 3.5×10^6 spores/ml spore suspension with diatomaceous earth	60.81	87.47	99.58	98.05	98.59	96.50	96.50	98.79
T_7 1.75×10^6 spores/ml spore suspension with diatomaceous earth	56.60	75.51	96.40	92.81	92.51	97.24	98.70	99.80
T_8 0.875×10^6 spores/ml spore suspension with diatomaceous earth	30.73	32.21	60.90	81.10	82.10	80.37	87.06	96.26
T_9 Water spray	94.17	79.73	55.12	32.19	53.59	45.97	33.21	65.32
T_{10} Quinalphos 0.05 %	98.86	97.96	61.28	57.05	80.58	33.19	46.38	24.02

increase during subsequent period and 94.7 per cent of aphids got killed at the end of 32 DAT. While in the corres-ponding water suspension the inhibition was only 70.04 per cent.

Water spray without insecticide and spores, was also inhibitory to the aphids immediately after application (4DAT) where 89.87 per cent reduction in the population of aphids over the initial count, was noticed. As the days advanced, the rate of reduction of aphids also, decreased and 48.70 per cent of aphids survived at the end of 32 DAT. The inhibitory effect of quinalphos was the best at the end of 4 DAT, with 97.71 per cent inhibition. This inhibition was 6 per cent more than the best inhibition noticed using fungal spore suspension in T_1 ($7x10^6$ spores/ml). However, in contrast to the other treatments the aphid population subsequently increased in this treatment. From 8 to 20 DAT the population count was less than the initial count. But, from 24th day onwards, the aphid population increased over the initial count and at 28 and 32 DAT the population counts were more than the pretreatment counts by 32.74 and 16.83 per cent respectively. In none of the treatments including water spray, increase in the aphid population was noticed at any stage of the experiment.

4.2 Effect of different formulations and doses of *F. pallidoroseum* on the yield of cowpea

Management of the aphids through biological means, had a significant effect on the yield of cowpea (Table 4; Fig. 5 and 6).

Table 4 Effect of different spore formulations and doses of *F. pallidoroseus* on the yield of cowpea (g/plant) (mean of three replications)

Treatments		Wt. of pod (g) Season I	Percentage increase over water spray	Wt. of pod (g) Season II	Percentage increase over water spray	Pooled mean	Percentage increase over water spray
T_1	7×10^6 spores/ml water suspension	194.00	24.64	265.22	12.81	229.612	17.41
T_2	3.5×10^6 spores/ml water suspension	210.33	34.83	258.45	9.93	234.39	19.86
T_3	1.75×10^6 spores/ml water suspension	178.11	14.17	252.00	7.18	215.055	9.97
T_4	0.875×10^6 spores/ml water suspension	168.78	8.19	246.34	4.78	207.56	6.14
T_5	7×10^6 spores/ml spore suspension with diatomaceous earth	215.11	34.89	262.56	11.68	238.84	22.13
T_6	3.5×10^6 spores/ml spore suspension with diatomaceous earth	206.78	32.55	254.44	8.22	230.61	17.92
T_7	1.75×10^6 spores/ml spore suspension with diatomaceous earth	174.56	11.89	234.89	0.09	204.73	4.69
T_8	0.875×10^6 spores/ml spore suspension with diatomaceous earth	161.55	3.56	238.00	1.23	199.78	2.16
T_9	Water spray	156.00	0	235.11	0	195.56	0
T_{10}	Quinalphos 0.05%	157.11	0.710	241.11	2.55	199.11	1.82
CD (0.05)		9.86	-	14.25	-	14.033	-

CD for comparing seasons - 6.28 (0.05)

Table 5 Mean yield of pods in different treatments

	I season		II season	
	Number of pods/plant	Wt. of pods g/plant	Number of pods/plant	Wt. of pods g/plant
T_1	22	194.00	33	265.22
T_2	23	210.33	30	258.45
T_3	20	178.11	29	252.00
T_4	21	168.78	29	246.34
T_5	23	215.11	31	262.56
T_6	24	206.78	32	254.44
T_7	20	174.56	27	234.89
T_8	19	161.55	28	238.00
T_9	19	156.00	29	235.11
T_{10}	20	157.11	29	241.11

Fig.(5).Effect of different spore formulations of F.pallidoroseum on the number and weight of pods produced per plant.(Season I).

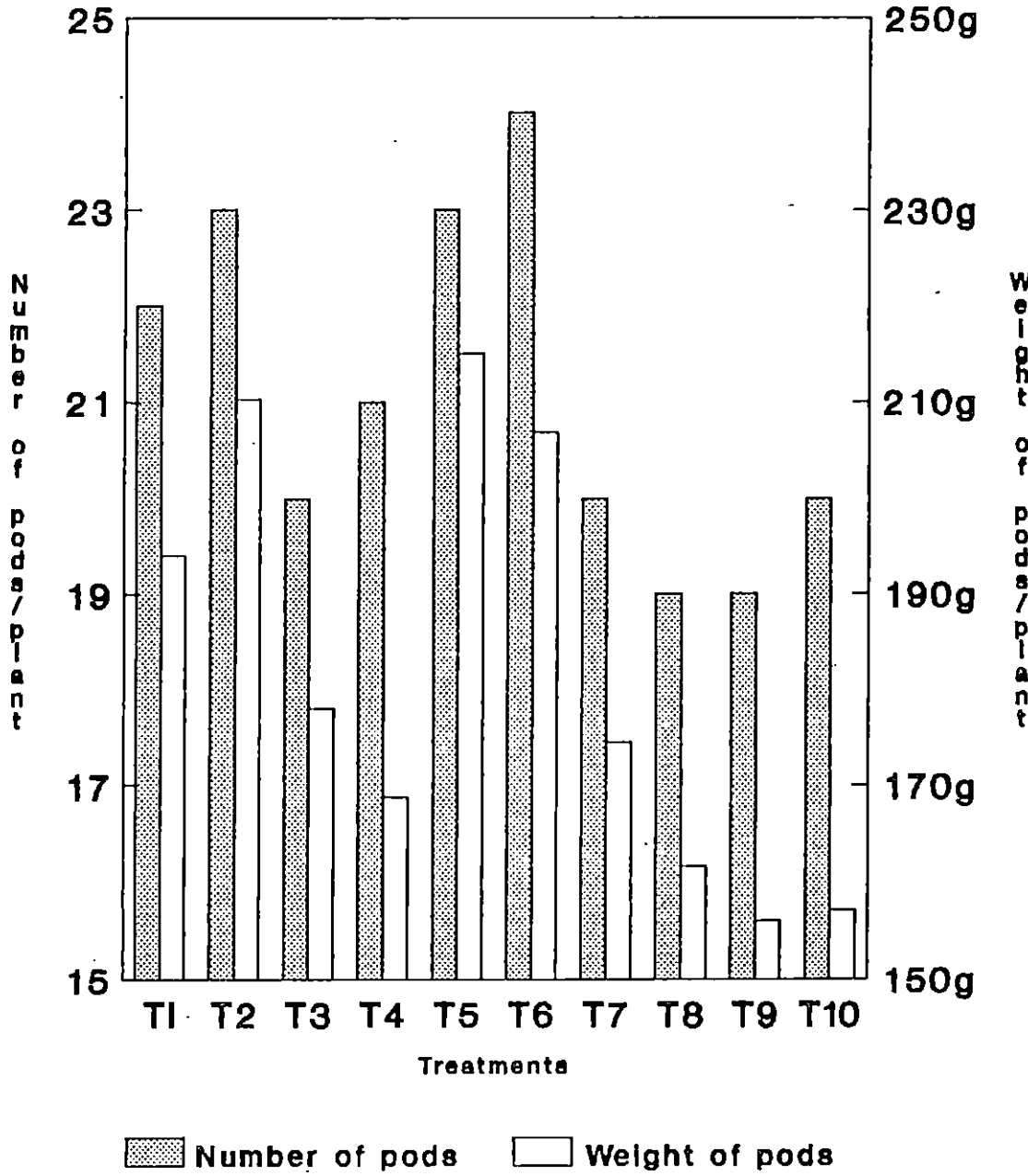
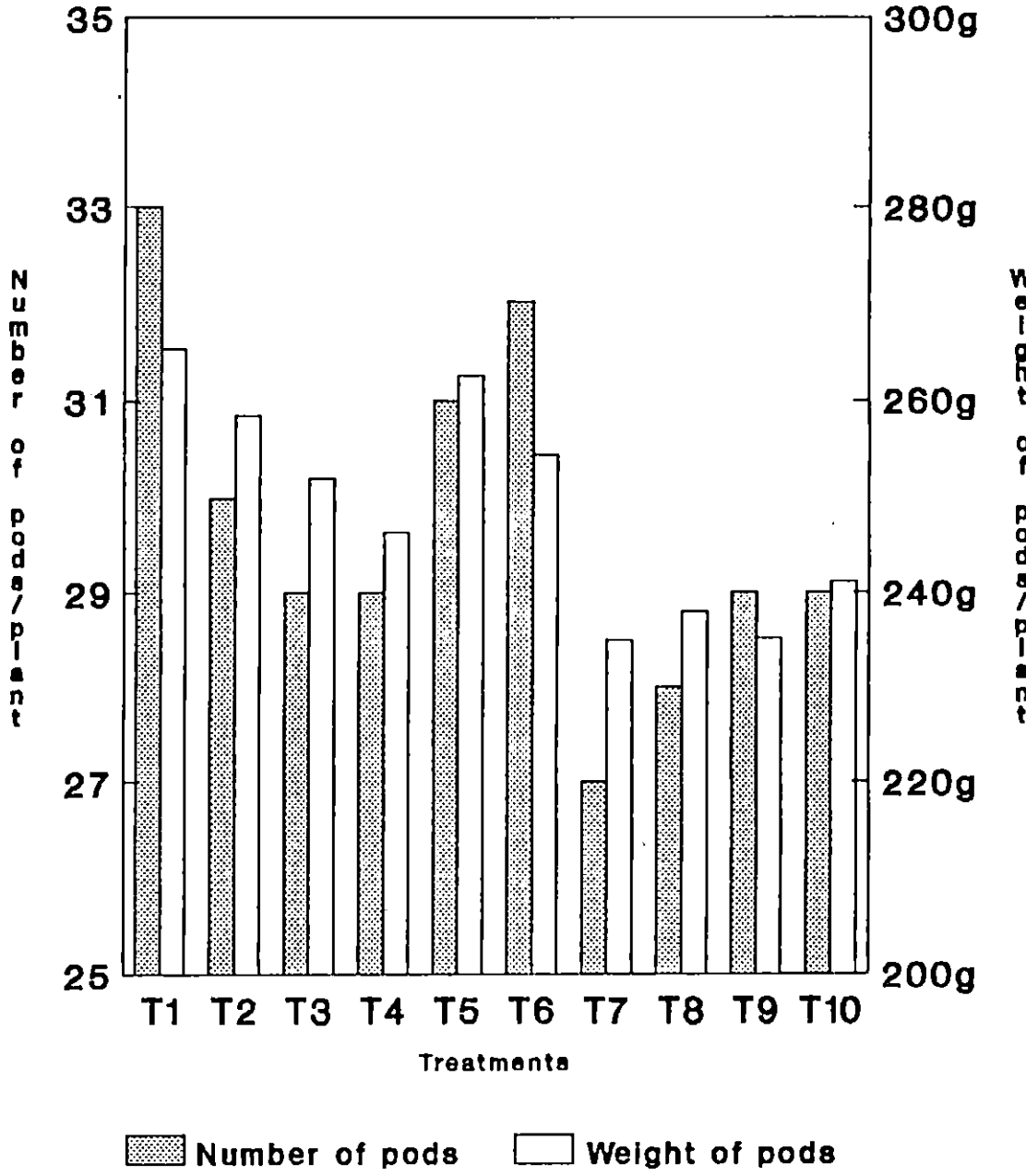


