MANAGEMENT OF WATER HYACINTH [*Eichhornia crassipes* (Mart.) Solms] USING FUNGAL PATHOGENS

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DECLARATION

I hereby declare that this thesis entitled "**Management of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] using fungal pathogens**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled "**Management of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] using fungal pathogens**" is a record of research work done independently by Ms. Praveena, R. (99–21–09)) 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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LIST OF ABBREVIATIONS

%	Per cent
@	At the rate of
^{0}C	Degree Celsius
ai	Active ingredient
CD	Critical difference
cfu	Colony forming unit
cm	Centimetre
CNSL	Cashew nut shell liquid
DAD	Days after dusting
DAI	Days after inoculation
DAS	Days after spraying
DAT	Days after treatment
et al.	And others
Fig.	Figure
g	Gram
h	Hour
ha	Hectare
kg	Kilogram
L	Litre
m	Metre
ml	Millilitre
mm	Millimetre
NMR	Nuclear magnetic resonance
ppm	Parts per million
sp.	Species
viz.	Namely
WAS	Weeks after spraying
WP	Wettable powder

INTRODUCTION

1. INTRODUCTION

With the advancement of international trade and tourism more plant species with potentially weedy traits are being moved around the world giving rise to new invasive weed problems particularly in non-agricultural or natural ecosystems. A typical example of this is the introduction of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] to India.

Water hyacinth is one of the most invasive and gregariously growing aquatic weeds in the world. It belongs to the family Pontederiaceae and its centre of origin is Amazon basin. This weed reproduces mostly by offsets, but seeds also play a role in its survival and colonization. It is capable of doubling its biomass within ten days forming thick mats of plants that cover water bodies, reducing light, oxygen and changing fauna and flora.

The weed was introduced to India from Brazil in 1896 as an ornamental pond plant. Now water hyacinth is the most serious aquatic weed in India infesting more than 2,00,000 ha of water surface (Singh, 1999). This weed is causing concern in 98 out of 246 districts in India (Harikumar and Madhavan, 2002).

Water hyacinth is used for biogas production, animal feed, for waste water treatments etc. However, disadvantages outweigh the merits as it interferes with production of hydroelectricity, blocks water flow in irrigation projects, facilitates breeding of mosquitoes and fosters water borne diseases. Also, water loss due to evapotranspiration from the luxuriant foliage of water hyacinth is a major concern where water shortages have become chronic.

The management of water hyacinth could be taken up by physical, chemical or biological means. However, the most widely followed practice among the farmers is by manual collection and destruction. Since

the multiplication rate of the weed is very fast and the expense involved in manual collection is very high, alternative methods are being investigated.

Chemical destruction of weed by herbicides is not feasible in most cases as the water in which the weed grows is being utilised for drinking purpose for animals and other household uses. Further, apart from polluting the environment, chemical herbicides are very expensive also.

Nowadays, increased interest has been generated in the biological control of weeds using microorganisms. An ideal microorganism used to control weed should be easy to produce and store, inexpensive to use, reliable at high infestation and with predictable level of control and safe for environment. Fungal biocontrol agents possess many of these characters.

Among the fungi *Cercospora rodmanii* Conway has shown the greatest promise as a biocontrol agent of water hyacinth (Conway, 1976b). Studies conducted by Santhi (1994) and Susha (1997) revealed that *Fusarium pallidoroseum* (Cooke) Sacc., *Fusarium equiseti* (Corda) Sacc. and *Colletotrichum gloeosporioides*(Penz.)Penz. and Sacc. are capable of restricting the multiplication of the weed and reducing the population.

Fungal weed pathogens are difficult to formulate into effective products because, as living organisms, their viability must be preserved throughout processing and storage. Other major limitation is the inconsistency in efficacy which is often observed with many biocontrol microorganisms when it reaches stages of large scale glasshouse or field testing.

Based on the foregoing considerations the aim of the present study is to explore the feasibility of utilizing fungi and their metabolites in the management of water hyacinth and to develop an effective formulation.

The study was taken up with the following objectives :

1. Survey to identify newer fungal pathogens of water hyacinth
2. Detailed study on the host range of the fungal pathogens

3. Testing the efficacy of cell free metabolites
4. Purification and characterization of the fungal metabolites
5. Testing the biocontrol agents and their metabolites against common flora and fauna present in the water ways
6. Mass production of the biocontrol agent on commonly available and cheap substrates
7. Effect of different concentration of herbicides on the growth and sporulation of the biocontrol agents
8. Development of an effective formulation of the biocontrol agent

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Water hyacinth is known to be infected by several fungal pathogens. A perusal of literature revealed that some of these pathogens have the capacity to restrict the multiplication and thus reduce the population of the weed.

2.1 PATHOGENS OF WATER HYACINTH

During a search for natural enemies of water hyacinth (*Eichhornia crassipes*), Nagraj and Ponnappa (1967) isolated *Corticium solani* (Prill. and Delacr.) Bourd. and Galz. in the *Rhizoctonia* phase and *Myrothecium roridum* Tode ex Fr. from the leaves.

Nagraj and Ponnappa (1970) found water hyacinth to be infected by several plant pathogens including *Uredo eichhorniae* Ciferri and Fragoso, *Fusarium equiseti* (Corda.) Sacc., *Corticium sasakii* (Shirai) Matsumoto, *Cephalosporium eichhorniae* Padwick, *Rhizoctonia solani* Kuhn, *Cercospora piaropi* Tharp, *Marasmiellus inoderma* (Berk.) Singh and *Alternaria eichhorniae* Nagraj and Ponnappa. According to them, of the several fungi recorded as pathogens of water hyacinth, *M. inoderma* and *A. eichhorniae* appear to hold some promise as possible agents of biological control.

Freeman and Zettler (1971) reported the occurrence of *Rhizoctonia solani* on *Eichhornia azurea* Kunth. which caused severe blighting of immersed portion of plants, resulting in death of the entire plant. The sclerotia of the fungus remained viable for about nine months in the lake water without loss of virulence. According to Charudattan (1973) among the 32 fungal isolates tested on water hyacinth the most pathogenic was *Myrothecium roridum* followed by *R. solani* and *A. eichhorniae*.

Freeman and Charudattan (1974) observed purplish black spot with a tan centre in the distal portion of the leaf blade of water hyacinth and

identified the pathogen as *Cercospora piaropi*. During the survey on water hyacinth diseases in Florida, Louisiana, Panama and Puerto Rico, Rintz (1974) observed *R. solani* as the most vigorous pathogen causing extensive lesions on aerial parts of the plant. He also found minor pathogens *viz.*, *Nigrospora sphaerica, Apiocarpella* sp., *Curvularia lunata* (Wakker) Boedjin and *Pestalotia* sp. during the survey.

Rakvidyasastra and Visarathanonth (1975) isolated thirteen fungi from water hyacinth and among them *A. eichhorniae*, *M. roridum* and *R. solani* were found pathogenic to the weed. Zettler *et al.* (1975) reported about 150 additional fungal and bacterial isolates from water hyacinth in India including isolates of *A. eichhorniae*, *M. roridum*, *Cephalosporium zonatum* Corda. and *Rhizoctonia* sp. Among these fungi *C. zonatum* readily infected *E. crassipes* in the field.

Mycoleptodiscus terrestris Gerdemann, a root and crown infecting fungus was reported for the first time to cause a necrotic leaf spot on water hyacinth by Charudattan and Conway (1976). The fungus *Cercospora rodmanii* Conway was isolated from declining water hyacinth in Rodman reservoir of Florida (Conway, 1976a). Conway (1976b) evaluated the biological control potential of *C. rodmanii* under greenhouse and field conditions. A combination of mycelia and conidia was applied to the plants and the plants were killed by the pathogen.

Charudattan *et al.* (1978) worked on the seasonal occurrence of rust, *U. eichhorniae* on water hyacinth in Argentina. They found this pathogen to be a promising biocontrol agent of the weed. *Acremonium zonatum* (Sawada) Gams. could be used as a biocontrol agent of water hyacinth (Martyn and Freeman, 1978). They observed that plants inoculated with the fungus, *A. zonatum* responded differently to infection depending on the plants morphotypic state of development.

In a search to find out an effective biocontrol agent against *E. crassipes*, Abdel-Rahim (1984) isolated *Phoma sorghina* Sacc.

constantly from the diseased plants. In the study conducted in Sudan by Abdel-Rahim and Tawfig (1984) to identify microorganisms pathogenic to water hyacinth, many bacteria and fungi were isolated from diseased plant parts of the weed. Out of the 25 fungal and bacterial isolates only five were found pathogenic *viz.*, *A. zonatum*, *Drechslera spicifera* (Bain) Nicof., *F. equiseti*, *P. sorghina* and *Bacillus* sp.

Of the fungi found to be associated with *E. crassipes* in the North Western and Western Provinces of Sri Lanka, *Penicillium oxalicum, Curvularia lunata, Fusarium* sp., *Helminthosporium* sp., *M. roridum* and a sterile fungus were the main colonizers of leaf surfaces (Balasooriya *et al.*, 1984). Caunter (1984) made isolations from diseased leaves and leaf stalks of *E. crassipes* from Penang Islands and Kedah. Pathogenicity tests revealed that species of *Helminthosporium, Myrothecium* and *Chaetomiella* were pathogenic to water hyacinth.

Galbraith and Thyagarajan (1984) reported *Cercospora piaropi, A. zonatum* and *Fusarium graminearum* as most virulent pathogens of *E. crassipes* in Eastern Australia. Jamil and Narsaiah (1984) observed that of the three fungal pathogens isolated from *E. crassipes* plants, *A. eichhorniae* caused more damage than *Cercospora* sp. or *Fusarium solani* (Mart.) Sacc. *F. solani*, however, showed remarkable selectivity in attacking the older leaves of *E. crassipes* and its use as a co-pathogen with *Cercospora* appeared feasible.

Abdel-Rahim and Tawfig (1985) studied the biocontrol potential of *A. zonatum* and *P. sorghina*. *E. crassipes* plants when inoculated with *A. zonatum*, the percentage of diseased leaf area was 45 compared with 37 per cent leaf area infected by *P. sorghina*. Singh *et al.* (1985) worked on the mycoflora associated with water hyacinth in India. Of the various fungi isolated, *A. eichhorniae*, *Corticium solani* Prill and Delacr, *Curvularia* sp., *Pestalotia* sp., *M. roridum*, *U. eichhorniae* and *C. piaropi* were found potentially pathogenic to the weed.

Abdel-Rahim (1986) isolated *D. spicifera* from diseased leaves of *E. crassipes*. A survey on the mycoflora of water hyacinth in Andhra Pradesh was conducted by Jamil and Rajagopal (1986). They reported *Fusarium oxysporum* Schlet, *F. semitectum* Berk. and Rav., *Alternaria* sp., *Curuvlaria* sp., *Helminthosporium* sp. and a sterile fungus from water hyacinth.

Galbraith (1987) observed that feeding by *Neochetina eichhorniae* increased infection by *Acremonium zonatum* and growth of plants was reduced by 49 per cent. Aneja *et al.* (1988) isolated *Alternaria alternata* causing leaf spot of *E. crassipes*.

Aneja *et al.* (1990) observed heavy infection on water hyacinth in different locations in India. Plants showing small punctate leaf spots with an ash coloured centre becoming elliptical to irregular shape yielded *Fusarium chlamydosporum* Wollen and Reinking. *Epicoccum nigrum* Link was isolated from plants with compact zonation starting from tip of leaf and spreading backwards. Plants showing small brown coloured leaf spots, forming lesions of irregular shapes on the leaves and petioles yielded *P. sorghina*

During a survey conducted in Haryana, Aneja and Srinivas (1990) observed a leaf spot disease on water hyacinth at Kurukshethra. The affected leaves had small punctate to circular dark coloured spots with necrosis of leaf tip and chlorosis of lamina and petiole. The fungus obtained from infested water hyacinth was identified as *C. rodmanii*

A survey of fungus associated with naturally infected water hyacinth was conducted at different sites in Egypt (Elwakil *et al.*, 1990). They could isolate 200 fungal isolates belonging to different genera and found *A. alternata* to be the best candidate for biological control of water hyacinth. Morris (1990) reported severe decline of water hyacinth plants in South Africa by *C. piaropi*.

Aneja *et al.* (1993) observed *F. chlamydosporum* causing small, punctate leaf spots with ash coloured centres. They also found that small and young leaves were less susceptible to infection than larger and older leaves.

Santhi (1994) conducted an experiment to screen the fungal pathogens of water hyacinth for its biocontrol and isolated *F. equiseti, F. pallidoroseum* (Cooke) Sacc. *F. solani, R. solani, Colletotrichum gloeosporioides* (Penz.) Penz and Sacc., *C. lunata* and a sterile fungus. She also observed that *F. equiseti, F. pallidoroseum* and *C. gloeosporioides* had efficient biocontrol potential.

Shabana *et al.* (1995) reported that among the different isolates of *A. eichhorniae*, isolate five was the most virulent and caused severe disease, characterized by discrete leaf lesions resulting in leaf blight. Caunter and Lee (1996) isolated *A. eichhorniae* and *M. roridum* from water hyacinth in Malaysia. During a survey conducted in Western Cape and Eastern Cape provinces of South Africa, Alana-den-Breeyen (2001) reported *C. piaropi*, *C. rodmanii*, *A. zonatum* and *A. eichhorniae* from water hyacinth plants.

2.2 HOST RANGE OF PATHOGENS OF WATER HYACINTH

Detailed host range information is a requirement for the use of pathogens as efficient biocontrol agents to avoid potential hazards associated with the introduction into an area where pathogens did not occur previously or to accommodate changes in cropping pattern.

Host range studies conducted by Nagraj and Ponnappa (1970) revealed that of the 42 genera of plants tested, *A. eichhorniae* was found to be pathogenic only to *Monochoria vaginalis* Prest. *Mycoleptodiscus terrestris*, a pathogen of water hyacinth was found to be pathogenic to 10 host plants including eight legumes (Charudattan and Conway, 1976),

susceptible to *Fusarium lateritium* Nees ex Fries a pathogen of spurred anoda, prickly sida and velvet leaf (Walker, 1981b). He also found that soybean, maize, cotton and 18 other representative crops and weed species in eight families were resistant to the pathogen. Nagalingam (1983) reported that *F. semitectum* Berk and Rav. was safe to plants like chilli, cabbage, brinjal and tobacco.

Abdel-Rahim and Tawfig (1984) tested the susceptibility of 31 genera of cultivated plants to four highly pathogenic fungi isolated from water hyacinth. *Dreschlera spicifera* attacked *Sorghum vulgaris* and *Triticum vulgaris.* With the exception of *S. vulgaris* and *Nicotiana tabacum,* all other plants were infected with *Acremonium zonatum. Phoma sorghina* was found to be pathogenic to sorghum, sugarcane and wheat. *Fusarium equiseti* infected 20 out of the 31 plants showing a wide host range.

F. lateritium was effective in suppressing the growth of velvet leaf and prickly sida and it did not infect corn, soybean and cotton (Boyette and Walker, 1984). Studies by Hareendranath (1989) on the safety aspect of *F. pallidoroseum* showed that it was not pathogenic to rice, bhindi, chilli and tomato. Bedi and Borg (1993) reported that *F. oxysporum* f. sp. *orthoceras*, a potential mycoherbicide of sunflower broom rape did not infect sunflower, wheat, maize, tomato, tobacco and carrot.

Boyette *et al.* (1993a) observed that *F. oxysporum* a potential mycoherbicide for senna, sickle pod and hemp sesbania was not pathogenic on cucumber, squash, corn, Johnson grass and rice. But slight reduction in plant stand occurred in tomato and soybean.

Host range studies of *F. equiseti, F. pallidoroseum* and *F. solani* on six crop plants and six weed plants by Santhi (1994) found that *Fusarium* spp. could not infect the cultivated plants tested whereas it was pathogenic to the weed *M. vaginalis*.

thus it was excluded from the list of potential biocontrol agents of water hyacinth.

Conway and Freeman (1977) tested the susceptibility of *C. rodmanii*, a potential biocontrol agent of water hyacinth on 85 selected plants representing 22 families. Results showed that only squash, cucumber and spinach were found to be susceptible to *C. rodmanii* and the pathogen had a limited host range.

Rakvidyasastra *et al.* (1978) studied the host range of fungi pathogenic to water hyacinth and found that *R. solani* was pathogenic to all the test plants *viz.,* rice, maize, sorghum, cotton, tobacco and *Hibiscus sabdariffa* L. It also caused post emergence damping off of all except those plants belonging to family poaceae at the seedling stage. All the tested plants except tobacco was susceptible to *M. roridum*. *A. eichhorniae* infected only *H. sabdariffa*.

A narrow host range of the Australian isolate of *Acremonium zonatum* was indicated by negative host pathogenicity tests on known hosts *viz.*, fig and coffee and economically important plants *viz.,* radish, cucumber, tomato, tobacco, carrot and cotton (Galbraith, 1987).

Shabana *et al.* (1995) reported that none of the 97 plant species representing 21 families was susceptible to a virulent Egyptian isolate of *A. eichhorniae*. The host ranges of *C. piaropi* and *A. zonatum* native to Mexico and pathogens of water hyacinth were evaluated by Jimenez and Lopez (2001) on 31 plant species representing 22 families with economic and ecological importance. The results showed that only water lettuce (*Pistia stratiotes*) was infected by *C. piaropi*.

2.2.1 Host Range of *Fusarium* spp.

Andrews and Hecht (1981) observed *Fusarium sporotrichoides* Sherb on the aquatic weed *Myriophyllum spicatum*. In host range studies, *Abutilon theophrasti, Hibiscus trionum,* okra and hollyhock were

Manisegarame and Letchoumanane (1996) on the safety aspect of *F. pallidoroseum*, a biocontrol agent of rice leaf folder showed that it was not pathogenic to rice, cotton, brinjal and okra.

Naseema and Balakrishnan (1999) observed that out of the 30 crop plants and 41 weed plants tested, *F. equiseti* was not pathogenic to any of the crop plants tested except amaranthus. Among the various weed plants tested, *F. equiseti* infected *Amaranthus viridis* L, *Commelina benghalensis* L., *C. jacobi* (C.) Fischer and *M. vaginalis*. *F. pallidoroseum* was pathogenic to napier grass and 14 species of weed plants.

Rejirani (2001) reported that *F. pallidoroseum,* a biopesticide against pea aphid was not pathogenic to crop plants such as amaranthus, pineapple, drumstick, mulberry, banana, passion fruit and sesamum.

2.2.2 Host Range of *Colletotrichum* spp.

Host range studies of *Colletotrichum xanthii* Halst a potential pathogen of Bathurst Burr, showed that none of the 20 crop plants tested developed symptoms (Butler, 1951). Daniel *et al.* (1973) tested 30 plant species and 46 cultivars of economic and wild plants and found that only the target weed *Aeschynomene virginica* L and a related weed *A. indica* L were susceptible to *C. gloeosporioides* f.sp. *aeschynomene*

Boyette *et al.* (1979) reported that *C. gloeosporioides* f.sp. *jussiaceae* var.*glabrescens*, a pathogen of *Jusssiaceae* (*Ludwigia*) *decurrens* Walt. was not pathogenic to *J. repens* var *glabrescens,* rice, soybean and cotton.

C. gloeosporioides f. s.p. *aeschynomene* could be used for the biocontrol of Northern jointvetch (*Aeschynomene virginia*) in rice fields (Smith, 1986). Trijillo *et al.* (1986) worked on biocontrol of *Clidemia hirta* (L.) D. Don. using *C. gloeosporioides* in Hawaii. Host range studies indicated, appressoria formation on leaves of all the 11 ornamental species of the family Melastomataceae tested.

Mortensen (1988) studied host susceptibility of *C. gloeosporioides* f. sp. *malvae* isolated from *Malva pusilla*, a species of Malvaceae. The fungus was found to be host specific to *Malva* spp. and *Abutilon theophrasti* and not pathogenic to okra and cotton. TeBeest (1988) tested the susceptibility of 77 plant species from 43 genera in 10 families to *C. gloeosporioides* f. sp. *aeschynomene* and found four genera *viz.*, *Aeschynomene, Lathyrus, Lupinus* and *Vicia* were susceptible to the fungus. *C. gloeosporioides* f. sp. *aeschynomene* and *C. truncatum* infected the genera *Lupinus, Cicer, Indigofera, Lathyrus, Lens, Vicia* and *Pisum* in leguminosae (Weidemann *et al.*, 1988).

Santhi (1994) conducted host range studies of *C. gloeosporioides* on weed plants *viz.*, *C. benghalensis, Fimbristylis miliaceae* L., *Hydrocotyl asiatica* Urban, *M. vaginalis, Panicum repens* L. and *Ludwigia parviflora* L. Of these the fungus infected *C. benghalensis, H. asiatica* and *L. parviflora.*

Susha and Naseema (1998) reported that out of 30 cultivated and 41 weed plants tested, *C. gloeosporioides* was found to be pathogenic to amaranthus, bhindi, chilli, *Euphorbia hirta, Hydrocotyl asiatica* and *Phyllanthus niruri.*

Chetti *et al.* (1999) reported that *C. gloeosporioides* isolated from *Chromolaena odorata* is host specific and safe to be used as a mycoherbicide. They found that none of the field crops tested *viz.*, rice, wheat, sunflower, groundnut, cowpea, greengram, soybean and cotton and plantation crops *viz.*, coconut, arecanut, pepper, betelvine, cocoa, coffee and cardamom were susceptible to *C. gloeosporioides* isolated from *C. odorata.*

2.3 EFFECT OF CULTURE FILTRATE OF FUNGI ON PLANTS

Secondary metabolites produced by plant pathogens are known to play an important role in plant pathogenesis and disease expression. Research on the potential of secondary metabolites from microorganisms

for use as herbicides has met with practical success in the development of herbicides.

Nagraj and Ponnappa (1970) reported that cell free culture filtrate of *Alternaria eichhorniae* when sprayed on water hyacinth plants showed necrosis within 24 h and in the next 24 h the leaves scorched and dried up. These metabolites were found to have selective action and had positive effects on water hyacinth and *Monochoria vaginalis*. Metabolites produced by *Fusarium roseum* caused vascular browning in *E. crassipes* leaves but the culture of the fungus showed weak pathogenic activity (Rintz, 1974).

Abdel-Rahim and Tawfig (1984) tested the toxicity of culture filtrates of four pathogenic fungi *viz.*, *F. equiseti, Phoma sorghina, A. zonatum* and *D. spicifera* to water hyacinth. Culture filtrates of *F. equiseti, A. zonatum* and *P. sorghina* produced blighting of water hyacinth whereas filtrate of *D. spicifera* was not toxic to the weed. The toxicity symptoms induced were similar to the necrotic reaction of the respective isolates except that they developed much earlier.

Botryodiplodia theobromae Pat causing die back disease of ornamental roses was found to produce toxic metabolites *in vitro* (Akhtar *et al.*, 1991). Drop of culture filtrate placed on the leaves induced necrosis within 8-12 hours and yellowing within 24-48 hours. Abbas *et al.* (1991) reported that culture filtrate of *Fusarium moniliforme* Sheld exhibited phytotoxicity symptoms of mild to severe necrosis on Jimson weed. Santhi (1994) observed that the culture filtrates of the three species of *Fusarium viz.*, *F. equiseti, F. pallidoroseum* and *F. solani* produced symptoms on water hyacinth as these contained toxin. Mathur (1995) reported that culture filtrates of *Colletotrichum capsicum* (Syd.) Butl. Bisby isolates inhibited seed germination, seedling wilt and fruit damage. Khare and Goswami (1996) observed that filtrates of *Alternaria porri* (Elis) Ciferri.

inhibited root development of onion seedlings and the effect increased with incubation period from 4 to 14 days.

Susha (1997) reported that culture filtrate of *F. pallidoroseum* produced maximum damage (96.28 per cent) on water hyacinth plants followed by *F. equiseti* (83.00 per cent) and *C. gloeosporioides* (26.28 per cent). Application of culture filtrate of *Alternaria alternata* on dolichos bean produced symptoms of *Alternaria* leaf spot (Maheshwari *et al.*, 2000).

Pandey *et al.* (2002) observed that the phytotoxicity of cell free culture filtrate of various fungal isolates varied significantly. Maximum and rapid damage to the shoots of *Lantana camara* L. was caused by cell free culture filtrate of *Phoma herbarum* where epinasty of lower leaves followed by necrosis were frequently observed even within six hours of treatment. Severe chlorosis and curling of leaves were noticed with increase in incubation time and reached maximum after 48 h.

2.3.1 Dilution

Vidhyasekaran (1977) reported that toxin produced by *Helminthosporium nodulosum* was highly active up to 1 : 10 dilution, but further dilution reduced its activity markedly and the dilution end point is 1 : 500.

Maji and Prasanta (1986) reported that on dilution of the culture filtrate of *Rhynchosporium oryzae,* appreciable degree of phytotoxicity was observed upto 40 per cent concentration. On further dilution, phytotoxic effect was greatly reduced, but could be traced even at five per cent concentration. Akhtar *et al.* (1991) observed that culture filtrate of *Botryodiplodia theobromae* induced strong inhibition of shoot and root elongation of germinating wheat seeds upto a dilution of 1 : 10.

Maheshwari *et al.* (2000) reported that culture filtrate of *Alternaria alternata* at 1 : 10 dilution also inhibited seed germination and seedling growth of Dolichos bean.

Cell free culture filtrate of *Phoma sorghina* was effective in producing symptoms on *Lantana camara* even at 10 per cent dilution. Time required for complete collapse of the shoots was reduced significantly at higher concentrations (Pandey *et al.*, 2002).

2.3.2 Temperature

Narain and Das (1970) reported that the fungus *Colletotrichum capsici* produced a thermostable toxin which causes toxicity to host cell protoplasm without affecting the cell wall. Maity and Samaddar (1977) isolated heat stable toxic metabolite from fourteen day old culture filtrate of *Alternaria eichhorniae*.

Effect of temperature on phytotoxicity of the culture filtrate of *Rhynchosporium oryzae* was studied by Maji and Prasanta (1986). At 5°C phytotoxic effect of culture filtrate was not manifested and it was pronounced at a temperature of 22-27°C. Akhtar *et al.* (1991) reported that toxic principle contained in the culture filtrate of *Botryodiplodia theobromae* was heat stable and non specific.

Mathur (1995) reported the thermostable nature of culture filtrate of *Colletotrichum capsici* stating that both autoclaved and unautoclaved filtrates inhibited seed germination of chilli. The seed germination and seedling growth of *Lablab purpureus* was inhibited by unheated culture filtrates of *Alternaria alternata* (Maheshwari *et al.*, 2000).

Thermal sensitivity test on cell free culture filtrate of *Phoma sorghina* at 121°C for 15 minutes indicated that the toxins produced by the fungus was temperature tolerant and thermostable (Pandey *et al*, 2002).

The toxin produced by *H. nodulosum* was stable upto a temperature of 90°C, but when autoclaved or heated at 100°C, the toxin activity was almost lost (Vidhyasekaran, 1977).

2.3.3 pH

Vidhyasekaran (1977) observed that toxin produced by *H. nodulosum* was stable to pH 3-7, but unstable at alkaline range. Khare and Goswami (1996) reported that the effect of culture filtrates of *Alternaria porri* increased with increase in pH from 4 to 8.

2.4 TOXINS PRODUCED BY PLANT PATHOGENIC FUNGI

Use of purified phytotoxins of microbial origin have certain advantages over living pathogens on biocontrol of weeds in their handling, quantification, application and biodegradability in the field.

Fusaric acid from *Fusarium* infected plants caused wilting of several plants (David, 1969). Maity and Samaddar (1977) isolated a toxic metabolite from 14 day old culture filtrate of *Alternaria eichhorniae*. This was heat stable and a partially purified toxin showed some degree of host specificity. At lower concentrations it reproduced typical blight symptoms on water hyacinth leaves.

A heat stable, high molecular weight toxin, isolated from culture filtrate of *Cladosporium fulvum* Cooke caused callus formation, host cell necrosis and ion leakage in tomato leaf tissue (Lazarovits *et al.*, 1979). Highly purified toxin contained a polydisperse glycoprotein with a molecular weight in the range of 3×10^4 to 2.5×10^3 daltons.

Stevens *et al.* (1979) made an attempt to isolate and characterise the phytotoxic substance produced by *Alternaria* sp. However, it was observed that the major metabolic constituent bostrycin obtained from *Alternaria eichhorniae* showed no herbicidal activity towards *E. crassipes*. A compound called maculosin was isolated from *Alternaria alternata* infecting spotted knap weed by Dinoor and Eshed (1984). They

also found that 19 other grasses and broad leaved plants tested were not injured by maculosin, even at a concentration of 1 μm.

Howell and Stiponovic (1984) demonstrated the potential of necrogenic toxin producing fungus *Gliocladium virens* Miller Giddens and Foster as mycoherbicide on various weeds. *Alternaria eichhorniae* a pathogen of water hyacinth, produced a phytotoxin alteichin, which caused necrotic lesions, analogous to the pathogen infection when the compound was applied at 1-10 μg/10 μl droplet (Robenson *et al.*, 1984).

Cutler (1986) isolated and characterized a mycotoxin with herbicidal activity from moniliformin molecule synthesized by *Fusarium moniliforme*. Moniliformin from *F. moniliforme* controlled *Setaria italica, Lolium prenne, Sinapsis alba* and *Stellaria media* by post emergent application. Tentoxin, a cyclic tetrapeptide showed potent chlorosis activity on a variety of weeds of soybean and maize (Lax *et al.*, 1988).

Abbas *et al.* (1991) isolated a toxin from *Fusarium moniliforme*, grown on rice and it caused soft rot, diffusing along veins in jimson weed. Only identifiable phytotoxin detected from the filtrate of *F. moniliforme* was fumonisin B_1 which was isolated in amounts of 400 μg/g. Members of the genus *Fusarium* produced a range of phytotoxic compounds that are chemically diverse (Hoagland, 1990). Moniliformin a fusarial toxin caused growth inhibition, necrosis and chlorosis in plants. Mirocha *et al.* (1990) reported that fumonisin B_1, a toxin produced by *Fusarium* spp. caused inhibition in the growth and development of corn callus and was toxic to tomato plants.

Strobel *et al.* (1990) isolated and identified a diketopiperazine compound, maculosin from a strain of *Alternaria alternata* infecting spotted knap weed (*Centaurea maculosa* Lam.). This toxin was phytotoxic and host specific at 10^{-3} and 10^{-5} concentration. Another phytotoxin tenuzoic acid was also produced by *Alternaria alternata*.

Fusaric acid isolated from several *Fusarium* spp. was found to be very active against weeds, including jimson weed and duck weed (Abbas *et al.*, 1991). Paterson and Rutherford (1991) described a simplified thin layer chromatography (TLC) extracellular technique for the detection of fusaric acid from *Fusarium* strains. Fusaric acid showed a dark blue spot with a Rf value of 0.16. Stierle *et al.* (1991) isolated cyperine, a phytotoxin from *Ascochyta cypericola*, a pathogen of *Cyperus rotundus*.

Vesonder *et al.* (1992) studied the phytotoxicity of fusarial toxins on chlorophyll synthesis in the aquatic weed *Lemna minor*. Fumonisin B_1 proved to be the most active toxin against the weed in reducing the growth by 53 per cent and its ability to synthesize chlorophyll by 59 per cent at a concentration of 0.7 µg/ml. The growth rate of *L. minor* was reduced by 50 per cent with fusaric acid at a concentration of 6.7 µg/ml. Moniliformin was found to be the best phytotoxic fusarial toxin.

Zeaneol, isolated from culture filtrates of *Dreschlera portulacae* was characterized using single crystal x-ray differential and nuclear magnetic resonance (Kim, 1994). When applied at 3×10^{-6} M, zeaneol inhibited root length in *Echinocloa crusgalli* and *Abutilon avecinnae* by 22.8 and 54.8 per cent respectively.

Abbas *et al.* (1995) detected the presence of fumonisins, fusaric acid and moniliformin in the isolates of *Fusarium moniliforme* and *F. oxysporum* but none of the isolates tested produced more than one phytotoxin. Altomare *et al.* (1995) isolated a toxic compound from extracts of maize grown culture of *Fusarium tumidum*.

Bilgrami *et al.* (1995) studied the synthesis of zearalenone, deoxynivalenol and T-2 mycotoxins by the isolates of *Fusarium* sp. under varied environmental conditions. Production of these toxins were maximum after six weeks of incubation at 25°C temperature when the grain moisture was 50 per cent. Tseng *et al.* (1995) reported that fumonisin producing strain of *Fusarium moniliforme* are widely

distributed among the economic crops such as corn, rice, sugarcane and sorghum in Taiwan.

Phytotoxic polypeptide was identified by Jin *et al.* (1996) in the culture filtrate of *Fusarium solani* after purification by gel filtration chromatography. Fractions containing the protein caused, browning of soybean calli, necrosis on detached soybean cotyledons and leaves.

Fusaric acid and 9,10-dehydro fusaric acid and their methyl esters were shown to be the main phytotoxins produced by *Fusarium nygamai*, a potential control agent of striga (Zonno *et al.*, 1996). The application of very low amount of toxin caused reduction of seed germination. The toxic effects of these metabolites on punctured leaves showed the appearance of large necrotic spots.

Moniliformin, a phytotoxin isolated from *Fusarium moniliforme* causes growth inhibition, necrosis and chlorosis in weeds such a jimsonweed (Abbas and Duke, 1997). They also reported that moniliformin is not suitable as a herbicide, because of its toxicity to animals.

Meredith *et al.* (1997) isolated and purified mycotoxin fumonisin B_2 from *Fusarium moniliforme* grown on rice. Fumonisins were extracted with acetonitrile.

Singh and Saxena (1997) observed that fusaric acid was produced by *Fusarium solani* and *F. equiseti* and a direct correlation exists between toxin production and virulence of the fungi. Cauliflower seedlings treated with fusaric acid solution produced typical wilting symptoms, early yellowing and defoliation indicating a positive role of toxin in disease development.

Susha (1997) isolated toxin from the cell free metabolite of *Fusarium* spp. which are pathogenic to water hyacinth and was identified as fusaric acid by paper chromatography.

Phytotoxin isolated from *Fusarium avenaceum* showed wilting symptom on broad bean, cucumber, okra and sesamum seedlings when the roots of the seedlings were soaked in culture filtrate for 24 h (Al-Hamdany, 1998). In thin layer chromatography plates the toxins gave single spot and pink colour when examined under UV light. Rf values of the toxins were 0.87 and 0.82 on Butanol : acetic acid : water and isopropanol : n butanol : water solvent systems respectively.

Gupta (1998) reported that *Fusarium* spp. isolated from sorghum grains were capable of producing zearalenone in variable amounts. Solvent fractions from the culture filtrate of *F. moniliforme* decreased germination and seedling vigour in sorghum (Niranjana and Shekhar, 1998). This was attributed to the secondary metabolite zearalenone.

Culture filtrate of *Drechslera rostrata* yielded a toxin complex (a dark red viscous substance) which was further purified by thin layer chromatography (Shukla *et al.*, 1998). TLC analysis showed a compound t_1 (Rf value – 0.39) which was found to be highly toxic producing necrosis and chlorosis of *Costus speciosus* leaves followed by yellowing and defoliation at 400 µg/ml. Symptoms produced by the phytotoxin (t_1) on leaves were similar to those of natural infection of the fungus on *Costus* sp.

Naseema *et al.* (2001a) reported that toxins isolated form the culture filtrates of *F. pallidoroseum* and *F. equiseti* produced brown spots on leaves and petiole of water hyacinth which later enlarged resulting in the blighting and drying up of the entire plant.

2.5 MASS PRODUCTION AND STORAGE OF FUNGI ON LOCALLY AVAILABLE SUBSTRATES

Large scale use of fungi for biological control requires mass production of the inoculum. Low input mass production systems utilizing agricultural or other waste materials may be of great value. Many

attempts have been made in the past to mass produce biocontrol fungi in naturally available substrates.

2.5.1 Solid substrate

Beevi (1979) reported that for mass production of *Fusarium moniliforme* var. *subglutinans*, sorghum and bajra grains appeared to be most suitable as they produced maximum spores with high virulence. Chlamydospores of *F. oxysporum* f. sp. *cannabis* produced on a mixture of barley straw, glycine-succinate and sodium nitrate retained their disease potential over a six month period at room temperature (Hildebrand and McCain, 1978).

Kuruvila and Jacob (1981) observed that greengram, wheat or sorghum could be used as substrates for easy production of the fungus *F. oxysporum*. Nagalingam (1983) observed broken maize grains plus blackgram husk or redgram husk at 4 : 1 w/w as a suitable media for the mass production of *F. semitectum*. Abundant sporulation of *F. glutinans* was observed on sorghum grains by Raghavendran *et al.* (1987).

Mathai *et al.* (1988) conducted studies on growth and sporulation of *F. pallidoroseum* on locally available and cheaper substrates *viz.*, rice bran, wheat bran, paddy straw bits, tapioca bits, tapioca stem peelings, vegetable waste and their different combinations of the substrates. Wheat bran and rice bran plus tapioca bits gave maximum spore, count followed by wheat bran plus straw bits. The growth of the fungus was very poor in vegetable waste and its combination and also on tapioca stem peelings.

Hareendranath (1989) reported broken maize grains 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. Morris (1989) observed wheat bran inoculum of *C. gloeosporioides* remained viable for four weeks and spore production started declining after extended periods of storage.

Horsten and Kempenaar (1994) reported wheat bran as the best substrate for spore production by *Ascochyta caulina* and the spores produced on wheat bran inoculum stored at 5°C were infectious even after eight months.

Santhi (1994) conducted an experiment to study the use of different carrier materials for storage of potential pathogens of water hyacinth and concluded that wheat and rice bran are good carrier materials for *Fusarium* spp. and *Colletotrichum gloeosporioides.*

Fravel *et al.* (1995) used pyrophyllite clay, corn cobs, milled chitin, fish meal, neem cake, peanut hulls, soyfibre and wheat bran to make alginate prill formulation of *Talaromyces flavus.*

Mehta *et al.* (1995) reported that solid state fermentation using rice bran and wheat bran was the cheapest method for the production of biomass of *Trichoderma harzianum.* Preparation of *T. harzianum*, *T. viride*, *T. koningii* and *Gliocladium virens* had been developed at Pantnagar in wheat bran-saw dust medium (Sharma and Basondrai, 1996).

An isolate of *F. oxysporum* (M12- 4A) grown on sorghum straw and incorporated into pots, successfully prevented the emergence of weed *Striga hermonthica* (Ciotola *et al.*, 1995). Diarra *et al.* (1996) reported sorghum straw for maximum production of micro conidia of *F. oxysporum* (M12-4A). When straw or glumes were soaked overnight, *F. oxysporum* sporulated abundantly producing micro and macro conidia and later chlamydospores. Isolate M12-4A grown on sorghum straw or glumes survived and remained viable for atleast 12 months when stored at room temperature.

Wheat and rice bran were found to be suitable substrates for the mass production of *F. pallidoroseum*, as maximum number of spores with higher virulence were produced in them within a short period (Faizal and Mathai, 1996). They reported that the best time for harvest of the spores

was 8th day after inoculation. Wheat bran showed white cushiony mycelium which covered the entire surface within the shortest time of ten days.

Evaluation of four substrates *viz.*, neem cake, farm yard manure, coffee husk and tea waste for the mass production of fungal biocontrol against *T. harzianum* and *T. virens* indicated that tea waste was the best medium (Prakash *et al.*, 1999). Cultures produced in tea waste could be stored for three months without much reduction in the populations of the fungus.

Three carrier materials *viz.*, talc, kaolin and bentonite were tested for the mass production and storage of *T. harzianum* (Prasad and Rangeshwaran, 2000). A significant decline in *Trichoderma* population was noticed in all the three carrier materials after 120 days. Talc and kaolin retained more than 10^6 viable propagules upto 90 days and by 120 days propagules declined below the optimum level. Kaolin and talc were concluded as better carriers of *T. harzianum*.

Rejirani (2001) reported that carrier materials like diatomaceous earth, charcoal powder, leaf mold and saw dust could maintain 50 per cent viability of *F. pallidoroseum* at room temperature, till the end of four months of storage. Viability of spores stored in bran, peat and vermicompost were greatly reduced within 2-3 months of storage at room temperature, whereas, neem and cowdung, did not support the growth and sporulation of *F. pallidoroseum*.

Tewari and Mukhopadhyay (2001) observed that *Gliocladium virens* grown on sorghum grains added with carboxy methyl cellulose showed spore viability upto nine months.

Susha and Naseema (2002) reported guinea grass straw as a good substrate for growth and sporulation of *F. pallidoroseum* and *F. equiseti*,

with maximum number of spores upto 40 days for *F. equiseti* and 20 days for *F. pallidoroseum*.

2.5.2 Liquid Substrates

Hildebrand and McCain (1978) reported that diffusates from alfalfa straw, cotton seed meal and soybean oil meal induced chlamydospore formation in *F. oxysporum* f. sp. *cannabis*, but few spores were formed with diffusates from sugar beet pulp, barley straw or safflower meal. Batista *et al.* (1989) attempted utilization of naturally available liquid substrates for mass production of *Beauveria bassiana*. They observed better conidial production in bran broth compared to rice and potato broth.

Among various liquid media tried for mass production of *Beauveria bassiana* and *Paecilomyces* sp., coconut water was found to yield maximum number of spores (Ibrahim and Low 1993). Manisekarame and Letchoumanane (1996) tried coconut water, par boiled rice water, par boiled rice gruel, raw rice water and raw rice gruel for the mass production of *F. pallidoroseum*. They observed maximum biomass production in coconut water compared to other substrates. Rejirani (2001) studied the effect of coconut water, boiled rice water and raw rice water on growth and sporulation of *F. pallidoroseum* and found that coconut water recorded maximum growth and sporulation compared to others.

2.6 INTERACTION OF FUNGI WITH HERBICIDES

By integrating biocontrol fungi with herbicides it is possible to increase the efficiency of fungi, reduce the chemical dosage and minimize effects on non target plants and the environment.

Yu and Templeton (1983) reported that trifluralin at 1 kg ai/ha was not toxic to *Fusarium solani* f. sp. *cucurbitae* and the tank mixture or sequential application of the fungus and trifluralin caused earlier disease incidence and higher seedling mortality to Texas gourd than that achieved by the application of the fungus alone.

Quimby (1985) concluded that tank mixture of *Fusarium lateritium* and acifluorfen had synergistic activity on prickly sida.

Spraying water hyacinth with the mycoherbicide *C. rodmanii* with sublethal rates of 2,4-D (154 ppm) resulted in improved weed control compared to using both alone (Charudattan, 1986). Liu and Lozano (1987) reported that sequential application of propanil (1 kg ai/ha) followed by the mycoherbicide Collego was not satisfactory in managing jointvetch and water primrose in rice fields and resulting in poor yield.

In greenhouse and field experiments, Boyette and Quimby (1988) observed that when *F. lateritium* was applied in tank mixtures or 2,4-DB (1.1 kg ai/ha) applied after the fungus, the control of *Abutilon theophrasti* (velvet leaf) was not effective. But when 2,4-DB was applied before *F. lateritium* the efficacy of bioherbicide was enhanced.

The combination of plant growth regulator, thidiazuron and plant pathogenic fungus *Colletotrichum coccodes* suppressed velvet leaf competition in soybean fields thereby increasing crop yield (Hodgson *et al.*, 1988). Tank mix combinations of *C. coccodes* (10^9 spores/ml) and thidiazuron 0.1 and 0.2 kg ai/ha were approximately twice as effective as the same rate of thidiazuron alone.

Sublethal doses of 2,4-D had no adverse effect on *in vitro* mycelial growth of *Myrothecium roridum* (Liyange and Gunasekara, 1989). Efficacy of *M. roridum* as a biocontrol agent of water hyacinth was enhanced when used sequentially with low rates of 2,4-D (5-10 per cent of recommended rate, 1 kg ai/ha). Spraying with tank mix combinations of the fungus *C. coccodes* and thidiazuron increased mortality, decreased biomass and height of the weed, *Abutilon theophrasti* and increased soybean yield (Wymore and Watson, 1989). Grant *et al.* (1990a) reported 20 per cent reduction in spore germination and appressorial formation of *C. gloeosporioides* f. sp. *malvae* with herbicides *viz.*, 2,4-D (1 kg ai/ha) under *in vitro* condition. Herbicides *viz.*, Cyanazine (1.4 kg a.i/ha) and

dicamba (0.4 kg a.i/ha) increased appressorial formation without reducing germination compared to control.

Grant *et al.* (1990b) demonstrated that the control of round leaved Mallow (*Malva pusilla*) can be improved by simultaneous application of the herbicides bromoxynil (0.28 kg a.i/ha) plus MCPA (0.28 kg ai/ha), metribuzin (0.21 kg a.i/ha) or imazethapyr (1.1 kg ai/ha) with *C. gloeosporioides* f. sp. *malvae*, whereas application of bioherbicide or herbicide alone did not achieve the same level of control. Herbicides *viz.*, 2,4-D (1 kg ai/ha), alachlor (1 kg ai/ha) and oxyfluorfen (1 kg ai/ha) at their respective recommended concentrations and at 0.5 and 1.5 times their concentration inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *cumini* (Patel and Patel, 1993). Basidiomycete fungus *Chondrosterum purpureum* was found to be a good potential biocontrol candidate for control of hard wood vegetation in forests (Rajprasad, 1994). 2,4-D at low concentration (0.01 per cent) was proved to be compatible with the fungus but bialaphos and glufosinate inhibited the growth of the fungus drastically.

Combination of imazaquin at 18 g ai/ha and *Alternaria helianthi* (4×10^5 conidia/ml) was neither synergistic nor anatognistic in killing the weed *Xanthium strumerium* (Abbas and Barrentine, 1995). Rayachhetry and Elliott (1997) reported that mixing inoculum of *Botryosphaeria ribis* with herbicides imazapyr at 12-60 mg ai/ml enhanced the efficacy of the fungus in managing the weed, *Melaleuca*. But glyphosate even at very low concentration reduced the inoculum viability of the fungus.

Susha and Naseema (1998) reported that under *in vitro* condition 2,4-D at 0.1 per cent concentration resulted in 68.5 and 77.8 per cent inhibition in the mycelial growth of *Fusarium equiseti* and *F. pallidoroseum* respectively.

When Collego was applied along with acifluorfen it resulted in the better management of northern joint vetch than the application of the Collego alone (Te Beest and Guerber, 2000).

2.7 FORMULATION OF BIOCONTROL FUNGI

The commercialization of a microbial biocontrol agent is possible only by developing a market acceptable formulation.

Walker (1981a) described a method for large scale production of granular formulation of *Alternaria macrospora* Zimm. consisting of spores, mycelia and vermiculite. This formulation of *A. macrospora* spores was applied as pre-emergent or post emergent herbicide to control spurred anoda.

Aqueous mixtures of one per cent sodium alginate and homogenized mycelia of *Alternaria cassiae* Jurair and Khan and *F. lateritium* pelletized by dropwise additions of each mycelial mixture into 0.25 M $CaCl_2$ (Walker and Connick, 1983). Ten per cent clay was incorporated into the pellets and the formulation was air dried.

Boyette and Walker (1984) found that a granular formulation of *F. lateritium* prepared by mixing with sodium alginate and kaolin clay was effective in suppressing the growth of velvet leaf and prickly sida. This formulation also controlled the weeds without affecting the crop. Walker and Boyette (1985b) developed a procedure for granulation of mycelial inoculum of *Cercospora kikuchi* Mats and Tommy, containing mixture of sodium alginate, kaolin, clay and mycelium in 0.25 M $CaCl_2$ solution containing 3.8×10^6 conidia/g of air dried granules.

Quimby (1985) developed a water dispersible spray product, by mixing more conidia of *F. lateritium* with hydrated silica powder, peptone and starch. Collego a post emergence mycoherbicide for the control of northern joint vetch in rice soybean fields is a two component product (Bowers, 1986). Component A is a water soluble spore rehydrating agent

while component B is a water suspendible dried spore preparation of the fungus *C. gloeosporioides* f. sp. *aeschynomene*.

Devine, marketed by Abbott Laboratories, USA is the first registered mycoherbicide. The formulation contained chlamydospores of *Phytophthora palmivora* in V-8 juice medium (Kenney, 1986). The same company developed an experimental formulation of *Cercospora rodmanii* against *E. crassipes*. The formulation named ABG-5003, consisted of mycelial fragments and spores and was applied as wettable powder (TeBeest, 1991).

Weidemann and Templeton (1988) formulated macroconidia of *Fusarium solani* f. sp. *cucurbitae* in alginate to control Texas gourd. Granules containing spores, mycelium and soyaflour (2 per cent w/v), applied to soil resulted in 70 per cent or higher mortality of the weed. Biomal, a microbial herbicide containing *C. gloeosporioides* f. sp. *malvae*, has been produced as wettable powder with silica gel carrier (Boyette *et al.*, 1991).

Connick *et al.* (1991) developed an oil phase emulsion of *Alternaria cassiae,* a pathogen of *Cassia obtusifolia.* The oil phase contained paraffin wax, paraffin spray oil and an unsaturated monoglyceride emulsifier. The oil phase was mixed with 1 : 1 w/w water.

A wettable powder formulation of *Fusarium pallidoroseum* with diatomaceous earth was developed by Faizal (1992) and found that the formulation containing 3.5 x 10^6 spores/ml was as effective as aqueous suspension of the spores against aphids. Also found that diatomaceous earth based wettable powder was better than talc based dust formulation.

Boyette *et al.* (1993b) reported that by the application of *Colletotrichum truncatum* in an invert emulsion formulation gave 100 per cent control of hemp sesbania. The oil phase of the invert emulsion consisted of paraffin oil, monoglyceride emulsifier, paraffin wax and lanolin.

Egley and Boyette (1995) reported that the water unrefined maize oil emulsion of *Colletotrichum truncatum* improved conidial germination and did not affect appressoria formation. The emulsion enhanced mycoherbicide efficacy by increasing conidial germination from 30 per cent in water alone to 92 per cent and protecting the conidia during dew free period.

Spores of *C. orbiculare* dried in kaolin and mixed with vegetable oil or mineral oil improved mycoherbicide activity in comparison with application of spores in water alone (Klein *et al.*, 1995). Jackson *et al.* (1996) reported that dried preparation of *C. truncatum* micro sclerotia encapsulated in pregelatinized corn flour increased the effectiveness of this bioherbicde.

Shabana (1997) prepared dried mycelium alginate (1 per cent) of *Alternaria eichhorniae* containing 10^{10} propagules/g was mixed with 15 per cent vegetable oil, four per cent soybean lecithin and 80 per cent water. The oil emulsion formulation induced higher level of disease.

Control of fire weed (*Epibolium angustifolium*) increased when *Colletotrichum dematium* f. sp. *epilobii* conidia were suspended in 25 per cent v/v canola oil/water emulsion. The formulation reduced the inoculum concentration and the required dew period was reduced to 12 h and also the effect of temperature during the dew period was minimised (Leger *et al.*, 2001).

2.7.1 Effect of Formulation Ingredients on Growth and Sporulation of Fungi

The mycoherbicidal efficiency of *A. cassiae* and *F. lateritium* were improved by mixing mycelia with one per cent sodium alginate (Walker and Connick, 1983). Andersen and Walker (1985) reported that Tween 80 at different concentrations, tried was compatible with *C. coccodes*.

Charudattan *et al.* (1985) used triton X-100 (0.05 per cent v/v) as wetting agent along with *Cercospora rodmanii* to improve its biocontrol efficiency.

Addition of sucrose to the formulation of *Alternaria macrospora* increased the severity of disease caused by fungi on spurred anoda (Walker and Boyette, 1985a). Similarly sucrose increased spore germination and disease severity on Florida beggar weed (*Desmodium tortuosum*) caused by *Colletotrichum truncatum* (Cardina, 1986).

Connick (1988) reported the formulation of weed killing fungi. *Alternaria* sp., *Fusarium* sp. and *Phyllosticta* sp. using alginate which has got excellent gel forming properties Grant *et al.* (1990a) observed that spore germination of *C. gloeosporioides* f. sp. *malvae* a mycoherbicide of *Malva* sp. was higher in suspensions with Tween 20 (0.1 and 0.5 per cent) and sucrose (0.1 and 0.5 per cent). But alcohol or ether based adjuvants like triton X-R and Agral 90 inhibited spore germination.

Formulation of *A. cassiae* spores containing the surfactant 0.1 – 1.0 per cent, Tween 80, 0.02 M potassium phosphate buffer and one per cent potato dextrose broth promoted germination of spores and thereby improved the mycoherbicidal efficacy (Daigle and Cotty, 1991). Rajprasad (1994) reported that an adjuvant, Bond and a sunscreen agent suntan gel-2 showed good compatibility with the fungus *Chondrostereum purpureum* at 0.01 and 0.1 per cent v/v.

Under *in vitro* conditions corn oil emulsion and surface active agents like Tween 20, Tween 40, Tween 60 and Tween 80 maintained the germination ability of *Alternaria helianthi* (Abbas and Egley, 1996). Inoculum viability of *Botryosphaeria ribis* remained unaffected by Dyne-Amic (surfactant) at 1, 5 and 10 per cent v/v for 24 h. But after 24 h, the viability reduced inconsistently (Rayachhetry and Elliott, 1997).

Alginate formulations supplemented with the hydrophilic polymer were more effective in promoting disease (Shabana *et al.*, 1997). Greaves

et al. (1998) reported that glycerol at 0.1 per cent concentration stimulated conidial germination and appressoria formation in *Colletotrichum dematium*. They found that both medium and high viscosity alginic acids at 0.1 per cent w/v inhibited germination of *C. dematium* conidia.

2.7.2 Shelf Life of Formulated Biocontrol Fungi

There are various reports on the shelf life of the biocherbicide formulations. Walker and Connick (1983) reported that sodium alginate-clay formulation of *A. cassiae* could be stored at 4°C for 6-8 months. *F. pallidoroseum* spores retained 75 per cent viability till four days storage and thereafter a marked decrease was noticed in the virulence of the fungus in water, talc and diatomaceous earth formations (Faizal, 1992).

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

Mortensen (1988) found spores in wettable powder can survive upto two years without unacceptable loss of viability and efficacy. Dried mycelial formulation of *Metarhizium* remained viable for more than a year at 4°C (Booth *et al.*, 2000).

Wettable powder comprising of a hydrophilic carrier material with hydrophobic spores was the most efficient formulation of fungal control agents (Butt *et al.*, 2001). Honeycutt and Benson (2001) reported that the shelf life of binucleate *Rhizoctonia* spp. declined to 68 per cent of the original propagule concentration after six months in pesta and rice flour formulation, with the maximum decline in the first two months. Spores of *Paecilomyces lilacinus* stored at 0°C on tapioca broth + talc formulation

and paddy grain + talc formulation showed more than 80 per cent viability even 150 days after storage (Nagesh *et al.*, 2001). Spore viability declined with increasing storage duration and temperature. The viability of spores in grain based formulation declined more rapidly than those in tapioca based formulation.

Conidial viability of *Metarhizium anisopliae* formulated with emulsifiable adjuvant oils and spreader declined at 27°C when stored for 40 weeks. But conidial viability was maintained above 40 per cent when temperature was at 10°C (Alves *et al.*, 2002).

2.8 SAFETY OF FORMULATED BIOCONTROL FUNGI TO AQUATIC FAUNA

Any biocontrol fungi to be a successful candidate in weed control should be safe on non target organisms.

Genthner *et al.* (1993) reported that eastern oysters exposed to the spores of *C. gloeosporioides* f. sp. *aeschynomene* showed no signs of infectivity. Srivastava *et al.* (1994) observed that *Achyla orion*, *Saprolegnia diclina* and *Pythium aphanidermatum* caused fungal infection of skin and eye of fish (*Chela laubauca* Ham.) showing destruction of skin and profuse hyphal growth in eyes.

Fusarium moniliforme and *F. udum* were found to cause mycoses and high mortality in fresh water fishes *viz.*, *Labeo rohita, Barbus rana* and *Channa punctatus* (Deepa *et al.*, 2000). Otto (2000) observed that during a four year period study, *Cladosporium, Saprolegnia, Candida* and *Penicillium* cause fungal infection on fishes.

Out of 1225 fishes screened for fungal infections, four species of fishes *Chanda ranga, Labeo rohita, Nandus nandus* and *Puntius sarana* showed varying degrees of infection on epidermis (Qureshi *et al.*, 2001). The infected skin showed the presence of fungal mycelia resulting in the necrosis of cells and formation of granuloma in some cases. The pathogenic fungi observed were *Achyla* sp., *Aphanomyces* sp. and *Dictyuchus* sp.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Laboratory and pot culture studies were carried out at College of Agriculture, Vellayani, Thiruvananthapuram, Kerala to explore the feasibility of utilizing fungi or their metabolites in the management of water hyacinth and to develop an effective mycoherbicide formulation. The field trial connected with the work was carried out at Aakkulam lake near Thiruvananthapuram.

3.1 SURVEY ON THE FUNGAL PATHOGENS OF WATER HYACINTH

The survey was conducted for a period of one year (February 2000 – March 2001) at quarterly intervals *viz.*, February – March (2000), June – July (2000), October – November (2000), February – March (2001) in the four southern districts of Kerala (Fig. 1) *viz.*, Thiruvananthapuram, Kollam, Alappuzha and Kottayam to identify fungal pathogens of water hyacinth. In each district observations were taken from five locations (Table 1).

Table 1 Locations surveyed and observatories from where the weather data collected

Sl. No.	District	Location	Observatory
1	Thiruvananthapuram	Aakkulam	Centre for Earth Science Studies, Aakkulam
		Ambalathara	College of Agriculture, Vellayani
		Muttathara	College of Agriculture, Vellayani
		Veli	Centre for Earth Science Studies, Aakkulam
		Venpalavattom	College of Agriculture, Vellayani

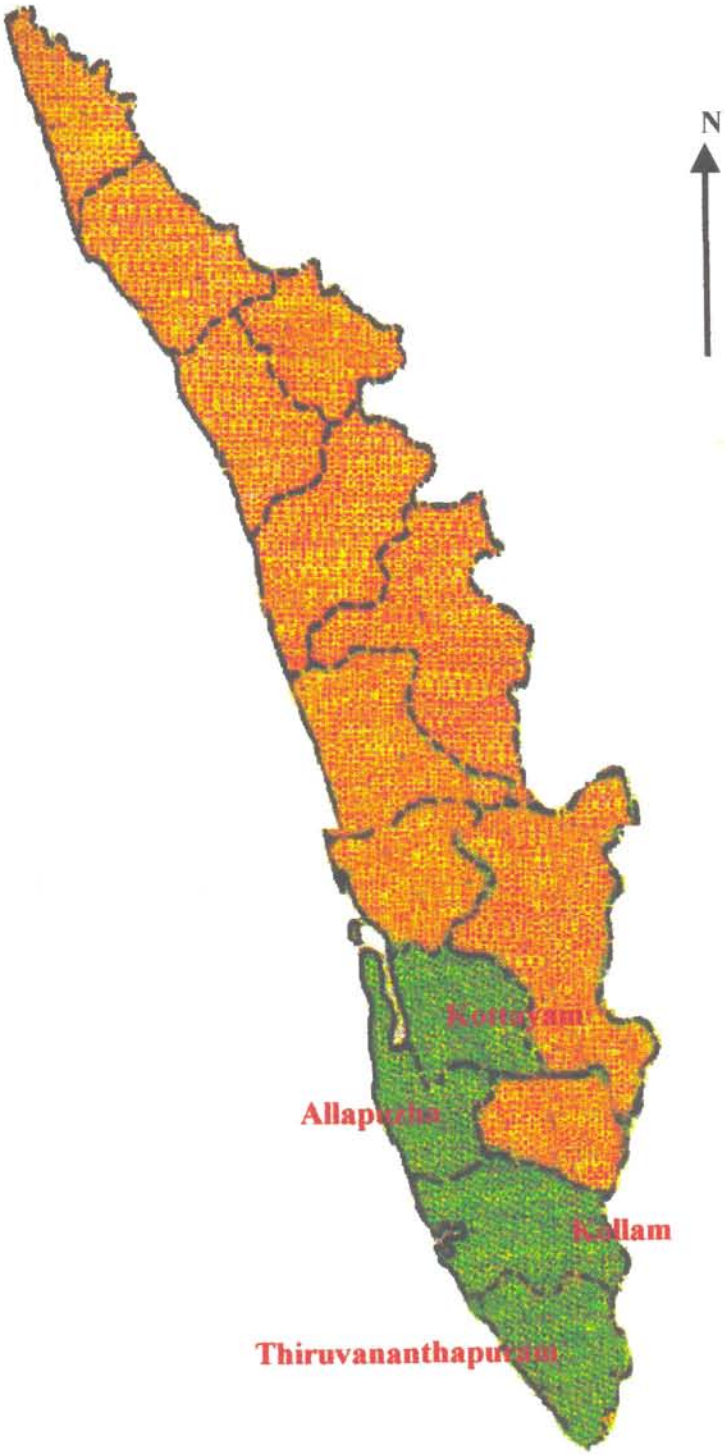


Fig. 1. Map showing districts surveyed in Kerala State

2	Kollam	Asramam	Farming Systems Research Station, Kottarakkara
		Chavara	"
		Kollam town	"
		Karunagapally	"
		Thevally	"
3	Alappuzha	Haripad	Rice Research Station, Moncombu
		Karumadi	Regional Agriculture Research Station, Onattukara
		Karuvatta	Rice Research Station, Moncombu
		Mullackal	Rice Research Station, Moncombu
		Nedumudi	Regional Agriculture Research Station, Onattukara
4	Kottayam	Karapuzha	Rubber Research Institute of India, Kottayam
		Kodimatha	Rubber Research Institute of India, Kottayam
		Kollad	Rubber Research Institute of India, Kottayam
		Kumarakom	Regional Agriculture Research Station, Kumarakom
		Parippu	Regional Agriculture Research Station, Kumarakom

From each location diseased specimens of water hyacinth were collected and isolations were made. The fungi isolated were maintained on potato dextrose agar slants.

3.1.1 Correlation Studies

The weather data *viz.*, maximum and minimum temperature, relative humidity, number of rainy days and rainfall of each locality surveyed were collected from observatories near to the area (Table 1).

The data was used to correlate environmental factors with the frequency of occurrence of different genera of fungi.

$$\text{Frequency of occurrence of fungi} = \frac{\text{No. of times a fungus occurs in a year}}{\text{Total locations surveyed (20)}} \times 100$$

3.1.2 Pathogenicity

The fungi isolated during the survey were tested for their pathogenicity. Water hyacinth plants used for pathogenicity studies were collected from Aakkulam lake. The plants were allowed to grow in pots (size 15 x 15 cm) filled with water. Once the plants were properly established they were used for artificial inoculation studies.

The leaves and petioles were given pin pricks before inoculation. Seven day old culture bits of the fungi were placed on the injured portion and covered with small bit of moist cotton wool. The entire plant was covered with polyethene cover to maintain sufficient humidity. Uninoculated plants served as the control.

Apart from the fungi isolated from different areas, *Fusarium pallidoroseum, F. equiseti* and *Colletotrichum gloeosporioides* which were already confirmed as fungal pathogens of water hyacinth by earlier studies in the Department of Plant Pathology, College of Agriculture, Vellayani were also used for the present investigation.

Observations on the nature of symptoms and time taken for development of the symptoms were recorded.

3.1.3 Extent of Damage

The extent of damage produced by each of the pathogenic fungi obtained from the survey and the three already identified fungi on water hyacinth were tested. Observations were recorded using a score chart (Fig 2).

Score

0 – No symptom

1 – Symptom development around the pin pricked area only

2 – Upto 10 per cent leaf area showing yellowing / browning symptom

3 – 11-25 per cent of leaf areas showing yellowing / browning symptom

4 – 26-50 per cent of leaf area including petiole showing symptom

5 – 50-75 per cent of leaf area including petiole showing symptom

6 – Complete drying of the plant

Disease index was calculated using the formula (Mayee and Datar, 1986).

$$\text{Disease index \% (DI)} = \frac{\text{Sum of the score of each leaf}}{\text{Number of leaves scored x Maximum score}} \times 100$$

3.1.4 Morphological Characters of Selected Fungi

Studies on the morphological characters of pathogenic fungi were limited to those producing more than 40 per cent damage. The fungi included for the study were

Fungi isolated from survey

1. *Fusarium pallidoroseum* isolate 1
2. *F. pallidoroseum* isolate 2
3. *F. oxysporum* isolate 1
4. *F. oxysporum* isolate 2
5. *F. moniliforme* isolate 1
6. *F. moniliforme* isolate 2
7. *Myrothecium advena*
8. *Alternaria eichhorniae*

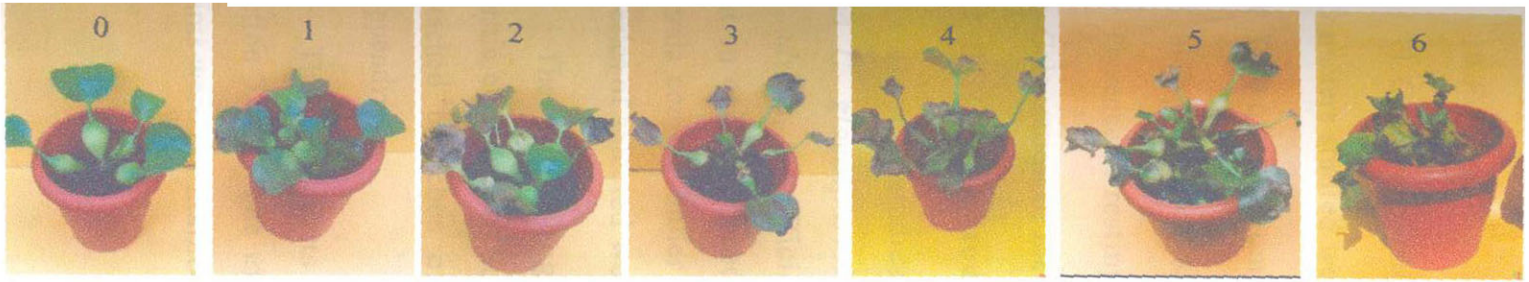


Fig. 2. Score chart for extent of damage

Fig. 3. Score chart for cell free metabolites

Fungi from previous studies

9. *F. pallidoroseum* isolate 3

10. *F. equiseti*

11. *C. gloeosporioides*

Characters studied were :

★ Type of mycelium, texture and colour

★ Size, colour and shape of conidia and conodiophore

★ Presence of resting structures, chlamydospores

★ Colouration of media

3.2 Host Range Studies

Detailed studies on the host range of *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides* (cultures maintained in the department) and *M. advena*, the efficient pathogen obtained from the survey, were carried out on common weeds seen near the waterways and cultivated plants in Kerala coming under 47 families (53 species of cultivated plants and 54 species of selected weeds) (Table 2).

The weeds, vegetables, pulses and oil seeds were grown in small plastic cups (8.5 x 8.5 cm). For plantation crops, fruits and forest trees, detached branches of the plants were maintained in nutrient solution (Appendix I).

The plant parts were inoculated with the fungi as described under section 3.1.2. The experiment was replicated thrice and suitable control plants were maintained.

Table 2 Host range studies

Sl. No.	Plants tested		
	Family	Scientific name	Common name / Vernacular name
1	Acanthaceae	*Ruellia tuberosa*	Ruellia
2		*Justicia diffusa* Willd	Justicia
3		*Justicia prostrata* Gamble N. Comb	Justicia
4	Amaranthaceae	*Amaranthus viridis* (Linn.) Notrysag	Slender amaranthus
5		*Amaranthus tricolor* L.	Amaranthus
6		*Aerva lanata* (L.) Juss	Balippovu
7		*Alternanthera sessilis*	Alligator weed, Vayal cheera
8	Anacardiceae	*Mangifera indica* L.	Mango
9		*Anacardium occidentale* L.	Cashew
10	Araceae	*Colocasia esculenta*	Taro
11		*Amorphophallus companulatus*	Elephant foot yam
12		*Anthurium andreanum* L.	Anthurium
13		*Pistia stratiotes*	Water lettuce, Muttapayal
14	Asclepiadiaceae	*Calotropis gigantea* R. Br.	Gigantic swallow weed, Erukku
15		*Hemidesmus indicus* R. Br.	Indian saraxparlke Naruneendi
16	Asteraceae	*Tridax procumbens* L.	Odiyan
17		*Vernonia cinaria* L.	Poovamkurunnu
18		*Synedrella nodiflora* L.	Venppacha
19		*Chromolaena odoratum* (L) King and Robinson	Communist pachha
20		*Emilia sonchifolia* (L.) DC	Muyalchevian
21		*Eclipta alba* (L.) Hassk	Kayonni
22		*Knoxia* sp.	Knoxia
23		*Ageratum conyzoides* L.	Goat weed / Appa
24	Boraginaceae	*Heliotropium indicum* L.	Venppacha
25	Capparidaceae	*Cleome viscosa* L.	Wild mustard, Kattukaduku
26	Caricaceae	*Carica papaya*	Papaya
27	Commelinaceae	*Commelina benghalensis* L.	Tropical spider wort Vazhappadathy

Table 2 Continued

Sl. No.	Plants tested		
	Family	Scientific name	Common name
28		*C. jacobi* Fischer	Vazhappadathy
29	Convolvulaceae	*Ipomoea batatus* (L.) Lam	Sweet potato
30		*Alyssicarpus vaginalis*	Alyssciarpus
31	Cucurbitaceae	*Momordica charantia* L.	Bittergourd
32		*Cucumis sativus* L.	Cucumber
33		*Trichosanthes anguina* L.	Snakegourd
34	Cyperaceae	*Bulbostylis barbata*	Sooryan
35	Dioscoriaceae	*Dioscorea alata*	Yam
36	Euphorbiaceae	*Phyllanthus niruri* (L.) Hoof F.	Keezharnelli
37		*Euphorbia geniculata* L.	Paloorippacha
38		*Euphorbia hirta* L.	Tharavu
39		*Manihot esculenta* L.	Tapioca
40		*Hevea brasiliensis*	Rubber
41	Labiatae	*Hyptis suaveolens* Poit.	Nattapoochedi
42	Lamiaceae	*Leucas aspera* Spreng.	Thumba
43	Lauraceae	*Cinnamomum zeylancium*	Cinnamon
44		*Jasminium sambac*	Jasmine
45	Leguminosae	*Clitoria ternatea* L.	Sankhupushpam
46		*Cassia occidentalis* L.	Thakara
47		*Vigna unguiculata* Savi	Cowpea
48		*Phaseolus mungo* L.	Blackgram
49		*Phaseolus aureus* Roxh.	Greengram
50		*Arachis hypogaea* L.	Groundnut
51		*Centrosema pubescens* (Benth.) Centro	Butterfly pea
52	Limnocharitaceae	*Limnochrius flava*	Malamkoovalam
53	Malvaceae	*Abelmoschus esculentus* L. Mench	Bhindi
54		*Sida acuta* Burm	Vellakurumthotti
55		*Marsilia quadrifoliata*	Ainy pepper Wort
56		*Abutilon theophrasti*	Velvet leaf
57	Marantaceae	*Maranta arundinaceae* L.	Arrowroot
58	Mimosaceae	*Acacia arabica* L.	Acacia
59	Moraceae	*Artocarpus integrifolia* L.	Jack
60	Musaceae	*Musa* sp.	Banana

Table 2 Continued

Sl. No.	Plants tested		
	Family	Scientific name	Common name
61	Myrtaceae	*Eugenia caryophyllus* L.	Clove
62		Psidium guajava	Guava
63	Myristacaceae	*Myristica fragrans* L.	Nutmeg
64	Nyctaginaceae	*Boerhaavia diffusa* L.	Thazhuthama
65	Nymphiaceae	*Nelumbo nucifera* Gaertn	Lotus
66		*Nymphaea nouchali* Burm. F.	Lily
67	Onagraceae	*Ludwigia parviflora* Roxb.	Ludwigia
68	Orchidaceae	*Dendrobium* sp.	Orchid
69	Oxalidaceae	*Oxalis corniculata* L.	Puliyarila
70	Palmae	*Cocos nucifera* L.	Coconut
71		*Areca catechu* L.	Arecanut
72	Pedaliaceae	*Sesamum indicum* L.	Sesamum
73	Piperaceae	*Piper nigrum* L.	Pepper
74		*Piper betle* L.	Betelvine
75		*Pepperomia* sp.	Kolumashi
76	Poaceae	*Oryza sativa* L.	Rice
77		*Eleusine coracana* Gaertn	Ragi
78		*Sorghum vulgare* Pers.	Sorghum
79		*Saccharum officinarum* L.	Sugarcane
80		*Panicum maximum* Jreq.	Guinea grass
81		*Pennisetum purpureum* Schum	Napier grass
82		*Axonopus compresses* (Sw.) Beauv	Carpet grass
83		*Cynodon dactylon* (L.) Pers.	Bermuda grass
84		*Eragrostis tenella*	Eragrostis
85		*Eleusine indica* (L.) Gaertn	Fowl foot grass
86		Alloteropsis cimicina (L.) Staff	Kannuneerthulli
87		*Echinochloa colonum* Beauv	Jumela rice, Kavada
88		*Brachiaria ramosa* (L) Staff	Palpullu
89		*Panicum repens* L.	Torpedo grass
90	Ponte deriaceae	*Monochoria vaginalis* Prest	Pickerel weed, Neerthamara

Table 2 Continued

Sl. No.	Plants tested		
	Family	Scientific name	Common name
91	Portulacaceae	*Portulaca oleraceae*	Indian purselane Karicheera
92	Punicaceae	*Punica granatum* L.	Pomegranate
93	Rubiaceae	*Oldenlandia umbellate* L.	Nonganam pullu
94		*Coffea arabica* L.	Coffee
95	Sapotaceae	*Achras sapota* L.	Sapota
96	Scrophulariaceae	*Scoparia dulcis* L.	Kallurukki
97			Brahmi
98	Solanaceae	*Solanum melongena* L.	Brinjal
99		*Capsicum annuum* L.	Chilli
100		*Lycopersicon esculentum* L.	Tomato
101	Umbelliferae	*Centlla asiatica* Urban	Pennywort Kudangal
102	Verbanaceae	*Lantana camara* L.	Large leaf lantana Kongini
103		*Tectonia grandis* L.	Teak
104		*Clerodendron infortunatum* L.	Peruvalam
105	Violaceae	*Ionidium suffruticosum*	Orilathamara
106	Zingiberaceae	*Curcuma longa* L.	Turmeric
107		*Zingiber officinale* L.	Ginger

3.3 EFFECT OF CELL FREE METABOLITES OF THE PATHOGENIC FUNGI ON WATER HYACINTH

The effect of cell free metabolite of *F. pallidoroseum*, *F. equiseti*, *C. gloeosporioides* and *M. advena* on water hyacinth plants were studied.

3.3.1 Preparation of Cell Free Metabolites

For preparing cell free metabolites of the fungi, they were grown in Richard's solution (Appendix I). Hundred ml of the medium was taken in 250 ml conical flasks and sterilized by autoclaving at 121°C for 20 minute (1.1 kg/ cm^2 Pressure). Each of the flasks were inoculated with 5 mm culture disc cut from actively growing seven day old culture of the fungi and incubated for 15 days at room temperature (28 ± 4°C). The fungal

growth was filtered through Whatman No.1 filter paper. This filtrate was then passed through 0.45 μm Sartorius membrane filters, in a sterilized filter assembly by applying pressure. The filtrate so obtained was cell free.

3.3.2 Testing the Efficacy of Cell Free Metabolites

The efficacy of cell free metabolite was tested by spraying it (@ 10 ml/plant) on water hyacinth plant maintained in small pots (15 x 15 cm) using an atomizer. Sterile water and 15 day old uninoculated Richard's solution served as control. Intensity of damage was recorded, seven days after spraying, by using a score chart described below (Fig. 3).

Score

0 – No scorching

1 – Upto 10 per cent leaf area showing scorching / blighting

2 – 11-25 per cent leaf area showing scorching / blighting

3 – 26-50 per cent of leaf area including bulbous portion showing scorching / blighting

4 – 50-75 per cent of leaf area showing scorching / blighting with shrinkage of bulbous portion

5 – Complete decay of the plant.

Index for intensity of damage was assessed as per details given under section 3.1.3

3.3.3 Host Range

In order to study the host range of cell free metabolites of *M. advena, F. pallidoroseum, F. equiseti* and *C. gloeosporioides*, the metabolites were sprayed on common weeds and cultivated plants listed under section 3.2.

Undiluted metabolites of the four fungi were sprayed separately on different host plants. As control, plants were sprayed with 15 day old

uninoculated Richard's solution. Three replications were maintained for the study. Observations on symptoms such as necrosis, chlorosis, scorching etc. were recorded on seventh day after spraying.

3.3.4 Effect of Dilution, Temperature and pH on Cell Free Metabolites

The experiments were conducted under glass house conditions.

3.3.4.1 Dilution

The cell free metabolites were diluted in the ratios 1 : 99, 5 : 95, 10 : 90, 25 : 75, 50 : 50 and 75 : 25 by adding required quantity of sterile distilled water.

3.3.4.2 Temperature

Cell free metabolites were taken in thin walled test tubes and heated in a water bath maintained at 50, 60, 70, 80, 90 and 100°C for 10 minutes. Immediately after the treatment the metabolites were cooled to room temperature. This was then used for further studies.

3.3.4.3 pH

The pH of the metabolites was adjusted to three and nine by adding 0.1 N HCl and 0.1 N NaOH respectively.

3.4 ISOLATION, PURIFICATION AND CHARACTERIZATION OF THE TOXIN

Methods suggested by Mahadevan and Sridhar (1974) were used for isolation, purification and characterization of the toxin produced by *M. advena, F. pallidoroseum, F. equiseti* and *C. gloeosporioides*.

3.4.1 Isolation

For isolating the toxin, each fungus was grown for 14 days in 75 ml of Richard's solution in 250 ml flask.

3.4.1.1 Exotoxin

The culture filtrate was centrifuged at 2000 rpm for 20 minutes using desktop centrifuge and from this clear supernatant was collected and this was reduced to $1/10^{th}$ of its original volume using flash evaporator. The concentrated filtrate thus obtained was combined with equal volume of methanol, stirred well and stored at 5°C overnight. This solution was clarified by filtering using Whatman No.1 filter paper and methanol removed by evaporation under vacuum. This aqueous solution was adjusted to pH 3.5 using 6 N HCl and shaken well with equal volume of ether in a separating funnel. The ether phase was collected. The aqueous phase was extracted twice with ether. The ether phase thus obtained was combined together and mixed with equal volume of five per cent sodium carbonate solution, shaken well and the aqueous phase was discarded and the ether phase was evaporated to dryness. The ash coloured precipitate thus obtained was again dissolved in ether and dried. Residue thus obtained is designated as exotoxin.

3.4.1.1.1 Qualitative Detection of Exotoxin

Paper chromatography technique was used for qualitative detection of exotoxin. Exotoxin obtained was redissolved in 2 ml acetone. The concentrated extract thus obtained was spotted on a Whatman No.1 sheet using a microsyringe (5 µl). The chromatography paper was developed ascendingly in a solvent mixture of butanol : formic acid : water (75 : 15 : 10 v/v). The paper was dried and sprayed with indicator bromophenol dissolved in acetone followed by one per cent copper sulphate (aqueous) solution to detect the coloured spot and Rf value calculated.

3.4.2 Purification of Toxin

Only the toxin separated from *F. pallidoroseum* was purified. *F. pallidoroseum* was cultured in 250 ml conical flask containing sterilized 75 ml of Richard's solution. One litre of culture filtrate was

collected after 14 days and centrifuged at 2000 rpm for 20 minutes. pH of the clear supernatant solution was adjusted to 4.0 with 5 N hydrochloric acid. The filtrate was extracted four times with ethyl acetate using 150 ml of the solvent for each extraction. A pinch of sodium chloride was added to the mixture for each extraction for breaking the emulsion. The extracts were pooled and passed through anhydrous sodium sulphate. It was then concentrated to 10 ml using flash evaporator at 45°C.

To this concentrated extract (10 ml), 5 ml of water was added and pH adjusted to eight using 0.1 N sodium hydroxide. This was again extracted repeatedly (four times) with diethyl ether. The extracts were pooled and dried using 20 g anhydrous sodium sulphate. The ether was evaporated to dryness and the toxin so obtained was weighed and dried for thin layer chromatography.

3.4.2.1 Thin Layer Chromatography

With the help of a micropipette, 5 µl of the precipitated toxin was spotted on TLC plates (coated with silica gel G, 0.5 mm thickness). The plates were placed in the solvent chamber containing butanol : formic acid : water (75 : 15 : 10 v/v) and was allowed to run till the solvent reached $^{3}/_{4}^{th}$ height of the plate. The plates were then air dried and observed for spots in an iodine chamber.

3.4.3 Characterization of Toxin

Structural confirmation of the toxin was done by Varian EM 360-L (60 MHz) nuclear magnetic resonance (NMR) spectrometer. Deuterated di methyl sulphoxide (DMSO) was used as solvent and tetra methyl xylene (TMX) as the internal standard. The chemical shifts were expressed as δ values (ppm) and coupling constant (J values) in Hertz.

3.4.1.2 Endotoxin

The fifteen day old mycelium grown in Richard's solution was separated by filtering it through a Whatman No. 1 filter paper and dried.

This was then homogenized with five volumes of water using a pestle and mortar. The homogenate was centrifuged at 1000 rpm for 15 minutes and the pellets discarded. The supernatant solution was again centrifuged for 15 minutes at 1000 rpm. The supernatant after second centrifugation is designated as endotoxin.

3.4.4 Testing the Efficacy of Toxins

Purified exotoxin obtained after extraction was dissolved in five ml of acetone. Filter paper discs of one cm diameter was dipped in this solution and placed on leaves and petiole of healthy water hyacinth plants grown in pots. Observations on time required for symptom development and type of symptom developed were recorded. The efficacy of endotoxin was also estimated using the same procedure.

3.5 MASS PRODUCTION AND STORAGE OF BIOAGENTS

3.5.1 Solid Substrates

In order to find out the best solid medium for mass multiplication and storage of *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides*, trials were conducted with the following materials.

T_1 – Coconut oil cake

T_2 – Gingelly oil cake

T_3 – Groundnut oil cake

T_4 – Guinea grass straw powder

T_5 – Cowdung (dried)

T_6 – Rice bran

T_7 – Neem oil cake

T_8 – Coirpith

T_9 – Neem oil cake + dried cowdung (1 : 1 w/w)

T_{10} – Coirpith + Rice bran (1 : 1 w/w)

Twenty gram each of the substrates was powdered and taken in 250 ml conical flask. In order to moisten, 25 ml of water was added to coir pith + rice bran and guinea grass straw powder. While for coconut oil cake, gingelly oil cake and cowdung, 20 ml of water was added. Groundnut oil cake, neem oil cake and neem oil cake + cowdung were moistened with 10 ml water. These substrates after sterilization (121 °C for one hour) were inoculated with five mm discs from seven day old culture of the test pathogens grown on potato dextrose agar medium and incubated at room temperature. Three replications were maintained for each treatment. The flasks were shaken daily for uniform growth of the fungi. Observations on nature and extent of growth and spore viability were recorded.

3.5.1.1 Extent of Growth

The score chart developed by Naseema (1989) was used for grading growth of the fungi.