Fig.(6).Effect of different spore formulations of F.pallidoroseum on the number and weight of pods produced per plant.(Season II).



Further a significant influence of season on yield was also observed. The yield of cowpea, in general was low in the first season (April to July) compared to the second season (September to December).

During the first season, the lowest yield of 156 g/plant was noticed in treatment 9 where the plants were sprayed with water alone, which was on par with T_{10} (quinalphos) and T_8 (0.875×10^6 spores/ml/ with diatomaceous earth). All other treatments were significantly better than the control. The highest yield of 215.11 g was obtained in treatment T_5 (7×10^6 spores/ml with diatomaceous earth) which was on par with T_2 (3.5×10^6 spores/ml water suspension) and T_6 (3.5×10^6 spores/ml with diatomaceous earth). The plants which received a spray containing 1.75×10^6 spores/ml without and with diatomaceous earth (T_3 and T_7) had almost the same yield. Similarly the yield from plants sprayed with a suspension containing 0.875×10^6 spores/ml, with and without diatomaceous earth (T_4 and T_8) also did not differ significantly. In general as the spore concentration increased there was a corresponding increase in the yield of cowpea. More than 32 per cent increase in the yield was noticed (except in T_1 where it was 24.64) in treatments which received 3.5×10^6 or more spores of *F. pallidoroseum* /ml of suspension.

During the second season the lowest yield of 234.89 g/plant was recorded in treatment T_7 (1.75×10^6 spores/ml with

diatomaceous earth) which was on par with water spray (T_4). Quinalphos (T_{10}), T_8 (0.875×10^6 spores/ml with diatomaceous earth) and T_4 (0.875×10^6 spores/ml water suspension). The highest yield of 265.22 was noticed in T_1 (7×10^6 spores/ml water suspension) which was on par with T_5 (7×10^6 spores/ml with diatomaceous earth), T_2 and T_6 (3.5×10^6 spores/ml water suspension and diatomaceous earth wettable powder) and T_3 (1.75×10^6 spores/ml water suspension). During this season the maximum yield received was only 12.81 per cent higher than that with water spray compared to 34.89 per cent increase obtained in T_5 during the first seaosn. Only two treatments T_1 and T_5 (7.5×10^6 spores/ml water suspension and diatomaceous earth wettable powder) had more than 10 per cent yield increase. Unlike in the first season, where the yield increase obtained in quinalphos was 0.71 per cent, during the second season, insecticidal spray increased the yield by 2.55 per cent over the control.

From the pooled mean of the yield (Table 4) obtained during the two seasons, it is clear that plants which received more than 3.5×10^6 spores/ml either in the form of water suspension or wettable powder, gave almost similar yields. The yield obtained in these treatments, 7×10^6 spores/ml water suspension and wettable powder, 3.5×10^6 spores/ml water suspension and wettable powder did not differ significantly from one another. Similarly, the yield obtained in T_9 (water spray)

was on par with T_{10} (quialphos) and T_4 and T_8 (0.875×10^6 spores/ml water suspension and diatomaceous earth wettable powder respectively). The highest increase of 22.13 per cent yield in the pooled mean was obtained in treatment T_5 (7×10^6 spores/ml wettable powder). T_2 (3.5×10^6 spores/ml water suspension), T_6 (3.5×10^6 spores/ml/wp) and T_1 (7×10^6 spores/ml/water suspension) also had an increase of more than 17 per cent over the control.

4.4 Influence of period of storage and storage temperature on the pathogenicity of aphids

In order to find out the sruvival ability and pathogenicity of *F. pallidoroseum* under room temperature and refrigerated condition an experiment was laid out where the spores of the fungus formulated as water suspension or wettable powder with diatomaceous earth as inert material were used. These two formulations were used to artificially inoculate *A. craccivora* at monthly intervals under laboratory conditions and the extent of mortality of the aphids was recorded. The concentration of spores in water suspension and wettable powder were maintained at 7×10^6 spore load. When water suspension was used for inoculation one month after storage cent per cent mortality of the aphids was noticed (Table 6; Fig. 7). The mortality rate however decreased from second month onwards (80 %) and only 43.3 per cent of the aphids got infected at the end of

ten months. Almost a similar trend was observed when wettable powder of spores stored at room temperature was sprayed on aphids. Here also the mortality was cent per cent, one month after storage, and got reduced to 50 per cent at the end of 10 months. However in wettable powder formulation stored for 4 months, the mortality rate was 90 per cent compared to 73.3 per cent in water suspension stored for the same period. The pathogenicity of *F. pallidoroseum* was better when they were stored under refrigerated condition. The water suspension of the fungus could cause 100 cent per cent mortality, even when a 3 month old culture was used for inoculation compared to 77 per cent mortality observed when a similar suspension maintained at room temperature was used as the inoculum. Even at the end of 5 months, the spore suspension maintained at refrigerated condition could inhibit 95 per cent of the aphids. At the end of 10 months, 35 per cent more aphids were killed by the water suspension under refrigeration (78.3) compared to water suspension at room temperature (43.3). The wettable powder formulation of the fungus stored under refrigerated condition gave highest mortality rate compared to other treatments. Here cent per cent mortality of the aphids was noticed upto the end of 5th month and during susbequent periods also more than 90 per cent of the aphids were infected. Even at the end of 10 months 91.4 per cent of the aphids were killed by this preparation. This was 13.3 per cent more than the mortality recorded when a 10 month old water suspension was used for spraying.

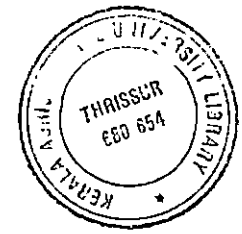


Table 6 Effect of period of storage and storage temperature on the pathogenicity of *F. pallidoroseum* on aphids

Treatment No.	Treatments	Percentage mortality of aphids Months after storage									
		1	2	3	4	5	6	7	8	9	10
T_1	Spore suspension at room tempeature	100	80.0	77	73.3	75	62.0	53.3	46.7	45.0	43.3
T_2	Spore suspension at refrigeration	100	98.4	100	98.4	95	88.4	85.0	81.7	80.0	78.3
T_3	Spore suspension at room temperature with diatomaceous earth	100	90.0	90	90.0	86.7	75.0	68.3	56.7	56.7	50.0
T_4	Spore suspension at refrigeration with diatomaceous earth	100	100.0	100	100.0	100.0	98.4	95.0	95.0	93.4	91.4

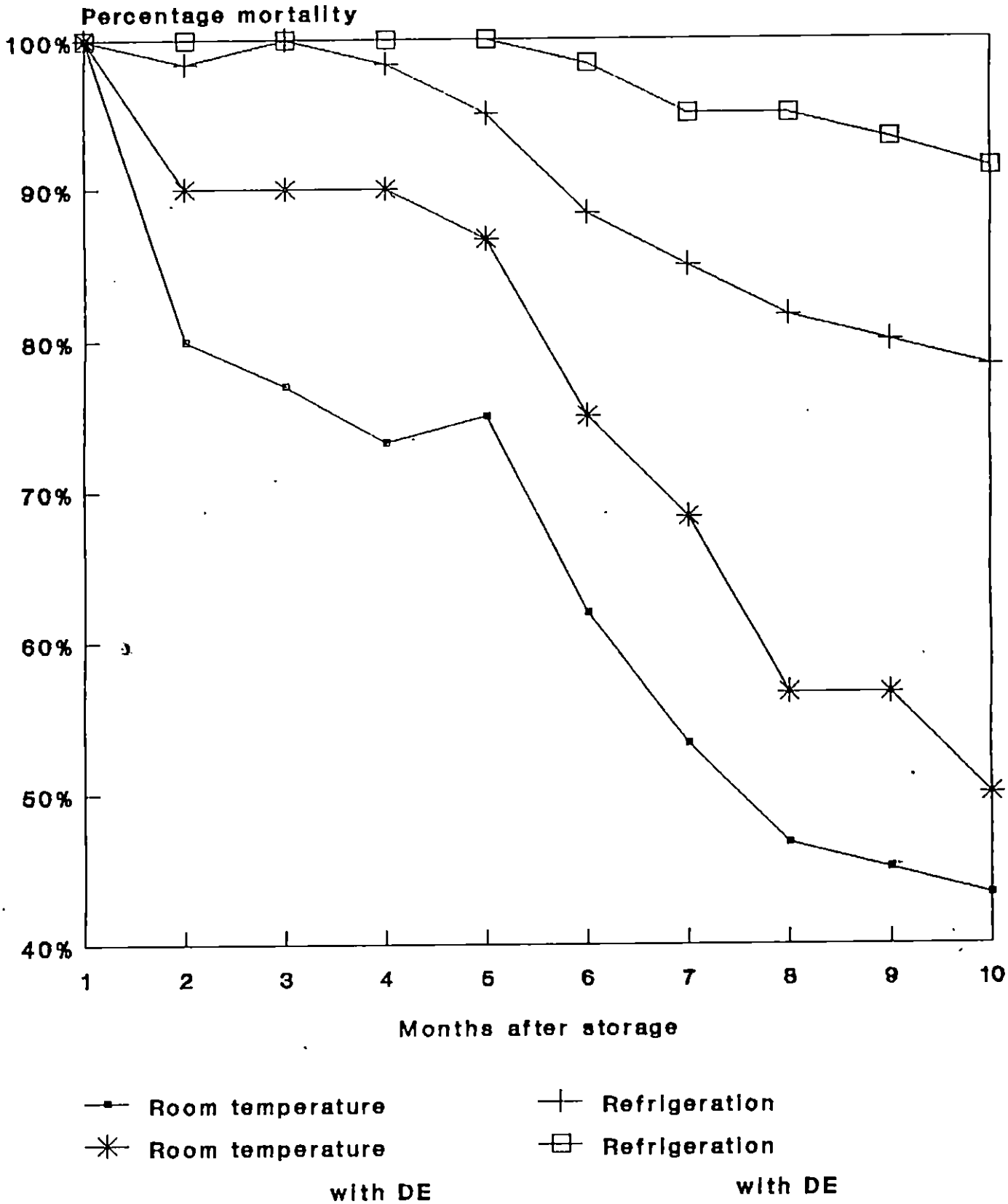
Table 7 Effect of period of storage on sporulation of *F. pallidoroseum*

Treatment No.	Treatments	Mean number of spores/ml at monthly intervals									
		1	2	3	4	5	6	7	8	9	10
T_1	Spore suspension at room tempeature	2.69×10^6	2.09×10^6	1.213×10^6	0.3466×10^6	0.033×10^6	Nil	Nil	Nil	Nil	Nil
T_2	Spore suspension on refrigeration	4.23×10^6	3.66×10^6	3.36×10^6	3.11×10^6	2.96×10^6	2.47×10^6	2.33×10^6	2.06×10^6	1.88×10^6	1.39×10^6
T_3	Spore suspension with diatomaceous earth at room temperature	2.97×10^6	2.43×10^6	1.63×10^6	0.68×10^6	0.12×10^6	0.027×10^6	Nil	Nil	Nil	Nil
T_4	Spore suspension with diatomaceous earth under refrigeation	4.57×10^6	4.51×10^6	4.02×10^6	3.99×10^6	3.85×10^6	3.64×10^6	3.06×10^6	2.76×10^6	2.28×10^6	1.45×10^6

Table 8 Effect of period of storage and storage temperature on the colony diameter of *F. pallidoroseum* on PDA.

Treatment	Mean diameter 7 days after inoculation (in cm) at monthly intervals																			
	1	Percentage inhibition over control	2	Percentage inhibition over control	3	Percentage inhibition over control	4	Percentage inhibition over control	5	Percentage inhibition over control	6	Percentage inhibition over control	7	Percentage inhibition over control	8	Percentage inhibition over control	9	Percentage inhibition over control	10	Percentage inhibition over control
$7x10^6$ spores/ml at room temperature	68.2	24.22	62.4	30.67	49.4	45.11	22.2	75.33	6.0	93.33	0	100	0	100	0	100.00	0	100.00	0	100.00
$7x10^6$ spores/ml at refrigeration	88.0	2.22	76.0	15.55	72.6	19.33	48.2	46.44	58.2	35.33	52.2	42	40.2	55.33	36.2	59.78	29.0	67.78	25.6	71.56
$7x10^6$ spores/ml with diatomaceous earth at room temperature	72.6	19.33	64.8	28.00	62.0	31.33	36.8	59.11	16.4	81.77	4.4	95.11	0	100.00	0	100.00	0.0	100.00	0	100.00
$7x10^6$ spores/ml with diatomaceous earth under refrigeration	88.2	2.00	83.2	7.56	80.0	11.11	72.0	20.00	64.4	28.44	58.6	34.89	48.6	46.00	38.8	56.89	33.4	62.89	30	66.66
Control		90.0	0	90.0	0	90.00	0	90.00	0	90.00	0	90	0	90	0	90	0	90	0	90

Fig.(7).Effect of period of storage and storage temperature of spore formulations of F.pallidoroseum on mortality of aphids.



Data on the sporulation presented in Table 7 shows gradual reduction in the number of spores produced from the stored formulation as the length of storage advanced. The formulations ie. water suspension kept at room temperature retained the ability for sporulation upto 5 months of storage whereas wettable powder retained the same for 6 months. The formulations that were stored under refrigeration showed moderate sporulation evenafter 10 months thus the wettable powder formulation under refrigeration appeared the best form of storage.