–	No visible growth
+	25 per cent area covered
++	50 per cent area covered
+++	More than 50 per cent area covered

Observations were taken at weekly intervals starting from third day after inoculation (DAI) for a period of three months.

3.5.1.2 Spore Viability

The effect of substrate on the viability of spores was taken by counting the number of colony forming units in one gram (cfu/g) sample. This was done by serial dilution technique (Timonin, 1940) using Martin's rose bengal agar medium with streptomycin (Appendix I).

Observations on the number of viable colonies were taken upto six months at weekly intervals and expressed as cfu/g of the substrate.

3.5.2 Liquid Substrates

Fourteen liquid substrates were tried for the study.

T_1 – Coconut oil cake extract

T_2 – Gingelly oil cake extract

T_3 – Groundnut oil cake extract

T_4 – Guinea grass straw powder extract

T_5 – Cowdung (dried) extract

T_6 – Rice bran extract

T_7 – Neem oil cake extract

T_8 – Coirpith extract

T_9 – Neem oil cake + cowdung (dried) extract

T_{10} – Coirpith + rice bran extract

T_{11} – Raw rice washings (Kadi vellam)

T_{12} – Jaggery water

T_{13} – Boiled rice water (Rice gruel)

T_{14} – Coconut water

The extracts (T_1 – T_{10}) were prepared by boiling and filtering 50 g of solid material with 400 ml water for 10 minute. Hundred ml of this was taken in 250 ml conical flask. Same quantity of coconut water, boiled rice water, raw rice washings and jaggery water (75 g jaggery dissolved in 300 ml water) were taken in 250 ml conical flask, sterilized (121°C for 20 minutes) and inoculated with five mm discs of seven day old culture bit of the fungi and incubated at room temperature (28 ± 4°C). Three replications were maintained. Mycelial growth and viability of spores were recorded.

3.5.2.1 Mycelial growth

Growth of fungi in liquid substrates was recorded by taking the mycelial dry weight 10 DAI. The fungal growth in the liquid substrates was filtered through a previously weighed Whatman No. 1 filter paper and oven dried at 60°C, till two consecutive weights agreed.

3.5.2.2 Spore Viability

Viability of spores was taken on alternate days starting from 3 DAI for a period of one month as described under section 3.5.1.2. In order to get a truly representative population of the spores, just before drawing samples, the flasks were shaken for 30 minutes in a mechanical shaker.

3.6 INTEGRATION OF BIOCONTROL AGENTS WITH HERBICIDES

3.6.1 Compatibility

Compatibility of five herbicides (paraquat, pretilachlor, glyphosate, 2,4-D sodium salt and the combination herbicide anilofos 24 % + 2,4-D 32 % EC) with biocontrol fungi (*F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides*), were tested under *in vitro* conditions. The herbicides were tried at field doses and three lower concentrations (Table 3).

Table 3 Herbicides and concentrations tested

Sl. No.	Herbicides		Concentrations	
	Common name	Trade name	Dose (kg ai/ha)	Quantity required (ml/L)
1	paraquat	Gramaxone 24 % SL	0.75, 0.19, 0.05, 0.01	2.90, 0.73, 0.18, 0.05
2	pretilachlor	Refit 50EC	1, 0.25, 0.06, 0.02	2, 0.50, 1.25, 0.03
3	glyphosate	Weed Off 41EC	0.80, 0.20, 0.05, 0.01	0.50, 0.13, 0.03, 0.01
4	anilofos 24 % EC + 2,4-D32 % EC	One shot 56EC	0.40, 0.10, 0.03, 0.01	0.71, 0.18, 0.05, 0.01
5	2,4-D sodium salt	Fernoxone 80WP	1, 0.25, 0.06, 0.02	1.25, 0.31, 0.08, 0.02

The effect of herbicides on growth of the fungi was studied by poisoned food technique (Zentmeyer, 1955). Hundred ml of Czapek's (Dox) broth (Appendix I) was dispensed in 250 ml conical flask and sterilized. Requisite quantity of herbicide was incorporated and mixed well. Five mm discs from seven day old culture of each fungi was inoculated in the broth and incubated at room temperature. Three replications were maintained. Czapek's broth without herbicide served as control. Mycelial growth and spore count were recorded 10 DAI.

3.6.1.1 Mycelial Growth

Observations on the effect of herbicides on the growth of fungi was recorded 10 DAI and expressed as dry weight of mycelium as described under section 3.5.2.1.

3.6.1.2 Spore Count

The population of spores in the liquid medium was recorded using haemocytometer 10 DAI.

3.6.2 Effect of Lower Concentrations of Herbicides on Water Hyacinth

The herbicides which are compatible with the biocontrol fungi at the concentrations tested under *in vitro* conditions were found to completely destroy the water hyacinth plants. Therefore another experiment was set up to find out still lower concentrations that will weaken the water hyacinth plants. These concentrations were obtained by trial and error spraying of herbicides at different lower concentrations to water hyacinth plants maintained under glasshouse conditions. Finally the herbicides and their lowest concentrations that was effective for integrating with biocontrol fungi was selected.

3.6.3 Combined Effect of Biological Agents and Herbicides on Water Hyacinth Plants

Studies were carried out for integrating biocontrol agents with lower concentrations of herbicides.

3.6.3.1 Glasshouse Condition

A pot experiment was set up under glasshouse condition to study the combined effect of fungi and different concentrations of herbicides. Fungi used for the experiment were *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides*. The herbicides (paraquat, glyphosate, anilofos 24 % + 2,4-D 32 % EC and 2,4-D sodium salt) were tested at concentrations 50, 30 and 10 ppm. For each fungus, three experiments were set up.

3.6.3.1.1 Experiment I – Simultaneous Application

Each of the fungus and herbicides were applied simultaneously on water hyacinth plants.

Treatment

T_1 – Fungus + paraquat (50 ppm)

T_2 – " + " (30 ppm)

T_3 – " + " (10 ppm)

T_4 – " + glyphosate (50 ppm)

T_5 – " + " (30 ppm)

T_6 – " + " (10 ppm)

T_7 – " + anilofos 24 % + 2,4-D 32 % EC (50 ppm)

T_8 – " + " (30 ppm)

T_9 – " + " (10 ppm)

T_{10} – " + 2,4-D sodium salt (50 ppm)

T_{11} – " + " (30 ppm)

T_{12} – " + " (10 ppm)

T_{13} – Fungus alone

3.6.3.1.2 Experiment II – Herbicide Followed by Biocontrol Fungus

The treatments were fixed in such a way that the herbicide at different concentrations (as in Experiment I) was sprayed first. Five days later, each of the fungus was inoculated.

3.6.3.1.3 Experiment III – Biocontrol Fungus Followed by Herbicides

In this set of experiment, the biocontrol fungus was inoculated on the water hyacinth plants and five days later the herbicides at the concentrations (as in experiment I) were sprayed.

Suitable control plants with fungus alone, for each fungus and herbicides alone for each herbicide concentration were also kept. Three replications were maintained for each treatment. Observations on the extent of damage was recorded seven days after treatment (DAT) by using score chart (Susha, 1997).

Score chart for *F. pallidoroseum* and *F. equiseti* (Fig. 4).

Score

0 – No symptom

1 – Blighting from the tip covering less than one per cent leaf area

2 – Blighting covering 25 per cent leaf area

3 – Blighting covering 26-50 per cent leaf area

4 – Blighting covering 51-75 per cent leaf area

5 – Blighting covering >75 per cent leaf area

Score chart for *C. gloeosporioides* (Fig. 5)

Score

0 – No symptom

1 – Small spots covering less than one per cent leaf area

2 – Small spots covering 1-10 per cent leaf area

3 – Large lesions, not coalescing, covering 1-25 per cent leaf area

4 – Lesions coalescing covering 26-50 per cent

5 – Blighting covering 51-75 per cent leaf area

6 – Blighting covering more than 75 leaf area

Disease index (DI)was calculated using the formula (section 3.1.3).

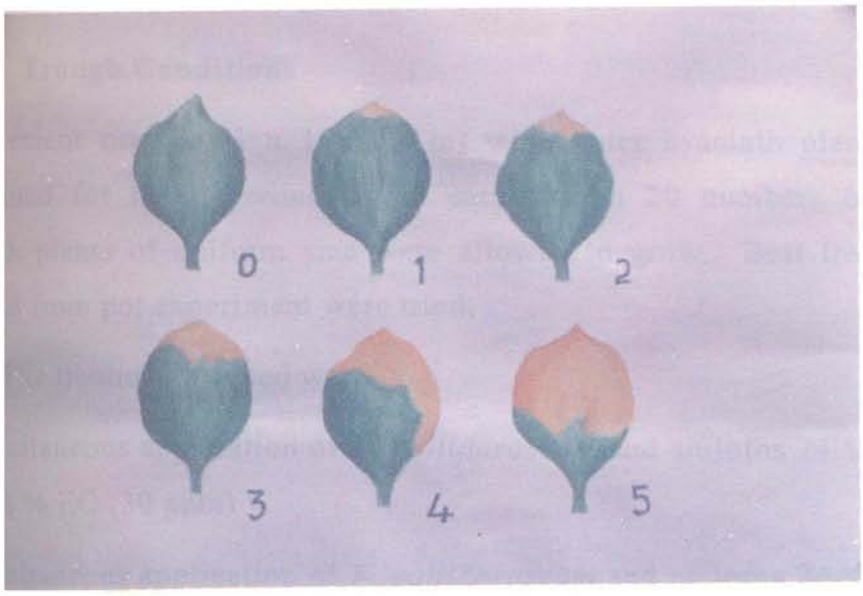


Fig. 4 Score chart for *Fusarium* spp

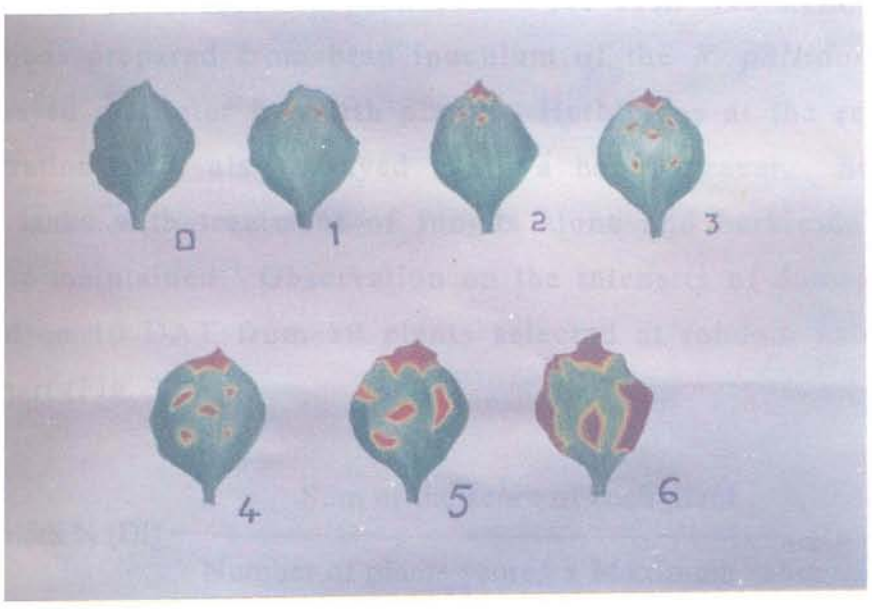


Fig. 5 Score chart for *C. gloeosporioides*

3.6.3.2 Trough Conditions

Cement troughs (1 x 1 x 0.4 m) with water hyacinth plants were maintained for the experiment. In each trough 20 numbers of water hyacinth plants of uniform size were allowed to grow. Best treatments obtained from pot experiment were tried.

The treatments tested were :

- ★ Simultaneous application of *F. pallidoroseum* and anilofos 24 % + 2,4-D 32 % EC (30 ppm)
- ★ Simultaneous application of *F. pallidoroseum* and anilofos 24 % + 2,4-D 32 % EC (50 ppm)
- ★ Simultaneous application of *F. pallidoroseum* and 2,4-D sodium salt(50 ppm)

Three replications were maintained for each treatment. Spore suspensions prepared from bran inoculum of the *F. pallidoroseum* was sprayed on water hyacinth plants. Herbicides at the required concentration were also sprayed using a hand sprayer. Suitable control tanks with treatment of fungus alone and herbicide alone were also maintained. Observation on the intensity of damage was recorded on 10 DAT from 10 plants selected at random using the score chart (Fig. 3).

$$\text{Disease index \% (DI)} = \frac{\text{Sum of the score of each plant}}{\text{Number of plants scored x Maximum score}} \times 100$$

3.7 EFFECT OF DIFFERENT OILS ON WATER HYACINTH

The oils and concentrations tested are given in Table 4.

Table 4 Oils and concentrations tested on water hyacinth plants

Sl. No.	Common name	Scientific name	Concentration tested (%)
1	Castor oil	*Ricinus communis* L.	1, 2, 3
2	Coconut oil	*Cocos nucifera* L.	1, 2, 3
3	Marotty oil	*Hydnocarpus wightiana* (Blume)	1, 2, 3
4	Neem oil	*Azadiracta indica* L.	1, 2, 3
5	Cashew nut shell liquid (CNSL)	*Anacardium occidentale* L.	1, 2, 3

To 100 ml of water, Teepol @ 1 ml/l was added and mixed well. Then the requisite quantity of the oil was added and mixed well to make an emulsion. The emulsions at different concentrations were thus prepared and sprayed on water hyacinth plants grown in pots. Three replications were maintained for each treatment. Control plants sprayed with water were also maintained. Observations on the type of symptoms produced by the oil were recorded.

3.8 DEVELOPMENT OF FORMULATION USING *F. PALLIDOROSEUM*

F. pallidoroseum, which was the most efficient fungal pathogen in managing water hyacinth was used for developing an effective formulation.

3.8.1 Compatibility of Formulation Ingredients with *F. pallidoroseum*

The basic ingredients tested for formulating *F. pallidoroseum* were

a) Humectant (Glycerol, sodium alginate, paraffin liquid)

b) Spreader (Tween-80)

c) Oil base [Cashew nut shell liquid (CNSL)]

In order to find out the ideal concentration of the ingredients for the formulation and to find out the action of these materials on *F. pallidoroseum* an experiment was laid out with the following treatments.

Formulation ingredient	Concentration tested (%)
Cashew nut shell liquid	1, 2, 3
Glycerol	1, 2, 3
Paraffin liquid	1, 2, 3
Sodium alginate	1, 2, 3
Tween-80	1, 2, 3

The effect of the ingredients on growth and sporulation of *F. pallidoroseum* in Czapek's (Dox) broth was studied using poisoned food technique (Zentmeyer, 1955). Observations on mycelial dry weight and sporulation were taken on 10 DAI.

3.8.1.1 Mycelial Growth

The different ingredients in required quantities were incorporated in sterilized Czapek's (Dox) broth and mixed thoroughly.

For uniform mixing of CNSL, teepol (@0.5 ml/l) was incorporated in Czapek's (Dox) broth before incorporation of CNSL. Flasks with media alone served as check. After thorough mixing of ingredients, flasks were inoculated with 5 mm discs of seven day old culture of the fungus and incubated at room temperature (28 ± 4°C). Experiment was replicated thrice. In order to find out the mycelial weight, the media with the fungal growth (10 DAI) was filtered through a previously weighed Whatman No.1 filter paper, dried at 60°C and weight recorded, till two consecutive weights become equal.

3.8.1.2 Spore Count

For this, the flasks containing the growth (10 DAI) was shaken in a rotary shaker for five minutes and the spores discharged into the medium was estimated using haemocytometer.

3.8.2 Preparation of Different Formulations

Dust, wettable powder and oil formulation were prepared using *F. pallidoroseum* culture.

3.8.2.1 Dust

The proportions of the various ingredients for the dust formulation is given in Table 5. Six different types of dust formulations (A to F) with varying concentrations of the fungal mycelium were tried.

Stock culture of *F. pallidoroseum* was maintained on potato dextrose agar. Discs of 5 mm diameter from seven day old culture of *F. pallidoroseum* was inoculated into 100 ml sterilized Czapek's (dox) broth in 250 ml conical flask. The flasks were incubated at room temperature for 10 days. At the end of incubation period the mycelium was separated from the broth by filtering through the filter paper. Fungal mats thus obtained were pressed between layers of sterile coarse filter paper to remove water and thoroughly ground using a blender. This was mixed in different proportions of Tween-80, glycerol, sucrose and sterilized talc powder and air dried.

Table 5 Ratio of ingredients in dust formulation

Dust formulation	Mycelium *F. pallidoroseum*	Talc w/w	Sucrose w/w	Tween-80 w/w	Glycerol w/w
A	10	84	2	2	2
B	8	86	2	2	2
C	5	89	2	2	2
D	10	86	-	2	2
E	8	88	-	2	2
F	5	91	-	2	2

3.8.2.1.1 Effect of Dust Formulation on Water Hyacinth

The six different formulated dust products were dusted uniformly on water hyacinth plants maintained in plastic pots @ 2 g/plant.

Treatments

T_1 – Talc alone

T_2 – Dusting formulated product A

T_3 – Dusting formulated product B

T_4 – Dusting formulated product C

T_5 – Dusting formulated product D

T_6 – Dusting formulated product E

T_7 – Dusting formulated product F

Three replications were maintained for each treatment. Observations on the extent of damage was recorded on 7 DAI using the score chart and disease index was calculated (section 3.6.3.2).

3.8.2.1.2 Effect of Dust Formulations on Mite Infected and Oil Sprayed Water Hyacinth Plants

Mite infected plants were separately collected and maintained under glasshouse conditions. Dust formulations A, B and C (section 3.8.2.1) @ 2 g/plant was dusted on these plants. Similarly the dust formulations were also applied on plants previously sprayed with 0.5, 1 and 2 per cent CNSL.

Treatments

T_1 – Dusting formulated product A

T_2 – Dusting formulated product B

T_3 – Dusting formulated product C

T_4 – Dusting formulated product A on 0.5 per cent CNSL sprayed plants

T_5 – Dusting formulated product B on 0.5 per cent CNSL sprayed plants

T_6 – Dusting formulated product C on 0.5 per cent CNSL sprayed plants

T_7 – 0.5 per cent CNSL spray

T_8 – Dusting formulated product A on 1 per cent CNSL sprayed plants

T_9 – Dusting formulated product B on 1 per cent CNSL sprayed plants

T_{10} – Dusting formulated product C on 1 per cent CNSL sprayed plants

T_{11} – 1 per cent CNSL spray

T_{12} – Dusting formulated product A on 2 per cent CNSL sprayed plants

T_{13} – Dusting formulated product B on 2 per cent CNSL sprayed plants

T_{14} – Dusting formulated product C on 2 per cent CNSL sprayed plants

T_{15} – 2 per cent CNSL spray

T_{16} – Dusting formulated product A on mite injured plants

T_{17} – Dusting formulated product B on mite injured plants

T_{18} – Dusting formulated product C on mite injured plants

T_{19} – Mite injured plants

T_{20} – Control (Dusting talc)

CNSL was applied 30 minutes before the dust application. The experiment was replicated thrice. Observations were recorded on seven days after dusting (DAD) based on the score chart (section 3.6.3.2).

3.8.2.1.3 Shelf Life of Dust Formulation

In order to find out the shelf life of the formulated products they were kept in sterilized polythene bags at room temperature (28 ± 4°C). Viability of spores was tested at weekly intervals by estimating the number of colony forming units in one gram (cfu/g) sample, by serial dilution technique (Timonin, 1940).

3.8.2.2 Wettable Powder

The ingredients used for making the wettable powder (WP) formulation were :

★ Mycelium of *F. pallidoroseum*

★ Talc

★ Sucrose

★ Tween-80

★ Glycerol

Mycelial mat required for formulation was prepared as described under Section 3.8.2.1. The mycelium thus obtained was air dried to 50 per cent moisture content. The mycelium was then ground using a blender after incorporating the following ingredients.

Mycelium – 40 g w/w

Talc – 54 g w/w

Sucrose – 2 g w/w

Tween-80 – 2 g w/w

Glycerol – 2 g w/w

After proper blending the WP formulation was air dried and stored in polythene covers at room temperature (28 ± 4°C) and refrigeration (4°C).

3.8.2.2.1 Effect of Wettable Powder Formulation on Water Hyacinth

The wettable powder formulation at different concentration was sprayed on water hyacinth plants (@ 10 ml/plant) grown in pots and maintained in glasshouse.

While testing dust formulation it was found that pretreatment of water hyacinth plants with CNSL spray gave better infection. Hence CNSL was also included in testing the efficacy of wettable powder formulation.

Treatments

T_1 – 40 per cent WP @ 1 g/100 ml

T_2 – 40 per cent WP @ 3 g/100 ml

T_3 – 40 per cent WP @ 5 g/100 ml

T_4 – 40 per cent WP @ 10 g/100 ml

T_5 – 40 per cent WP @ 15 g/100 ml

T_6 – 40 per cent WP @ 20 g/100 ml

T_7 – 40 per cent WP @ 25 g/100 ml

T_8 – 0.5 per cent CNSL spray

T_9 – T_8 + T_1

T_{10} – T_8 + T_2

T_{11} – T_8 + T_3

T_{12} – T_8 + T_4

T_{13} – T_8 + T_5

T_{14} – T_8 + T_6

T_{15} – T_8 + T_7

T_{16} – 1.0 per cent CNSL spray

T_{17} – T_{16} + T_1

T_{18} – T_{16} + T_2

T_{19} – T_{16} + T_3

T_{20} – T_{16} + T_4

T_{21} – T_{16} + T_5

T_{22} – T_{16} + T_6

T_{23} – T_{16} + T_7

T_{24} – 2.0 per cent CNSL spray

T_{25} – T_{24} + T_1

T_{26} – T_{24} + T_2

T_{27} – T_{24} + T_3

T_{28} – T_{24} + T_4

T_{29} – T_{24} + T_5

T_{30} – T_{24} + T_6

T_{31} – T_{24} + T_7

T_{32} – Talc spray

T_{33} – Water + teepol + glycerol

T_{34} – Control (water spray)

CNSL spray was given 30 minutes before WP treatment. Each treatment was replicated thrice. Observations were recorded on seven days after spraying based on the score chart and disease index was calculated (section 3.6.3.2).

3.8.2.2.2 Shelf Life of Wettable Powder

The cfu/g of the WP formulation of *F. pallidoroseum*, kept under room temperature (28 ± 4°C) and refrigeration (4°C) was estimated at weekly intervals (Timonin, 1940).

3.8.2.3 Oil Based Formulation

The spore suspensions of the test pathogen was prepared and mixed with CNSL at different concentrations. For preparing the spore suspensions *F. pallidoroseum* was grown in moistened rice bran taken in 250 ml flask, sterilized at 121°C for one hour and inoculated with 5 mm culture disc from seven day old culture of the fungus. The flasks were incubated at room temperature and were shaken daily for uniform growth of the fungus.

Twenty day old bran inoculum was used for preparing different concentrations of spore suspensions. For this, bran inoculum was mixed with water and filtered through a muslin cloth to remove the bran particles. The spore concentration of the suspension was adjusted to 10^7, 10^9 and 10^{11} spores/ml. Tween-80 and glycerol were added @ 1 ml/ 100 ml of the spore suspension.

For preparing the oil phase of the formulation, CNSL at three concentration *viz.*, 0.5 per cent, 1.0 per cent and 2.0 per cent were used. CNSL, at the required concentration was added to 100 ml of spore suspension mixture, drop by drop with constant stirring. This emulsion was used within two hours.

3.8.2.3.1 Effect of Oil Based Formulation on Water Hyacinth

Oil based formulation at different concentrations was sprayed on water hyacinth plants grown in pots and maintained under glass house condition.

Treatments

T_1 – Spore suspension (10^7 spores/ml) + Glycerol + Tween-80

T_2 – Spore suspension (10^9 spores/ml) + Glycerol + Tween-80

T_3 – Spore suspension (10^{11} spores/ml) + Glycerol + Tween-80

T_4 – T_1 + CNSL (0.5 per cent)

T_5 – T_2 + CNSL (0.5 per cent)

T_6 – T_3 + CNSL (0.5 per cent)

T_7 – CNSL 0.5 per cent spray

T_8 – T_1 + CNSL (1.0 per cent)

T_9 – T_2 + CNSL (1.0 per cent)

T_{10} – T_3 + CNSL (1.0 per cent)

T_{11} – CNSL 1.0 per cent spray

T_{12} – T_1 + CNSL (2.0 per cent)

T_{13} – T_2 + CNSL (2.0 per cent)

T_{14} – T_3 + CNSL (2.0 per cent)

T_{15} – CNSL 2.0 per cent spray

T_{16} – Water + Glycerol + Tween-80

T_{17} – Water spray

Three replications were maintained for each treatment. Observations were recorded on seventh day after spraying (DAS) based on the score chart and disease index was calculated (Section 3.6.3.2).

3.8.3 Effect of Wettable Powder Formulation on Water Hyacinth Grown in Troughs

From the glasshouse studies WP was found to be the most efficient formulation for managing water hyacinth. Hence this formulation was tested on water hyacinth grown in troughs, which resembled the growth conditions in natural habitat.

Troughs of size (1 x 1 x 0.4 m) was filled with mud and water collected from lake where water hyacinth was noticed. The water level in the trough was always maintained so that the plants grew and multiplied normally. Each trough contained twenty plants of uniform age.

Treatments

T_1 – WP @ 5 g/100 ml

T_2 – WP @ 10 g/100 ml

T_3 – 2.0 per cent CNSL + T_1

T_4 – 3.0 per cent CNSL + T_1

T_5 – 4.0 per cent CNSL + T_1

T_6 – 2.0 per cent CNSL + T_2

T_7 – 3.0 per cent CNSL + T_2

T_8 – 4.0 per cent CNSL + T_2

T_9 – 2.0 per cent CNSL

T_{10} – 3.0 per cent CNSL

T_{11} – 4.0 per cent CNSL

T_{12} – Control (water spray)

CNSL was applied 30 minutes before WP spraying. The experiment was replicated thrice. Observations on extent of damage was recorded 10 days after spraying from 10 plants selected at random using the score chart and disease index calculated (section 3.6.3.2).

Under glasshouse condition 0.5, 1.0 and 2.0 per cent CNSL was used but preliminary studies under trough condition did not induce appreciable extent of damage. Hence two higher concentrations *viz.*, three and four per cent were also tried.

3.8.4 Field Trial

The experiment was laid out at the Aakkulam Lake. Water hyacinth infested area was divided into plots of size 2 x 2 sqm area and was demarcated using rope. The plants were selected at random from each plot and observations were taken before application of treatments.

The most effective concentration of WP (@ 5 g/100 ml) from the trough trial (section 3.8.3) was used for the field experiment. The water hyacinth plants were sprayed with CNSL (5.0 per cent) @ 50 ml/sqm and it was allowed to dry for a period of 30 minutes. A second round spray with wettable powder (@ 5 g/100 ml) was given @ 50 ml/sqm.

Suitable replications (9 numbers) were maintained for the treatment. The control plots were laid out away from the experiment area so that the spray fluid did not contaminate plants.

The extent of damage was scored (section 3.6.3.2) on the fourth and seventh day after application of formulation from previously tagged plants and control plants and disease index was calculated.

3.9 EFFECT OF FORMULATED PRODUCT, METABOLITES OF *F. PALLIDOROSEUM* AND CNSL ON AQUATIC FAUNA AND FLORA

In order to find out the effect of the formulated product, metabolites of *F. pallidoroseum* and CNSL on commonly seen aquatic flora and fauna

in lakes, where management of water hyacinth is taken up, a study was conducted. The flora associated with water hyacinth *viz.*, water lily, Pistia, Hydrilla, Salvinia and Lemna were grown in the troughs along with water hyacinth and into that fresh water fishes, frogs, snails and water skaters were introduced (Table 6). The trough was covered by chicken mesh to prevent escape of frogs.

Table 6 Aquatic flora and fauna tested

Sl. No.	Scientific name	Common name
1	Fishes	
	Aplocheilus lineatus	Poochutti/Manathukanni
	Etroplus maculatus	Pallathi/Orange chronid
	Puntius filamentosus	Valechutti Paral
	Puntius denisoni	Poovali/Indian tiger parb
2	*Pila* sp.	Snail
3	*Geocorris* sp.	Water skater
4	*Rana hexadactyla*	Frog

One week time interval was given for the flora and fauna to get acclimatized with the surroundings and then the treatments were applied.

Treatments

★ Metabolites of *F. pallidoroseum*

★ Formulated product (WP @ 10 g/100 ml)

★ CNSL (4.0 per cent)

★ Application of WP on CNSL (4.0 per cent) sprayed plants

★ Control (water spray)

Three replications were maintained for each treatment. Visual observations on toxicity symptoms if any produced on the flora and fauna were recorded at regular intervals for a period of one month.

3.9.1 Observations Recorded

Fauna

★ Any behavioural changes, every 15 minutes during the first hour, every 30 minutes during next four hour and at the end of 24 h. Observations were repeated at one day interval for a period of one month

★ Lesion development on the skin of fishes, snails and frogs

★ Mortality rate by taking initial and final population.

Flora

Symptoms such as yellowing, scorching, or drying up of the flora were recorded daily for a period of 10 days.

3.10 STATISTICAL ANALYSIS

Data relating to each experiment was analysed by applying the Analysis of Variance Technique (ANOVA) (Panse and Sukhatme, 1967). Correlation was also worked out to determine the relation between disease incidence and weather parameters.

RESULTS

4. RESULTS

4.1 SURVEY ON THE FUNGAL PATHOGENS OF WATER HYACINTH

Survey was conducted for a period of one year at quarterly intervals in four southern districts of Kerala to isolate different fungi infecting water hyacinth. Fungi associated with the diseased water hyacinth were isolated and purified.

Observations on the occurrence of various fungi on water hyacinth and their frequency of occurrence were also worked out (Table 7 and Fig. 6).

Thiruvananthapuram District

Twelve different fungi were found associated with water hyacinth in Thiruvananthapuram district. The frequency of occurrence of *Myrothecium advena* was maximum (85 per cent) followed by *Curvularia lunata* (70 per cent). *M. advena* was a new report on water hyacinth (Plate 1). *M. advena* and *C. lunata* were found to occur irrespective of the seasons (Table 8).

Three species of *Fusarium* was invariably found associated with water hyacinth. *F. pallidoroseum* was seen during all the season while *F. moniliforme* was limited to rainy season only.

Eventhough, *Colletotrichum gloeosporioides* was present during all the seasons of the year, its frequency of occurrence (35 per cent) was ≤ 50 per cent of that of *C. lunata* and *M. advena*. *Altenaria eichhorniae* was present in only one location (Venpalavattom) during February – July.

Kollam District

The waterways in Kollam district was less frequently infested with water hyacinth. Generally during February – March and June – July seasons the waterways were free from this weed. Even when the

Table 7 Frequency of occurrence of fungi on water hyacinth

Fungi	Frequency of occurrence (%)			
	Thiruvananthapuram	Kollam	Alappuzha	Kottayam
Alternaria eichhorniae	10	-	5	5
Aspergillus sp.	15	-	-	5
Colletotrichum gloeosporioides	35	-	25	30
Curvularia lunata	70	-	60	65
Fusarium moniliforme	20	-	15	15
F. pallidoroseum	45	-	30	10
F. oxysporum	30	5	5	10
Helminthosporium sp.	-	-	10	-
Myrothecium advena	85	-	75	65
Nigrospora sp.	5	-	5	-
Penicillium sp.	15	-	-	-
Pestalotia sp.	-	-	5	-
Rhizoctonia solani	-	-	15	10
Trichoderma sp.	5	-	-	-
Sterile fungus	10	5	5	5

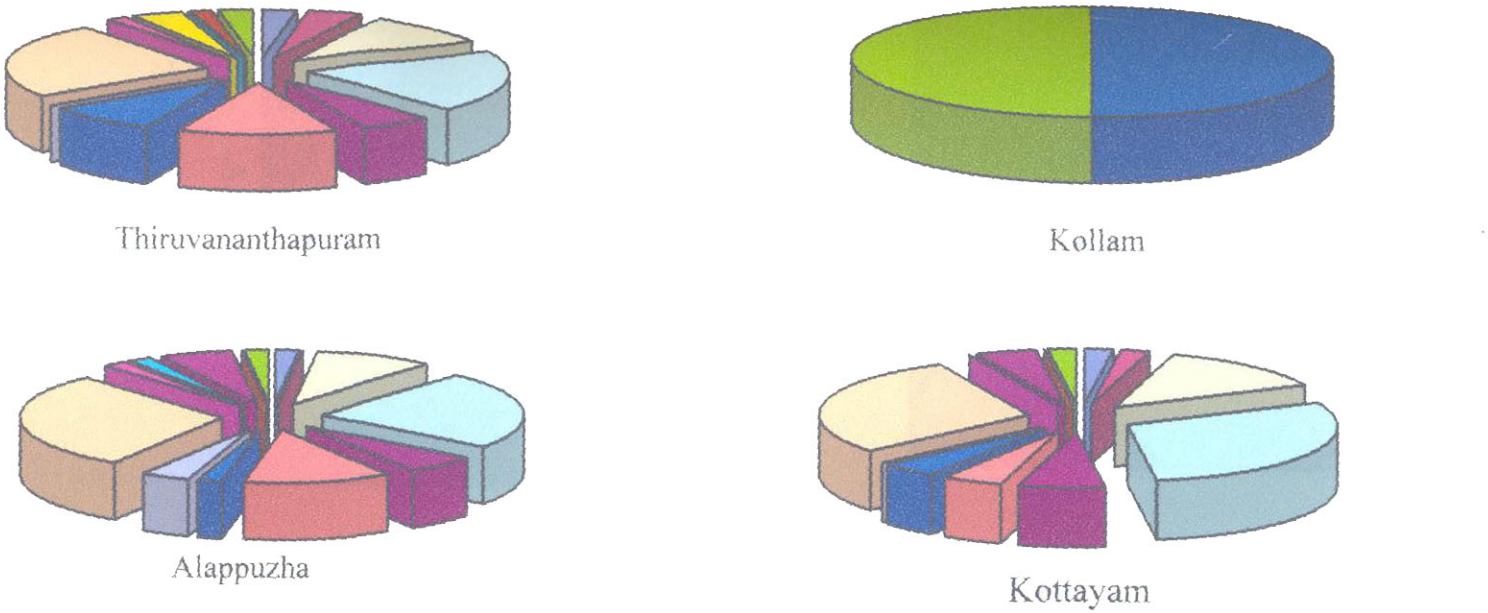


Fig. 6 Frequency of occurrence of fungi on water hyacinth

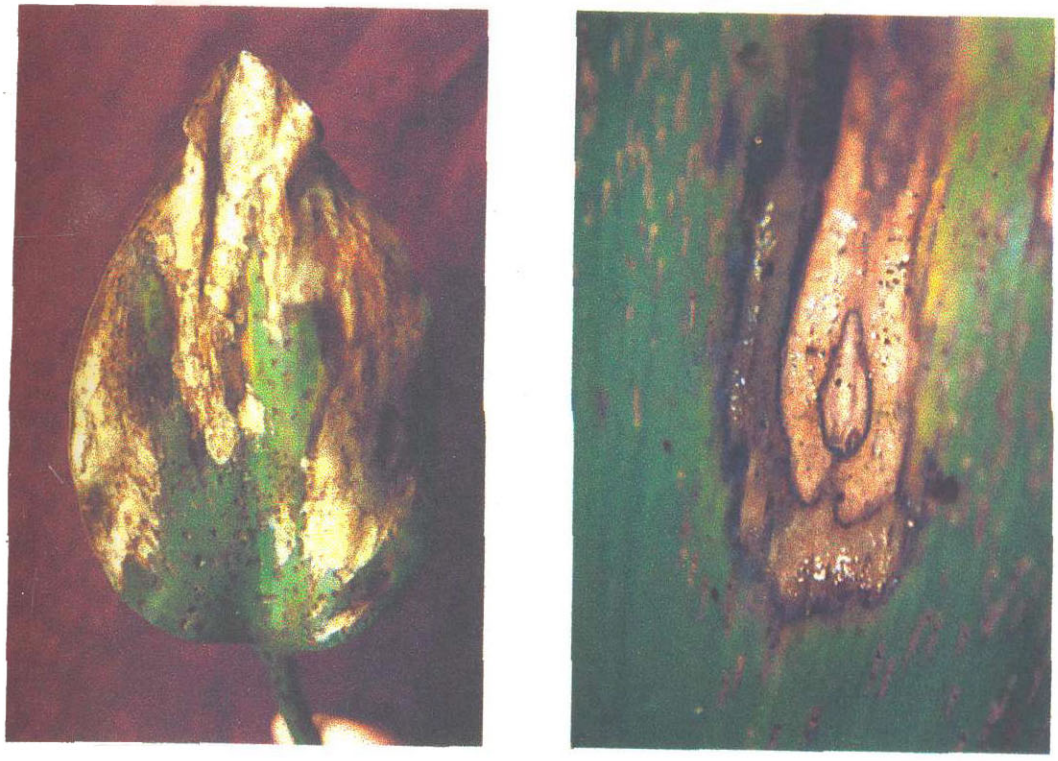
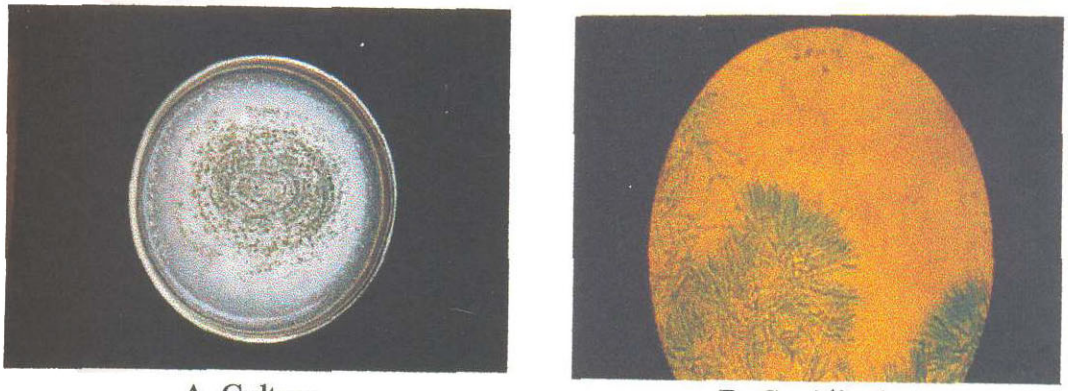


Plate 1. Symptom produced by *Myrothecium advena* on water hyacinth leaves



A. Culture

B. Conidiophores

Plate 2. *M. advena*

Table 8 Seasonal occurrence of fungi on water hyacinth in Thiruvananthapuram district

Period	Location	Temperature (°C)		Relative humidity (%)	Total rain fall (cm)	No. of rainy days	Fungi isolated
		Maximum	Minimum				
February – March (2000)	Akkulam	32.65	23.20	75.26	36.20	3	*Curvularia lunata, Colletotrichum gloeosporioides, Fusarium oxysporum, F. pallidoroseum, Myrothecium advena,*
	Ambalathara	31.33	23.40	86.58	160.20	6	*C. lunata, F. pallidoroseum, M. advena,*
	Muttathara	31.35	23.12	88.91	171.21	6	*C. lunata., C. gloeosporioides,* non sporulating fungus
	Veli	32.34	25.47	73.21	41.34	3	*C. lunata, M. advena, Penicillium* sp.
	Venpalavattom	31.44	23.05	77.25	55.50	4	*C. lunata, Alternaria eichhorniae, M. advena,*
June – July (2000)	Akkulam	30.20	23.80	89.57	171.20	18	*C. lunata, F. oxysporum, F. pallidoroseum, M. advena, Aspergillus* sp., *Penicillium* sp.
	Ambalathara	29.69	22.56	81.71	159.65	16	*C. lunata., F. moniliforme, F. pallidoroseum, M. advena*
	Muttathara	28.34	22.50	90.57	175.75	14	*C. gloeosporioides, M. advena, F. pallidoroseum*
	Veli	30.19	24.16	90.65	331.24	19	*C. lunata, F. moniliforme, C. gloeosporioides*
	Venpalavattom	29.01	22.40	89.80	181.21	14	*C. lunata, A. eichhorniae, F. oxysporum, M. advena,*

Table 8 Continued

Period	Location	Temperature (°C)		Relative humidity (%)	Total rain fall (cm)	No. of rainy days	Fungi isolated
		Maximum	Minimum				
October – November (2000)	Akkulam	28.70	23.03	91.90	270.70	11	*C. lunata., F. pallidoroseum, M. advena, F. oxysporum*
	Ambalathara	30.14	21.73	85.00	170.24	12	*F. pallidoroseum, M. advena, F. moniliforme, Nigrospora* sp., *Curvularia*
	Muttathara	30.56	22.01	88.32	196.21	13	*F. moniliforme, M. advena*
	Veli	30.78	24.89	88.83	231.32	13	*C. lunata, M. advena, Penicillium* sp., *C. gloeosporioides*
	Venpalavattom	29.92	23.12	89.10	221.12	13	*M. advena, F. oxysporum, Aspergillus* sp. non sporulating fungi, *Trichoderma* sp.
February – March (2001)	Akkulam	32.01	23.19	78.21	101.23	3	*Aspergillus* sp., *F. pallidoroseum, Penicillium* sp., *F. oxysporum*
	Ambalathara	31.89	21.48	75.00	16.20	1	*F. pallidoroseum, M. advena*
	Muttathara	31.21	22.41	77.12	18.10	1	*C. lunata, C. gloeosporioides, M. advena*
	Veli	31.89	22.21	80.16	111.21	3	*C. lunata, M. advena*
	Venpalavattom	30.01	21.57	76.50	14.23	1	*C. gloeosporioides, M. advena,*

waterways were infected during October – November the infection by fungal pathogen was minimum and only *Fusarium oxysporum* and a sterile fungus were noticed (Table 9).