Regarding the diameter of the colony (Table 8) maximum diameter was given by $7x10^6$ spores/ml with diatomaceous earth under refrigeration which gave 30 mm diameter even at the end of 10 months. This is followed by water suspension which gave 25.6 mm diameter at the end of 10 months. Water suspension at room temperature produced a diameter of 6.0 mm at the end of 5 months and thereafter there was no growth of the fungus, whereas the wettable powder at room temperature gave a diameter of 4.4 mm, six months after storage.

4.6 Influence of host plants of *A. craccivora* on the efficacy of *F. pallidoroseum*

Table 9 shows that the host plants did not influence the mortality rate of aphids when *F. pallidoroseum* was sprayed on aphids harbouring on different host plants (Plate 5, 6, 7 and 8).

Plate 5 *Aphis craccivora* killed by *Fusarium pallidoroseum* remaining attached to grain cowpea.

Plate 6 *Aphis craccivora* killed by *Fusarium pallidoroseum* remaining attached to green gram.

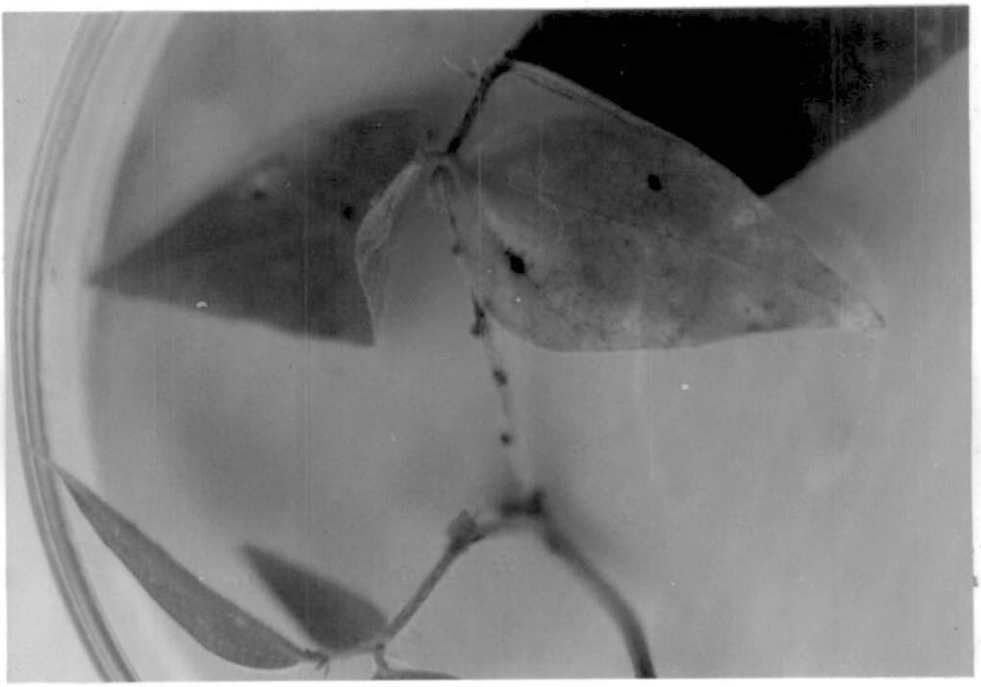


PLATE 5

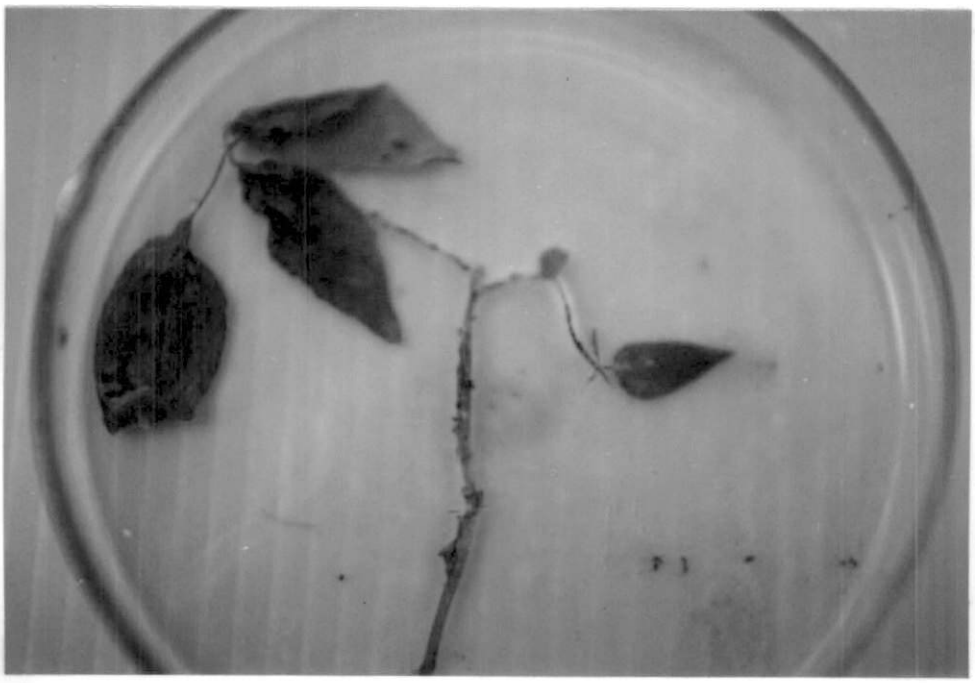


PLATE 6

Plate 7 *Aphis craccivora* killed by *Fusarium pallidoroseum* remaining attached to black gram.

Plate 8 *Aphis craccivora* killed by *Fusarium pallidoroseum* remaining attached to cluster beans.

PLATE 7

PLATE 8

Table 9 Influence of host plants on the pathogenicity of *F. pallidoroseum* on *Aphis craccivora*

	Crop	Mean number of aphids per plant (Mean of 6 replications)						
		Pretreatment	2 DAS	% reduction over pretreatment	3 DAS	% reduction over pretreatment	4 DAS	% reduction over pretreatment
Spraying spore formulation containing 7×10^6 spores/ml	Black gram	156.34	101.76	34.91	15.34	90.19	0	100.00
	Green gram	111.80	78.40	29.88	21.00	81.23	0	100.00
	Cluster bean	254.00	157.60	37.95	28.11	88.93	0	100.00
	Cowpea	287.53	164.30	42.86	30.12	89.53	0	100.00
Control	Black gram	189.91	208.11	-9.58	170.43	10.26	144.55	23.88
	Green gram	124.87	136.64	-9.43	141.40	-13.24	110.70	11.35
	Cluster bean	246.81	212.37	13.95	287.73	-16.58	186.66	24.37
	Cowpea	264.00	249.83	5.37	278.44	-5.46	267.87	-1.47

DAS - Days after spraying

Two days after spraying the percentage mortality ranged from 29.88 (green gram) to 42.86 (grain cowpea). During the subsequent day there was a marked increase in the mortality of aphids. Thus 81.23 per cent of aphids found on green gram was killed while the highest mortality of 90.1 per cent was noticed on aphids attacking black gram. On the 4^{th} day, all the aphids in the 4 different host plants were killed.

Population fluctuation of aphids on the 4 different untreated host plants were also studied. In black gram and green gram a 9 per cent increase in population of aphids was noticed after 2 days. While in cluster bean and grain cowpea there was a decrease in aphid count. During third day population of aphids on all the plants except black gram showed an increase, while on black gram 10.2 per cent decrease in population was noticed. On the 4^{th} day the population of aphids on black gram and cluster bean, was seen reduced by 23.88 and 24.37 per cent respectively, while that of grain cowpea, there was an increase of 1.4 per cent.

4.5 Effect of spraying spore formulation of *F. pallidoroseum* on the predator fauna of *Aphis craccivora* Koch under field conditions

The effect of *F. pallidoroseum* spray on the natural enemies of aphids found on 4 different host plants were studied up to a period of 12 days after spraying with the fungal spores (Table 10; Fig 8). The common natural enemies noticed during the present investigations were coccinellids, syrphids and spiders. On the 4^{th} day after spraying there was a marked increase in the population of natural enemies and in green gram and cluster beans, more than 150 per cent increase was noted in the natural enemy population. There was a decrease in the population of natural enemies on the 8^{th} day compared to that observed on the 4th day and in black gram and grain copwea plants, there was a reduction (12.78 and 33.33 % respectively) in the natural enemy population. In green gram and cluster bean a marginal increase was noticed (13.67 and 25.56%). The population of natural enemies on 12^{th} day, the count was much less than that observed on the day of spraying. The reduction ranged from 62.41 in cluster bean to 87.97 per cent in black gram. On the contrary in untreated plants the population of natural enemies increased during the entire period of observation. During the 4^{th} day after treatment the increase ranged from 15 (Black gram) to 65 per cent (grain cowpea) and on 8^{th} day after treatment it ranged from 25 per cent (grain cowpea) to 99.15 per cent (green gram).

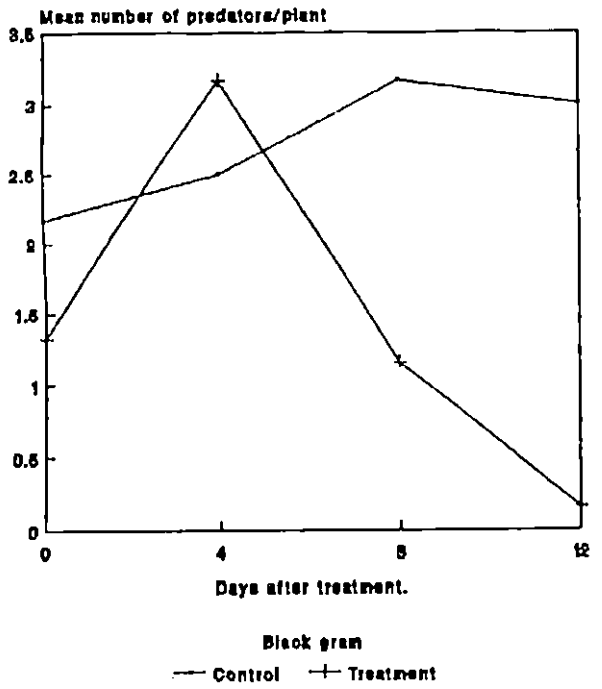
Table 10 Effect of *F. pallidoroseum* on the natural enemies of *Aphis craccivora*

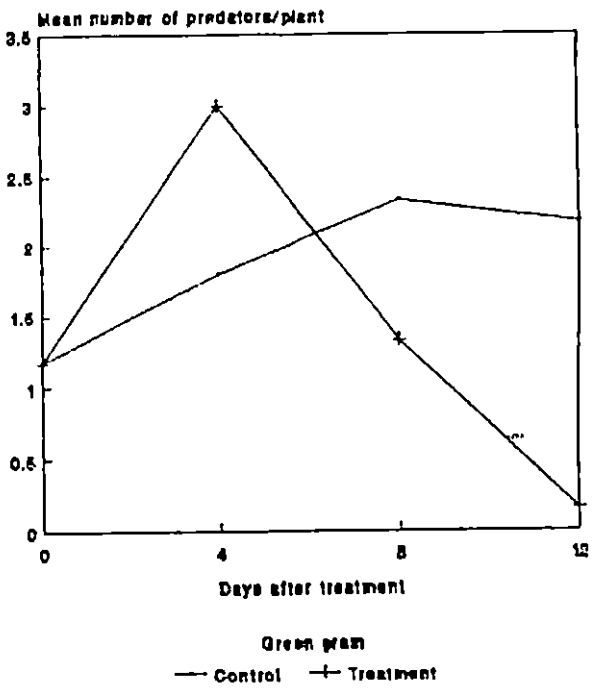
Treatments	Crop	Number of predators per plant (mean of 6 replications)						
		Pre-treatment	4 DAT	Percentage increase over pre-treatment	8 DAT	Percentage increase over pre-treatment	12 DAT	Percentage increase over pre treatment
Spraying	Black gram	1.33	3.17	138.00	1.16	-12.78	0.16	-87.97
Spore suspension containing $7x10^6$ spores/ml	Green gram	1.17	3.00	156.41	1.33	13.67	0.16	-86.32
	Cluster bean	1.33	3.33	150.37	1.67	25.56	0.50	-62.41
	Cowpea	1.50	2.67	78.00	1.00	-33.33	0.33	-78.00
Control	Black gram	2.17	2.50	15.20	3.17	46.08	3.00	38.25
	Green gram	1.17	1.80	53.85	2.33	99.15	2.17	85.47
	Cluster bean	2.17	2.67	23.04	2.83	30.42	2.83	30.42
	Cowpea	2.00	3.30	65.00	2.50	25.00	2.83	41.50

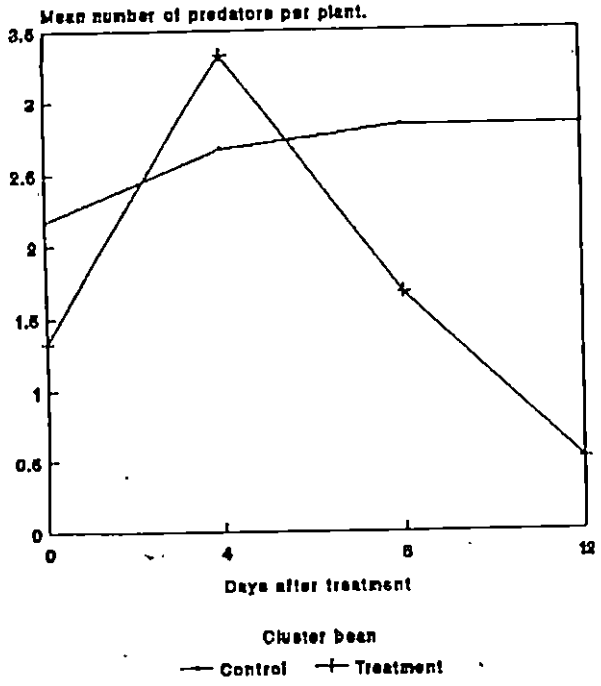
(-ve sign indicate population reduction over pretreatments counts)

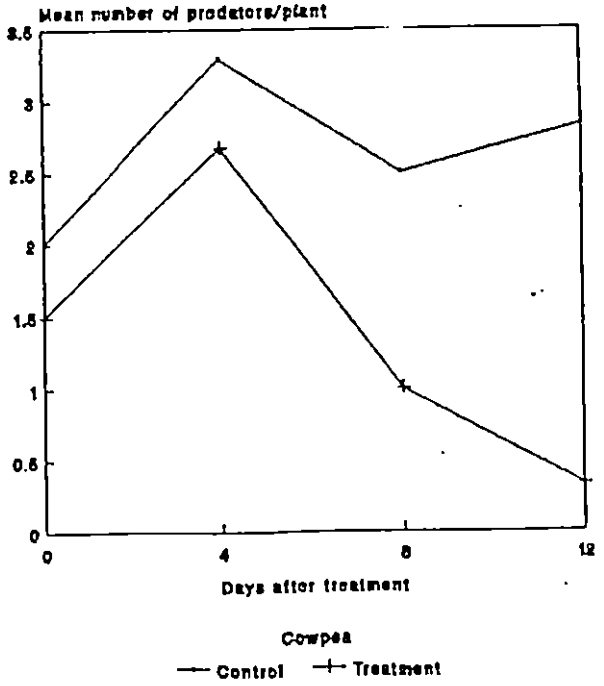
* Coccinellids
Syrphids
Spiders

Fig.(6).Effect of F.pallidoroseum on the natural enemies of aphids in different host plants









Natural enemies
Coccinellids
Syrphids and Spiders

On 12th day, except in grain cowpea where the population of natural enemies increased from 25 per cent (8 DAT) to 41.5 per cent (12 DAT), in all other treatments the population during this period was either the same (cluster bean) or less than that observed on 8th day, though the population was more than the initial value.

4.7 Pathogenicity of *F. pallidoroseum* to certain selected crop plants

Seven vegetables viz. chillies, tomato, brinjal, bhindi, amaranthus, bittergourd, snakegourd and medicinal plants like adathoda, notchi and Tulsi were selected for the study. None of these plants showed any symptom of fungal infection upto two weeks in leaf inoculated plants and upto 3 months in root inoculated ones though most of these crops have been reported to be susceptible to certain species of fusarium causing damping off and wilt diseases.