Alappuzha District

Maximum number of pathogenic fungi (12 nos.) were found associated with water hyacinth in Alappuzha district. Here also, *M. advena* was the most commonly observed fungi (75 per cent) followed by *C. lunata* (60 per cent).

Among the different species of *Fusarium* isolated, *F. pallidoroseum* was found to occur irrespective of season and with a maximum frequency of occurrence of 30 per cent (Table 10).

Helminthosporium sp. and *Pestalotia* sp. infection was recorded only from Alappuzha district. *Helminthosporium* was seen during rainy season while *Pestalotia* was recorded both during summer and rainy season.

Alternaria eichhorniae was recorded from one location (Karuvatta) during February – March season and its frequency of occurrence was only 5 per cent.

Kottayam District

The frequency of occurrence of the different species of fungi noticed in Kottayam was almost similar to that recorded from Thiruvananthapuram. The only difference was the presence *Rhizoctonia solani* in Kottayam which was not observed in Thiruvananthapuram (Table 11).

C. lunata and *M. advena* was found to occur irrespective of season in the frequency of 65 per cent. Eventhough *C. gloeosporioides* was present during summer and rainy season, its frequency of occurrence was low (30 per cent).

Table 9 Seasonal occurrence of fungi on water hyacinth in Kollam district

Period	Location	Temperature (°C)		Relative humidity (%)	Total rain fall (cm)	No. of rainy days	Fungi isolated
		Maximum	Minimum				
February – March (2000)	Asramam	35.67	21.71	77.37	62.01	4	-
	Chavara	34.89	22.32	76.51	61.87	4	-
	Kollam town	35.59	21.06	77.70	61.65	5	-
	Karunagapally	34.91	22.07	77.01	61.91	5	-
	Thevally	35.02	21.08	76.56	61.64	4	-
June – July (2000)	Asramam	30.02	20.32	82.01	169.12	12	-
	Chavara	29.89	19.13	82.32	181.32	13	-
	Kollam town	30.71	20.85	81.30	170.30	12	-
	Karunagapally	29.91	19.89	82.19	191.23	14	-
	Thevally	30.56	20.12	81.21	167.81	12	-
October – November (2000)	Asramam	31.23	20.08	76.51	77.21	4	-
	Chavara	30.89	20.87	78.29	97.23	5	-
	Kollam town	31.02	19.41	77.49	62.50	4	*F. oxysporum*, non sporulating fungus
	Karunagapally	31.43	21.23	78.08	118.41	5	-
	Thevally	30.87	19.57	77.02	63.10	4	-
February – March (2001)	Asramam	33.23	21.04	75.30	61.03	3	-
	Chavara	33.81	21.21	77.10	81.42	4	-
	Kollam town	34.12	21.40	76.30	55.17	3	-
	Karunagapally	34.01	21.53	77.20	90.91	4	-
	Thevally	34.25	21.68	78.90	51.24	3	-

Table 10 Seasonal occurrence of fungi on water hyacinth in Alappuzha district

Period	Location	Temperature (°C)		Relative humidity (%)	Total rain fall (cm)	No. of rainy days	Fungi isolated
		Maximum	Minimum				
February – March (2000)	Haripad	33.89	23.71	91.03	101.32	8	*C. lunata, C. gloeosporioides, M. advena*
	Karumadi	34.25	23.98	92.06	94.20	7	*C. lunata, C. gloeosporioides, M. advena*
	Karuvatta	33.72	23.13	91.72	108.39	7	*C. lunata, F. pallidoroseum, M. advena*
	Mullackal	34.22	23.32	91.89	107.13	8	*F. pallidoroseum, Pestalotia* sp., non sporulating fungus
	Nedumudi	34.01	23.01	92.13	114.31	8	*M. advena*
June – July (2000)	Haripad	31.21	23.23	92.30	455.07	23	*C. lunata, F. pallidoroseum, M. advena*
	Karumadi	30.99	23.81	91.30	421.35	23	*C. lunata, C. gloeosporioides, Helminthosporium* sp., *Nigrospora* sp.
	Karuvatta	30.61	22.89	91.89	521.05	24	*C. lunata, F. moniliforme, M. advena*
	Mullackal	31.23	22.68	92.28	428.17	24	*Pestalotia,* Non sporulating fungus
	Nedumudi	30.87	22.71	92.41	537.34	25	*F. moniliforme, M. advena,*

Table 10 Continued

Period	Location	Temperature (°C)		Relative humidity (%)	Total rain fall (cm)	No. of rainy days	Fungi isolated
		Maximum	Minimum				
October – November (2000)	Haripad	32.41	23.89	91.33	221.30	12	*C. lunata, M. advena*
	Karumadi	32.03	24.28	92.63	182.40	11	*C. lunata, C. gloeosporioides, M. advena, Helminthosporuim* sp
	Karuvatta	32.31	24.01	91.31	231.13	11	*C. lunata, F. moniliforme, F. pallidoroseum*
	Mullackal	31.89	23.92	92.01	253.10	11	*F. oxysporum, M. advena*
	Nedumudi	31.78	24.31	91.78	212.43	13	*M. advena,* non sporulating culture
February – March (2001)	Haripad	34.13	23.78	80.93	33.31	3	*C. lunata, F. pallidoroseum, M. advena*
	Karumadi	33.30	23.91	81.22	38.30	3	*C. lunata, F. pallidoroseum, M. advena*
	Karuvatta	34.01	24.31	81.89	35.32	3	*A. eichhorniae, C. lunata, M. advena*
	Mullackal	33.78	24.10	81.57	29.32	3	*C. lunata, M. advena, Phoma* sp.
	Nedumudi	33.81	23.48	81.13	22.31	2	*C. gloeosporioides, M. advena,*

Table 11 Seasonal occurrence of fungi on water hyacinth in Kottayam district

Period	Location	Temperature (°C)		Relative humidity (%)	Total rain fall (cm)	No. of rainy days	Fungi isolated
		Maximum	Minimum				
February – March (2000)	Karapuzha	33.72	24.13	75.81	79.13	4	*C. lunata, M. advena, F. pallidoroseum*
	Kodimatha	34.48	24.31	76.53	61.23	3	*F. moniliforme, M. advena*
	Kollad	34.51	24.02	76.12	58.13	3	–
	Kumarakom	33.45	23.90	75.00	60.45	4	*C. lunata, M. advena, C. gloeosporioides*
	Parippu	33.87	23.72	75.71	68.13	3	*C. lunata*
June – July (2000)	Karapuzha	30.91	23.71	86.72	351.02	22	*C. lunata, M. advena, C. gloeosporioides*
	Kodimatha	31.23	23.31	85.87	331.43	21	Non sporulating fungus
	Kollad	31.72	24.12	84.81	221.32	21	*C. lunata, F. moniliforme, M. advena*
	Kumarakom	30.55	23.65	85.00	394.40	22	*C. lunata, F. moniliforme, F. oxysporum, M. advena, Rhizoctonia* sp.
	Parippu	31.23	23.81	85.72	371.31	22	*Aspergillus* sp., *C. gloeosporioides*

Table 11 Continued

Period	Location	Temperature (°C)		Relative humidity (%)	Total rain fall (cm)	No. of rainy days	Fungi isolated
		Maximum	Minimum				
October – November (2000)	Karapuzha	32.57	23.72	81.32	143	2	*C. lunata, C. gloeosporioides, M. advena*
	Kodimatha	33.31	24.12	81.51	141	3	*C. lunata*
	Kollad	33.13	24.01	81.23	121	2	*C. lunata, M. advena*
	Kumarakom	32.21	23.63	80.00	134	1	*C. lunata, F. oxysporum, M. advena, Rhizoctonia* sp.
	Parippu	32.41	23.12	80.95	131	2	*M. advena*
February – March (2001)	Karapuzha	33.01	23.23	75.50	35.12	2	*C. lunata, C. gloeosporioides, F. pallidoroseum, M. advena,*
	Kodimatha	34.23	24.32	75.20	37.23	2	*M. advena, C. gloeosporioides*
	Kollad	34.13	24.41	76.23	33.10	2	*C. lunata, A. eichhorniae*
	Kumarakom	33.94	24.20	75.00	26.30	2	*C. lunata, M. advena*
	Parippu	33.71	23.51	76.10	29.10	2	*M. advena*

R. solani (10 per cent) was isolated only during rainy season. *F. pallidoroseum* (5 per cent) and *A.eichhorniae* (10 per cent) were found to occur during summer season.

4.1.1 Correlation Studies

On statistical analysis of the weather data and number of fungi recorded, it was observed that there was a positive correlation between the occurrence of fungi and weather parameters *viz.*, minimum temperature, relative humidity, total rainfall and number of rainy days and negative correlation with maximum temperature (Table 12).

Correlation observed between occurrence of fungi and maximum temperature was highly significant and negative while it was highly significant and positive for minimum temperature and relative humidity.

The results of the study also revealed that the number of fungal genera isolated was more during the period June – July followed by October – November period when the number of rainy days was more. During summer season lesser number of fungal genera was isolated from diseased water hyacinth plants (Table 8, 9, 10 and 11).

4.1.2 Pathogenicity

The fungi isolated during the survey (18 nos) and three potential biocontrol agents *viz.*, *Fusarium pallidoroseum, F. equiseti* and *C. gloeosporioides* collected from the Department of Plant Pathology,College of Agriculture,Vellayani were tested for its pathogenicity on water hyacinth.

Based on the time taken for development of symptoms the pathogens were grouped into four categories highly virulent, virulent, moderately virulent and avirulent.

The highly virulent pathogens developed symptoms within three days of artificial inoculation while virulent isolates took 3 – 7 days,

Table 12 Coefficient of correlation between weather parameters and occurrence of fungi on water hyacinth

	Maximum temperature	Minimum temperature	Relative humidity	Rainfall	Number of rainy days	Occurrence of fungi
Maximum temperature	1.0000					
Minimum temperature	0.2303	1.0000				
Relative humidity	-0.4152	0.2693	1.0000			
Rainfall	-0.5411	0.1233	0.7133	1.0000		
Number of rainy days	-0.5808	0.0655	0.7087	0.9221	1.0000	
Occurrence of fungi	-0.3360**	0.5825**	0.4168**	0.2511*	0.2713*	1.0000

*significant at 5 per cent level
**significant at 1 per cent level

moderately virulent above seven days and avirulent did not produce any symptom.

M. advena was the only fungus which was grouped under highly virulent category (Table 13). This fungus developed symptom within two days of artificial inoculation.

C. gloeosporioides, C. lunata, F. equiseti and *Pestalotia* sp. were grouped under moderately virulent as it took more than seven days for symptom development. All other pathogenic fungi *viz., A. eichhorniae, Fusarium* spp. *Helminthosporium* sp and *R. solani* were grouped under virulent category. Four fungi *viz., Aspergillus, Penicillium, Nigrospora* and *Trichoderma* sp. failed to initiate symptoms.

The symptoms produced by *M. advena* were characterized by dull greenish water soaked lesion resulting in blighting of the leaves as well as petiole. Both older and younger leaves were infected uniformly (Table 13). All *Fusarium* spp. except *F. moniliforme* produced brown spots with yellow halo both on lamina as well as petiole which resulted in yellowing of the leaf lamina. However, *F. moniliforme* failed to produce yellow halo. In the case of *Fusarium* spp. infection was more pronounced on older leaves eventhough younger leaves were also susceptible to infection.

Symptom development by *C. gloeosporioides* was limited to older leaves. On these leaves the symptoms exhibited as dark brown spots. Unlike in the case of fusarium infection general yellowing of leaves was not a symptom associated with this pathogen.

All other pathogenic fungi produced brown or straw coloured spots which usually did not kill the infected leaves.

4.1.3 Extent of Damage

The intensity of infection of the fungi on water hyacinth ranged from 16.67 (*C. lunata* and sterile fungus) to 61.11 per cent (*M. advena*) (Table 14 and Fig. 7).

Table 13 Variation in symptom development by fungi on water hyacinth

Sl. No.	Pathogenic fungi	Time taken for symptom development (days)	Type of symptom
	Survey		
1	*Alternaria eichhorniae*	5-6	Initially minute brown spots on leaves which coalesced to form large brown spots with a faint yellow halo, later enlarged to form irregular blotches. Small brown spots were seen on petiole also.
2	*Curvularia lunata*	10	Brownish black coloured pin head sized spots on leaf lamina and petiole
3	*Colletotrichum gloeosporioides* isolate1	7-10	Started as dark brown spots on leaf lamina, gradually enlarged and coalesced to form large patches. Brown spots were observed on petiole also but the spots remained as such.
4	*Rhizoctonia solani*	7	Initially irregular straw coloured spots with a dark brown margin on leaf lamina and petiole, later enlarged to form lesions.
5	Sterile fungus	7	Small light brown coloured spots with a prominent yellow halo developed on leaf lamina
6	*Pestalotia* sp.	8-10	Isolated small pin head sized brown spots on the leaf lamina only
7	*Helminthosporium* sp.	7	Light brown coloured irregular spots appeared on leaf lamina, but these spots remained as such. Small light brown spots developed on petiole also

Table 13 Continued

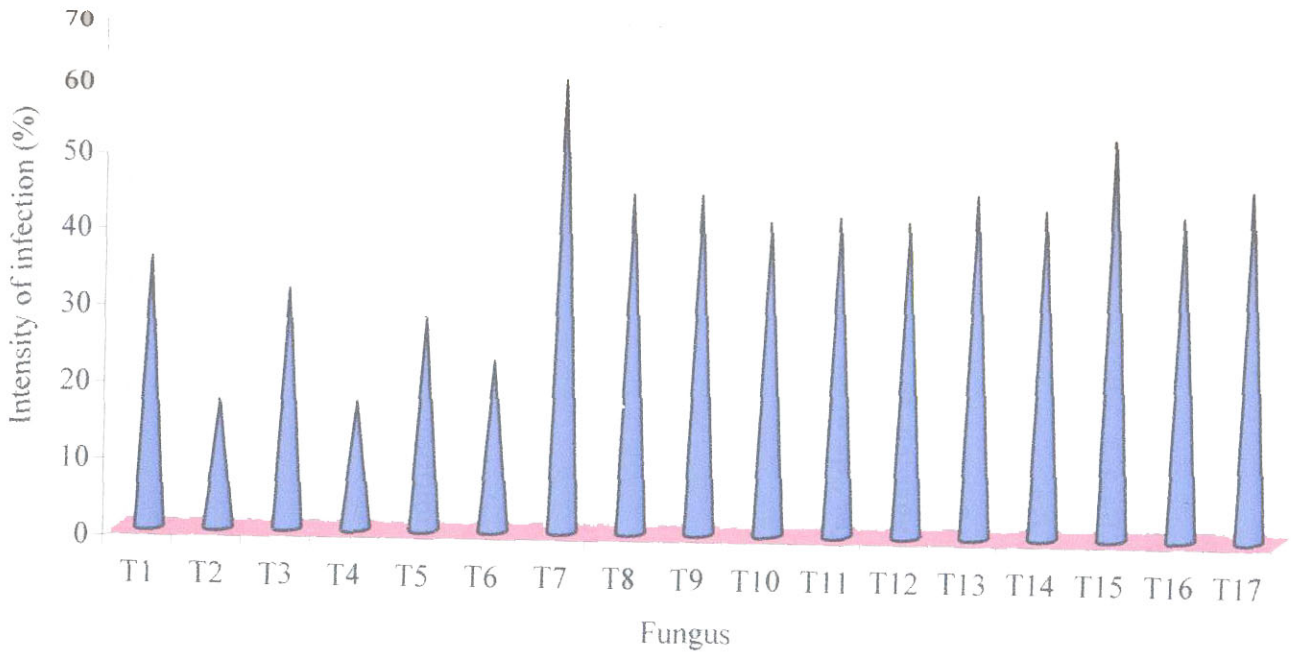
Sl. No.	Pathogenic fungi	Time taken for symptom development (days)	Type of symptom
8	*Myrothecium advena*	2	Water soaked spots developed initially, which later enlarged and coalesced to form dull greyish water soaked lesions. Spores were formed near margins later spread inwards. Water soaked lesions were produced on petiole also. These lesions later enlarged in size and had spreading nature.
9	*Fusarium oxysporum* isolate 1	5 – 7	Small light brown spots with dark margin and enlarged to form irregular shaped lesions. Same type of lesions were noticed on petiole also.
10	*F. oxysporum* isolate 2	5 – 7	Brown spots enlarged to form dark brown lesions with a faint yellow halo. On petiole also brown spots appeared which enlarged in size to form large spots.
11	*F. moniliforme* isolate 1	6 – 8	Brown spots on leaf lamina and petiole which enlarged to form lesions.
12	*F. moniliforme* isolate 2	6 – 8	Brown spots on leaf lamina and petiole which enlarged to form lesions.
13	*F. pallidoroseum* isolate 1	5 – 7	Brown spots with prominent yellow halo developed on leaf lamina and petiole. Later complete yellowing of the leaf lamina with brown spots coalesced to form large brown lesions.

Table 13 Continued

Sl. No.	Pathogenic fungi	Time taken for symptom development (days)	Type of symptom
14	*F. pallidoroseum* isolate 2	5 – 7	Brown spots enlarged to form large lesions with yellow halo. Similar symptoms were observed on petiole also.
15	*Aspergillus* sp.	-	No symptom development
16	*Penicillium* sp.	-	No symptom development
17	*Nigrospora* sp.	-	No symptom development
18	*Trichoderma* sp.	-	No symptom development
	Previous studies		
19	*F. pallidoroseum* isolate 3	4 – 5	On older leaves small brown spots of 3-4 mm size with characteristic yellow halo developed towards tips and margins of leaves. Later enlarged to form large brown irregular lesions spreading from the tip downwards, covering major area of the leaves, resulting in blighting and drying up of leaves. On younger leaves the symptoms developed as large dark brown irregular lesions. Symptoms were observed on the petiole and swollen portion as brown coloured spots which later enlarged to form lesions.
20	*F. equiseti*	10	Initially as small brownish spots of 2-3 mm size with a prominent yellow halo. As, the disease advanced, the spots enlarged to form large brown lesions on older leaves. On younger leaves the spots remained limited in size. The symptoms were confined to leaf lamina only.
21	*C. gloeosporioides* isolate 2	7 – 10	On leaf lamina, small dark brown spots (3-4 mm) with a prominent yellow halo. These spots gradually enlarged and adjacent spots coalesced to form large patches. The infection was more on older leaves. Small isolated brown specks were produced on petiole and swollen portion but they remained as such.

Table 14 Extent of damage produced by fungi on water hyacinth

Sl. No.	Fungus	Intensity of infection (%)
	Survey	
1	*Alternaria eichhorniae*	44.44
2	*Colletotrichum gloeosporioides* isolate 1	35.24
3	*Curvularia lunata*	16.67
4	*Fusarium moniliforme* isolate 1	41.72
5	*F. moniliforme* isolate 2	41.21
6	*F. oxysporum* isolate 1	44.44
7	*F. oxysporum* isolate 2	40.87
8	*F. pallidoroseum* isolate 1	45.09
9	*F. pallidoroseum* isolate 2	43.14
10	*Helminthosporium* sp.	27.77
11	*Myrothecium advena*	61.11
12	*Pestalotia* sp.	22.22
13	*Rhizoctonia solani*	31.24
14	Sterile fungus	16.67
	Previous studies	
15	*C. gloeosporioides* isolate 2	46.21
16	*F. equiseti*	42.44
17	*F. pallidoroseum* isolate 3	53.44



T1 – *Colletotrichum gloeosporioides* isolate 1
T2 – *Curvularia lunata*
T3 – *Rhizoctonia solani*
T4 – Sterile fungus
T5 – *Helminthosporium sp.*
T6 – *Pestalotia sp.*
T7 – *Myrothecium advena*
T8 – *Alternaria eichhorniae*
T9 – *Fusarium oxysporum* isolate 1
T10 – *F. oxysporum* isolate 2
T11 – *F. moniliforme* isolate 1
T12 – *F. moniliforme* isolate 2
T13– *F. pallidoroseum* isolate 1
T14– *F. pallidoroseum* isolate 2
T15– *F. pallidoroseum* isolate 3
T16– *F. equiseti*
T17– *C. gloeosporioides isolate 2*

Fig. 7 Extent of damage produced by fungi on water hyacinth

Only two fungi *viz*., *M. advena* and *F. pallidoroseum* (53.44 per cent) caused more than 50 per cent infection.

Intensity of infection of *A. eichhorniae, F. equiseti, F. moniliforme*, *F. oxysporum* and *C. gloeosporioides* (Isolate 2) ranged from 40 – 50 per cent. All the other fungi could induce only < 40 per cent infection on water hyacinth. *C. gloeosporioides* isolate 1 which was collected from diseased water hyacinth from Thiruvananthapuram district caused 35.24 per cent infection while isolate 2 collected from the Department of Plant Pathology could induce a higher infection of 46.21 per cent.

4.1.4 Morphological Characters of the Fungi

The morphological characters of those pathogenic fungi having more than 40 per cent intensity of damage were studied. This includes six isolates of *Fusarium, Alternaria* sp., *Myrothecium* sp. along with the three already identified fungi of water hyacinth *viz*., *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides*

Alternaria eichhorniae ITCC No. 5349

It produced velvetty mycelial growth with brownish white coloured mycelium. In culture, the fungus produced intense red pigment which darkened with age. Conidiophores simple, at times branched with conidia round to ovate in shape, 3-6 longitudinal septa and 1-4 transverse septa, having cylindrical beak which narrowed towards apex and 31.2 – 65 x 10 – 13.2 µm in size.

***C. gloeosporioides* (Penz.) Penz and Sacc. (Isolate 2)** IMI No. 357143

Aerial mycelium abundant, white in colour later changed to greyish white. Hyphae branched and septate. Fungus produced large number of acervuli in culture. Acervuli globose,dark brown to black coloured. Conidiophores non septate and hyaline. Conidia single celled, hyaline, straight with blunt ends, oil globule in the centre and 12.2 – 17.5 µm x 3-8 µm in size.

F. equiseti **(Corda) Sacc.** IMI No. 357141

Aerial mycelium abundant woolly and white, later became cream coloured. Macroconidia and microconidia abundant. Macroconidia were larger in size, long, hyaline, rounded at the tips and 35-60 x 3-5 μm size. Microconidia hyaline, small, oval and 5-14 x 3.5 – 5.0 μm size.

F. moniliforme **Isolate 1**

Aerial mycelium white initially, later a rosy peach colour and powdery growth seen towards the centre of the colony. Conidiophores simple and hyaline. No chlamydospores seen. Macroconidia delicate, somewhat straight, tapering towards either ends, hyaline, 3-5 septate and 3.6 – 10.89 x 1.8 – 3 μm in size. Microconidia abundant, hyaline, one celled, egg shaped and 21.2 – 47 x 2 – 4.5 μm size.

F. moniliforme **Isolate 2**

Aerial mycelium initially white, later yellow coloured. No chlamydospore production, macroconidia few in number, hyaline, somewhat falcate and 10.12-20.00 x 1.5-3.5 μm in size. Microconidia produced in large number, small, hyaline, oval, one celled and 3.6 – 10 x 4.8 – 7.2 μm in size.

F. oxysporum **Isolate 1**

In culture, mycelium white and puffy with no colouration form below. Conidiophore simple. Chlamydospores present, few in number. Macroconidia sub cylindrical with narrow tip, round base, hyaline, 3-4 septate and 21-42 x 2.5 – 4.5 μm. Microconidia produced abundantly, hyaline, oval and 4-6.72 x 2-3.1 μm size.

F. oxysporum **Isolate 2**

Cultures at first white, later peach coloured from below with aerial mycelium. Conidiophores hyaline and branched. Chlamydospores abundant and present in chains. Macroconidia abundant in number, hyaline, falcate

shaped, tip pointed and base slightly round, 5-7 septate and 19.21 – 54 x 2.5 – 5 μm in size. Macroconidia single celled, hyaline, oval and 7.26 – 13.98 x 2 – 3.63 μm size.

***F. pallidoroseum* Isolate 1**

Aerial mycelium dull white coloured, profuse, loose in growth later deep yellow coloured at the centre of colony, from below. Conidiophores branched. Macroconidia falcate in shape. Gradually tapering at one end but bluntly rounded at the apex, 3-4 septate and 14.52 – 44.14 x 2.5 – 4.5 μm size. Microconidia hyaline, single celled, oval and 4.23 – 14.52 x 1.87 – 7.3 μm in size.

***F. pallidoroseum* Isolate 2**

This isolate had white vigorously growing aerial mycelium which later changed to dark purplish peach in culture from below with powdery growth seen towards the centre of the colony. Conidiophores branched. Chlamydospores were large, rounded and seen in chains. Macroconidia falcate shaped, tapering towards one end and blunt at the other end, hyaline, 3-4 septate and 12.13 – 39.13 x 2.5 – 4.5 μm size. Microconidia were single celled, small, hyaline, oval and 3.63 – 7.26 x 1.8 – 3.63 μm in size.

***F. pallidoroseum* (Cooke.) Sacc. Isolate 3** IMI No. 357140

Cultures at first white later slight purple colouration from below. Conidiophores branched, non septate and hyaline. Macroconidia and microconidia were abundant. Macroconidia curved with slightly pointed ends, hyaline, 3-4 septate and 27-46 x 3-5 μm size. Microconidia hyaline, oval, single celled and 5.1 – 8.3 μm x 1.8 – 3.4 μm.

***Myrothecium advena* Sacc.** ITCC No. 5336

The fungus had off white mycelium initially and changed to dark green with spore masses formed on the surface of mycelial mat. The

fungus produced sporodochia in cultures, sometimes, globose due to the piling up of the conidial mass. The spore masses were at first green in colour later became dark (Plate 2). The conodiophores were fasiculate, cylindrical and rod shaped arising in clusters from a main hypha and pale greenish in colour. Conidia were cylindrical, rounded at the ends, one celled, smooth, pale greenish and measured 5.5 – 6.5 x 1.5 µm.

4.2 HOST RANGE STUDIES

M. advena which was the most virulent species observed in the survey (61.11 per cent) and three other promising cultures maintained in the Department of Plant Pathology *viz.*, *F. pallidoroseum, F. equiseti* and *C. gloeosporioides* were also included in the study.

4.2.1 *M. advena*

M. advena eventhough highly pathogenic to water hyacinth it was also pathogenic to a wide range of cultivated and weed plants.

Out of the 53 cultivated plants and 54 weeds tested, M. advena was pathogenic to 27 cultivated plants and 45 weeds (Table 15). The fungus was pathogenic to some of the important crop plants belonging to the family *Amaranthaceae, Araceae, Cucurbitaceae, Euphorbiaceae, Leguminosae, Malvaceae, Piperaceae, Poaceae, Solanaceae* etc.

Among the plants belonging to *Poaceae*, *M. advena* was highly pathogenic to rice, while it was not pathogenic to several plants belonging to the same family *viz.*, sorghum, sugarcane, guinea grass etc.

Most of the important crop plants in Kerala like pepper, rubber, rice etc were susceptible to *M. advena*. Two important crops *viz.*, cowpea and arecanut which are very common in Kerala were not susceptible.

The symptoms produced by *M. advena* on susceptible host plants were almost similar to that observed on water hyacinth (Plate 3).

Table 15 Host range of pathogens of water hyacinth

Sl. No.	Plants tested				Pathogen		
	Family	Scientific name	Common name / Vernacular name	M.a	F.p	F.e	C.g
1	Acanthaceae	*Ruellia tuberosa*	Ruellia	+	–	+	–
2		*Justicia diffusa* Willd	Justicia	+	–	–	+
3		*Justicia prostrata* Gamble N. Comb	Justicia	+	–	–	+
4	Amaranthaceae	*Amaranthus viridis* (Linn.) Notrysag	Slender amaranthus	+	+	+	–
5		*Amaranthus tricolor* L.	Amaranthus	+	+	+	+
6		*Aerva lanata* (L.) Juss	Balippovu	+	–	–	–
7		*Alternanthera sessilis*	Alligator weed, Vayal cheera	+	–	–	–
8	Anacardiceae	*Mangifera indica* L.	Mango	–	–	–	–
9		*Anacardium occidentale* L.	Cashew	–	+	+	+
10	Araceae	*Colocasia esculenta*	Taro	+	+	+	+
11		*Amorphophallus companulatus*	Elephant foot yam	+	–	–	–
12		*Anthurium andreanum* L.	Anthurium	–	–	–	–
13		*Pistia stratiotes*	Water lettuce, Muttapayal	+	–	–	–
14	Asclepiadiaceae	*Calotropis gigantea* R. Br.	Gigantic swallow weed, Erukku	+	+	–	–
15		*Hemidesmus indicus* R. Br.	Indian saraxparlke Naruneendi	+	–	–	–
16	Asteraceae	*Tridax procumbens* L.	Odiyan	+	+	–	–
17		*Vernonia cinaria* L.	Poovamkurunnu	+	+	–	–
18		*Synedrella nodiflora* L.	Venppacha	+	+	–	–
19		*Chromolaena odoratum* (L) King and Robinson	Communist pachha	+	–	–	+
20		*Emilia sonchifolia* (L.) DC	Muyalchevian	+	+	–	+
21		*Eclipta alba* (L.) Hassk	Kayonni	+	–	+	–
22		*Knoxia* sp.	Knoxia	+	–	–	–
23		*Ageratum conyzoides* L.	Goat weed / Appa	+	–	–	–
24	Boraginaceae	*Heliotropium indicum* L.	Venppacha	+	–	+	–

Table 15 Continued

Sl. No.	Plants tested			Pathogen			
	Family	Scientific name	Common name	M.a	F.p	F.e	C.g
25	Capparidaceae	*Cleome viscosa* L.	Wild mustard, Kattukaduku	+	+	–	–
26	Caricaceae	*Carica papaya*	Papaya	+	+	+	–
27	Commelinaceae	*Commelina benghalensis* L.	Tropical spider wort Vazhappadathy	+	+	+	+
28		*C. jacobi* Fischer	Vazhappadathy	+	+	+	+
29	Convolvulaceae	*Ipomoea batatus* (L.) Lam	Sweet potato	–	–	+	–
30		*Alyssicarpus vaginalis*	Alyssciarpus	+	–	–	–
31	Cucurbitaceae	*Momordica charantia* L.	Bittergourd	+	–	–	–
32		*Cucumis sativus* L.	Cucumber	+	–	–	–
33		*Trichosanthes anguina* L.	Snakegourd	+	–	–	–
34	Cyperaceae	*Bulbostylis barbata*	Sooryan	–	–	–	–
35	Dioscoriaceae	*Dioscorea alata*	Yam	–	–	–	–
36	Euphorbiaceae	*Phyllanthus niruri* (L.) Hoof F.	Keezharnelli	+	+	–	+
37		*Euphorbia geniculata* L.	Palooripppacha	+	–	–	–
38		*Euphorbia hirta* L.	Tharavu	+	+	+	+
39		*Manihot esculenta* L.	Tapioca	+	–	–	–
40		*Hevea brasiliensis*	Rubber	–	–	–	+
41	Labiatae	*Hyptis suaveolens* Poit.	Nattapoochedi	+	–	–	–
42	Lamiaceae	*Leucas aspera* Spreng.	Thumba	+	–	+	–
43	Lauraceae	*Cinnamomum zeylancium*	Cinnamon	–	–	–	+
44		*Jasminium sambac*	Jasmine	+	–	–	–
45	Leguminosae	*Clitoria ternatea* L.	Sankhupushpam	+	–	–	–
46		*Cassia occidentalis* L.	Thakara	+	–	+	–
47		*Vigna unguiculata* Savi	Cowpea	+	–	–	–
48		*Phaseolus mungo* L.	Blackgram	+	–	–	–
49		*Phaseolus aureus* Roxh.	Greengram	+	–	–	–
50		*Arachis hypogaea* L.	Groundnut	+	–	–	–
51		*Centrosema pubescens* (Benth.) Centro	Butterfly pea	–	–	–	–
52	Limnocharitaceae	*Limnocharis flava*	Malamkoovalam	+	–	–	–

Table 15 Continued

Sl. No.	Plants tested			Pathogen			
	Family	Scientific name	Common name	M.a	F.p	F.e	C.g
53	Malvaceae	*Abelmoschus esculentus* L. Mench	Bhindi	+	–	–	–
54		*Sida acuta* Burm	Vellakurumthotti	+	–	–	–
55		*Marselia quadrifolia*	Ainy pepper Wort	+	–	–	+
56		*Abutilon theophrasti*	Velvet leaf	+	–	–	–
57	Marantaceae	*Maranta arundinaceae* L.	Arrowroot	–	–	–	–
58	Mimosaceae	*Acacia arabica* L.	Acacia	–	–	–	–
59	Moraceae	*Artocarpus integrifolia* L.	Jack	–	–	–	–
60	Musaceae	*Musa* sp.	Banana	–	+	+	–
61	Myrtaceae	*Eugenia caryophyllus* L.	Clove	+	–	–	–
62		*Psidium guajava* L.	Guava	–	–	–	–
63	Myristacaceae	*Myristica fragrans* L.	Nutmeg	–	–	–	–
64	Nyctaginaceae	*Boerhaavia diffusa* L.	Thazhuthama	+	+	–	–
65	Nymphiaceae	*Nelumbo nucifera* Gaertn	Lotus	+	–	–	–
66		*Nymphaea nouchali* Burm. F.	Lily	+	–	+	–
67	Onagraceae	*Ludwigia parviflora* Roxb.	Ludwigia	+	–	–	–
68	Orchidaceae	*Dendrobium* sp.	Orchid	–	–	–	–
69	Oxalidaceae	*Oxalis corniculata* L.	Puliyarila	+	–	–	–
70	Palmae	*Cocos nucifera* L.	Coconut	–	–	–	–
71		*Areca catechu* L.	Arecanut	–	–	–	–
72	Pedaliaceae	*Sesamum indicum* L.	Sesamum	+	–	–	–
73	Piperaceae	*Piper nigrum* L.	Pepper	+	–	–	–
74		*Piper betle* L.	Betelvine	+	–	–	–
75		*Pepperomia* sp.	Kolumashi	+	–	–	+
76	Poaceae	*Oryza sativa* L.	Rice	–	–	–	–
77		*Eleusine coracana* Gaertn	Ragi	–	–	–	–
78		*Sorghum vulgare* Pers.	Sorghum	–	–	–	–
79		*Saccharum officinarum* L.	Sugarcane	–	–	–	–
80		*Panicum maximum* Jreq.	Guinea grass	–	–	–	–

Table 15 Continued

Sl. No.	Plants tested			Pathogen			
	Family	Scientific name	Common name	M.a	F.p	F.e	C.g
81		*Pennisetum purpureum* Schum	Napier grass	–	–	–	–
82		*Axonopus compresses* (Sw.) Beauv	Carpet grass	–	+	–	+
83		*Cynodon dactylon* (L.) Pers.	Bermuda grass	–	+	–	–
84		*Eragrostis tenella*	Eragrostis	–	–	–	–
85		*Eleusine indica* (L.) Gaertn	Fowl foot grass	–	–	–	–
86		*Alloteropsis cimicina* (L.) Staff	Kannuneerthulli	+	+	+	+
87		*Echinochloa colonum* Beauv	Jumela rice, Kavada	–	–	+	–
88		*Brachiaria ramosa* (L) Staff	Palpullu	+	+	–	–
89		*Panicum repens* L.	Torpedo grass	–	+	+	–
90	Ponte deraceae	*Monochoria vaginalis* Prest	Pickerel weed, Neerthamara	+	+	+	+
91	Portulacaceae	*Portulaca oleraceae*	Indian purselane Karicheera	–	–	–	–
92	Punicaceae	*Punica granatum* L.	Pomegranate	+	–	–	–
93	Rubiaceae	*Oldenlandia umbellata* L.	Nonganam pullu	+	–	–	–
94		*Coffea arabica* L.	Coffee	–	–	–	–
95	Sapotaceae	*Achras sapota* L.	Sapota	–	–	–	+
96	Scrophulariaceae	*Scoparia dulcis* L.	Kallurukki	+	+	–	–
97			Brahmi	+	–	+	–
98	Solanaceae	*Solanum melongena* L.	Brinjal	+	–	–	+
99		*Capsicum annuum* L.	Chilli	+	–	–	–
100		*Lycopersicon esculentum*	Tomato	+	+	+	–
101	Umbelliferae	*Centella asiatica* Urban	Pennywort Kudangal	+	+	+	+
102	Verbanaceae	*Lantana camara* L.	Large leaf lantana Kongini	+	–	–	–
103		*Tectonia grandis* L.	Teak	+	–	–	–
104		*Clerodendron infortunatum* L.	Peruvalam	–	–	–	–
105	Violaceae	*Ionidium suffruticosum*	Orilathamara	+	–	–	+
106	Zingiberaceae	*Curcuma longa* L.	Turmeric	+	–	–	+
107		*Zingiber officinale* L.	Ginger	–	–	–	–

M.a – *M. advena*, F.p – *F. pallidoroseum*, F.e – *F. equiseti*, C.g – *C. gloeosporioides*
+ : Susceptible, – : Non susceptible

A. *Amaranthus viridis*

B. *Marselia quadrifolia*

C. *Monochoria vaginalis*

D. *Eclipta alba*

Plate 3. Symptom produced by *Myrothecium advena* on different hosts

4.2.2 *F. pallidoroseum*

Among the four promising fungal biocontrol agents against water hyacinth, *F. pallidoroseum* had limited host range of six cultivated plants (Amaranthus, tomato, banana, cashew, colocasia and papaya) and 20 weed plants (Table 15).

F. pallidoroseum had wider host range among the weed plants (20 nos.) compared to *F. equiseti* and *C. gloeosporioides*. The weed plants which were the host of *F. pallidoroseum* belonged either to dryland (*Cleome viscosa, Emilia sonchifolia, Euphorbia hirta, Calotropis gigangtea* etc) or wet land (*Centella asiatica, Monochoria vaginalis, Brachiaria ramosa* etc).The symptoms produced by *F. pallidoroseum* were small brown spots, brown lesions and yellowing of leaves (Table 16 and Plate 4).

4.2.3 *F. equiseti*

The host range of *F. equiseti* included cultivated plants such as amaranthus, tomato, taro, sweet potato, banana, papaya, cashew and brahmi and 15 weed plants. Among the weeds excepting four (*Leucas aspera, Ruellia tuberosa, Cassia occidentalis* and *Amaranthus viridis*), remaining (10 nos.) all were wet land weeds (Table 15).

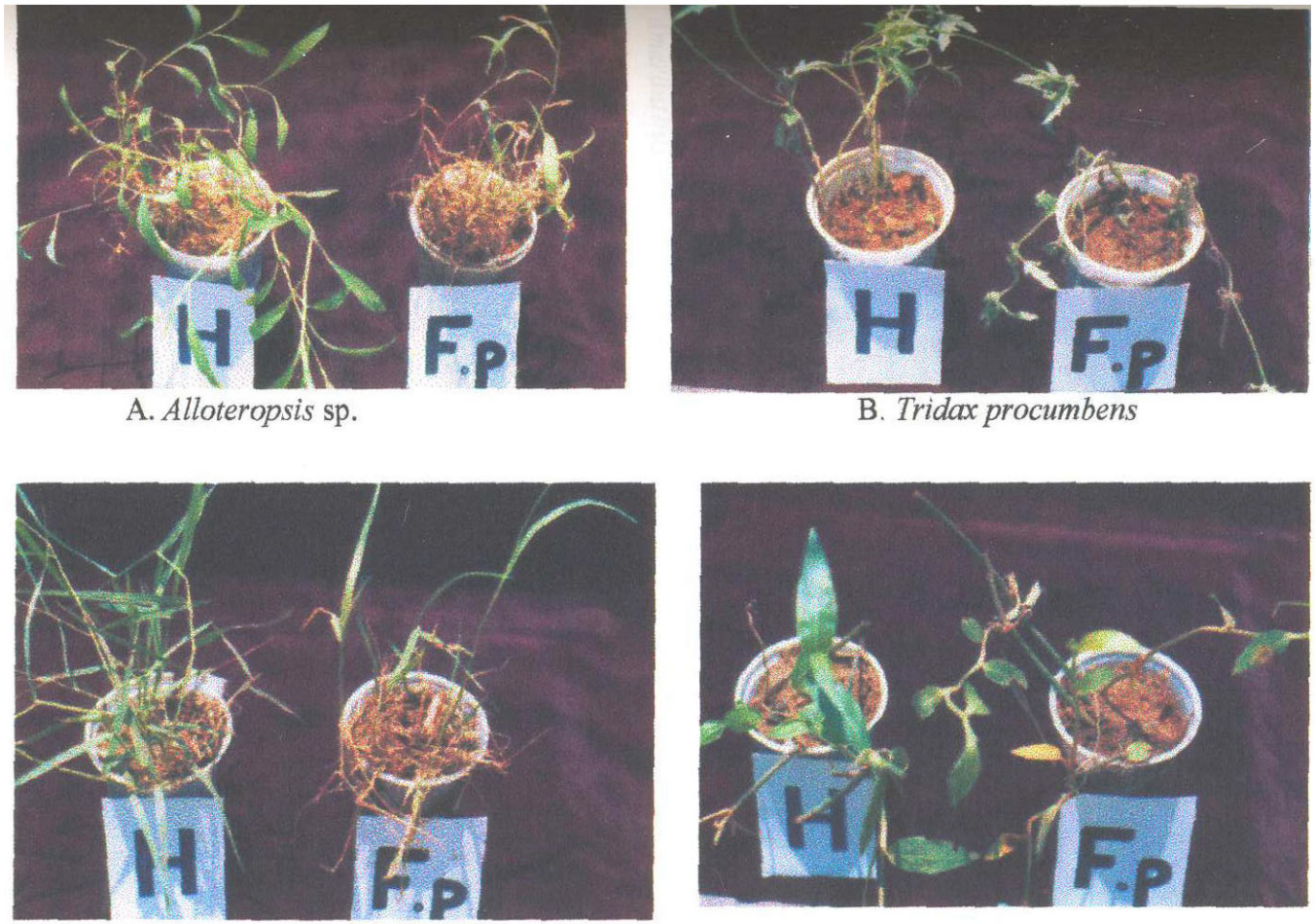
The fungus produced brown specks (Banana, lily etc), spots (tomato, cashew etc.), light brown lesions (*Commelina jacobi*), dull green coloured irregular lesions (*Amaranthus viridis*) or yellowing and drying up of leaves (*Panicum repens*) (Table 17 and Plate 5). Usually the infected leaves were not shed from plants.

4.2.4 *C. gloeosporioides*

Eventhough, *C. gloeosporioides* has a wide host range the isolate used for the study infected only eight cultivated plants and 15 weeds, out of 107 plants screened for infection (Table 15). Among the cultivated plants, vegetables like amaranthus, brinjal and colocasia, spices like turmeric and cinnamon and plantation crops like rubber and cashew are

Table 16 Symptoms produced by *F. pallidoroseum* on cultivated and weed plants

Host	Symptom produced
Cultivated plants	
Amaranthus	Dull green coloured spots developed on leaf lamina which enlarged to form lesions
Tomato	Light brown spots appeared on leaves and stem which later enlarged to form lesions
Banana, cashew, colocasia, papaya	Initially dark brown spots appeared on leaves which enlarged to form irregular lesions
Weed plants	
Axonopus compresses, Panicum repens, Cynodon dactylon, Alloteropsis cimicina	Yellowing of the infected part, which spread to the whole leaf, resulted in drying up of leaves and stalk
Phyllanthus niruri	Water soaked light brown lesions were produced on leaves and resulted in defoliation
Commelina jacobi, C. benghalensis	Brown areas surrounded by distinct yellow halo and resulted in the rotting of the leaf
Cleome viscosa, Vernonia cinarea, Emilia sonchifolia	Dark brown lesions were produced on leaves,later dried up and resulted in defoliation
Euphorbia hirta	Brown spots developed on the infected area, followed by general yellowing of the infected leaves.
Centella asiatica, Boerhaavia diffusa, Monochoria vaginalis, Calotropis gigantea, Scoparia dulcis, Synedrella nodiflora, Brachiaria ramosa, Amaranthus viridis, Tridax procumbens	Small brown spots with yellow halo were produced on leaves



A. *Alloteropsis* sp.

B. *Tridax procumbens*

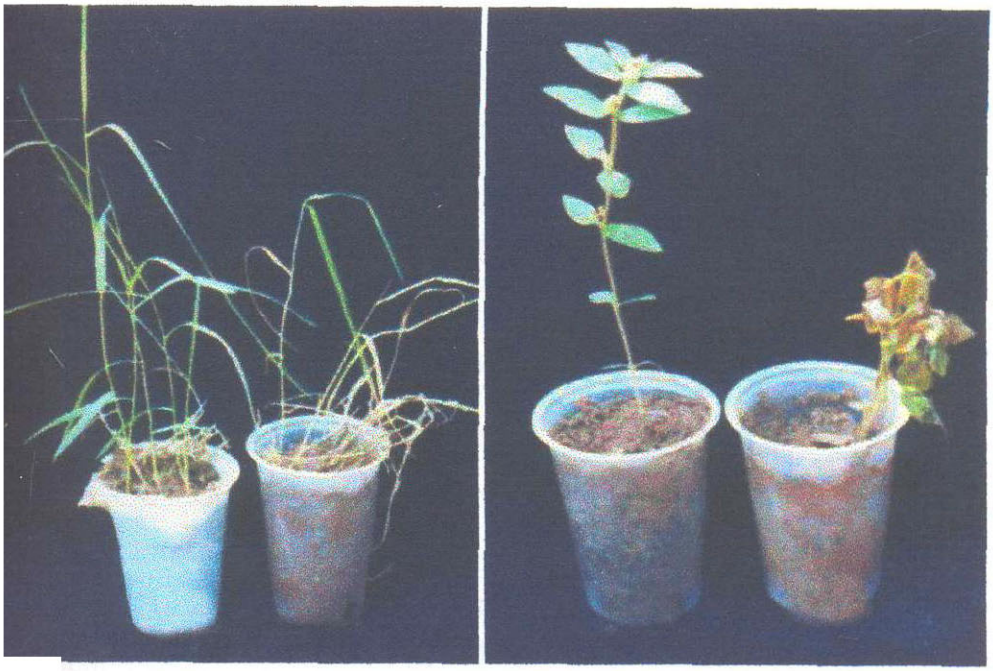
C. *Panicum repens*

D. *Commelina benghalensis*

Plate 4. Symptom produced by *Fusarium pallidoroseum* on different hosts

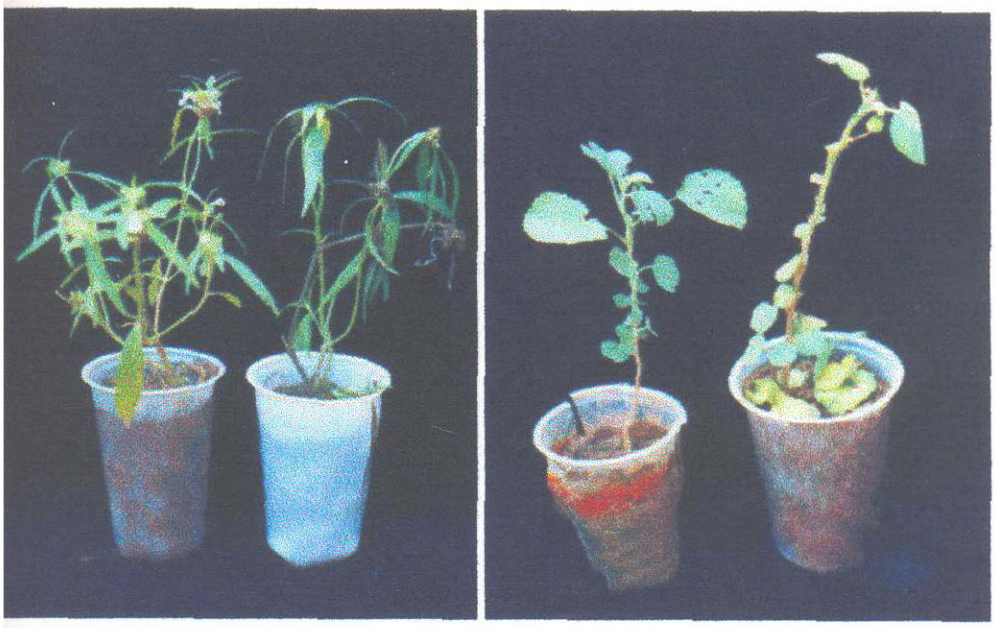
Table 17 Symptoms produced by *F. equiseti* on cultivated and weed plants

Host	Symptom produced
Cultivated plants	
Amaranthus, Cashew, Taro, Papaya, Sweet potato, Tomato	Brown spots developed in the leaf lamina, which later enlarged to form lesions
Banana, Brahmi	Brown specks appeared on leaves
Weed plants	
Commelina jacobi, C. benghalensis	Small light brown lesions with yellow halo appeared on leaves, which later resulted in rotting of leaves
Panicum repens, Alloteropsis cimicina	Yellowing of the infected part which spread to the whole leaf and resulted in drying up of leaves and stalks
Amaranthus viridis	Dull green coloured irregular lesions appeared on infected leaves and later defoliation was observed
Leucas aspera	Water soaked lesions appeared on leaves, later caused defoliation and wilting of plants.
Eclipta alba, Centella asiatica, Ruella tuberosa, Heliotropium indicum, Euphorbia hirta, Cassia occidentalis, Echinochloa colonum, Monochoria vaginalis, Nymphaea nouchali	Small brown pin head shaped spots appeared on leaves which remained as such



A. *Panicum repens*

B. *Euphorbia hirta*



C. *Leucas aspera*

D. *Abutilon* sp.

Plate 5. Symptom produced by *Fusarium equiseti* on different hosts

found to be susceptible. Among the weeds *C. gloeosporioides* was pathogenic on both wet land (*Marselia quadrifolia, Centella asiatica, Monochoria vaginalis*) and dry land weeds (*Phyllanthus niruri, Tridax procumbens, Ionidium suffruticosum* etc.) which are seen in Kerala. Symptoms produced by *C. gloeosporioides* on susceptible plants are given in Table 18 and Plate 6.

4.3 EFFICACY OF CELL FREE METABOLITES

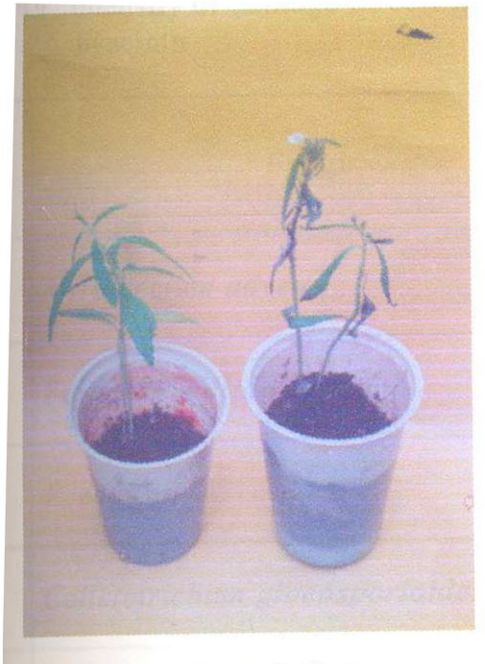
Undiluted cell free metabolic products of four fungi when sprayed on water hyacinth produced damage on the leaf surface (10.88 to 97.75 per cent) one week after spraying (Table 19 and Fig. 8). When cell free metabolites of *M. advena* was sprayed on water hyacinth leaves it almost completely scorched the leaves and petiole (97.75 per cent) (Plate 7). The initial symptoms were observed in less than two days, as spreading brown patches. These subsequently covered the entire leaf within a period of one week.

Intensity of damage by *F. pallidoroseum* (48.84 per cent) and *C. gloeosporioides* (42.17 per cent) were on par. The symptom development on leaves, sprayed with *F. pallidoroseum* started from the third day onwards. The initial symptom was scorching coupled with yellowing of older leaves. Yellowing was not observed on younger leaves. Mild scorching was also observed on the petiole.

Leaves exposed to *C. gloeosporioides* metabolites showed scorching symptoms without yellowing. The cell free metabolites of *F. equiseti* could induce only 10.88 per cent damage on the sprayed leaf surface. It took more than four days for symptom development. The symptom consisted of minute irregular patches scattered on the leaf surface. These patches did not cover wider area subsequently.

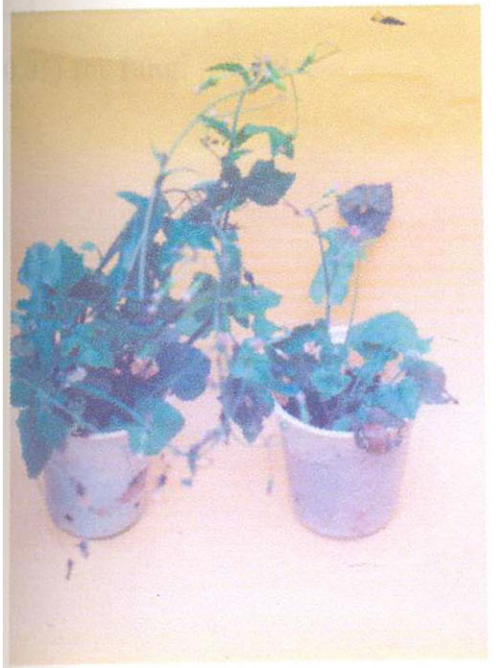
Table 18 Symptoms produced by *C. gloeosporioides* on cultivated and weed plants

Host	Symptom produced
Cultivated plants	
Rubber, Cinnamon, Cashew	Dark brown lesions appeared on leaves, later enlarged and resulted in blighting
Amaranthus	Dull straw coloured irregular spots appeared on leaf lamina resulted in defoliation of older leaves
Turmeric, Colocasia	Initially as brown coloured spots with yellowing of leaf and stalk. Later the leaves showed blighting and complete drying up of plants
Brinjal, Sapota	Dark brown spots developed in the infected area
Weeds	
Marselia quadrifolia	Initially brown lesions developed on leaves. Later blighting of leaves and complete drying up of plants
Euphorbia hirta, Ionidium suffruticosum	Small brown spots appeared on leaves, later enlarged and resulted in defoliation
Phyllanthus niruri, *Pepperomia* sp.	Small light brown lesions on the infected part, which gradually enlarged and resulted in the drying up of the leaves and resulted in leaf shedding
Emilia sonchifolia, Justicia diffusa, J. prostrata, Monochoria vaginalis, Alloteropsis cimicina, Axonopus compresses, Chromolaena odorata, Centella asiatica, Commelina jacobi, C. benghalensis	Small brown spots, which enlarged to form lesions.



A. *Oldenlandia umbellata*

B. *Chromolaena odorata*



C. *Emilia sonchifolia*

D. *Phyllanthus niruri*

Plate 6. Symptom produced by *Colletotrichum gloeosporioides* on different hosts

Table 19 Intensity of damage of cell free metabolite of fungi on water hyacinth

Sl. No.	Fungi	Mean intensity of damage (%)
1	*Myrothecium advena*	97.75 (9.94)
2	*Fusarium pallidoroseum*	48.84 (7.06)
3	*F. equiseti*	10.88 (3.45)
4	*Colletotrichum gloeosporioides*	42.17 (6.57)
	Mean	49.91

CD (0.05) for fungi – 0.69

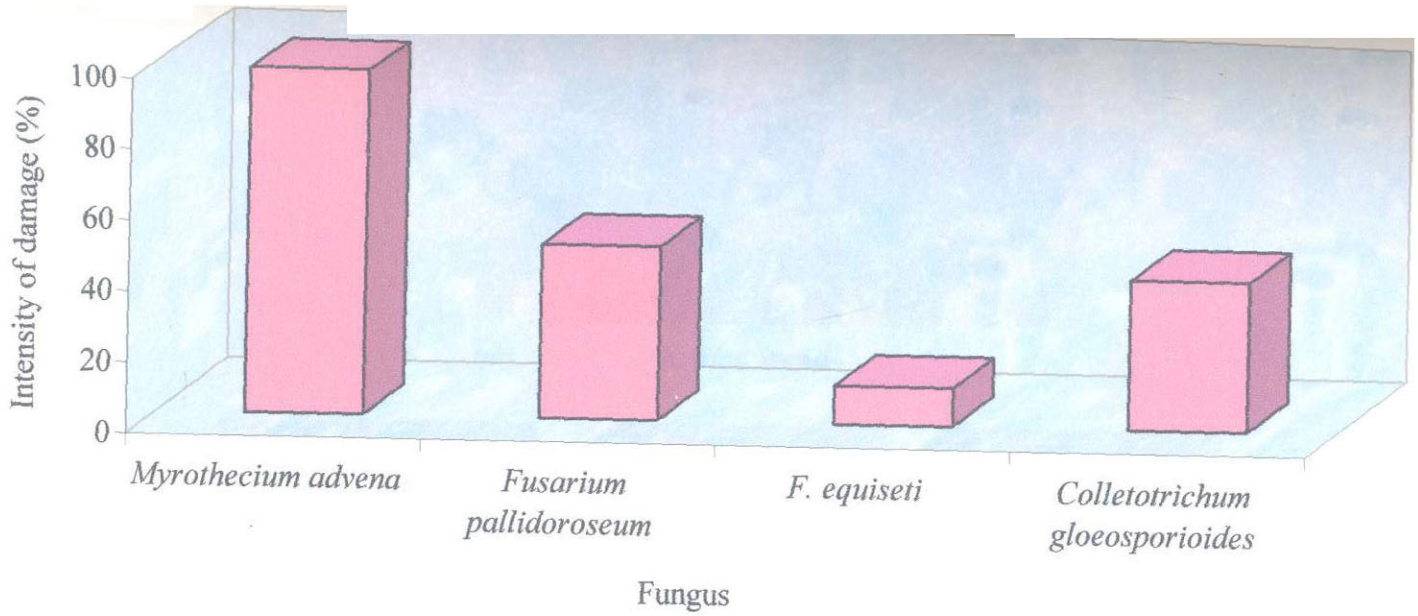
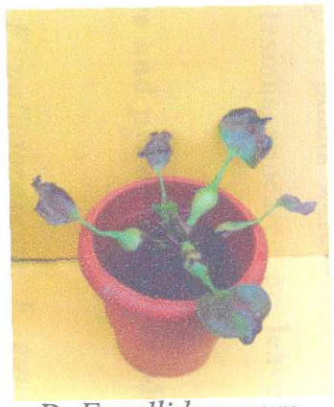
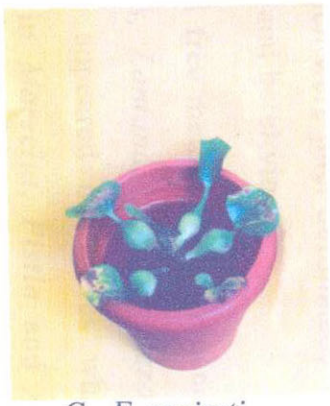


Fig. 8 Intensity of damage of cell free metabolite of fungi on water hyacinth

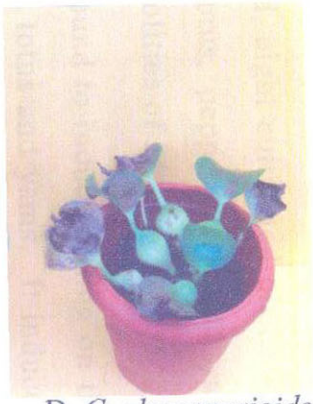
A. *M. advena*



B. *F. pallidoroseum*



C. *F. equiseti*



D. *C. gloeosporioides*

Plate 7. Symptom produced by cell free metabolite on water hyacinth

4.3.1 Host Range

The results of the study on the effect of cell free culture filtrates on 107 plants revealed that filtrates of *C. gloeosporioides* and *F. equiseti* had no effect on the plants tested (Table 20).

In the case of *F. pallidoroseum*, of the 53 cultivated and 54 weed plants tested, only *Monochoria vaginalis* was found sensitive to the culture filtrate. It produced scorching on the leaves of *M. vaginalis*.

Of the 53 cultivated plants tested, eight cultivated plants *viz.*, black gram, betelvine, clove, greengram, lotus, pepper, teak and yams were found to be sensitive to cell free metabolities of *M. advena*.

The filtrates of *M. advena* was found to induce rotting on the leaves and petioles of blackgram, greengram, lotus and yams. It induced brown lesions on leaves of teak and clove which were later defoliated. Browning of older leaves was observed in pepper and betel vine.

Of the 54 weed plants tested cell free metabolites produced damage to nine weed plants. On *Calotropis, Lantana, Hyptis, Clerodendron, Euphorbia geniculata* brown lesions appeared on leaves which later enlarged and defoliated. Where as in *Marselia*, *Pistia* and *Axonopus* yellowing of leaves and later drying up of plants were observed. But on *Monochoria* dull green water soaked lesions appeared on leaves, which later dried up.

4.3.2 Effect of Dilution, Temperature and pH on Cell Free Metabolite of the Fungi

The metabolites of *M. advena, F. pallidoroseum, F. equiseti,* and *C. gloeosporioides* were subjected to different dilutions, temperature and pH.

Table 20 Host range of cell free metabolites

Sl. No.	Plants tested				Pathogen		
	Family	Scientific name	Common name / Vernacular name	M.a	F.p	F.e	C.g
1	Acanthaceae	*Ruellia tuberosa*	Ruellia	–	–	–	–
2		*Justicia diffusa* Willd	Justicia	–	–	–	–
3		*Justicia prostrata* Gamble N. Comb	Justicia	–	–	–	–
4	Amaranthaceae	*Amaranthus viridis* (Linn.) Notrysag	Slender amaranthus	–	–	–	–
5		*Amaranthus tricolor* L.	Amaranthus	–	–	–	–
6		*Aerva lanata* (L.) Juss	Balippovu	–	–	–	–
7		*Alternanthera sessilis*	Alligator weed, Vayal cheera	–	–	–	–
8	Anacardiceae	*Mangifera indica* L.	Mango	–	–	–	–
9		*Anacardium occidentale* L.	Cashew	–	–	–	–
10	Araceae	*Colocasia esculenta*	Taro	–	–	–	–
11		*Amorphophallus companulatus*	Elephant foot yam	–	–	–	–
12		*Anthurium andreanum* L.	Anthurium	–	–	–	–
13		*Pistia stratiotes*	Water lettuce, Muttapayal	+	–	–	–
14	Asclepiadiaceae	*Calotropis gigantea* R. Br.	Gigantic swallow weed, Erukku	+	–	–	–
15		*Hemidesmus indicus* R. Br.	Indian saraxparlke Naruneendi	–	–	–	–
16	Asteraceae	*Tridax procumbens* L.	Odiyan	–	–	–	–
17		*Vernonia cinaria* L.	Poovamkurunnu	–	–	–	–
18		*Synedrella nodiflora* L.	Venppacha	–	–	–	–
19		*Chromolaena odoratum* (L) King and Robinson	Communist pachha	–	–	–	–
20		*Emilia sonchifolia* (L.) DC	Muyalchevian	–	–	–	–
21		*Eclipta alba* (L.) Hassk	Kayonni	–	–	–	–
22		*Knoxia* sp.	Knoxia	–	–	–	–
23		*Ageratum conyzoides* L.	Goat weed / Appa	–	–	–	–
24	Boraginaceae	*Heliotropium indicum* L.	Venppacha	–	–	–	–
25	Capparidaceae	*Cleome viscosa* L.	Wild mustard, Kattukaduku	–	–	–	–
26	Caricaceae	*Carica papaya*	Papaya	–	–	–	–
27	Commelinaceae	*Commelina benghalensis* L.	Tropical spider wort Vazhappadathy	–	–	–	–

Table 20 Continued

Sl. No.	Plants tested			Pathogen			
	Family	Scientific name	Common name	M.a	F.p	F.e	C.g
28		*C. jacobi* Fischer	Vazhappadathy	–	–	–	–
29	Convolvulaceae	*Ipomoea batatus* (L.) Lam	Sweet potato	–	–	–	–
30		*Alyssicarpus vaginalis*	Alyssciarpus	–	–	–	–
31	Cucurbitaceae	*Momordica charantia* L.	Bittergourd	–	–	–	–
32		*Cucumis sativus* L.	Cucumber	–	–	–	–
33		*Trichosanthes anguina* L.	Snakegourd	–	–	–	–
34	Cyperaceae	*Bulbostylis barbata*	Sooryan	–	–	–	–
35	Dioscoriaceae	*Dioscorea alata*	Yam	+	–	–	–
36	Euphorbiaceae	*Phyllanthus niruri* (L.) Hoof F.	Keezharnelli	–	–	–	–
37		*Euphorbia geniculata* L.	Palooripppacha	+	–	–	–
38		*Euphorbia hirta* L.	Tharavu	–	–	–	–
39		*Manihot esculenta* L.	Tapioca	–	–	–	–
40		*Hevea brasiliensis*	Rubber	–	–	–	–
41	Labiatae	*Hyptis suaveolens* Poit.	Nattapoochedi	+	–	–	–
42	Lamiaceae	*Leucas aspera* Spreng.	Thumba	–	–	–	–
43	Lauraceae	*Cinnamomum zeylancium*	Cinnamon	–	–	–	–
44		*Jasminium sambac*	Jasmine	–	–	–	–
45	Leguminosae	*Clitoria ternatea* L.	Sankhupushpam	–	–	–	–
46		*Cassia occidentalis* L.	Thakara	–	–	–	–
47		*Vigna unguiculata* Savi	Cowpea	–	–	–	–
48		*Phaseolus mungo* L.	Blackgram	+	–	–	–
49		*Phaseolus aureus* Roxh.	Greengram	+	–	–	–
50		*Arachis hypogaea* L.	Groundnut	–	–	–	–
51		*Centrosema pubescens* (Benth.) Centro	Butterfly pea	–	–	–	–
52	Limnocharitaceae	*Limnocharis flava*	Malamkoovalam	–	–	–	–
53	Malvaceae	*Abelmoschus esculentus* L. Mench	Bhindi	–	–	–	–
54		*Sida acuta* Burm	Vellakurumthotti	–	–	–	–
55		*Marselia quadrifolia*	Ainy pepper Wort	+	–	–	–
56		*Abutilon theophrasti*	Velvet leaf	–	–	–	–
57	Marantaceae	*Maranta arundinaceae* L.	Arrowroot	–	–	–	–

Table 20 Continued

Sl. No.	Plants tested			Pathogen			
	Family	Scientific name	Common name	M.a	F.p	F.e	C.g
58	Mimosaceae	*Acacia arabica* L.	Acacia	–	–	–	–
59	Moraceae	*Artocarpus integrifolia* L.	Jack	–	–	–	–
60	Musaceae	*Musa* sp.	Banana	–	–	–	–
61	Myrtaceae	*Eugenia caryophyllus* L.	Clove	+	–	–	–
62		*Psidium guajava* L.	Guava	–	–	–	–
63	Myristacaceae	*Myristica fragrans* L.	Nutmeg	–	–	–	–
64	Nyctaginaceae	*Boerhaavia diffusa* L.	Thazhuthama	–	–	–	–
65	Nymphiaceae	*Nelumbo nucifera* Gaertn	Lotus	+	–	–	–
66		*Nymphaea nouchali* Burm. F.	Lily	–	–	–	–
67	Onagraceae	*Ludwigia parviflora* Roxb.	Ludwigia	–	–	–	–
68	Orchidaceae	*Dendrobium* sp.	Orchid	–	–	–	–
69	Oxalidaceae	*Oxalis corniculata* L.	Puliyarila	–	–	–	–
70	Palmae	*Cocos nucifera* L.	Coconut	–	–	–	–
71		*Areca catechu* L.	Arecanut	–	–	–	–
72	Pedaliaceae	*Sesamum indicum* L.	Sesamum	–	–	–	–
73	Piperaceae	*Piper nigrum* L.	Pepper	–	–	–	–
74		*Piper betle* L.	Betelvine	+	–	–	–
75		*Pepperomia* sp.	Kolumashi	–	–	–	–
76	Poaceae	*Oryza sativa* L.	Rice	–	–	–	–
77		*Eleusine coracana* Gaertn	Ragi	–	–	–	–
78		*Sorghum vulgare* Pers.	Sorghum	–	–	–	–
79		*Saccharum officinarum* L.	Sugarcane	–	–	–	–
80		*Panicum maximum* Jreq.	Guinea grass	–	–	–	–
81		*Pennisetum purpureum* Schum	Napier grass	–	–	–	–
82		*Axonopus compresses* (Sw.) Beauv	Carpet grass	+	–	–	–

Table 20 Continued

Sl. No.	Plants tested			Pathogen			
	Family	Scientific name	Common name	M.a	F.p	F.e	C.g
83		*Cynodon dactylon* (L.) Pers.	Bermuda grass	–	–	–	–
84		*Eragrostis tenella*	Eragrostis	–	–	–	–
85		*Eleusine indica* (L.) Gaertn	Fowl foot grass	–	–	–	–
86		Alloteropsis cimicina (L.) Staff	Kannuneerthulli	–	–	–	–
87		*Echinochloa colonum* Beauv	Jumela rice, Kavada	–	–	–	–
88		*Brachiaria ramosa* (L) Staff	Palpullu	–	–	–	–
89		*Panicum repens* L.	Torpedo grass	–	–	–	–
90	Ponte deriaceae	*Monochoria vaginalis* Prest	Pickerel weed, Neerthamara	+	+	–	–
91	Portulacaceae	*Portulaca oleraceae*	Indian purselane Karicheera	–	–	–	–
92	Punicaceae	*Punica granatum* L.	Pomegranate	–	–	–	–
93	Rubiaceae	*Oldenlandia umbellata* L.	Nonganam pullu	–	–	–	–
94		*Coffea arabica* L.	Coffee	–	–	–	–
95	Sapotaceae	*Achras sapota*	Sapota	–	–	–	–
96	Scrophulariaceae	*Scoparia dulcis* L.	Kallurukki	–	–	–	–
97			Brahmi	–	–	–	–
98	Solanaceae	*Solanum melongena* L.	Brinjal	–	–	–	–
99		*Capsicum annuum* L.	Chilli	–	–	–	–
100		*Lycopersicon esculentum*	Tomato	–	–	–	–
101	Umbelliferae	*Centella asiatica* Urban	Pennywort Kudangal	–	–	–	–
102	Verbanaceae	*Lantana camara* L.	Large leaf lantana Kongini	+	–	–	–
103		*Tectonia grandis* L.	Teak	+	–	–	–
104		*Clerodendron infortunatum* L.	Peruvalam	+	–	–	–
105	Violaceae	*Ionidium suffruticosum*	Orilathamara	–	–	–	–
106	Zingiberaceae	*Curcuma longa* L.	Turmeric	–	–	–	–
107		*Zingiber officinale* L.	Ginger	–	–	–	–

M.a – *M. advena*, F.p – *F. pallidoroseum*, F.e – *F. equiseti*, C.g – *C. gloeosporioides*
+ : Susceptible, – : Non susceptible

4.3.2.1 Dilution

Diluting cell free metabolites of the fungi had a pronounced effect on the symptom development on water hyacinth (Table 21 and Fig. 9). As the dilution increased the extent of scorching on water hyacinth leaves decreased (Plate 8).

4.3.2.1.1 *M. advena*

Among the toxic metabolites tried, the most potent was the one produced by *M. advena*. This metabolite could induce a damage to the extent of 17.64 per cent even at five per cent concentration. And at 75 per cent concentration the intensity of damage was 75.50 per cent. This was more than the damage observed when undiluted metabolites of other fungi was applied on water hyacinth.

4.3.2.1.2 *F. pallidoroseum*

Damage inciting ability of metabolites of *F. pallidoroseum* was affected by dilution. However, this metabolite could induce 1.53 per cent damage even at a concentration of one per cent. Upto 50 per cent dilution the metabolite could induce only < 10 per cent damage while at 75 per cent the intensity of damage increased upto 17.64 per cent.

4.3.2.1.3 *F. equiseti*

Upto 50 per cent dilution the metabolites of *F. equiseti* did not produce any detectable symptom on the sprayed leaves. At 75 per cent concentration the metabolites of the fungus could induce 50 per cent of the damage compared to the undiluted metabolite.

4.3.2.1.4 *C. gloeosporioides*

At one per cent concentration the metabolites did not produce any symptom on water hyacinth. Intensity of infection was less than five per cent upto 25 per cent dilution. Even at 75 per cent dilution, intensity of damage (13.33 per cent) was less than 30 per cent of the undiluted control.

Table 21 Effect of dilutions of metabolites of fungi on water hyacinth

Sl. No.	Concentration	*M. advena*		*F. pallidoroseum*		*F. equiseti*		*C. gloeosporioides*		Mean
		Mean intensity of damage (%)	Inhibition over control (%)	Mean intensity of damage (%)	Inhibition over control (%)	Mean intensity of damage (%)	Inhibition over control (%)	Mean intensity of damage (%)	Inhibition over control (%)	
1	1 : 99	0 (1)	100	1.53 (1.59)	96.87	0 (1)	100	0 (1)	100	0.38
2	5 : 95	17.64 (4.32)	81.95	1.53 (1.59)	96.87	0 (1)	100	1.53 (1.59)	96.37	5.18
3	10 : 90	17.64 (4.32)	81.95	3.75 (2.18)	92.33	0 (1)	100	1.53 (1.59)	96.37	5.73
4	25 : 75	20 (4.58)	79.54	6.67 (2.77)	86.36	0 (1)	100	3.75 (2.18)	91.07	7.61
5	50 : 50	35.49 (6.04)	63.69	8.66 (3.11)	82.29	2.89 (1.97)	73.44	10.88 (4.58)	74.20	14.48
6	75 : 25	75.50 (8.75)	22.76	17.64 (4.32)	63.92	5.89 (2.62)	45.86	13.33 (6.04)	68.39	28.09
7	100 (Un diluted control)	97.75 (9.94)		48.89 (7.06)		10.88 (3.45)		42.17 (8.75)		49.92
	Mean	37.72		12.67		2.81		10.46		

Figures in parentheses indicate $\sqrt{x+1}$ transformation
CD (0.05) for fungi – 0.37
" dilution – 0.46
" interaction – 0.91

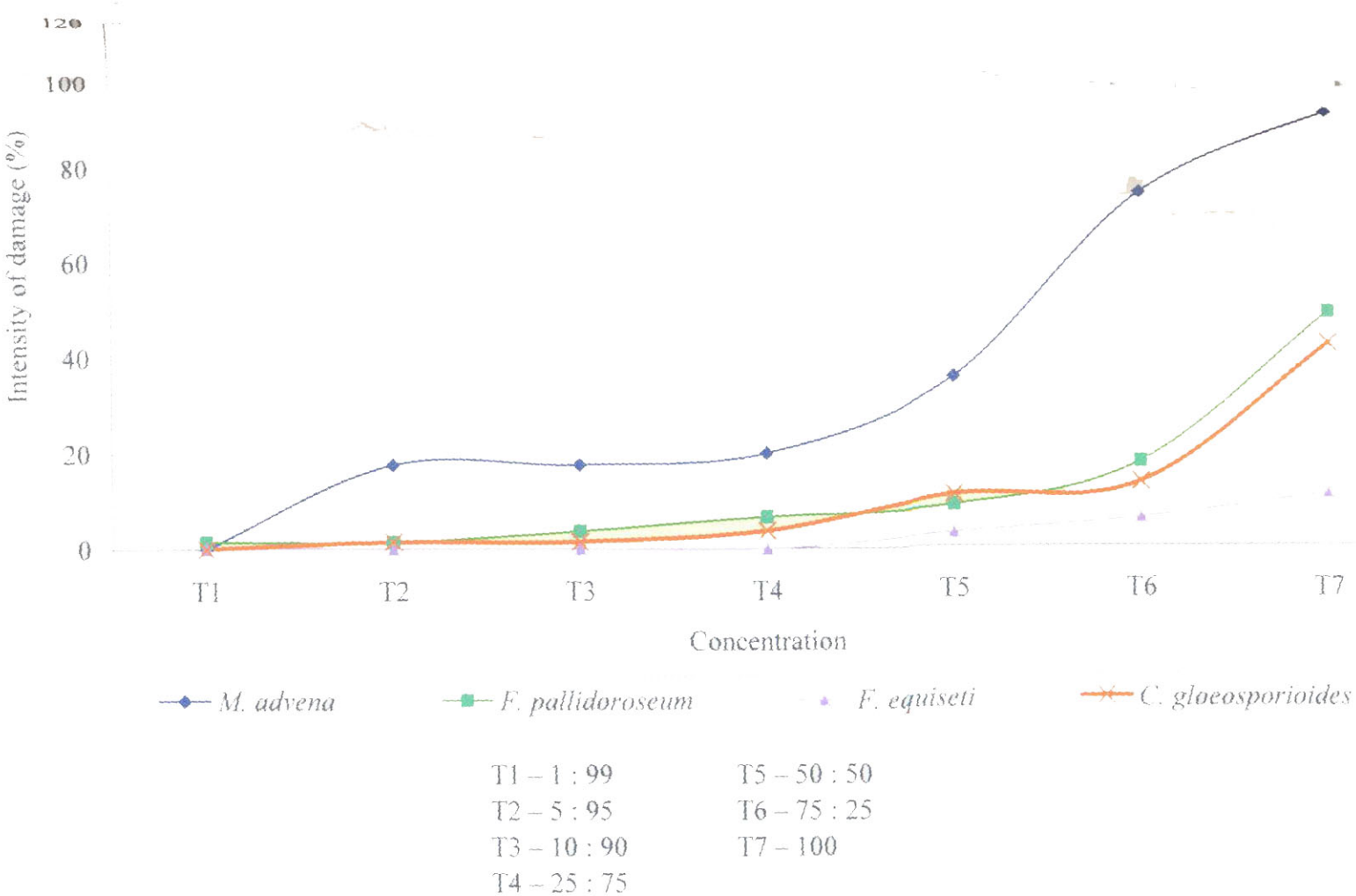
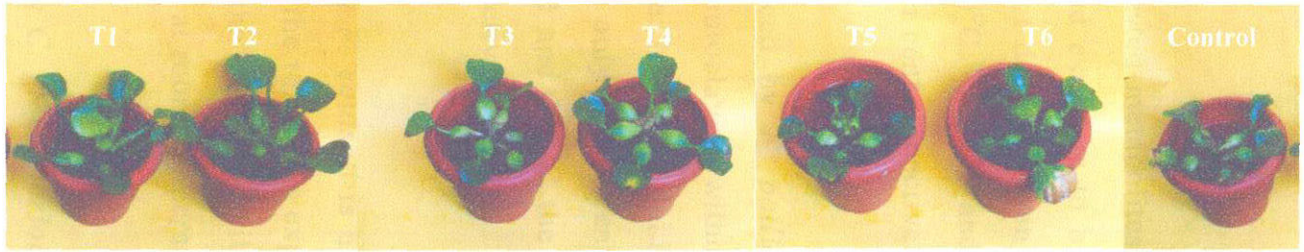


Fig. 9 Effect of dilutions of metabolites of fungi on water hyacinth

A. *M. advena*

B. *F. pallidoroseum*



C. *F.equiseti*

D. *C. gloeosporioides*

Plate 8. Symptom produced by different dilutions of metabolites on water hyacinth

4.3.2.2 Temperature

The effect of different temperature on the cell free metabolites were studied (Table 22). It was observed that in general with increase in temperature, there was decrease in the intensity of damage produced by fungi (Fig. 10 and Plate 9).

4.3.2.2.1 *M. advena*

Among the toxic metabolic tested metabolites from *M. advena* was the most stable and could withstand exposure to high temperature. The damage incited by this toxin did not decrease even when the metabolites were heated to a temperature of 60^0C. At 100 and 121^0C the inhibition over control was 81.96 and 88.87 per cent respectively.

4.3.2.2.2 *F. pallidoroseum*

The toxic metabolites produced by *F. pallidoroseum* was highly stable and it could even withstand autoclaving without completely loosing the ability to cause damage on water hyacinth. Inhibition in the symptom development of this metabolite was only 50 per cent when it was exposed to a temperature of 90^0C. At 100 and 121^0C the corresponding values were 68.45 and 96.87 per cent respectively.

4.3.2.2.3 *F. equiseti*

The metabolites produced by *F. equiseti* is highly thermolabile. It could not induce any damage when the toxin was exposed to a temperature of more than 50^0C. Even at 50^0C the mean intensity of damage was 6.67 per cent compared to 10.88 per cent at room temperature.

4.3.2.2.4 *C. gloeosporioides*

The metabolites produced by *C. gloeosporioides* could withstand a temperature upto 50^0C without any inhibition in the development of damage. As the temperature increased from 60 to 100^0C there was a gradual reduction in the intensity of damage and at 100^0C, 96.37 per cent

Table 22 Effect of temperature of metabolites of fungi on water hyacinth

Sl. No.	Temperature (°C)	*M. advena*		*F. pallidoroseum*		*F. equiseti*		*C. gloeosporioides*		Mean
		Mean intensity of damage (%)	Inhibition over control (%)	Mean intensity of damage (%)	Inhibition over control (%)	Mean intensity of damage (%)	Inhibition over control (%)	Mean intensity of damage (%)	Inhibition over control (%)	
1	50	97.78 (9.94)	–	46.67 (6.90)	4.44	6.67 (2.77)	38.69	42.17 (6.57)	–	48.32
2	60	97.78 (9.94)	–	37.71 (6.22)	22.79	0 (1)	100	28.81 (5.46)	31.68	41.02
3	70	64.41 (8.09)	34.13	28.81 (5.46)	41.01	0 (1)	100	22.12 (4.81)	47.55	28.84
4	80	44.39 (6.74)	54.60	28.81 (5.46)	41.01	0 (1)	100	22.12 (4.81)	47.55	23.83
5	90	42.17 (6.57)	56.87	24.41 (5.03)	50.02	0 (1)	100	13.33 (3.79)	68.39	19.98
6	100	17.64 (4.32)	81.96	15.41 (4.05)	68.45	0 (1)	100	1.53 (1.59)	96.37	8.65
7	121	10.88 (3.45)	88.87	1.53 (1.59)	96.87	0 (1)	100	0 (1)	–	3.10
8	Room temperature (28 ± 4°C) (control)	97.78 (9.94)		48.84 (7.06)		10.88 (3.45)		42.17 (6.57)		49.92
	Mean	59.10		29.02		2.19		21.53		

Figures in parentheses indicate $\sqrt{x + 1}$ transformation

CD (0.05) for fungi – 0.25
" temperature – 0.40
" interaction – 0.70

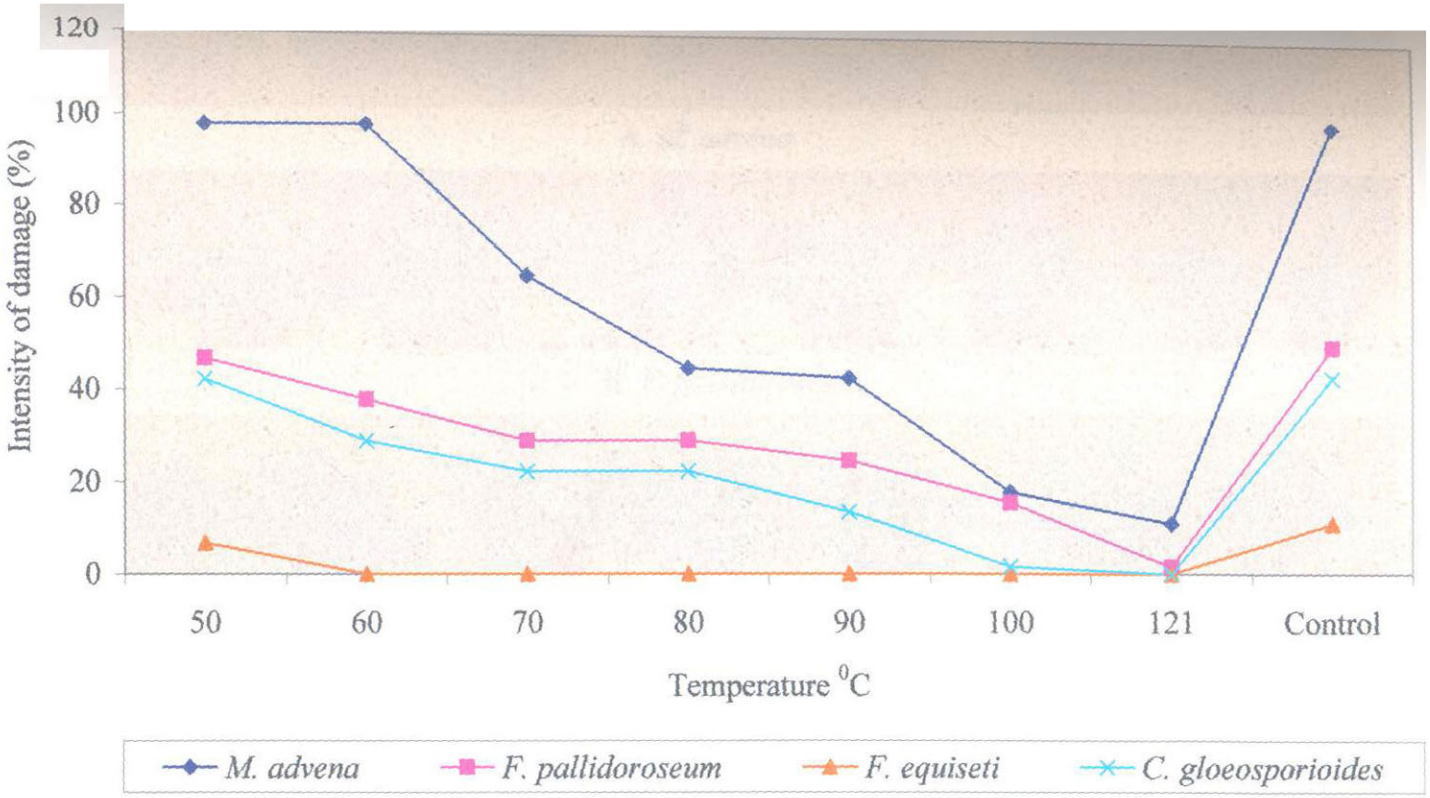


Fig. 10 Effect of temperature of metabolites of fungi on water hyacinth

A. *M. advena*

B. *F. pallidoroseum*

C. *F.equiseti*

D. *C. gloeosporioides*

Plate 9. Symptom produced by metabolites exposed to different temperature on water hyacinth

reduction in symptom over the control was observed. The toxin was inactivated when it was autoclaved (121^0C).