4.8 Infectivity of *F. pallidoroseum* to three species of coccinellid predators under laboratory conditions:-

Young grubs (second instar) of *Coccinella septumpunctata*, *Menochilus sexmaculatus* and *Scymnus* sp. were sprayed with fungal spore suspension containing $7x10^6$ spores/ml and the treated grubs were reared in specimen tubes and were observed daily. The treated grubs pupated and healthy adults

Table 11 Pathogenicity of *F. pallidoroseum* to crop plants

Crops Vegetables	Method of inoculation	Symptoms of disease if any	Percent mortality of plants	Trans-mission
Bhindi	Soil treatment	Nil	Nil	-ve
	Leaf treatment	Nil	Nil	-ve
Brinjal	Soil treatment	Nil	Nil	-ve
	Leaf treatment	Nil	Nil	-ve
Amaranthus	Soil treatment	Nil	Nil	-ve
	Leaf treatment	Nil	Nil	-ve
Tomato	Soil treatment	Nil	Nil	-ve
	Leaf treatment	Nil	Nil	-ve
Chillies	Soil treatment	Nil	Nil	-ve
	Leaf treatment	Nil	Nil	-ve
Snakegourd	Soil treatment	Nil	Nil	-ve
	Leaf treatment	Nil	Nil	-ve
Bitter gourd	Soil treatment	Nil	Nil	-ve
	Leaf treatment	Nil	Nil	-ve
Medicinal plants				
Adathoda	Soil treatment	Nil	Nil	-ve
	Leaf treatment	Nil	Nil	-ve
Tulsi	Soil treatment	Nil	Nil	-ve
	Leaf treatment	Nil	Nil	-ve
Notchi	Soil treatment	Nil	Nil	-ve
	Leaf treatment	Nil	Nil	-ve

In control there was no infection

emerged as in control showing that *F. pallidoroseum* did not infect these coccinellids.

4.9 Pathogenicity of the Fungus *F. pallidoroseum* to productive insects

1) Honeybees (*Apis mellifera*)

The worker bees of *Apis mellifera* were sprayed with water suspension containing $7x10^6$ spores/ml suspension and were observed for the occurrence of fungal growth up to two weeks. No symptoms suggestive of infection or mortality were observed in any of the treated bees.

Silkworm (*Bombyx mori*)

Second and fourth instar larvae were used for safety tests. They were sprayed with the formulation containing $7x10^6$ spores/ml. Then they were kept separately in petridishes and fed with chopped leaves. All the larvae got pupated and healthy adults emerged.

4.10 Pathogenicity of *F. pallidoroseum* to other aphid species

Pathogenicity tests were conducted on 3 species of aphids viz. *A. gossypii*, *A. malvae* and *Toxoptera aurantii*. Results showed that *F. pallidoroseum* was highly host specific and would not infect other species of aphids.

4.11 Effect of *F. pallidoroseum* isolated from *Eichhornia crassipes* on aphid, *A. craccivora*

The plant pathogen *F. pallidoroseum* isolated from water hyacinth failed to produce any pathological symptoms on pea aphid *A. craccivora* showing that it was an entirely different strain. So also *F. pallidoroseum* (Cooke) Sacc could not produce disease in the water hyacinth.

DISCUSSION

5. DISCUSSION

Pulse crops constitute the major source to meet the protein requirements of Indian diet. The most important insect pest affecting almost all the pulse crops in India is the black pea aphid *Aphis craccivora* Koch. This pest is of regular occurrence especially during the vegetative stage of the crops. Earlier studies carried out at College of Agriculture, Vellayani have revealed the occurrence of *Fusarium pallidoroseum* (Cooke) Sacc as a fungal pathogen of the pea aphid *A. craccivora* Koch (Hareendra Nath *et al*. 1989). Present studies were undertaken to find out the suitability of using *F. pallidoroseum* as a biocontrol agent against *A. craccivora*. The four main lines of work undertaken during the study are (1) field tests using different formulations and doses of the pathogen (2) Pot culture experiments to assess the influence of host plants on the efficiency of the pathogen and (3) Laboratory studies on the viability of spores under room temperature and under refrigeration. (4) Safety tests to natural enemies, productive insects, and cron plants.

Studies conducted by Hareendra Nath *et al*. (1989) showed that maximum population of pea aphid occurred in the crop planted during October, November and December. A decline in population was observed in crops planted from February onwards

with lowest population during March. Based on these findings, in the present investigation the field experiments were carried out during two seasons viz. April-July 1994 corresponding to the lowest population of aphids and September-December 1994 corresponding to the peak population of aphids.

The field studies conducted using two formulations of *Fusarium pallidoroseum* ie. diatomaceous earth wettable powder and water suspension of spores revealed that there was no difference in the efficacy of these two formulations. Similar observations were also recorded by Faizal (1992). Working with *F. pallidoroseum* against *A. craccivora*. In order to find out the ideal spore concentration of *F. pallidoroseum* for the effective control of *A. craccivora*, 4 different spore concentrations were used. Spray fluid with a concentration of 7×10^6 spores/ml was found to be as effective in controlling aphid infection as that of commonly used insecticide quinalphos at 0.05 per cent. When long term effect of the insecticide was compared with that of the spore suspension it was found that the fungus spray was even more effective than the insecticide. At this concentration cent per cent mortality of aphids was obtained with in 12 days of treatment. Further reinfestation was not noticed on the sprayed, plants. Beevi (1979) reported that *F. moniliformae* var *subglutinans* at a concentration of 7.5×10^5 conedia/ml caused 96.97 per cent control of epilachna beetles under field conditions. Kuruvilla and Jacob (1970) observed

complete control of *N. lugans* affecting rice when *F. oxysporum* at a concentration of 6.025×10^6 spores/ml was used. The spray caused cent per cent mortality of the plant hopper.

Compared to the high spore concentration of 7×10^6 the effectiveness of spore suspension containing 3.5×10^6 spores/ml was less effective during the initial stage. However it was found to be equally effective at the later intervals, that is 20 DAT. The spore concentration, 1.75×10^6 spores/ml was inferior and 0.875×10^6 was not effective in checking the aphid population. These observations clearly indicate that concentration of spores in the fungal inoculum has a significant role in initiating epizootics and bringing effective check on the pest population. Hareendra Nath *et al.* (1989) also found that *F. pallidoroseum* spores at a concentration of 1.75×10^6 spores/ml was inferior and 0.875×10^6 spores/ml was not at all effective in controlling aphid population.

Fungal pathogen is able to control the aphids only if the concentration of the fungus in the spray fluid is enough to cause successful infection. For the subsequent infection of the insect the fungus should multiply and cause fresh infection of the newly emerging aphids. When the spore concentration in the spray fluid is not adequate the time taken for multiplication may be prolonged resulting in a reduced control of the aphids.

The results of yield data showed that, as the concentration of spores increased there was a corresponding decrease in aphid population and a consequent increase of yield in cowpea, indicating that aphid population had a negative influence on the yield of the crop. Singh and Allan (1980) reported *Aphis craccivora* as a major pest of cowpea in Asia causing an estimated loss of 20-40 per cent in yield.

An yield increase of 17-22 per cent over the control (water) spray was noticed in treatments with spray fluid containing 3.5×10^6 spores/ml or more, compared to 1.82 per cent increase recorded with insecticidal spray. Even at a lower concentration of spore suspension the increase in yield was better than that obtained with quinalphos treatment.

Observations on the weight and number of pods in different treatments indicated that the wieght of pods were significantly different among the treatmens. While such a difference was not noticed with respect to the number of pods. This indicate that the aphid infestation causes a reduction in the weight of pods rather than the number of pods/plant. Van (1973) reported that aphids suck the plant sap, resulting in depletion of assimilates coupled with an increase in the respiration of the plant that caused a reduction in yield.

One of the major impediments in the commercial acceptance of a biocontrol agent is the reduction in viability of spores on storage Ferron (1981) considered short life of formulation as one of the factors which limits the use of microbial pesticides. In this study two formulations viz. water suspension and diatomaceous earth wettable powder were stored at room temperature and under refrigeration. In general there was a decrease in the effectiveness of the spores on storage. When incubated under room temperature there was marked reduction in the effectiveness of the pathogen even with in 5-6 months. However both the formulations could be stored for more than ten months under refrigerated condition with almost one third of the initial sporulation and colony growth. Zhang *et al.* (1992) formulated *B. bassiana* as wettable powder and found that this formulation after storage for about 8 months under refrigeration at 10-20°C gave a spore germination rate of more than 85 per cent.

Even though the spores failed to grow in artificial media 40 to 50 per cent mortality was recorded at the end of 10 months, when formulation stored under room temperature was sprayed on the aphids. One of the possible factors responsible for this observation may be fungistasis.

The term fungistasis describes the phenomenon where by viable propagules not under the influence of endogenous or

constitutive dormancy, fail to germinate some times even when conditions of temperature and moisture favourable for germination are given. This phenomenon had also been reported in *Fusarium* sp. (Watson and Ford 1972). The inability of the Fusarial formulations to germinate after 5 to 6 months under room temperature might be due to this phenomenon. While the mortality of aphids, when the suspension was sprayed may be due to the release from fungistasis by factors of biotic origin, produced by the fungus (Singh 1984).

Another possible reason for mortality of aphids by spraying apparently, non germinating fungal spores may be due to of the presence of fungal toxins in the formulation. Toscano and Reever (1973) noted that culture filtrate of *Aspergillus flavus* isolated from a moribund mosquito larva contained two chloroform extractable compounds toxic to *culex* larvae, by the addition to their habitat water. According to Claydon *et al*. (1977) a strain of *F. solani* pathogenic to lobster, *Homarus americanus*, produced in liquid media substance toxic to adult bower files. Similarly Mori and Takaishi (1989) isolated 'monocerin' an insecticidal constituent from entomogenous fungi, *F. larvarum*. Irrespective of storage temperature, the diatomaceous earth wettable powder was found to be slightly more effective than the water suspension at the corresponding temperature. Kabana (1975) reported diatomaceous earth as a suitable inert material for the formulation of mycofungicide with *Trichoderma harzianum*, used against *Sclerotium rolfsi*.

The pea aphid *A. craccivora* Koch is a serious pest of pulses and several other crop plants causing serious damage to the crop. Nair *et al.* (1976) observed the pea aphid *A. cracivora* as a serious pest of *Lab lab*, *Niger*, *Arachis hypogea*, *Gliricidia maculata*, *Phaseolus mungo*, *P. radiatus* and other pulse crops Gosh (1981) reported it on red gram.

A pot culture study was hence conducted to find out the efficiency of the fungus *F. pallidoroseum* on *A. craccivora* on other host plants. The results of the study using grain cowpea, green gram, black gram, and cluster beans clearly revealed that the host had no influence on the pathogenic effect of *F. pallidoroseum*. Further there was no reinfestation of the aphids in the plants, indicating its efficiency in controlling *A. craccivoa* irrespective of the host plant.

Another netable observation from the pot culture experiment was that by spraying *F. pallidoroseum*, it not only controlled *A. craccivora* but also caused an increase in the. population of natural enemies of the aphid. The common predators viz. coccinellids, syrphids and spiders were found attracted more towards the crops sprayed with the fungal spore formulation than the un sprayed crops. Among these the number of coccinellids and syrphids increased more rapidly than in control. However as the days advanced a gradual decline in natural enemy population was noticed. Immediately after spraying with *F. pallidoroseum* the

aphids became morbid and served as ideal source for the natural enemies. This enhanced food source might have increased the population of natural enemies of the aphids. But as the reinfestation with the aphid was not noticed in *F. pallidoroseum* sprayed plants, the natural enemies also declined in due course for want of suitable food source.

Before popularising any biocontrol agent against any crop pest its effect on other crop plants, especially on those cultivated along with the major host plant must be studied. From the present investigation it was proved that *F. pallidoroseum* was not pathogenic to vegetables like bhindi, brinjal, amaranthus, tomato, chillies, snake gourd and bitter gourd and to medicinal plants like adathoda, ocimum and notchi even though some of them have been recorded as susceptible to other species of *Fusarium* causing damping off and wilt disease. Thus the present strain of *F. pallidoroseum* used in the investigation is not pathogenic to crop plants and can be safely used as a pest control agent. Studies of Kuruvilla and Jacob (1970) with *F. oxysporum* on cotton, tomato and rice; Beevi *et al.* (1982) with *F. moniliformae* var subglutinans on cotton, tomato, bitter gourd, snake guard and brinjal and Hareendranath *et al.* (1989) with *F. pallidoroseum* on rice, bhindi, chillies and tomato, showed that they are non pathogenic to the respective crop plants.

Non-pathogenicity to common predators and parasites associated with the environment of the host insect is an ideal atribute for any candidate entomopathogen. *Menochilus sexmaculatus* has been reported as one of the most efficient coccinellid predators preving upon pea aphid (Saharia, 1981). To find out whether *F. pallidoroseum* is pathogenic to coecinellids three species of coccinellids viz *Menochilus sexmaculata*, *Coccinella septumpunctata* and *scymnus* sp, were tested. The results clearly showed that it is non pathogenic to the coccinellids. Similar observations with respect to *M. sexmaculata* was also made by Hareendranath *et al*. (1989) working with *F. pallidoroseum*.

Pathogenicity tests using *F. pallidoroseum* was also conducted against important productive insects like honey bees and silk worms. The results revealed that this fungus is non-pathogenic to worker honey bees and second and IVth instar larvae of silk worm. Nagalingam (1986) found that *F. semitectum* was safe to mulberry silk worm and adult honey bees.

It was also revealed that this pathogen was highly host specific and would not infect even other species of aphids viz. *A. gossypii*, *A. malvae* and *Toxoptera aurantii*.

One strain *F. pallidoroseum* has been identified as a biocontrol agent of water hyacinth (Jamil and Rajagopal 1986). However the strain of *F. pallidoroseum* which is used for this

study could not infect water hyacinth; further the strain which was isolated from water hyacinth could not attack the aphids. Thus it is clear from the above observation that the strain of *F. pallidoroseum* used in this study is different from the one that is noticed in water hyacinth.

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

According to the package of practices recommendations of the Kerala Agricultural University (1993) at least 3 sprayings with quinalphos 0.05 per cent have to be given at an interval of 15 days during the cropping period, to control the aphid population. For protecting $40m^2$ area of cowpea from aphids, 18 ml Ekalux (100 ml ekalux costs Rs. 48) is required for a season. The cost of the insecticide alone is Rs.8.64 (Rs. 9/-) cost of 3 spraying approximately equals to Rs.15/-. Thus the total cost involved works out to Rs. 24 per 40 m^2.

Instead of three sprays of ekalux only one spray of *F. pallidoroseum* at a concentration of 7×10^6 spores/ml is required. From a 7 day old 900 g rice bran culture of the fungus spore suspension of 7×10^6 spores/ml can be prepared which is enough for one spray. The cost required for spraying one cent of cowpea, the cost of rice bran including sterilization etc. works

out to Rs.15/-. Cost of spraying one cent land approximately equals Rs.5/-. Thus the cost of control of aphids using *F. pallidoroseum* works out to Rs.20/-, compared to Rs. 24/- when insecticide was used.

F. pallidoroseum strain used in the present study is a highly effective agent against *A. craccivora*. Besides it is harmless to natural enemies of aphids and productive insects like honey bees and silkworm. This is also non-pathogenic to commonly cultivated crop plants. Further the cost involved in controlling the aphid using the biocontrol agent is less, compared to insecticidal control, thus making *F. pallidoroseum* as an ideal candidate for biocontrol of black pea aphid, *A. craccivora*.

SUMMARY

6. SUMMARY

The study of "*Fusarium pallidoroseum* (Cooke) Sacc as a biocontrol agent for the pea aphid *Aphis craccivora* Koch' was conducted at the Department of Entomology, College of Agriculture, Vellayani during 1993-95 to find out the effect of different formulations and doses of the fungus in aphid control. The study also included the influence of period of storage and storage temperature on the pathogenicity of the fungus, the effect of *F. pallidoroseum* on various host plants of *A. craccivora*, the effect of the fungus on the predator fauna, the pathogenicity of the fungus to certain crop plants, productive insects and to other species of aphids. The effect of an other strain of *F. pallidoroseum* isolated from water hiacinth was also studied.

Influence of different formulations and doses of *F. pallidoroseum* on *A. craccivora* during two seasons viz., April-July and September-December, revealed that among the four different spore concentrations tried $7x10^6$ spores/ml as water suspension and wettable powder were equally effective as they produced maximum aphid control within a short period. The formulations having 3.5×10^6 spores/ml took more than 20 days while the lower spore concentrations viz., $1.75x10^6$ spores/ml and $0.875x10^6$ spores/ml took more than a month for complete control

of aphids. In insecticide sprayed plants reinfestation of the aphids was noticed after a few weeks whereas this phenomenon was not observed in plants sprayed with *F. pallidoroseum* spores.

Management of aphids had a significant effect on the yield of cowpea. The highest yield was obtained in treatments where higher concentration of spore suspension was used while there was no significant difference in the yield between treatments which received insecticidal spray and lowest concentration of spore suspension.

Viability and germination of the spores of *F. pallidoroseum* decreased as the period of storage increased. Both water suspension and wettable powder formulations of the spores when stored at room temperature lost the viability within five and six months respectively, whereas both these formulations retained viability for more than 10 months, when stored in refrigerator. Wettable powder formulation was found to be better than water suspension for prolonged storage.