4.3.2.3 pH

Damage inciting ability of metabolites was affected with change in pH. In general all the fungal metabolites preferred a neutral pH (Table 23).

4.3.2.3.1 *M. advena*

The cell free metabolites of *M. advena* at neutral pH produced 97.77 per cent intensity of damage. A drastic reduction in the activity by 68.26 and 61.43 per cent respectively was recorded at acidic and alkaline pH.

4.3.2.3.2 *F. pallidoroseum*

The extent of damage produced by the metabolites of *F. pallidoroseum* at neutral and acidic pH were statistically on par. At alkaline pH, reduction in activity by 50.16 per cent was recorded.

4.3.2.3.3 *F. equiseti*

The cell free metabolites of *F. equiseti* with neutral pH produced 10.88 per cent intensity of damage. A reduction in the activity by 45.96 and 38.69 per cent respectively was recorded at acidic and alkaline pH.

4.3.2.3.4 *C. gloeosporioides*

Cell free metabolites of *C. gloeosporioides* under neutral pH gave 42.17 per cent intensity of damage which was found to be statistically on par with the damage produced by the metabolites at acidic pH (35.49 per cent). But under alkaline condition (pH – 9), 58.19 per cent reduction in intensity of damage was observed.

4.3.3 Isolation and Purification of Toxin

The 14 day old culture filtrate of the fungi grown in Richard's broth was used for the studies

Table 23 Effect of pH of metabolites of fungi on water hyacinth

Sl. No.	pH	*M. advena*		*F. pallidoroseum*		*F. equiseti*		*C. gloeosporioides*		Mean
		Mean intensity of damage (%)	Inhibition over control (%)	Mean intensity of damage (%)	Inhibition over control (%)	Mean intensity of damage (%)	Inhibition over control (%)	Mean intensity of damage (%)	Inhibition over control (%)	Mean intensity of damage (%)
1	Acidic	37.71 (6.22)	61.43	24.34 (5.03)	50.16	6.67 (2.77)	38.69	35.49 (6.04)	15.84	21.59
2	Alkaline	31.03 (5.66)	68.26	42.17 (6.57)	13.67	5.88 (2.62)	45.96	17.63 (4.32)	58.19	28.64
3	Neutral (control)	97.77 (9.94)		48.84 (7.06)		10.88 (3.46)		42.17 (6.57)		49
4	Mean	55.50		38.45		7.81		31.76		

Figures in parentheses indicate $\sqrt{x+1}$ transformation

CD (0.05) for fungi – 0.33
" pH – 0.28
" interaction – 0.57

4.3.3.1 Exotoxin

No exotoxin could be extracted from the metabolites of *C. gloeosporioides* by the ether extraction method. From the culture filtrate of *F. equiseti* and *M. advena* a brownish precipitate was obtained on extraction. However it could not be detected on paper chromatograph.

On extraction of culture filtrate of *F. pallidoroseum*, needle shaped white crystals were obtained. When these crystals were spotted on paper chromatograph a bluish pink coloured spot with rf value 0.15 was produced indicating the presence of a toxic compound (Plate 10a). Hence for purification only the toxin obtained the *F. pallidoroseum* was used. From one litre of the culture filtrate of *F.pallidoroseum* grown in Richard's broth, 40 mg of toxin could be purified. This purified toxin was again spotted in thin layer chromatography. A bluish coloured spot was developed on the plates when exposed to iodine vapours.

The toxin isolated in crystalline form from the active cultures of *F. pallidoroseum* as per the procedure outlined in section 3.4.2 was subjected to structural confirmation by a 60 MHz proton NMR spectrometer (Plate 10b).

Interpretation of the spectral peaks revealed that peaks were obtained at δ 0.9 – 1.0, 1.2 – 1.4, 2.9, 4.4 –4.5 and 7.6 – 7.8. The peaks obtained towards the up field of the spectrum at δ 0.9 as a triplet. A peak was also observed at δ 1.2 – 1.5 in the form of a multiplet.

An analysis of the integration curve revealed, that the triplet obtained at δ 1.2 – 1.5 contains double the number of protons than when compared to the first triplet at δ 0.9.

Multiplets were also obtained at δ 7.7 – 7.8 and 4.4 – 4.6. The integration curve of these peaks indicated the presence of three protons. NMR spectral peak analysis of pure crystalline toxin, confirmed the presence of fusaric acid.

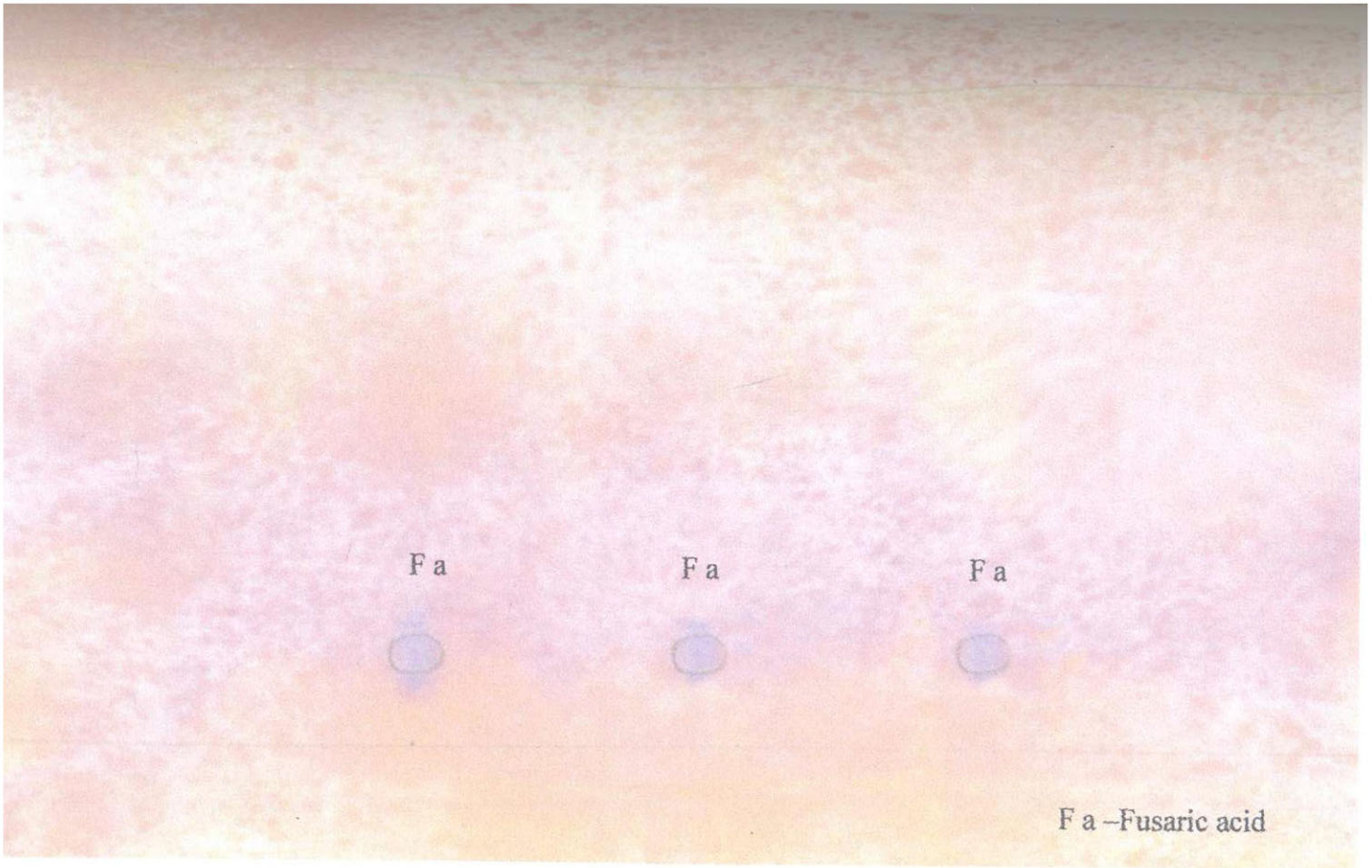


Plate 10a. Qualitative detection of fusaric acid

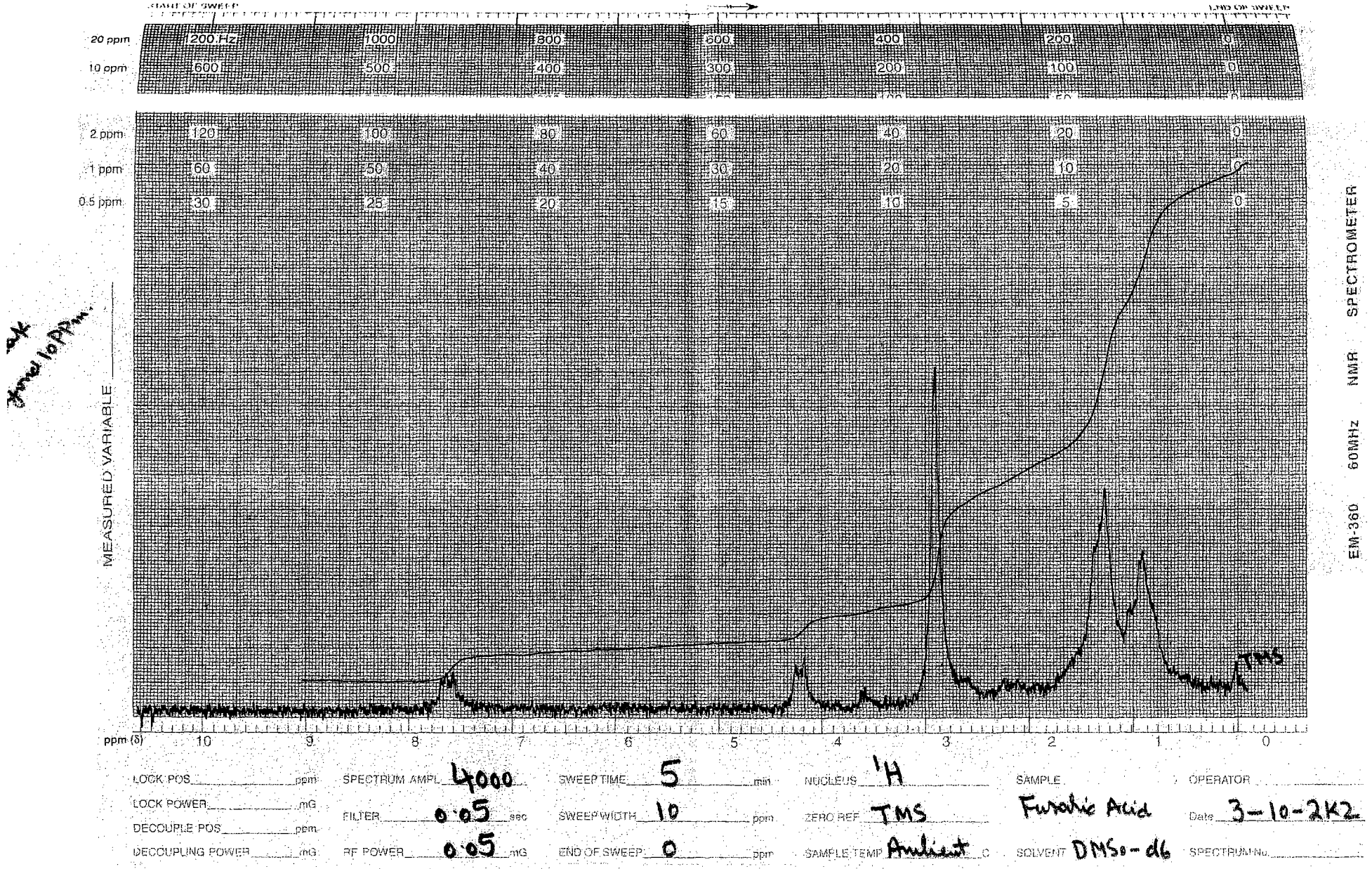


Plate 10b NMR spectrograph

4.3.3.2 Endotoxin

In order to detect the production of endotoxin by four species of fungi, the mycelial mat obtained after 14 day growth was filtered, dried and macerated using a blender. This was centrifuged and supernatent was used for testing the presence of endotoxin by applying it on water hyacinth plants.

4.3.4 Efficacy of Toxin

The biological efficacy of toxins (exo and endotoxin) obtained from the different fungi were tested on water hyacinth.

4.3.4.1 M. advena

Appearance of dull greenish water soaked lesions both on the leaves and petiole within 24 h, was the typical symptom associated with exotoxin of *M. advena*. The toxin took only 24 h for the symptom initiation, five days for complete blighting of the leaves and ten days for rotting of plants. Symptom expression with endotoxin was noticed only three days after application and unlike with exotoxin, rotting of the leaves and petiole were not observed.

4.3.4.2 F. pallidoroseum

When the purified exotoxin was applied on the leaves and petiole of water hyacinth typical symptoms resembling fungal infection appeared within 48 h. The initial detectable symptom was the appearance of water soaked brown lesions with a prominent yellow halo. These enlarged and covered the entire leaf surface. On the petiole even though brown spreading lesions were noticed, it was not surrounded by a yellow halo (Plate 11). The entire plant blighted within 10 days of the application of toxin.

Symptoms similar to that of exotoxin was observed when endotoxin was applied on the leaf surface. However it took more time for symptom

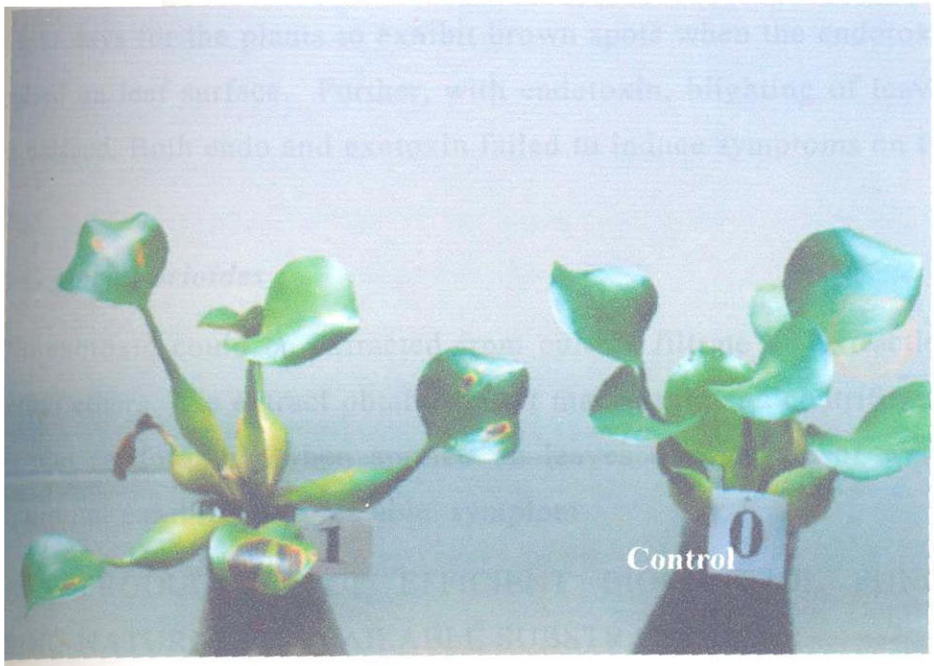


Plate 11. Symptom produced by fusaric acid on water hyacinth

expression (4 days) and the typical yellow halo was not observed. Further the time taken for blighting of the plants (14 days) was more.

4.3.4.3 F. equiseti

The toxin (exo and endo) of *F. equiseti* when applied on water hyacinth produced symptoms similar to that observed after artificial inoculation with the fungus. When exotoxin was applied by placing the filter paper discs saturated with toxin, brown spots appeared within 24 h which gradually enlarged in size resulting in blighting of leaves. However it took four days for the plants to exhibit brown spots when the endotoxin was applied on leaf surface. Further, with endotoxin, blighting of leaves was not noticed. Both endo and exotoxin failed to induce symptoms on the petiole.

4.3.4.4 C. gloeosporioides

No exotoxin could be extracted from culture filtrate on extraction with diethyl ether. The extract obtained after macerating and centrifuging mycelial mat (endotoxin), when applied on leaves and petiole of water hyacinth did not exhibit any detectable symptom.

4.4 MASS PRODUCTION OF EFFICIENT BIOCONTROL FUNGI USING NATURALLY AVAILABLE SUBSTRATES

An experiment was conduced to find suitable substrate for mass multiplication and storage of fungi *viz., F. pallidoroseum, F. equiseti* and *C. gloeosporioides. M. advena* was not included in the study as it was found to be pathogenic to several crop plants commonly cultivated in Kerala.

4.4.1 Solid Substrates

The different substrates tested are coconut oil cake, gingelly oil cake, groundnut oil cake, guinea grass straw powder, neem oil cake, rice bran, coir pith, dried cowdung, neem cake + cowdung(dried) and coirpith + rice bran. Observations on nature and extent of growth for a period of

three months and spore viability of the fungi for a period of six months were recorded (Plate 12).

4.4.1.1 F. pallidoroseum

4.4.1.1.1 Extent of Growth

Visible mycelial growth was observed on the third day of inoculation (DAI) of coconut oil cake, guinea grass straw powder, rice bran and coirpith + rice bran. In rice bran and guinea grass straw powder by 2 weeks after storage (WAS), mycelial growth covered 50 per cent of the surface area of the substrate (Table 24).

However, in general in coconut oil cake, guinea grass straw powder and rice bran profuse mycelial growth was observed through out the period of observation of 12 weeks. Eventhough dried cowdung, neem oil cake alone and in combination supported the growth of *F. pallidoroseum*, the extent of growth was very sparse. Coirpith in combination with rice bran was found to be a better substrate for growth of *F. pallidoroseum* than coirpith alone.

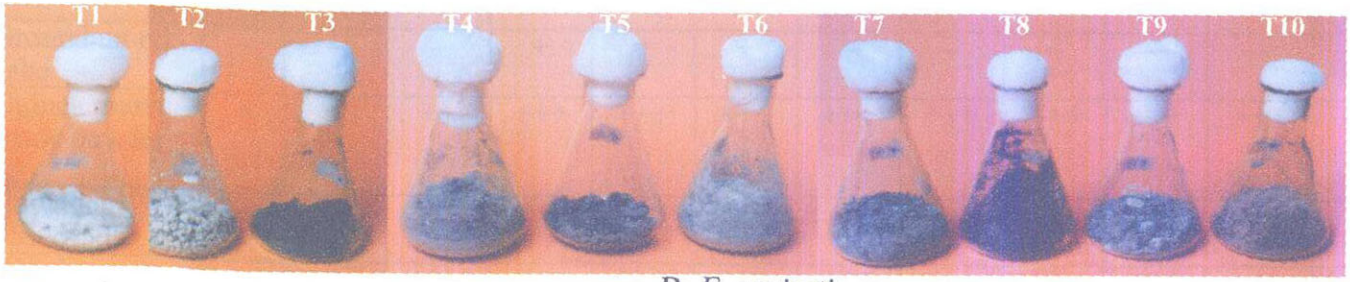
Eventhough slight visible growth was observed in groundnut oil cake at 3 WAS, it remained as such upto 5 WAS and thereafter no visible growth was observed.

4.4.1.1.2 Spore Viability

Significant difference between the effect of substrates on the number of viable spores and period of storage was observed when *F. pallidoroseum* was grown on different substrates (Table 25 and Fig. 11).

Maximum mean spore count was recorded in rice bran (1.07×10^8 cfu / g) followed by coirpith + rice bran and gingelly oil cake with 9.73×10^7 cfu / g and 6.97×10^7 cfu / g respectively. The least mean spore count was recorded in groundnut oil cake (3.37×10^6 cfu / g).

A. *F. pallidoroseum*



B. *F. equiseti*

C. *C. gloeosporioides*

Plate 12. Growth of pathogenic fungi on solid substrates

Table 24 Effect of solid substrates on the growth of F. pallidoroseum

Treatments		3rd day	Extent of growth (WAS)												
			1	2	3	4	5	6	7	8	9	10	11	12	Nature of growth
T_1	Coconut oil cake	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	White puffy
T_2	Gingelly oil cake	–	+	+	++	+++	+++	+++	+++	+++	–	–	–	–	Thick white velvetty
T_3	Groundnut oil cake	–	–	–	+	+	+	–	–	–	–	–	–	–	Thread like
T_4	Guinea grass straw powder	+	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Thick white velvetty
T_5	Cowdung (dried)	–	+	+	+	+	+	++	++	++	+	+	+	+	Scanty
T_6	Rice bran	+	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Thick white velvetty
T_7	Neem oil cake	–	+	+	+	++	++	++	+	++	+	+	–	–	Scanty
T_8	Coirpith	–	+	+	+	+	++	++	+	++	+	+	+	+	Scanty
T_9	Neem oil cake + cowdung (dried)	–	+	+	+	++	++	++	++	++	++	++	++	++	Scanty
T_{10}	Coirpith + rice bran	+	+	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Thick white velvetty

– No visible growth, + 25 % area covered, ++ 50 % area covered, +++ >50 % area covered
WAS : weeks after storage

Table 25 Effect of solid substrates on the spore viability of *F. pallidoroseum*

Treatments	Mean cfu/g x 10^5 (WAS)							
	1	2	3	4	5	6	7	8
Coconut oil cake	2188.17 (3.34)	1442.42 (3.16)	2673.17 (3.43)	5692.89 (3.76)	26.05 (1.43)	3.22 (0.62)	4.31 (0.73)	3.13 (0.62)
Gingelly oil cake	2791.95 (3.44)	3795.35 (3.58)	4099.62 (3.61)	2714.35 (3.43)	2977.54 (3.47)	267.32 (2.43)	42.51 (1.64)	31.33 (1.51)
Groundnut oil cake	719.06 (2.86)	66.94 (1.83)	20.33 (1.33)	1.20 (0.34)	0.31 (0.12)	0.13 (0.05)	0.0003 (0.0001)	0.001 (0.0004)
Guinea grass straw powder	414.41 (2.61)	253.99 (2.41)	119.73 (2.08)	305.99 (2.49)	701.66 (2.85)	373.26 (2.57)	3083.02 (3.49)	2114.40 (3.33)
Cowdung (dried)	189.31 (2.28)	729.88 (2.86)	790.03 (2.90)	411.74 (2.62)	16.86 (1.25)	17.55 (1.27)	860.03 (2.93)	179.27 (2.26)
Rice bran	445.60 (2.65)	369.94 (2.57)	409.47 (2.61)	8555.79 (3.93)	6746.41 (3.83)	1754.59 (3.24)	3474.65 (3.54)	2046.46 (3.31)
Neem oil cake	0.67 (0.22)	2.56 (0.55)	696.52 (2.84)	240.24 (2.38)	7266.41 (3.86)	236.64 (2.38)	288.54 (2.46)	449.37 (2.65)
Coirpith	–	–	–	0.012 (0.005)	0.21 (0.08)	13.57 (1.16)	405.01 (2.61)	332.37 (2.52)
Neem oil cake + cowdung (dried)	625.09 (2.79)	2128.42 (3.33)	3269.67 (3.51)	10.97 (1.07)	78.31 (1.90)	87.13 (1.95)	39.93 (1.61)	98.18 (1.90)
Coirpith + rice bran	327.84 (2.52)	3199.29 (3.50)	3859.14 (3.59)	2263.41 (3.36)	7758.78 (3.89)	3993.07 (3.60)	597.94 (2.78)	293.09 (2.47)
Mean	770.21	1198.88	1539.77	2019.66	2557.25	674.65	879.60	554.79

Table 25 Continued

Treatments	Mean cfu/g x 10^5 (WAS)							
	9	10	11	12	13	14	15	16
Coconut oil cake	0.87 (0.27)	0.79 (0.25)	0.77 (0.25)	0.72 (0.24)	0.049 (0.03)	0.028 (0.01)	0.018 (0.007)	0.016 (0.007)
Gingelly oil cake	7.93 (0.95)	1.2 (0.34)	0.11 (0.05)	0.27 (0.10)	0.075 (0.03)	0.001 (0.0004)	0.001 (0.0004)	–
Groundnut oil cake	0.001 (0.0002)	–	–	–	–	–	–	–
Guinea grass straw powder	346.67 (2.54)	723.27 (2.86)	872.78 (2.94)	932.33 (2.97)	39.23 (1.6)	37.97 (1.59)	28.00 (1.46)	1.03 (0.31)
Cowdung (dried)	80.0 (1.91)	7.63 (0.94)	6.76 (0.89)	1.40 (0.38)	1.22 (0.33)	0.23 (0.09)	0.12 (0.02)	4.9 (0.77)
Rice bran	760 (2.88)	913.33 (2.96)	82.54 (1.92)	76.45 (1.89)	0.15 (0.06)	0.01 (0.015)	0.030 (0.01)	0.07 (0.03)
Neem oil cake	323.33 (2.51)	6.69 (0.87)	1.13 (0.33)	0.76 (0.25)	0.15 (0.06)	0.16 (0.07)	0.033 (0.014)	0.01 (0.004)
Coirpith	150 (2.17)	223.01 (2.35)	179.83 (2.26)	0.19 (0.08)	1.89 (0.46)	39.00 (1.60)	3.13 (0.62)	2633.33 (3.42)
Neem oil cake + cowdung (dried)	72.64 (1.87)	27.82 (146)	65.57 (1.82)	5.81 (0.83)	8.73 (0.98)	3.33 (0.84)	3.33 (0.64)	5.13 (0.79)
Coirpith + rice bran	690.00 (2.84)	293.09 (2.47)	47.32 (1.68)	13.28 (1.15)	5.70 (0.83)	0.49 (0.16)	0.49 (0.17)	0.53 (0.18)
Mean	243.14	219.68	125.68	103.17	5.72	8.1	3.5	264.5

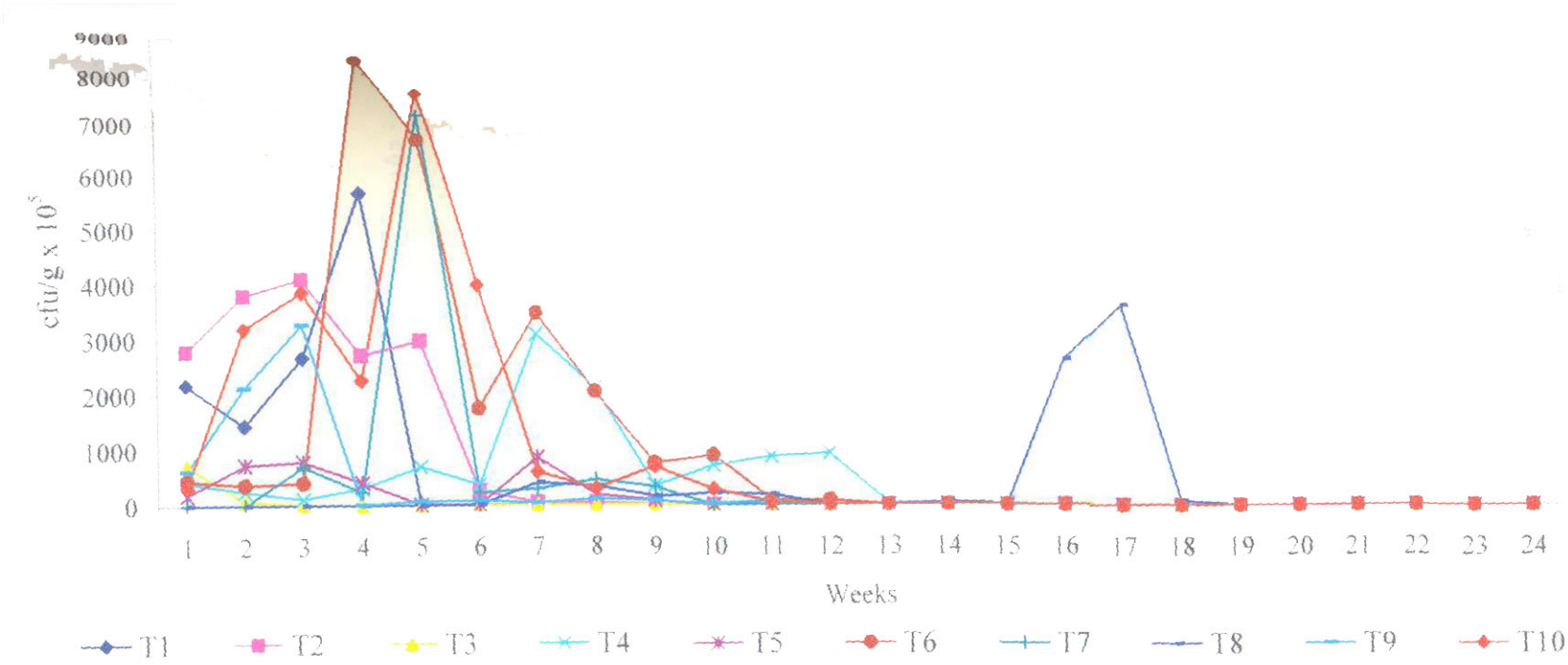
Table 25 Continued

Treatments	Mean cfu/g x 10^5 (WAS)								Mean
	17	18	19	20	21	22	23	24	
Coconut oil cake	0.001 (0.0004)	–	–	–	–	–	–	–	501.53
Gingelly oil cake	–	–	–	–	–	–	–	–	697.06
Groundnut oil cake	–	–	–	–	–	–	–	–	33.67
Guinea grass straw powder	2.06 (0.49)	6.8 (0.89)	3.5 (0.65)	1.17 (0.34)	0.004 (0.002)	0.002 (0.001)	0.001 (0.0004)	–	431.72
Cowdung (dried)	4.57 (0.75)	0.011 (0.005)	0.003 (0.001)	0.002 (0.001)	–	–	–	–	137.56
Rice bran	0.043 (0.02)	0.043 (0.02)	0.051 (0.02)	0.007 (0.003)	0.002 (0.001)	0.001 (0.0004)	0.001 (0.0004)	–	1068.17
Neem oil cake	0.001 (0.0004)	–	–	–	–	–	–	–	396.38
Coirpith	3633.33 (3.56)	75.67 (1.88)	4.47 (0.74)	0.038 (0.02)	0.002 (0.001)	0.001 (0.0004)	–	–	320.64
Neem oil cake + cowdung (dried)	5.57 (0.82)	0.11 (0.05)	0.014 (0.006)	0.056 (0.02)	0.002 (0.001)	0.001 (0.0004)	–	–	272.33
Coirpith + rice bran	0.16 (0.06)	0.12 (0.05)	0.16 (0.06)	0.002 (0.001)	0.001 (0.0004)	0.001 (0.0004)	–	–	972.66
Mean	364.57	8.30	0.82	0.13	0.001	0.0006	0.0002	–	

Figures in parentheses indicate logarithmic transformation

CD (0.05) for period of storage – 0.03
" for treatments – 0.03
" for interaction – 0.05

cfu : colony forming units, WAS : weeks after storage



cfu/g – colony forming units per gram

T1–Coconut oil cake
T2–Gingelly oil cake
T3–Groundnut oil cake
T4–Guinea grass straw powder
T5–Cowdung (dried)
T6–Rice bran
T7–Neem oil cake
T8–Coirpith
T9– Neem oil cake + cowdung (dried)
T10–Coirpith + rice bran

Fig. 11 Effect of solid substrates on the spore viability of *F. pallidoroseum*

In rice bran, the spore count was 4.46 x 10^7 cfu / g on 1 WAS, which increased and reached a maximum of 8.56 x 10^8 cfu / g at 4 WAS. Thereafter a declining trend was noticed and by 24 WAS a count of 1 x 10^2 cfu / g was recorded.

The spore count of coirpith + rice bran was 3.28 x 10^7 cfu / g, by 1 WAS and it increased to 3.86 x 10^8 cfu / g at the end of three weeks. Thereafter a reducing trend was noticed and the population reached 5.98 x10^7 cfu / g by 7 WAS and no colonies could be detected at 23 WAS.

Coconut oil cake supported a population of 2.19 x10^8 cfu / g, after one week of inoculation and the population remained more than 10^6 upto 6 weeks of storage. But the population suddenly decreased and no viable count was detected 18 WAS and remained like that till the end of observation.

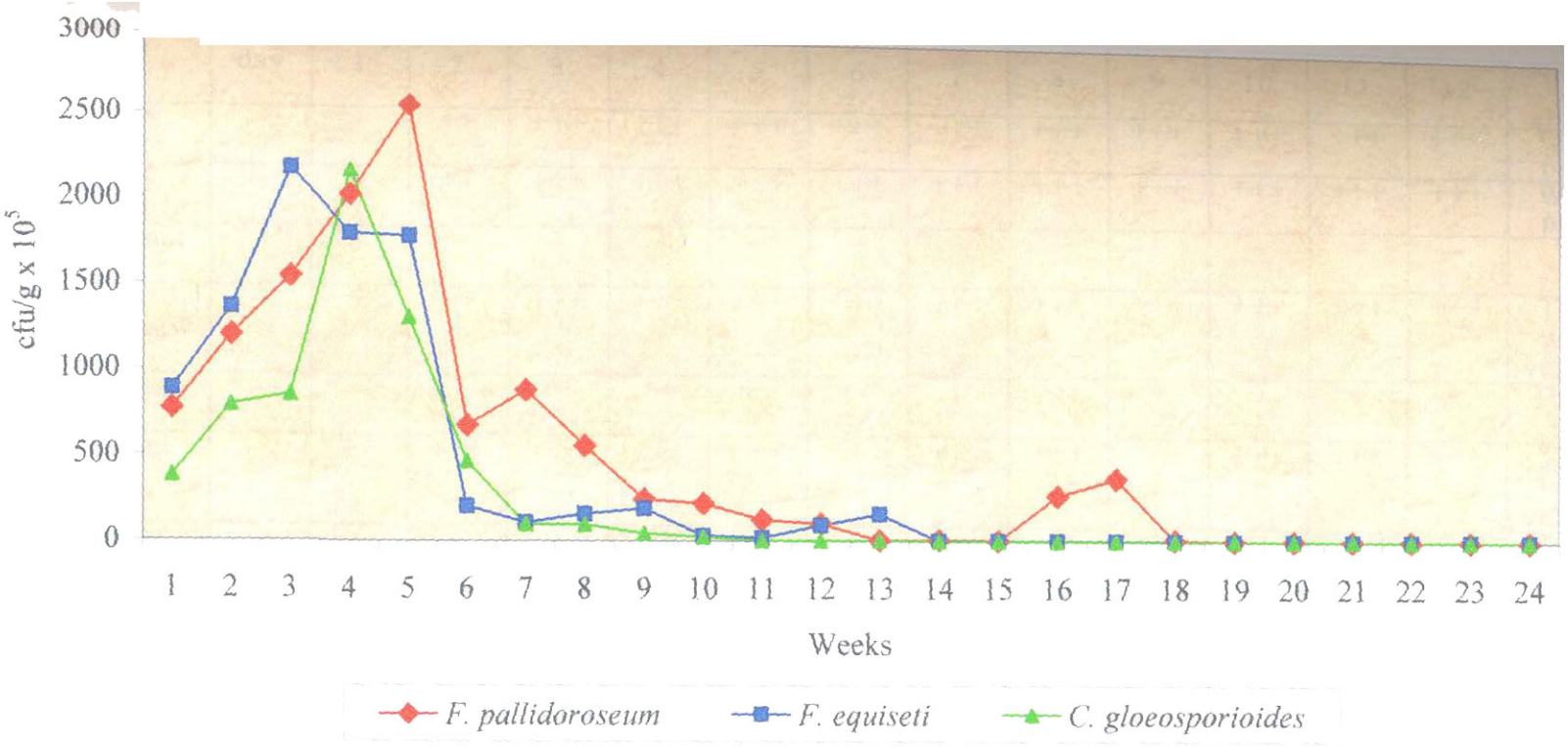
Groundnut oil cake recorded the least mean spore count of 3.37 x 10^2 cfu / g, however the initial spore count was 7.19 x 10^7 cfu / g which decreased gradually and reached zero by 10 WAS.

In general, spore count of *F. pallidoroseum* increased from 7.7 x 10^7 cfu/g (1 WAS) to 2.56 x10^8 cfu / g within seven weeks (Fig. 14). A declining trend was observed thereafter and even at the end of 12^{th} week the population was 1.03 x 10^7 cfu / g. At the end of 24 weeks, a population of 200 could be recorded.

4.4.1.2 F. equiseti

4.4.1.2.1 Extent of Growth

No visible growth was observed in all the solid substrates tested upto three days (Table 26). In coconut and gingelly oil cakes, white puffy mycelial growth was observed covering more than 50 per cent area of the substrate within three weeks and it remained as such. Colour of mycelium became dull, clumped together and whole mass separated from the walls of the flask by 6 WAS.



cfu/g – colony forming units per gram

Fig. 14 Effect of storage period on spore viability of fungi in solid substrates

Table 26 Effect of solid substrates on the growth of F. equiseti

Treatments		3rd day	Extent of growth (WAS)												
			1	2	3	4	5	6	7	8	9	10	11	12	Nature of growth
T_1	Coconut oil cake	–	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	White puffy
T_2	Gingelly oil cake	–	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	White puffy
T_3	Groundnut oil cake	–	–	–	–	–	–	–	–	–	–	–	–	–	–
T_4	Guinea grass straw powder	–	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Thick white velvetty
T_5	Cowdung (dried)	–	–	+	++	++	++	++	+	+	+	+	+	+	Thread like
T_6	Rice bran	–	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Thick white velvetty
T_7	Neem oil cake	–	–	–	+	++	++	+	+	+	+	–	–	–	Sparse
T_8	Coirpith	–	–	+	+	+	++	++	++	+	+	+	+	+	Thread like
T_9	Neem oil cake + cowdung (dried)	–	–	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Scanty
T_{10}	Coirpith + rice bran	–	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Thick white velvetty

– No visible growth, + 25 % area covered, ++ 50 % area covered, +++ >50 % area covered
WAS : weeks after storage

In case of guinea grass straw powder, rice bran and coirpith + rice bran by 3 WAS thick white velvetty growth of the fungus covered more than 50 per cent of the substrates. In neem oil cake + cowdung, eventhough at 3 WAS more than 50 per cent area was covered, the growth was scanty in nature through out the period of observation.

Growth of *F. equiseti* was sparse in cowdung and neem oil cake. In groundnut cake no visible mycelial growth of the fungus was noticed through out the period of observation.

4.4.1.2.2 Spore Viability

Among the different solid substrates tested, rice bran gave maximum mean spore count of 7.93 x 10^7 cfu / g followed by coconut oil cake and coirpith + rice bran (7.82 x 10^7 and 7.4 x 10^7 cfu / g respectively) (Table 27 and Fig. 12). The spore count was least in coirpith (5.76 x 10^6 cfu / g). No viable count of spores was recorded in groundnut oil cake throughout the period of observation.

In rice bran a spore count of 1.5 x 10^5 cfu / g was recorded on 1 WAS and gradually increased to 3.45 x10^8 cfu / g by 4 WAS. Thereafter a drastic increase in spore count of 1.12 x 10^9 cfu / g was observed at 5 WAS then suddenly declined and at the end of study the population count was zero.

In coconut oil cake, the spore count of 7.6 x10^6 cfu / g observed at the end of one week increased to 7.93 x 10^8 cfu / g by three weeks. Then declined to 5.07 x 10^5 cfu / g at the end of seven weeks and no viable propagules could be detected at the end of 18 weeks.

Coirpith + rice bran, the count of viable spores was 8.27 x 10^6 cfu / g noticed 1 WAS increased to 6.93 x 10^8 cfu / g by 3 WAS. Thereafter the spore count decreased gradually and reached 7.01 x 10^6 cfu / g by 10 WAS and to 40 by 23WAS.

Table 27 Effect of solid substrates on the spore viability of F. equiseti

Treatments	Mean cfu/g x 10^5 (WAS)							
	1	2	3	4	5	6	7	8
Coconut oil cake	76 (1)	53.32 (1.73)	7933.26 (3.89)	7065.45 (3.84)	3399.37 (3.53)	221.91 (2.35)	5.07 (0.78)	0.69 (0.23)
Gingelly oil cake	660 (2)	7530.03 (3.87)	271.31 (2.44)	15.29 (1.21)	26.29 (1.43)	15.13 (1.21)	2.92 (0.59)	0.28 (0.11)
Groundnut oil cake	–	–	–	–	–	–	–	–
Guinea grass straw powder	7433.33 (3)	729.88 (2.86)	423.18 (2.63)	299.91 (2.46)	169.82 (2.23)	1.73 (0.45)	1.68 (0.43)	0.030 (0.013)
Cowdung (dried)	19.66 (1)	18.77 (1.29)	16.67 (1.25)	64.43 (1.82)	310.86 (2.49)	69.73 (1.85)	15.82 (1.23)	55.67 (1.75)
Rice bran	1.5 (0)	70.34 (1.85)	710.03 (2.85)	3447.18 (3.54)	11262.32 (4.05)	888.45 (2.95)	700.76 (2.85)	1033.33 (3.01)
Neem oil cake	–	736.44 (2.87)	467.79 (2.67)	1598.07 (3.20)	522.32 (2.72)	4.85 (0.77)	0.46 (0.16)	0.37 (0.14)
Coirpith	–	7.20 (0.91)	38.65 (1.60)	328.41 (2.51)	636.60 (2.80)	43.02 (1.64)	49.22 (1.70)	29.67 (1.48)
Neem oil cake + cowdung (dried)	576.67 (2)	448.44 (2.65)	4932.3 (3.69)	3680.88 (3.57)	246.37 (2.39)	11.27 (1.09)	68.99 (1.85)	16.00 (1.22)
Coirpith + rice bran	82.67 (1)	4012.29 (3.60)	6931.08 (3.84)	1432.70 (3.17)	1197.35 (3.07)	778.99 (2.89)	243.11 (2.39)	440 (2.64)
Mean	884.98	1360.67	2172.43	1793.23	1777.13	203.51	108.80	157.60

Table 27 Continued

Treatments	Mean cfu/g x 10^5 (WAS)							
	9	10	11	12	13	14	15	16
Coconut oil cake	0.41 (0.15)	0.39 (0.15)	0.30 (0.11)	0.11 (0)	0.56 (0.5)	0.32 (0.12)	0.006 (0.003)	0.004 (0.002)
Gingelly oil cake	0.31 (0.12)	0.46 (0.16)	0.016 (0.007)	0.49 (0)	0.083 (0.17)	0.047 (0.02)	0.001 (0.0004)	0.001 (0.0004)
Groundnut oil cake	–	–	–	–	–	–	–	–
Guinea grass straw powder	63.51 (1.81)	1.13 (0.33)	2.51 (0.55)	7.97 (0)	2.99 (0.60)	0.411 (0.15)	3.88 (0.69)	4.30 (0.72)
Cowdung (dried)	814.35 (2.91)	149.79 (2.18)	50.87 (1.71)	32 (1)	3.49 (0.65)	5.99 (0.84)	1.36 (0.37)	2.26 (0.51)
Rice bran	820.62 (2.91)	76.97 (1.89)	2.77 (0.44)	7 (0)	4.19 (0.72)	0.69 (0.23)	0.28 (0.12)	0.29 (0.11)
Neem oil cake	0.78 (0.25)	0.60 (0.20)	0.045 (0.02)	0.04 (0.0)	0.01 (0.004)	0.07 (0.03)	0.047 (0.02)	0.042 (0.02)
Coirpith	86.60 (1.94)	17.33 (1.26)	14.07 (1.18)	41.33 (1)	62.81 (1.8)	6.2 (0.86)	7.26 (0.92)	3.37 (0.64)
Neem oil cake + cowdung (dried)	45.60 (1.67)	4.10 (0.71)	2.57 (0.55)	0.45 (0)	3.94 (0.69)	1.1 (0.32)	1.96 (0.47)	3.06 (0.61)
Coirpith + rice bran	73.48 (1.87)	70.19 (1.85)	135.99 (2.14)	840 (2)	1497.92 (3.18)	3.3 (0.63)	0.38 (0.14)	0.45 (0.16)
Mean	190.57	32.10	20.97	92.94	157.59	1.81	1.52	1.38

Table 27 Continued

Treatments	Mean cfu/g x 10^5 (WAS)								Mean
	17	18	19	20	21	22	23	24	
Coconut oil cake	0.002 (0.001)	–	0.001 (0.0004)	–	–	–	–	–	781.55
Gingelly oil cake	0.001 (0.004)	0.001 (0.004)	–	–	–	–	–	–	355.11
Groundnut oil cake	–	–	–	–	–	–	–	–	–
Guinea grass straw powder	1.91 (047)	2.9 (0.59)	0.87 (0.27)	0.847 (0.27)	0.0390 (0.02)	0.042 (0.02)	0.036 (0.02)	–	381.37
Cowdung (dried)	1.09 (0.32)	1.53 (0.40)	1.2 (0.34)	0.035 (0.01)	0.001 (0.0004)	–	–	–	68.12
Rice bran	0.22 (0.09)	0.16 (0.06)	0.13 (0.05)	0.017 (0.007)	0.012 (0.005)	0.014 (0.006)	0.0013 (0.001)	–	792.80
Neem oil cake	0.004 (0.002)	0.004 (0.002)	0.0027 (0.001)	0.001 (0.0004)	–	–	–	–	138.83
Coirpith	2.67 (0.56)	4.17 (0.71)	3.33 (0.64)	0.387 (0.14)	0.078 (0.03)	0.039 (0.02)	0.0057 (0.002)	–	57.60
Neem oil cake + cowdung (dried)	1.8 (0.45)	4.57 (0.75)	0.82 (0.26)	0.813 (0.26)	0.046 (0.02)	0.001 (0.0004)	–	–	418.82
Coirpith + rice bran	6.4 (0.87)	3.3 (0.63)	2.2 (0.51)	0.019 (0.008)	0.0027 (0.001)	0.001 (0.0004)	0.001 (0.0004)	–	739.66
Mean	1.41	1.66	0.86	0.212	0.02	0.01	0.004		

Figures in parentheses indicate logarithmic transformation

CD (0.05) for period of storage – 0.002
" for treatments – 0.02
" for interaction – 0.07

cfu : colony forming units, WAS : weeks after storage

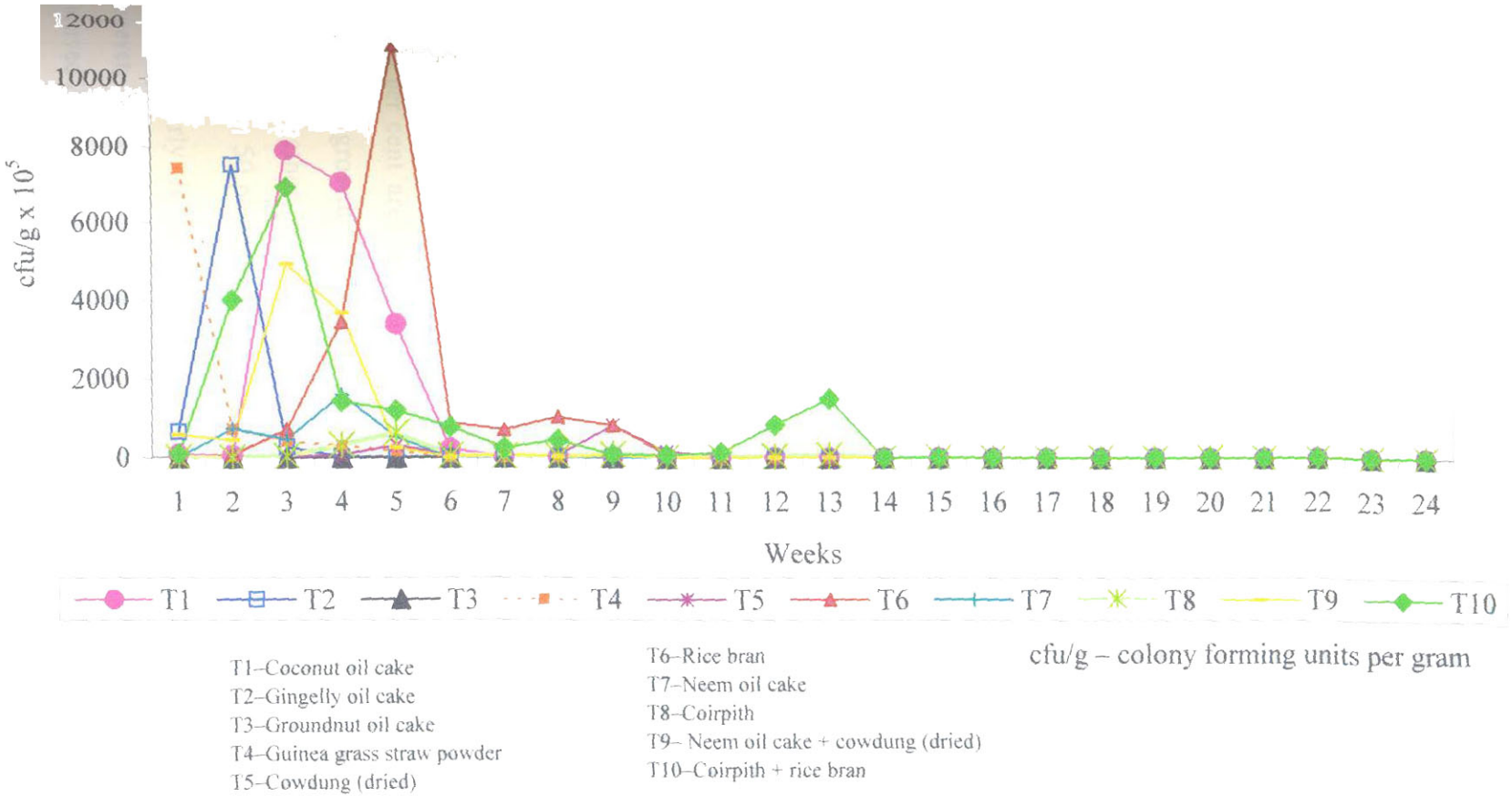


Fig. 12 Effect of solid substrates on the spore viability of *F. equiseti*

In general, spore count of 8.85 x 10^7 cfu / g which was recorded at the end of one week of inoculation increased to 2.17 x 10^8 cfu / g after three weeks and remained without significant change till 5 WAS (Fig. 14). After that a gradual decrease in spore count was recorded and it reached zero by the end of 24 WAS.

4.4.1.3 C. gloeosporioides

4.4.1.3.1 Extent of Growth

There was variation in the extent of growth of fungi tested, between substrates (Table 28).

Visible mycelial growth was observed in coconut oil cake, gingelly oil cake, guinea grass powder and rice bran 3 WAS. In guinea grass straw powder and rice bran, velvetty mycelial growth fully covered more than 50 per cent area of the substrates by 2 WAS.

White puffy mycelial growth covered more than 50 per cent area of coconut oil cake and gingelly oil cake by 3 WAS. In coconut oil cake, gingelly oil cake, guinea grass straw powder, rice bran, coirpith + rice bran, visible mycelial growth fully covered the substrates by 3 WAS and remained as such through out the period of observation of 12 weeks.

In cowdung, neem oil cake and coirpith upto 2 WAS no visible mycelial growth and there after scanty growth was observed covering less than 25 per cent area upto 12 WAS. Eventhough, in neem oil cake and cowdung, no growth was observed third day after inoculation, when they were used together, visible growth was noted 1 WAS and which covered and more than 50 per cent area of the substrates by 4WAS.

Similarly in coirpith, the growth was scanty and thread like, however, when mixed with bran profuse white velvetty growth was observed 2 WAS and continued as such through out the observation period of 12 weeks.

Table 28 *Effect of solid substrates on the growth of C. gloeosporioides*

Treatments		3rd day	Extent of growth (WAS)												
			1	2	3	4	5	6	7	8	9	10	11	12	Nature of growth
T_1	Coconut oil cake	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	White puffy
T_2	Gingelly oil cake	+	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	White puffy
T_3	Groundnut oil cake	–	–	+	+	+	++	++	++	++	++	++	++	++	Cream puffy
T_4	Guinea grass straw powder	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	White velvetty
T_5	Cowdung (dried)	–	–	-	+	+	+	+	+	++	++	++	++	++	Scanty
T_6	Rice bran	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	White velvetty
T_7	Neem oil cake	–	–	–	+	+	+++	+++	+++	+++	+++	+++	+++	+++	Scanty
T_8	Coirpith	–	–	–	+	+	+	+	+	+	+	+	+	+	Thread like
T_9	Neem oil cake + cowdung (dried)	–	+	++	++	+++	+++	+++	+++	+++	++	++	++	++	White thick threads
T_{10}	Coirpith + Rice bran	–	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	White velvetty

– No visible growth, + 25 % area covered, ++ 50 % area covered, +++ >50 % area covered

WAS : Weeks after storage

4.4.1.3.2 Spore Viability

Statistical analysis of viable spore count of *C. gloeosporioides* revealed that there was significant difference between the effects of solid substrates tested on sporulation and time of storage (Table 29 and Fig. 13).

The mean spore count was maximum in coconut oil cake (1.07×10^8 cfu / g) followed by gingelly oil cake (6.68×10^7 cfu / g). The average spore count in rice bran and coirpith + rice bran was 2.93×10^7 cfu / g and 2.92×10^7 cfu / g respectively which were statistically on par. The least was observed in neem oil cake (1.37×10^2 cfu/g).

In the case of gingelly oil cake which recorded maximum mean spore count had a count of 5.17×10^6 cfu/g, by 1 WAS, which increased to a maximum count of 8.26×10^8 cfu/g on 4 WAS and then declined to zero by 19WAS.

Coconut oil cake recorded a spore count of 7.4×10^6 cfu/g 1 WAS and increased to 8.13×10^8 cfu/g at the end of 4 WAS. After that spore count declined to 100 cfu/g 20 WAS and thereafter no count was recorded.

Rice bran which recorded the highest initial count of 1.7×10^8 cfu/g at the end of one week, declined to 7.21×10^7 cfu/g by 3 WAS and it gradually reduced to 100 by 22 WAS thereafter zero count was recorded.

In coirpith + rice bran, the initial count was 7.83×10^7 cfu/g and reached a maximum count of 1.7×10^8 cfu/g by 2 WAS. Then the spore count declined gradually and reached 3.46×10^6 cfu/g by 7 WAS.

In general, spore count of 3.79×10^7 cfu/g noticed by 1 WAS, increased and reached a maximum of 2.16×10^8 cfu/g at the end of four weeks (Fig. 14). A decreasing trend continued from then onwards and at the end of seven weeks, the colony count reached $9.b \times 10^6$ cfu/g. A gradual decrease in the spore count was noted thereafter and the population count after 11 and 24 weeks were 3.75×10^5 and 20 cfu/g respectively.

Table 29 Effect of solid substrates on the spore viability of C. gloeosporioides

Treatments	Mean cfu/g x 10^5 (WAS)								
	1	2	3	4	5	6	7	8	9
Coconut oil cake	74 (1.88)	862.42 (2.94)	793.30 (2.90)	8132.47 (3.91)	5129.80 (3.71)	436.21 (2.64)	146.90 (2.17)	283.54 (2.45)	167.57 (2.22)
Gingelly oil cake	51.67 (1.72)	601.83 (2.79)	4829.57 (3.68)	8261.08 (3.92)	6898.84 (3.84)	3764.98 (3.58)	696.52 (2.84)	489.17 (2.69)	6.89 (0.89)
Groundnut oil cake	3.1 (0.61)	0.19 (0.08)	0.003 (0.001)	–	–	–	–	–	–
Guinea grass straw powder	476.67 (2.68)	46.49 (1.68)	32.46 (1.52)	31.33 (1.51)	26.64 (1.44)	20.35 (1.33)	28.86 (1.48)	27.3 (1.45)	58.32 (1.77)
Cowdung (dried)	–	–	2.79 (0.58)	5.53 (0.82)	4.53 (0.74)	3.10 (0.61)	0.25 (0.09)	0.19 (0.08)	0.074 (0.03)
Rice bran	1700 (3.23)	3729.08 (3.57)	720.84 (2.86)	685.51 (2.84)	27.96 (1.46)	70.68 (1.86)	19.63 (1.31)	21.99 (1.36)	35.65 (1.56)
Neem oil cake	4.6 (0.75)	50.83 (1.71)	472.2 (2.68)	244.99 (2.39)	30.16 (1.49)	23.91 (1.39)	12.91 (1.14)	2.7 (0.57)	0.48 (0.17)
Coirpith	–	8.66 (0.98)	85.59 (1.94)	84.65 (1.93)	49.29 (1.70)	51.59 (1.72)	6.62 (0.88)	5.07 (0.78)	1.03 (0.31)
Neem oil cake + cowdung (dried)	703.33 (2.85)	865.96 (2.94)	252.02 (2.40)	2534.46 (3.40)	57.19 (1.76)	50.98 (1.72)	36.56 (1.57)	1.53 (0.40)	29.60 (1.49)
Coirpith + Rice bran	783.33 (2.89)	1742.82 (3.24)	1327.74 (3.12)	1655.54 (3.22)	813.14 (2.91)	239.01 (2.38)	34.57 (1.55)	140.34 (2.15)	152.08 (2.18)
Mean	379.67	790.83	851.65	2163.56	1303.76	466.08	98.28	97.19	44.97

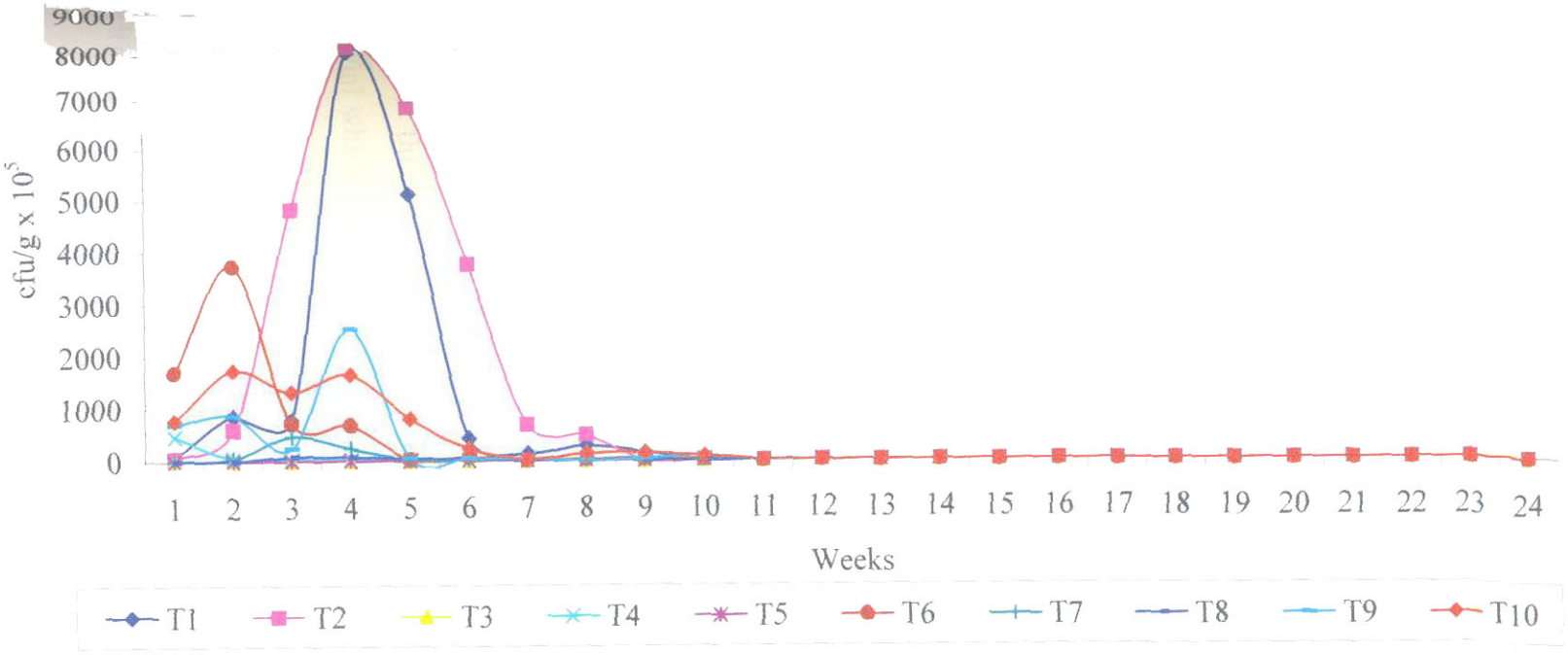
Table 29 Continued

Treatments	Mean cfu/g x 10^5 (WAS)					
	10	11	12	13	14	15
Coconut oil cake	8.26 (0.96)	6.06 (0.85)	0.15 (0.06)	0.04 (0.02)	0.017 (0.007)	0.007 (0.003)
Gingelly oil cake	3.06 (0.61)	0.25 (0.096)	0.11 (0.05)	0.067 (0.03)	0.038 (0.02)	0.006 (0.003)
Groundnut oil cake	–	–	–	–	–	–
Guinea grass straw powder	28.31 (1.47)	15.44 (1.22)	20 (1.32)	3.55 (0.66)	2.53 (0.55)	1.43 (0.39)
Cowdung (dried)	0.04 (0.02)	0.02 (0.009)	0.007 (0.003)	0.003 (0.001)	0.003 (0.001)	0.002 (0.001)
Rice bran	18.98 (1.3)	0.19 (0.08)	0.49 (0.17)	0.16 (0.06)	0.27 (0.1)	0.075 (0.03)
Neem oil cake	0.039 (0.02)	0.016 (0.007)	0.013 (0.006)	0.026 (0.01)	0.001 (0.0004)	0.001 (0.0004)
Coirpith	1.09 (0.32)	4.5 (0.74)	2.98 (0.59)	0.68 (0.03)	0.55 (0.19)	0.37 (0.17)
Neem oil cake + cowdung (dried)	103.24 (2.02)	0.67 (0.22)	0.33 (0.12)	0.20 (0.08)	0.012 (0.005)	0.002 (0.001)
Coirpith + Rice bran	87.66 (1.95)	10.33 (1.05)	8.29 (0.97)	1.42 (0.38)	1.06 (0.31)	0.22 (0.09)
Mean	25.07	3.75	3.24	6.15	0.45	0.21

Table 29 Continued

Treatments	Mean cfu/g x 10^5 (WAS)									Mean
	16	17	18	19	20	21	22	23	24	
Coconut oil cake	0.004 (0.0002)	0.001 (0.0004)	0.001 (0.0004)	0.001 (0.0004)	0.001 (0.0004)	–	–	–	–	668.28
Gingelly oil cake	0.003 (0.001)	0.002 (0.001)	0.001 (0.0004)	–	–	–	–	–	–	1066.83
Groundnut oil cake	–	–	–	–	–	–	–	–	–	0.137
Guinea grass straw powder	0.51 (0.18)	0.32 (0.12)	0.27 (0.10)	0.24 (0.09)	0.029 (0.01)	0.027 (0.01)	0.003 (0.001)	0.002 (0.001)	–	34.22
Cowdung (dried)	0.001 (0.0004)	–	–	–	–	–	–	–	–	7.08
Rice bran	0.08 (0.03)	0.045 (0.02)	0.038 (0.02)	0.004 (0.002)	0.002 (0.001)	0.002 (0.001)	0.001 (0.0004)	–	–	292.99
Neem oil cake	–	–	–	–	–	–	–	–	–	35.12
Coirpith	0.049 (0.02)	0.042 (0.02)	0.037 (0.02)	0.002 (0.0001)	0.001 (0.0004)	0.001 (0.0004)	0.001 (0.0004)	–	–	12.62
Neem oil cake + cowdung (dried)	0.001 (0.0004)	–	–	–	–	–	–	–	–	193.17
Coirpith + Rice bran	0.47 (0.17)	0.36 (0.13)	0.037 (0.02)	0.034 (0.001)	0.002 (0.001)	0.002 (0.001)	0.001 (0.0004)	0.001 (0.0004)	–	291.60
Mean	0.11	0.08	0.04	0.03	0.00	0.003	0.001	0.0003		

Figures in parentheses indicate logarithmic transformation
CD (0.05) for period storage – 0.02
" treatments – 0.02
" interaction – 0.06
cfu : colony forming units, WAS : weeks after storage



T1–Coconut oil cake
T2–Gingelly oil cake
T3–Groundnut oil cake
T4–Guinea grass straw powder
T5–Cowdung (dried)
T6–Rice bran
T7–Neem oil cake
T8–Coirpith
T9– Neem oil cake + cowdung (dried)
T10–Coirpith + rice bran

cfu/g – colony forming units per gram

Fig. 13 Effect of solid substrates on the spore viability of *C. gloeosporioides*

4.4.2 Liquid Substrates

4.4.2.1 F. pallidoroseum

4.4.2.1.1 Mycelial growth

Statistical analysis of mycelial dry weight of *F. pallidoroseum* in different liquid substrates revealed that there was significant difference between the treatments tested (Table 30). Among the 14 substrates tried, extracts of groundnut oil cake yielded maximum mycelial dry weight of 1.86 g followed by coconut water (0.86 g). Extracts of guinea grass straw yielded the least mycelial weight of 0.04 g. No growth was observed in the extracts of coirpith (Plate 13).

4.4.2.1.2 Spore Viability

Statistical analysis of colony forming units of *F. pallidoroseum* showed that there was significant difference between the effects of liquid substrates tested and time of storage (Table 31 and Fig. 15)

The viable spore count was maximum in jaggery water (5.57×10^7 cfu/ml) followed by boiled rice water which had 3.61×10^7 cfu/ml. The least count was observed in coirpith extract, being 2.1×10^4 cfu/ml.

In jaggery water, viable count of 1.6×10^4 cfu/ml was recorded on 3 DAI and increased gradually to a maximum of 6.33×10^8 cfu/ml after 13 days. Thereafter the viable count decreased and reached zero on 27 DAI.

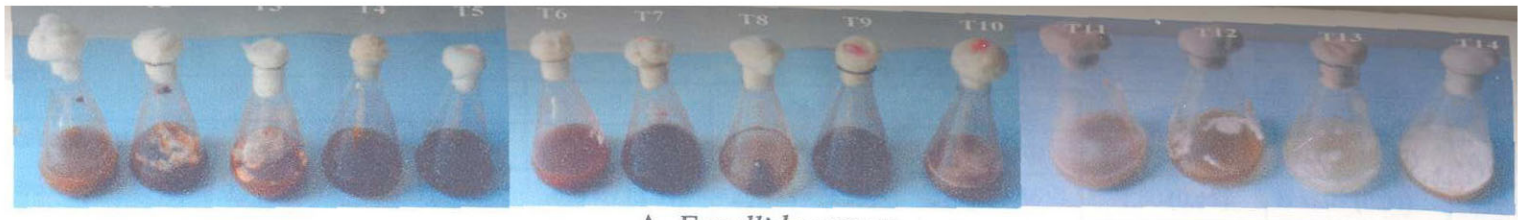
On the third day of observation, rice gruel recorded a count of 2.3×10^4 cfu/ml which increased to a maximum of 3.79×10^8 cfu/ml by 13 DAI and then declined and reached to 100 cfu/ml by 25 DAI.

In general, the initial mean spore count of 5.3×10^4 cfu/ml gradually increased and reached maximum of 7.81×10^7 cfu/ml on the 13^{th} day and then it gradually declined and reached 4×10^2 cfu/ml after 27 days (Fig. 18).

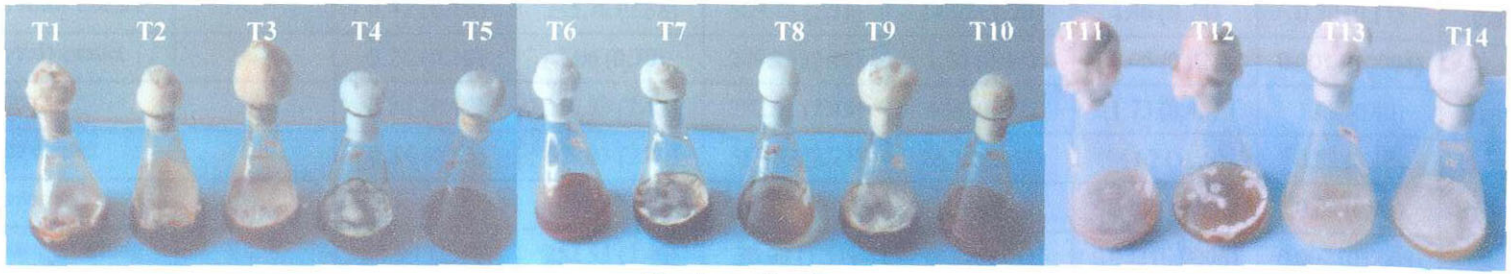
Table 30 Effect of liquid substrates on the growth of fungi

Treatments		Mean mycelial dry weight (g)		
		F. pallidoroseum	*F. equiseti*	*C. gloeosporioides*
T_1	Coconut oil cake extract	0.39 (1.18)	1.11 (1.45)	0.98 (1.41)
T_2	Gingelly oil cake extract	0.54 (1.24)	0.76 (1.33)	0.61 (1.27)
T_3	Groundnut oil cake extract	1.86 (1.69)	1.31 (1.52)	1.48 (1.57)
T_4	Guinea grass straw extract	0.04 (1.01)	0.18 (1.09)	0.13 (1.06)
T_5	Cowdung (dried) extract	0.16 (1.06)	0.09 (1.05)	0.12 (1.06)
T_6	Rice bran extract	0.29 (1.14)	0.40 (1.18)	0.35 (1.16)
T_7	Neem oil cake extract	0.20 (1.10)	0.56 (1.25)	0.47 (1.21)
T_8	Coir pith extract	– (1.00)	0.13 (1.06)	0.09 (1.04)
T_9	Neem oil cake + cowdung (dried) extract	0.30 (1.14)	0.42 (1.19)	0.40 (1.18)
T_{10}	Coir pith + rice bran extract	0.37 (1.17)	0.23 (1.11)	0.30 (1.14)
T_{11}	Raw rice washings (Kadivellam)	0.11 (1.05)	0.12 (1.06)	0.14 (1.07)
T_{12}	Jaggery water	0.54 (1.24)	0.41 (1.19)	1.23 (1.49)
T_{13}	Rice gruel (Kanchivellam)	0.17 (1.08)	0.03 (1.02)	0.95 (1.40)
T_{14}	Coconut water	0.86 (1.37)	0.64 (1.28)	1.18 (1.48)
	CD (0.05)	0.03	0.06	0.05

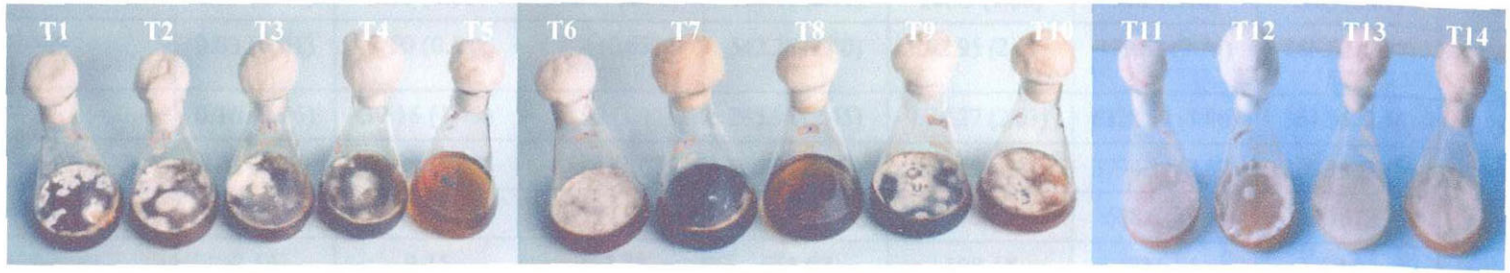
Figures in parentheses indicate square root transformation



A. *F. pallidoroseum*



B. *F. equiseti*



C. *C. gloeosporioides*

Plate 13. Growth of pathogenic fungi on liquid substrates

Table 31 Effect of liquid substrates on the spore viability of F. pallidoroseum

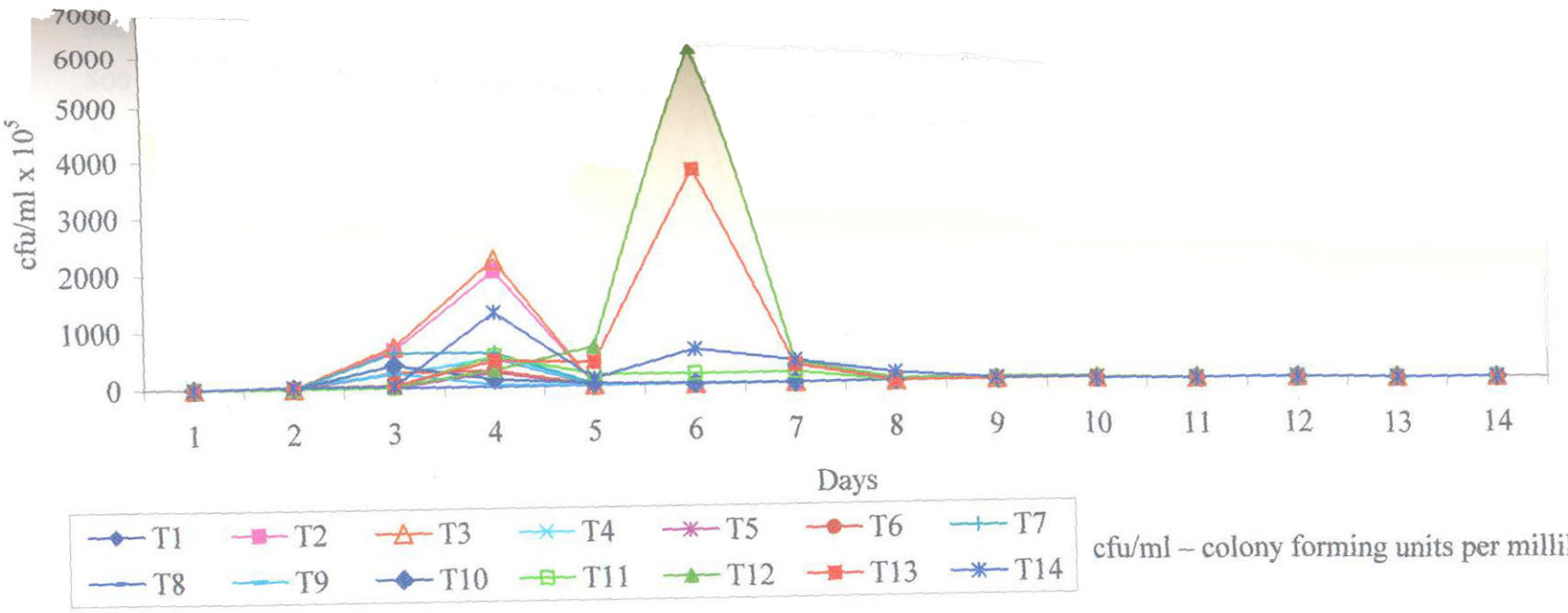
Treatments	Mean cfu/ml x 10^5 (DAI)							
	3	5	7	9	11	13	15	17
Coconut oil cake extract	–	0.30 (0.12)	239.88 (2.38)	498.33 (2.70)	6.93 (0.70)	4.00 (0.66)	0.057 (0.02)	0.001 (0.0004)
Gingelly oil cake extract	2.23 (0.51)	1.56 (0.40)	653.07 (2.81)	2026.19 (3.31)	21.99 (1.36)	1.80 (0.45)	0.09 (0.04)	0.001 (0.0004)
Groundnut oil cake extract	2.50 (0.54)	2.52 (0.55)	716.30 (2.86)	2224.20 (3.35)	5.06 (0.79)	–	–	–
Guinea grass straw extract	–	0.47 (0.16)	235.78 (2.38)	509.18 (2.71)	14.92 (1.20)	4.46 (0.74)	0.30 (0.11)	0.054 (0.02)
Cowdung (dried) extract	–	0.26 (0.10)	4.06 (0.70)	249.12 (2.40)	1.76 (0.44)	0.05 (0.02)	0.001 (0.0004)	–
Rice bran extract	–	0.22 (0.10)	266.39 (2.43)	282.62 (2.45)	4.90 (0.77)	1.73 (0.45)	1.49 (0.40)	0.055 (0.02)
Neem oil cake extract	–	0.34 (0.13)	609.83 (2.79)	589.82 (2.77)	26.30 (1.44)	14.62 (1.19)	0.53 (0.18)	0.003 (0.001)
Coir pith extract	–	–	–	–	–	2.90 (0.59)	0.04 (0.06)	0.002 (0.001)
Neem oil cake + cowdung (dried) extract	–	28.99 (1.48)	252.53 (2.40)	30.99 (1.51)	0.03 (0.01)	0.002 (0.0008)	0.002 (0.0008)	–
Coir pith + rice bran extract	1.93 (0.47)	6.03 (0.85)	393.17 (2.60)	122.11 (2.71)	38.99 (1.60)	0.52 (0.18)	0.03 (0.01)	0.002 (0.001)
Raw rice washings (Kadivellam)	0.03 (0.01)	2.50 (0.54)	3.4 (0.65)	542.25 (2.40)	192.95 (2.29)	179.83 (2.26)	186.62 (2.27)	1.50 (0.40)
Jaggery water	0.16 (0.06)	30.16 (1.49)	23.99 (1.40)	285.94 (2.45)	674.37 (2.83)	6328.67 (3.80)	361.56 (2.56)	39.00 (1.59)
Rice gruel (Kanchivellam)	0.23 (0.09)	28.99 (1.48)	45.64 (1.67)	455.97 (2.77)	406.59 (2.61)	3794.39 (3.57)	308.19 (2.49)	2.40 (0.53)
Coconut water	0.29 (0.11)	31.31 (1.51)	24.89 (1.41)	1297.55 (3.11)	119.73 (2.08)	608.6 (2.79)	383.16 (2.58)	143.33 (2.12)
Mean	0.53	9.35	247.78	651.02	108.18	781.55	88.72	13.31

Table 31 Continued

Treatments	Mean cfu/ml x 10^5 (DAI)						Mean
	19	21	23	25	27	29	
Coconut oil cake extract	–	–	–	–	–	–	53.54
Gingelly oil cake extract	–	–	–	–	–	–	193.35
Groundnut oil cake extract	–	–	–	–	–	–	210.76
Guinea grass straw extract	0.054 (0.02)	0.002 (0.001)	0.001 (0.0004)	–	–	–	54.66
Cowdung (dried) extract	–	–	–	–	–	–	18.23
Rice bran extract	0.032 (0.01)	0.002 (0.001)	0.001 (0.0004)	–	–	–	39.82
Neem oil cake extract	–	–	–	–	–	–	88.67
Coir pith extract	–	–	–	–	–	–	0.21
Neem oil cake + cowdung (dried) extract	–	–	–	–	–	–	22.32
Coir pith + rice bran extract	0.001 (0.0004)	–	–	–	–	–	40.20
Raw rice washings (Kadivellam)	1.97 (0.47)	3.77 (0.68)	0.032 (0.01)	0.003 (0.001)	–	–	79.61
Jaggery water	38.67 (1.60)	17.33 (1.26)	0.410 (0.15)	0.001 (0.0004)	–	–	557.16
Rice gruel (Kanchivellam)	4.07 (0.71)	1.77 (0.44)	0.050 (0.02)	0.001 (0.0004)	–	–	360.59
Coconut water	15.33 (1.21)	4.43 (0.73)	0.570 (0.20)	0.350 (0.13)	0.05 (0.02)	–	187.83
Mean	4.29	1.95	0.076	0.025	0.004		

Figures in parentheses indicate logarithmic transformation
CD (0.05) for period storage – 0.014, treatments – 0.022, interaction – 0.054

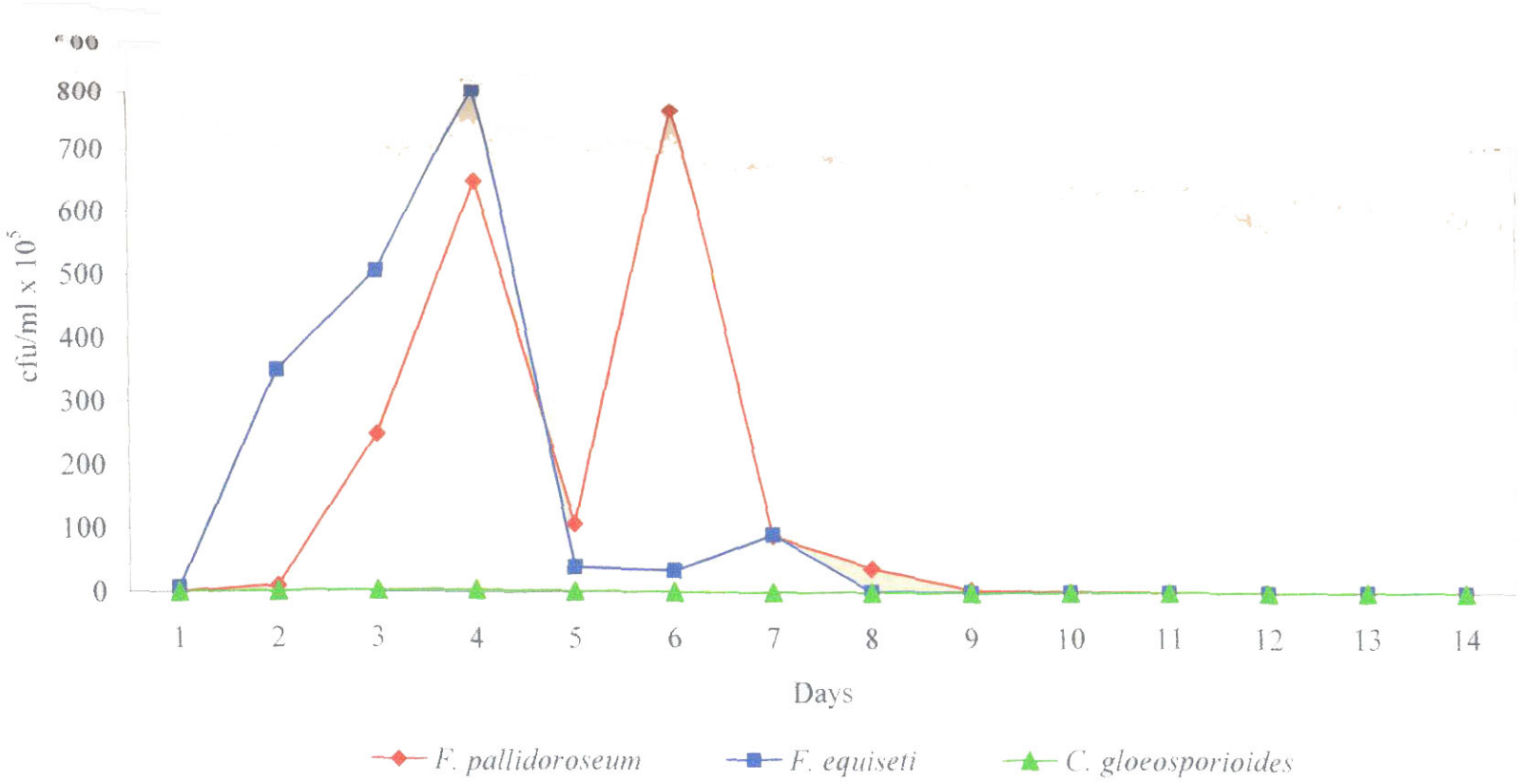
cfu : colony forming units, DAI : days after inoculation



cfu/ml – colony forming units per millilitre

T1–Coconut oil cake extract
T2–Gingelly oil cake extract
T3–Groundnut oil cake extract
T4–Guinea grass straw extract
T5–Cowdung (dried) extract
T6–Rice bran extract
T7–Neem oil cake extract
T8–Coir pith extract
T9–Neem oil cake + cowdung (dried) extract
T10–Coir pith + rice bran extract
T11–Raw rice washings
T12–Jaggery water
T13–Rice gruel
T14–Coconut water

Fig. 15 Effect of liquid substrates on the spore viability of *F. pallidoroseum*



cfu/ml – colony forming units per millilitre

Fig. 18 Effect of storage period of spore viability of fungi in liquid substrates

4.4.2.2 F. equiseti

4.4.2.2.1 Mycelial Growth

Statistical analysis of the dry weight of mycelium *F. equiseti* grown in different liquid substrates revealed that there was significant difference between the substrates tested (Table 30).