Pot culture experiment was conducted to assess the efficacy of *F. pallidoroseum* on *A. craccivora* on different changed with host plants. Four different host plants viz., black gram, green gram, grain cowpea, and cluster bean were selected for this trial. The spores at a concentration of 7×10^6 spores/ml was found to control the pest completely irrespective of the host plant. An increase in the number of

natural enemies just after spraying the fungal spore formulation was noticed in all these crops.

The safety tests conducted on vegetables viz., bhindi, brinjal, amaranths, tomato, chillies, snakegourd and bittergourd and medicinal plants adathoda, ocimum and notchi revealed that the spores of *F. pallidoroseum* at a concentration of 7×10^6 spores/ml did not cause any disease in these plants.

F. pallidoroseum was also nonpathogenic to the common coccinellid predators of aphids viz., *Coccinella septumpunctata*, *Menochilus sexmaculatus* and *Scymnus* sp., and productive insects like silkworm and honey bees. This fungus was highly species specific to *A. craccivora* and was not pathogenic to other species of aphids *Toxoptera aurantii*, *Aphis gossypii* and *Aphis malvae*.

F. pallidoroseum strain identified from the weed water hyacinth was not pathogenic to *A. craccivora* and the *F. pallidoroseum* strain used in the study did not cause disease on water hyacinth.

The field use of *F. pallidoroseum* as a biocontrol agent was found to be cost efficient. Thus this fungus was found to be an ideal candidate for the control of black pea aphid *A. craccivora*.

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* Originals not seen

APPENDICES

APPENDIX - I

Effect of various treatments on the mean number of aphids at different intervals

April - July - Season I (mean of 3 replications)

Treatments	Pre treatment mean	Post treatment means				
		4 DAT	8 DAT	12 DAT	16 DAT	20 DAT
$7x10^6$ spores/ml water suspension	62.67	45.82	23.30	2.25	1.76	9.97
$3.5x10^6$ spores/ml water suspension	182.27	32.78	1.79	7.96	22.20	0.44
$1.75x10^6$ spores/ml water suspension	121.37	123.63	100.35	18.16	20.23	17.70
$0.875x10^6$spores/ml water suspension	93.00	137.55	180.30	27.53	15.70	46.29
$7x10^6$ spores/ml with diatomaceous earth as inert material	170.83	-16.52	-7.25	-0.56	-0.82	0.047
$3.5x10^6$ with diatomaceous earth as inert material	247.67	86.65	25.08	-2.40	1.01	0.85
$1.75x10^6$ with diatomaceous earth as inert material	188.10	75.34	48.46	3.59	9.13	0.95
$0.875x10^6$ with diatomaceous earth as inert material	175.80	153.51	192.78	42.10	30.36	37.55
Water spray	65.47	41.56	32.96	24.04	62.63	101.33
Quinalphos 0.05%	56.83	45.49	29.57	40.53	109.60	111.75
CD (0.05)		50.23	75.57	36.36	55.89	50.93

DAT - Days after treatment

APPENDIX - II

Effect of various treatments on the mean number of aphids at different intervals

September-December - Season II (mean of 3 replications)

Treatments	Pre treatment mean	Post treatment means				
		4 DAT	8 DAT	12 DAT	16 DAT	20 DAT
$7x10^6$ spores/ml water suspension	200.37	16.58	8.56	1.58	0.43	0.18
$3.5x10^6$ spores/ml water suspension	174.46	34.49	2.97	-0.91	-0.76	-0.31
$1.75x10^6$ spores/ml water suspension	245.79	113.18	91.81	40.39	30.54	15.19
$0.875x10^6$ spores/ml water suspension	229.17	170.01	153.37	116.67	116.39	109.07
$7x10^6$ spores/ml with diatomaceous earth as inert material	209.92	3.22	0.25	1.51	0.87	0.35
$3.5x10^6$ with diatomaceous earth as inert material	182.39	33.39	2.72	0.14	4.02	4.25
$1.75x10^6$ with diatomaceous earth as inert material	185.59	58.19	27.82	10.46	21.35	17.48
$0.875x10^6$ with diatomaceous earth as inert material	142.54	92.80	69.81	72.46	26.27	19.64
Water spray	201.94	19.28	46.10	114.71	84.43	106.13
Quinalphos 0.05%	135.71	6.10	5.98	10.16	31.41	95.03
CD (0.05)		38.07	61.57	73.96	44.33	41.64

DAT - Days after treatment

APPENDIX - III

Weather data during the cropping period April 02,1994 to July 29, 1994

Period from	to	Rainfall mm	Maximum temperature °C	Minimum temperature °C	Relative humidity
April 02	86	8.2	31.5	24.1	80.2
09	15	6.0	31.0	24.0	85.4
16	22	1.5	32.1	24.6	79.1
23	29	1.4	32.6	25.5	86.9
April 30	May 06	9.1	32.6	26.2	79.2
May 07	13	-	28.2	26.0	84.2
14	20	-	33.0	26.4	84.3
21	27	23.8	31.3	24.1	87.4
May 28	June 03	20.9	29.9	23.0	91.4
04	10	15.2	29.4	23.6	87.8
11	17	7.8	29.9	23.2	90.5
18	24	0.4	30.0	24.8	83.3
25	July 01	0.3	30.3	24.2	77.0
July 02	08	1.1	29.6	23.6	81.8
09	15	7.3	29.7	23.7	88.8
16	22	10.6	29.2	22.9	82.6
23	29	10.0	29.8	23.5	83.2

APPENDIX - IV

Weather data during the cropping period September 10, 1994 to December 31

Period from	to	Rainfall mm	Maximum temperature °C	Minimum temperature °C	Relative humidity
September					
10	16	-	30.4	25.1	86.9
17	23	0.7	30.2	23.4	85.9
24	30	-	30.9	24.0	84.4
October					
01	07	24.4	28.0	23.7	96.9
08	14	1.5	30.5	22.3	83.6
15	21	16.1	29.5	23.3	82.0
22	28	2.1	30.3	23.9	84.3
October	November				
29	04	15.7	29.7	23.2	85.2
05	11	3.3	30.5	23.5	80.2
12	18	3.8	29.9	23.3	84.2
19	25	-	30.4	23.0	85.7
	December				
26	02	4.1	31.2	23.8	84.1
December					
03	09	-	30.8	22.1	80.4
10	16	-	30.5	21.5	78.5
17	23	-	31.5	23.1	81.5
24	31	-	31.8	22.0	77.2

Fusarium pallidoroseum (Cooke) Sacc AS A BIOCONTROL AGENT FOR THE PEA APHID *Aphis craccivora* Koch

By

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ABSTRACT OF A THESIS

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ABSTRACT

Detailed investigations were made on the entomogenous fungus *Fusarium pallidoroseum* (cooke) Sacc infecting cowpea aphid *Aphis craccivora* koch.

Field studies showed that spore suspension and wettable powder formulations were equally effective in controlling the pea aphid. 7 x 10^6 spores per ml suspension in water or a similar suspension of a wettable powder or quinalphos 0.05 per cent were found to be equally effective in controlling the aphids under field conditions. Spore suspension containing 3.5 x 10^6 spores per ml have shown similar results during the later half of the experimental period.

Pot culture studies conducted to assess the influence of host plants of aphid on the efficacy of the pathogen revealed that there was no influence of host plant on the pathogenecity of *F. pallidoroseum*. The germination and viability of *F. pallidoroseum* in formulation was found to decrease with increase in storage period and the formulations retained its capacity for germination and viability for more than ten months under refrigeration.

The fungus was found nonpathogenic to vegetables viz. as bhindi, brinjal, amaranths, tomato, chillies, snakegourd,

bittergourd and medicinal plants, viz., adathoda, ocimum and notchi under field conditions. Besides it was found safe to natural enemies of aphids viz., *Coccinella septumpunctata*, *Menochilus sexmaculata* and *Scymnus sp*. The productive insects, honey bees and silkworms were not at all affected by this fungus.

Cross inoculation studies using this pathogen showed that three species of aphids, viz., *Toxoptera aurantii*, *Aphis gossypii* and *Aphis malvae* were not infected.

A strain of *F. pallidoroseum* isolated from a weed plant water hyacinth (*Eichhornia crassipes*) was found to be nonpathogenic to the aphid *Aphis crassivora*. Similarly *F. pallidoroseum* was observed nonpathogenic to water hyacinth. These observations indicated that the above two strains of *F. pallidorosem* are entirely different.