Groundnut oil cake extract recorded maximum mycelial growth of 1.31 g followed by coconut oil cake extract (1.11 g). This was followed by gingelly oil cake extract and coconut water with 0.76 and 0.64 g mycelial dry weight respectively and were found to be statistically on par. Lowest mycelial dry weight was noticed in rice gruel (0.03 g) (Plate 13).

4.4.2.2.2 Spore Viability

Statistical analysis of the viable spore count of *F. equiseti* in different liquid substrates revealed that there was significant difference between the liquid substrates tested and periods of storage on sporulation (Table 32 and Fig. 16).

Maximum mean viable spore count was observed in coconut water (4.18×10^7 cfu/ml) followed by extracts of gingelly oil cake extract with a spore count of 2.69×10^7 cfu/ml. Least spore count was observed in jaggery water (4.28×10^6 cfu/ml).

In coconut water a spore count of 3.2×10^3 cfu/ml was observed at the end 3 DAI. Then the spore count increased to 5.46×10^8 cfu/ml on 9 DAI and even at the end of 15 days, spore count of 2.86×10^7 cfu/ml was observed and subsequently it reduced to 300 cfu/ml by 29 DAI.

Eventhough in gingelly oil cake extract the no colonies were observed on 3 DAI, subsequently viable colonies of 1.59×10^8 cfu/ml was recorded at the end of 5 DAI. The count was more than 10^7 even at 9DAI and the count of viable fungal colonies reached zero at the end of 17 DAI.

Table 32 Effect of liquid substrates on the spore viability of F. equiseti

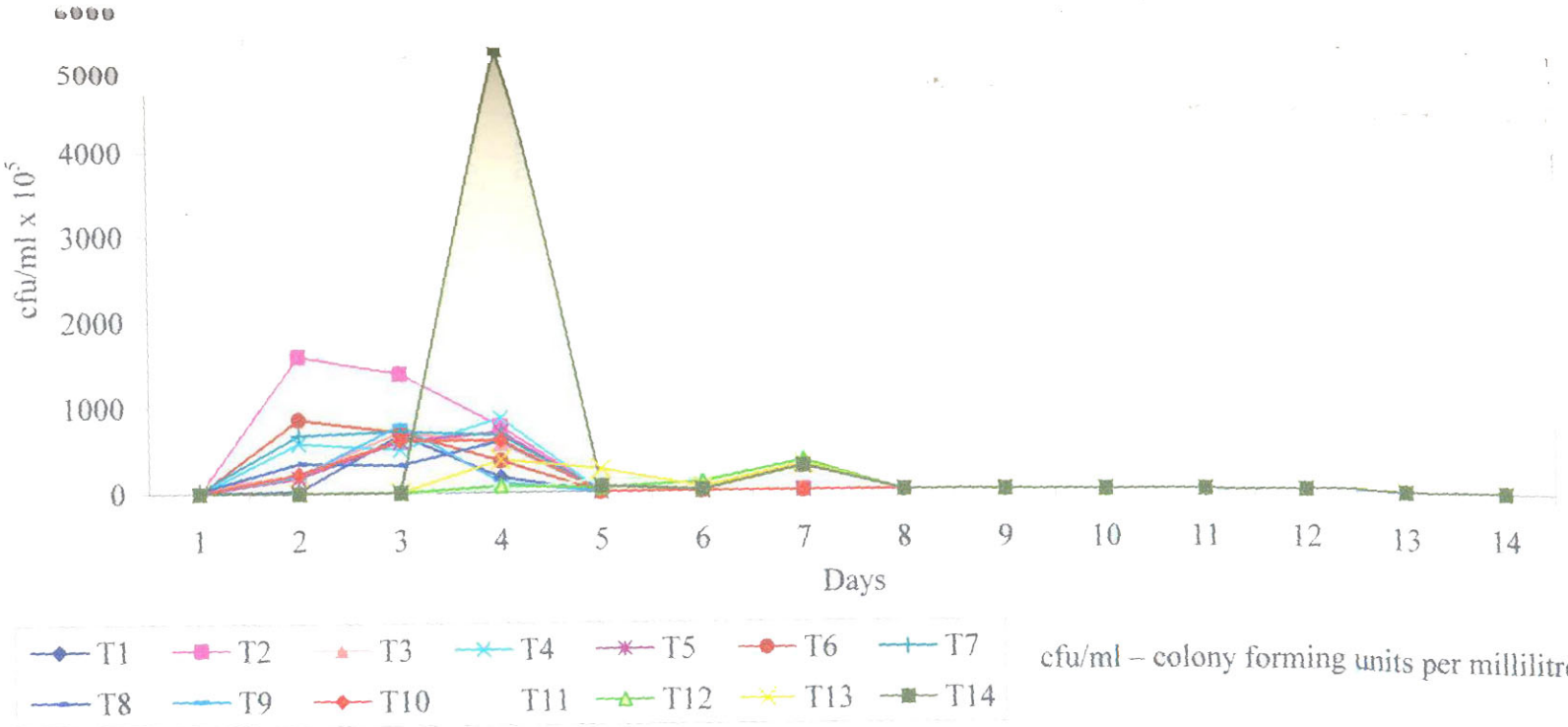
Treatments	Mean cfu/ml x 10^5 (DAI)							
	3	5	7	9	11	13	15	17
Coconut oil cake extract	0	20.98 (1.34)	680.01 (2.8)	179.83 (2.26)	0.18 (0.07)	0.17 (0.06)	0.14 (0.06)	0.032 (0.014)
Gingelly oil cake extract	0	1598.07 (3.20)	1393.75 (3.15)	773.20 (2.89)	2.03 (0.48)	0.036 (0.02)	0.001 (0.0004)	–
Groundnut oil cake extract	50.33 (1.74)	219.86 (2.34)	710.01 (2.85)	573.24 (2.76)	0.43 (0.18)	0.002 (0.001)	0.001 (0.0004)	–
Guinea grass straw extract	–	579.89 (2.76)	509.96 (2.71)	870.04 (2.94)	2.26 (0.51)	0.050 (0.021)	0.04 (0.02)	0.003 (0.001)
Cowdung (dried) extract	–	179.82 (2.26)	603.36 (2.78)	710.01 (2.85)	0.29 (0.11)	0.002 (0.001)	0.002 (0.001)	–
Rice bran extract	–	860.03 (2.94)	706.61 (2.85)	369.94 (2.57)	0.51 (0.18)	0.62 (0.21)	0.31 (0.12)	0.049 (0.02)
Neem oil cake extract	25.33 (1.42)	673.27 (2.83)	720.01 (2.86)	670.01 (2.83)	2.86 (0.59)	0.89 (0.28)	0.071 (0.03)	0.002 (0.01)
Coir pith extract	23.33 (1.39)	349.65 (2.54)	326.75 (2.51)	603.36 (2.78)	5.23 (0.79)	0.29 (0.11)	0.001 (0.004)	–
Neem oil cake + cowdung (dried) extract	–	199.85 (2.30)	790.03 (2.89)	115.99 (2.07)	2.23 (0.51)	0.32 (0.12)	0.001 (0.004)	–
Coir pith + rice bran extract	–	219.86 (2.34)	613.26 (2.79)	609.99 (2.79)	1.23 (0.35)	0.32 (0.12)	0.052 (0.02)	0.047 (0.02)
Raw rice washings (Kadivellam)	0.002 (0.001)	0.17 (0.068)	2.73 (0.57)	28.99 (1.48)	145.73 (2.17)	153.33 (2.19)	326.26 (2.51)	1.90 (0.46)
Jaggery water	–	0.12 (0.049)	3.83 (0.68)	79.00 (1.90)	50.27 (0.71)	110.00 (2.04)	352.77 (2.55)	1.20 (0.36)
Rice gruel (Kanchivellam)	0.001 (0.0004)	0.34 (0.13)	7.99 (0.95)	383.16 (2.59)	269.90 (2.43)	52.00 (1.72)	309.90 (2.49)	4.01 (0.70)
Coconut water	0.032 (0.014)	0.43 (0.15)	7.46 (0.93)	5463.37 (3.74)	67.97 (1.84)	16.00 (1.23)	286.41 (2.46)	4.80 (0.76)
Mean	7.07	350.17	505.39	816.44	39.37	23.86	91.14	0.87

Table 32 Continued

Treatments	Mean cfu/ml x 10^5 (DAI)						Mean
	19	21	23	25	27	29	
Coconut oil cake extract	0.001 (0.0004)	–	–	–	–	–	62.95
Gingelly oil cake extract	–	–	–	–	–	–	269.08
Groundnut oil cake extract	–	–	–	–	–	–	110.99
Guinea grass straw extract	0.002 (0.001)	0.001 (0.0004)	0.001 (0.0004)	–	–	–	140.16
Cowdung (dried) extract	–	–	–	–	–	–	106.68
Rice bran extract	0.002 (0.001)	0.002 (0.001)	0.001 (0.0004)	–	–	–	138.43
Neem oil cake extract	–	–	–	–	–	–	149.46
Coir pith extract	–	–	–	–	–	–	93.45
Neem oil cake + cowdung (dried) extract	–	–	–	–	–	–	79.17
Coir pith + rice bran extract	0.002 (0.00)	0.002 (0.001)	–	–	–	–	103.20
Raw rice washings (Kadivellam)	1.67 (0.43)	1.70 (0.43)	0.27 (0.10)	0.005 (0.002)	–	–	47.34
Jaggery water	1.30 (0.36)	1.27 (0.36)	0.017 (0.007)	0.003 (0.001)	0.002 (0.001)	–	42.85
Rice gruel (Kanchivellam)	4.0 (0.70)	3.80 (0.68)	0.03 (0.013)	0.005 (0.002)	0.003 (0.001)	0.001 (0.0004)	73.94
Coconut water	4.1 (0.71)	3.20 (0.62)	0.29 (0.11)	0.007 (0.003)	0.004 (0.002)	0.003 (0.001)	418.15
Mean	0.79	0.71	0.044	0.0014	0.0006	0.0003	

Figures in parentheses indicate logarithmic transformation
CD (0.05) for period storage – 0.08, treatments – 0.01, interaction – 0.03

cfu : colony forming units, DAI : Days after inoculation



T1–Coconut oil cake extract
T2–Gingelly oil cake extract
T3–Groundnut oil cake extract
T4–Guinea grass straw extract
T5–Cowdung (dried) extract
T6–Rice bran extract
T7–Neem oil cake extract
T8–Coir pith extract
T9–Neem oil cake + cowdung (dried) extract
T10–Coir pith + rice bran extract
T11–Raw rice washings
T12–Jaggery water
T13–Rice gruel
T14–Coconut water

Fig. 16 Effect of liquid substrates on the spore viability of *F. equiseti*

In general, population of *F. equiseti* 3 DAI was 7.07 x 10^5 cfu/ml, gradually increased to a maximum of 8.16 x 10^7 cfu/ml after nine days and it declined to 30 by 29th day (Fig. 18).

4.4.2.3 C. gloeosporioides

4.4.2.3.1 Mycelial Growth

Statistical analysis of mycelial dry weight of the fungi grown in different liquid substrates revealed that there was significant difference between the substrates (Table 30).

Extracts of groundnut oil cake gave maximum mean mycelial weight of 1.48 g followed by jaggery water (1.23 g) which was on par with coconut water (1.18 g) (Plate 13).

4.4.2.3.2 Spore Viability

Statistical analysis of the viable spore count of *C. gloeosporioides* recorded a significant difference between the substrates and periods of storage (Table 33 and Fig. 17).

Maximum average spore count was observed in coconut oil cake extract (5.08 x 10^5 cfu/ml) followed by guinea grass straw extract and jaggery water with a count of 6.45 x 10^4 cfu/ml and 6.30 x 10^4 cfu/ml respectively and were statistically on par. Least mean count was recorded in the extracts of coirpith (2.3 x 10^3 cfu/ml).

Viable colonies of *F. equiseti* were not observed at the end of 3 DAI when the fungus was grown in coconut oil cake extract. But count of 2 x 10^4 cfu/ml was recorded by 5 DAI which reached a maximum count of 5.2 x 10^6 cfu/ml at 9 DAI. No detectable colonies were observed at the end of 21 days.

Jaggery water yielded a count of 1 x 10^2 cfu/ml at 3 DAI and it increased to a maximum spore count of 3.45 x 10^5 cfu/ml by 7 DAI and then reduced to zero by 27 DAI.

Table 33 Effect of liquid substrates on the spore viability of *C. gloeosporioides*

Treatments	Mean cfu/ml x 10^5 (DAI)						
	3	5	7	9	11	13	15
Coconut oil cake extract	–	0.20 (0.08)	0.37 (0.14)	52 (1.72)	18 (1.28)	0.38 (0.14)	0.16 (0.06)
Gingelly oil cake extract	0.41 (0.15)	0.57 (0.20)	5.47 (0.81)	0.032 (0.01)	0.029 (0.01)	0.01 (0.0004)	0.001 (0.0004)
Groundnut oil cake extract	0.32 (0.12)	7.3 (0.92)	0.54 (0.19)	0.042 (0.02)	0.038 (0.02)	0.001 (0.003)	0.005 (0.002)
Guinea grass straw extract	–	0.39 (0.14)	2.7 (0.57)	4.8 (0.76)	0.70 (0.23)	0.35 (0.13)	0.049 (0.02)
Cowdung (dried) extract	–	–	3 (0.60)	0.29 (0.11)	0.056 (0.02)	0.035 (0.01)	0.001 (0.0004)
Rice bran extract	–	0.26 (0.1)	5.2 (0.79)	0.48 (0.17)	0.43 (0.16)	0.039 (0.02)	0.005 (0.002)
Neem oil cake extract	–	0.04 (0.02)	0.32 (0.12)	0.26 (0.10)	0.033 (0.01)	0.002 (0.001)	0.001 (0.0004)
Coir pith extract	0.21 (0.08)	0.054 (0.02)	0.04 (0.02)	0.003 (0.001)	0.002 (0.001)	0.002 (0.001)	0.002 (0.001)
Neem oil cake + cowdung (dried) extract	–	0.22 (0.09)	0.081 (0.03)	0.058 (0.02)	0.003 (0.001)	0.001 (0.0004)	– –
Coir pith + rice bran extract	–	0.06 (0.02)	0.32 (0.12)	0.039 (0.02)	0.037 (0.02)	0.007 (0.003)	0.005 (0.002)
Raw rice washings (Kadivellam)	0.003 (0.001)	0.71 (0.23)	3.45 (0.65)	0.89 (0.28)	0.5 (0.18)	0.011 (0.005)	0.35 (0.13)
Jaggery water	0.001 (0.0004)	0.41 (0.15)	3.1 (0.61)	1.73 (0.45)	1.7 (0.43)	0.38 (0.14)	0.094 (0.04)
Rice gruel (Kanchivellam)	0.022 (0.009)	0.50 (0.18)	0.19 (0.07)	0.057 (0.02)	0.018 (0.008)	0.068 (0.03)	0.008 (0.003)
Coconut water	0.012 (0.005)	0.26 (0.10)	1.33 (0.35)	0.69 (0.23)	0.15 (0.06)	0.043 (0.02)	0.027 (0.02)
Mean	0.069	0.784	1.865	4.384	1.55	0.095	0.051

Table 33 Continued

Treatments	Mean cfu/ml x 10^5 (DAI)						Mean
	17	19	21	23	27	29	
Coconut oil cake extract	0.035 (0.001)	0.001 (0.0004)	0 (–)	–	–	–	5.079
Gingelly oil cake extract	–	–	0 (–)	–	–	–	0.465
Groundnut oil cake extract	0.002 (0.001)	–	0 (–)	–	–	–	0.59
Guinea grass straw extract	0.039 (0.002)	0.002 (0.001)	0.001 (0.0004)	–	–	–	0.645
Cowdung (dried) extract	–	–	–	–	–	–	0.24
Rice bran extract	0.004 (0.002)	0.002 (0.001)	0.002 (0.001)	–	–	–	0.459
Neem oil cake extract	–	–	–	–	–	–	0.047
Coir pith extract	–	–	–	–	–	–	0.023
Neem oil cake + cowdung (dried) extract	–	–	–	–	–	–	0.026
Coir pith + rice bran extract	0.003 (0.001)	0.001 (0.0004)	0.001 (0.0004)	–	–	–	0.034
Raw rice washings (Kadivellam)	0.39 (0.14)	0.36 (1.83)	0.16 (0.06)	0.003 (0.001)	–	–	0.53
Jaggery water	0.084 (0.04)	0.55 (0.81)	0.14 (0.06)	0.002 (0.001)	–	–	0.63
Rice gruel (Kanchivellam)	0.35 (0.13)	0.11 (0.32)	0.36 (0.13)	0.001 (0.0004)	–	–	0.13
Coconut water	0.3 (0.11)	0.91 (1.00)	0.002 (0.001)	0.002 (0.001)	–	–	0.29
Mean	0.90	0.14	0.048	0.0006	–	–	

Figures in parentheses indicate logarithmic transformation

CD (0.05) for period of storage – 0.003

" treatments – 0.005

" interaction – 0.001

cfu : colony forming units, DAI : days after inoculation

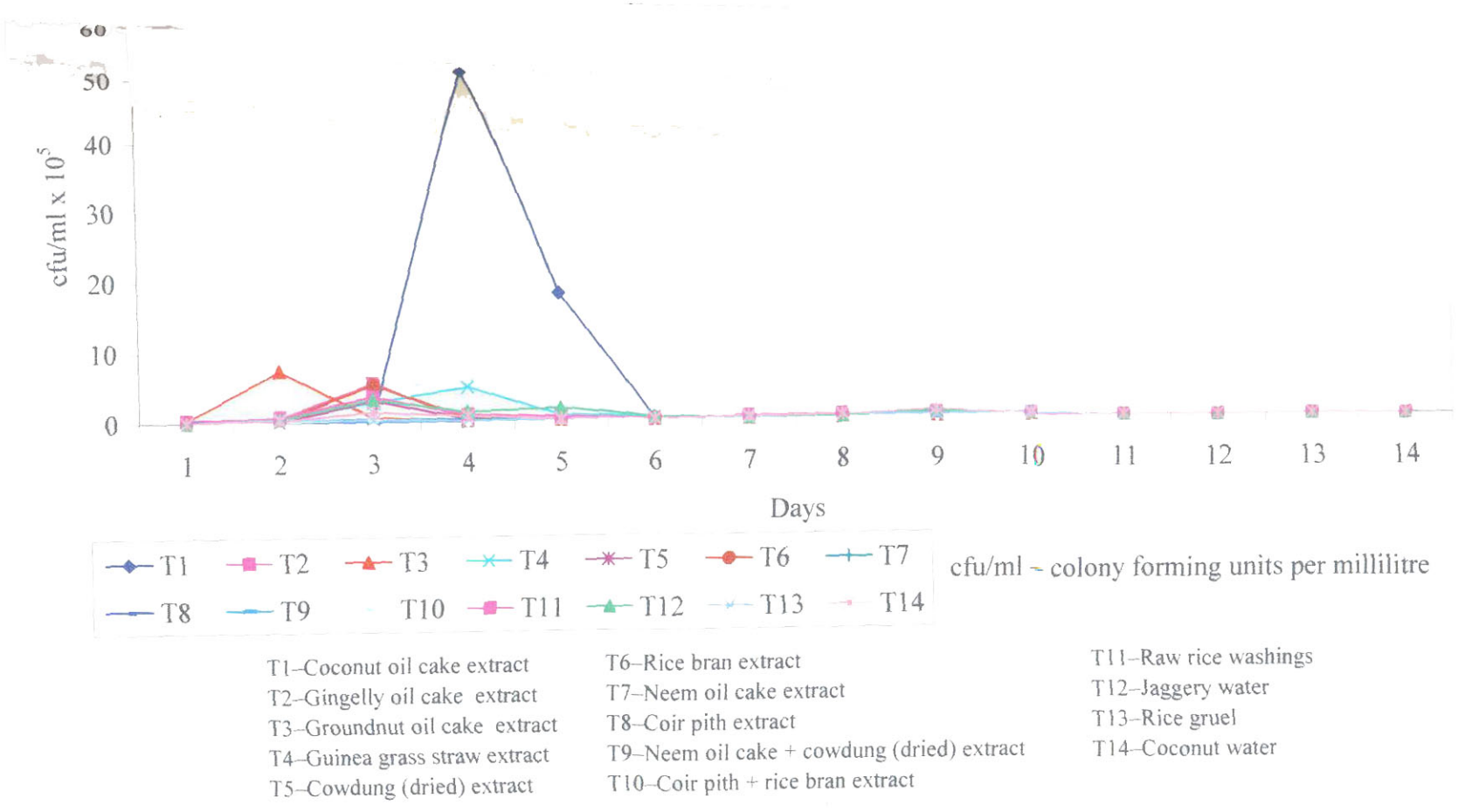


Fig. 17 Effect of liquid substrates on the spore viability of *C. gloeosporioides*

In guinea grass straw extract the spore count was 3.9 x 10^4 cfu/ml by 5 DAI, then the count increased to a maximum of 4.8 x 10^5 cfu/ml by 9 DAI and it decreased to 100 by 21 DAI and thereafter no viable count was recorded.

In general, a spore count of 6.99 x 10^3 cfu/ml increased to 4.38 x 10^5 cfu/ml 9 DAI and then the spore count declined drastically to 8.6 x 10^3 cfu/ml by 17 DAI and reduced to 60 after 23 days and thereafter it reached zero by 27th day (Fig. 18).

4.5 INTEGRATION OF BIOCONTROL AGENTS WITH HERBICIDES

4.5.1 Compatibility under *In vitro* Condition

Effect of five herbicides *viz.*, paraquat, glyphosate, anilofos 24 % + 2,4-D 32 % EC, 2,4-D sodium salt and pretilachlor on *F. pallidoroseum*, *F. equiseti, C. gloeosporioides* were assayed by poisoned food technique. Observations on mycelial growth and spore count were recorded.

4.5.1.1 F. pallidoroseum

4.5.1.1.1 Mycelial Growth

The effect of herbicides at different concentrations on the mycelial growth of *F. pallidoroseum* is presented in Table 34. It was observed that there was significant difference in the percentage inhibition of mycelial growth over control due to the different concentrations of herbicides tested (Fig. 19).

Among the five herbicides tested pretilachlor at all concentrations, 2.4-D and anilofos 24 % + 2,4-D 32 % EC at two higher concentrations tried completely inhibited the growth of the fungus. These two herbicides even at the lowest concentration (0.02 and 0.01 kg ai/ha respectively) could inhibit growth of the fungus by 60.68 and 69.58 per cent.

Paraquat and glyphosate at all concentrations tested recorded more than 60 per cent inhibition in the mycelial growth of *F. pallidoroseum*.

Table 34 *In vitro* effect of herbicides on growth and sporulation of *F. pallidoroseum*

Sl. No.	Herbicides (kg ai/ha)	Mean mycelial dry weight (g)	Per cent inhibition over control	Mean spore count	Per cent inhibition over control
1	Pretilachlor				
	1.00	–	100 (90)	–	100 (90)
	0.25	–	100 (90)	–	100 (90)
	0.06	–	100 (90)	–	100 (90)
	0.02	–	100 (90)	–	100 (90)
2	Anilofos 24 % + 2,4-D 32 % EC				
	0.40	–	100 (90)	–	100 (90)
	0.10	–	100 (90)	–	100 (90)
	0.03	0.07	92.24 (73.80)	295	70.35
	0.01	0.37	60.68 (51.15)	273	64.24
3	2,4-D				
	1.00	–	100 (90)	–	100 (90)
	0.25	–	100 (90)	–	100 (90)
	0.06	0.17	81.39 (64.42)	255.67	60.00
	0.02	0.37	69.58 (56.50)	222.67	52.94
4	Para quat				
	0.75	0.18	81.13 (64.22)	–	100 (90)
	0.19	0.19	78.85 (62.60)	–	100 (90)
	0.05	0.25	72.95 (58.63)	2.56	99.39
	0.01	0.30	67.93 (55.49)	2.93	99.31
5	Glyphosate				
	0.80	0.15	84.27 (66.61)	242	57.18
	0.20	0.40	61.87 (51.84)	227.67	54.11
	0.05	0.39	62.12 (51.99)	210	49.41
	0.01	0.33	62.42 (52.18)	131.67	30.59
	CD (0.05)		2.30	0.09	

Figures in parentheses indicate arc sine transformation

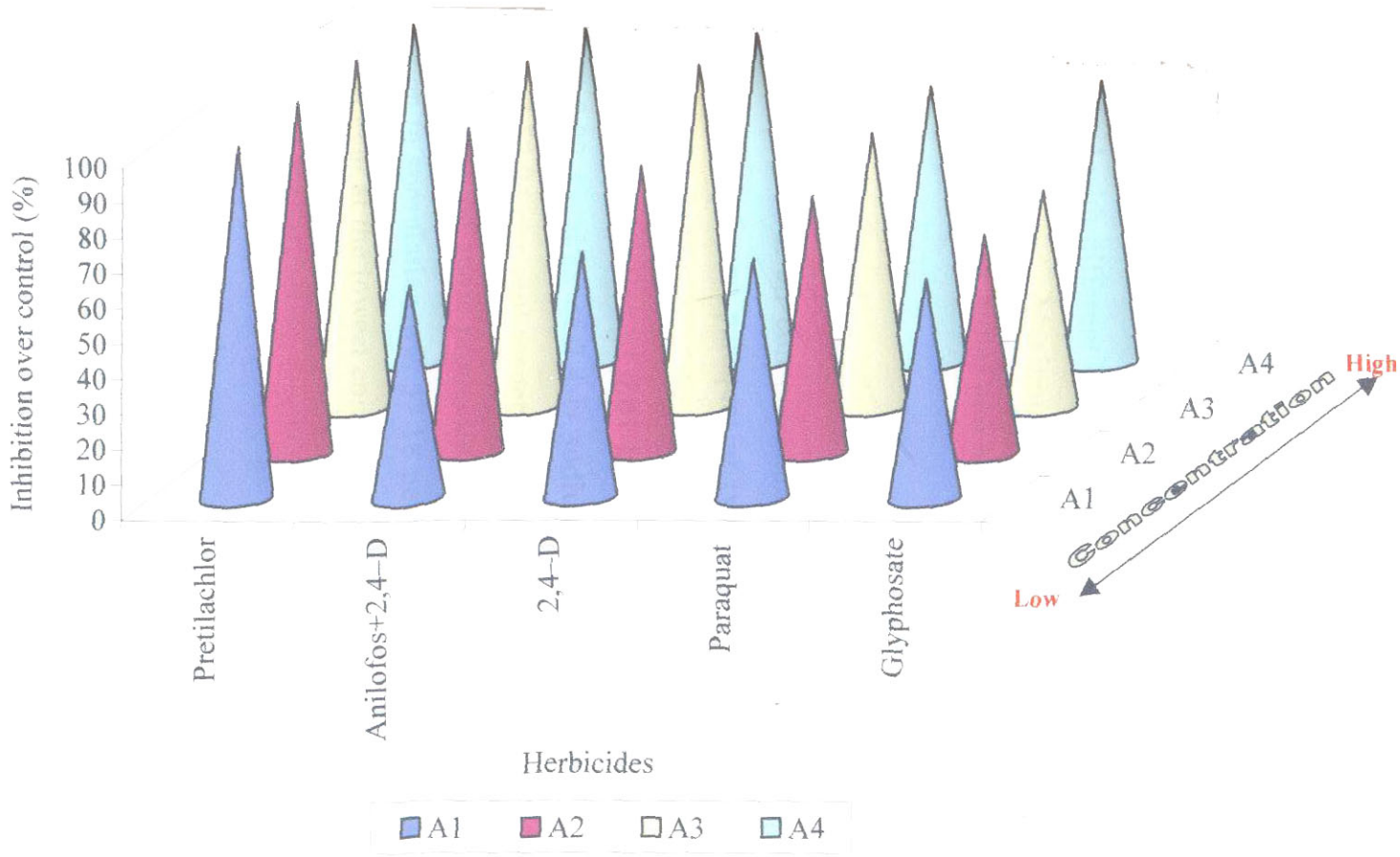


Fig. 19 *In vitro* effect of herbicides on growth of *F. pallidoroseum*

Paraquat at the recommended rate (0.75 kg ai/ha) showed 81.13 per cent inhibition of mycelial growth over control. The extent of reduction of growth of the fungus did not commensurate with reduction in concentration. Paraquat even at lowest concentration of 0.01 kg ai/ha gave only 67.93 per cent inhibition of mycelial growth.

The reduction in the growth of the fungus in liquid media incorporated with different concentration of glyphosate was similar to that observed in paraquat. Maximum inhibition of 84.27 per cent was observed at field dose (0.80 kg ai/ha) and at 0.01 kg ai/ha inhibition was 62.42 per cent.

4.5.1.1.2 Spore Count

Pretilachlor at all concentrations, anilofos 24 % + 2,4-D 32 % EC, 2,4-D sodium salt and paraquat at two higher concentrations completely inhibited the sporulation of the fungus in liquid media (Table 34). Even at the lowest concentration of 0.01 kg ai/ha of anilofos 24 % + 2,4-D 32 % EC and 0.02 kg ai/ha of 2,4-D sodium salt, inhibited the sporulation by 64.24 and 52.94 per cent respectively. Among the five herbicides tried the least inhibition on spore production was noticed with glyphosate. Here the reduction in sporulation was 57.18 per cent at field dose (0.80 kg ai/ha) to 30.59 per cent at the lowest concentration (0.01 kg ai/ha).

4.5.I.2 F. equiseti

4.5.1.2.1 Mycelial Growth

There was significant difference in the per cent inhibition of mycelial growth over control due to the different concentrations of herbicides tested (Table 35 and Fig. 20).

Pretilachlor at all concentrations, 2,4-D sodium salt and anilofos 24 % + 2,4-D 32 % EC at three higher concentration showed cent per cent inhibition in the mycelial growth of *F. equiseti*. Anilofos 24 % + 2,4-D 32

Table 35 *In vitro* effect of herbicides on growth and sporulation of *F. equiseti*

Sl. No.	Herbicides (kg ai/ha)	Mean mycelial dry weight (g)	Per cent inhibition over control	Mean spore count	Per cent inhibition over control
1	Pretilachlor				
	1.00	–	100 (90)	–	100 (90)
	0.25	–	100 (90)	–	100 (90)
	0.06	–	100 (90)	–	100 (90)
	0.02	–	100 (90)	–	100 (90)
2	Anilofos 24 % + 2,4-D 32 % EC				
	0.40	–	100 (90)	–	100 (90)
	0.10	–	100 (90)	–	100 (90)
	0.03	–	100 (90)	–	100 (90)
	0.01	0.13	85.29 (67.42)	0.16	99.61
3	2,4-D				
	1.00	–	100 (90)	–	100 (90)
	0.25	–	100 (90)	–	100 (90)
	0.06	–	100 (90)	–	100 (90)
	0.02	0.17	80.06 (63.45)	–	100 (90)
4	Para quat				
	0.75	0.24	72.37 (58.26)	–	100 (90)
	0.19	0.26	70.05 (56.80)	–	100 (90)
	0.05	0.28	67.82 (55.44)	0.31	99.24
	0.01	0.69	20.10 (26.56)	0.32	99.21
5	Glyphosate				
	0.80	0.33	62.31 (52.11)	–	100 (90)
	0.20	0.67	22.51 (28.31)	0.13	99.68
	0.05	0.84	2.44 (8.99)	0.27	99.34
	0.01	0.99	1.20 (6.28)	0.37	99.09
	CD (0.05)		1.04	0.01	

Figures in parentheses indicate arc sine transformation

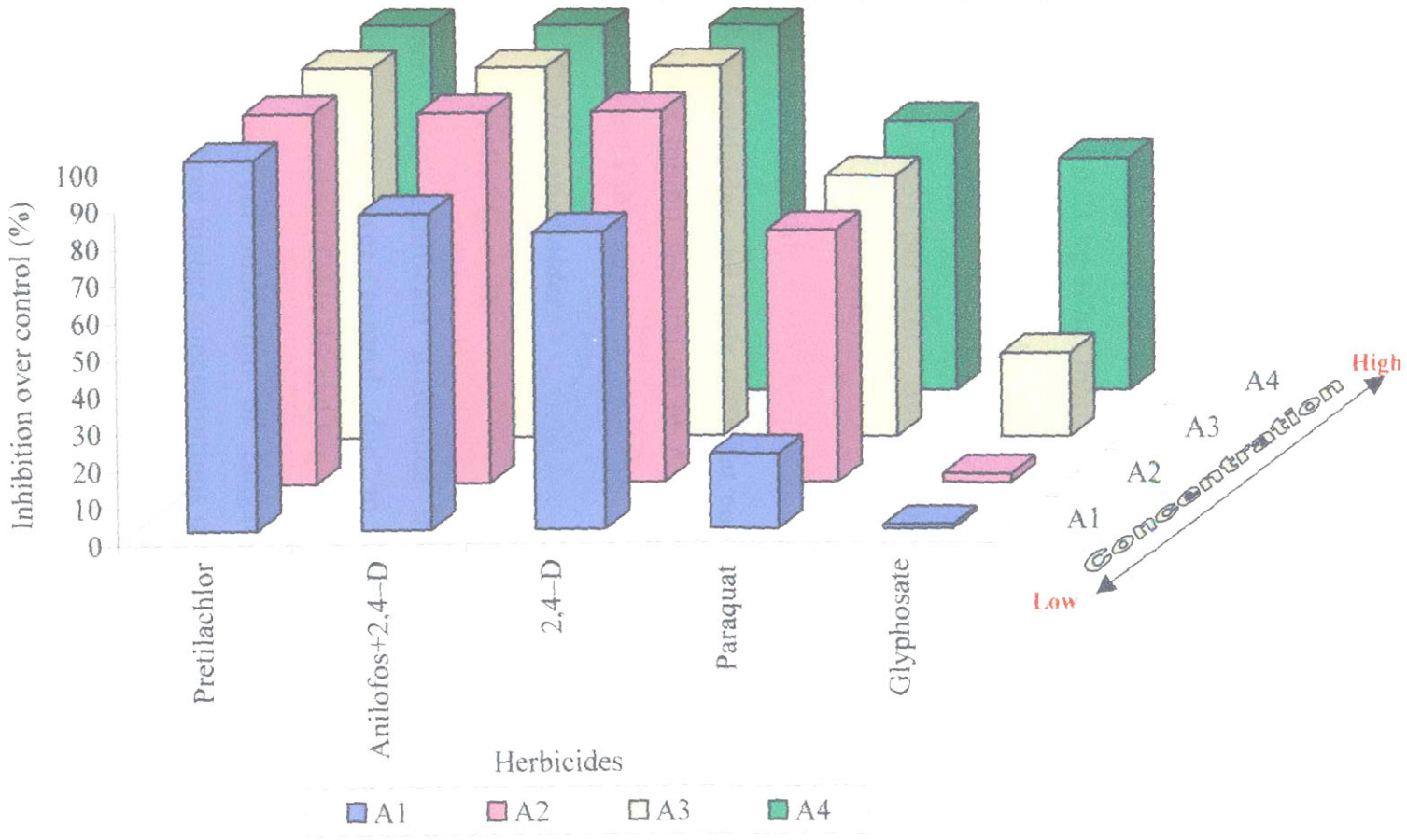


Fig. 20 *In vitro* effect of herbicides on growth of *F. equiseti*

% EC and 2,4-D sodium salt at lowest concentration (0.01 and 0.02 kg ai/ha respectively) inhibited the growth by 85.29 and 80.06 per cent respectively.

At the field dose of paraquat (0.75 kg ai/ha) the growth inhibition was 72.37 per cent and almost similar inhibition (70.05 per cent) was noticed even when the dosage was reduced by $\frac{1}{4}^{th}$. At lowest concentration of 0.01 kg ai/ha, inhibition of 20.10 per cent was recorded.

Among the five herbicides tried the lowest inhibition of *F. equiseti* was with glyphosate. At the highest concentration (0.80 kg ai/ha) the reduction was 62.31 per cent. But at 0.05 kg ai/ha, it could inhibit the growth only by 2.44 per cent.

4.5.1.2.2 Spore Count

Five herbicides and their different concentrations tested were found to completely inhibit sporulation of *F. equiseti* in liquid media under *in vitro* condition (Table 35).

4.5.1.3 C. gloeosporioides

4.5.1.3.1 Mycelial Growth

There was significant difference in the per cent inhibition of mycelial growth over control due to the herbicides at different concentrations tested (Table 36 and Fig. 21).

Cent per cent inhibition of fungal growth was recorded with pretilachlor at three higher concentrations of 1.00, 0.25 and 0.06 kg ai/ha. Even at the lowest concentration (0.02 kg ai/ha) the inhibition of growth was very high (96.34 per cent).

Anilofos 24 % + 2,4-D 32 % EC at all concentrations except the lowest of 0.01 kg ai/ha (82.22 per cent), inhibited the fungal growth completely. Eventhough 2,4-D sodium salt at field dose (1 kg ai/ha) and two lower concentrations caused cent per cent inhibition in mycelial

Table 36 *In vitro* effect of herbicides on growth and sporulation of *C. gloeosporioides*

Sl. No.	Herbicides (kg ai/ha)	Mean mycelial dry weight (g)	Per cent inhibition over control	Mean spore count	Per cent inhibition over control
1	Pretilachlor				
	1.00	–	100 (90)	–	100 (90)
	0.25	–	100 (90)	–	100 (90)
	0.06	–	100 (90)	–	100 (90)
	0.02	0.05	96.34 (78.93)	–	100 (90)
2	Anilofos 24 % + 2,4-D 32 % EC				
	0.40	–	100 (90)	0.22	95.14
	0.10	–	100 (90)	0.15	96.19
	0.03	–	100 (90)	0.27	94.04
	0.01	0.22	82.22 (65.03)	0.29	93.59
3	2,4-D				
	1.00	–	100 (90)	–	100 (90)
	0.25	–	100 (90)	–	100 (90)
	0.06	–	100 (90)	–	100 (90)
	0.02	0.77	38.33 (38.24)	0.03	99.34
4	Para quat				
	0.75	0.28	78.01 (62.01)	–	100 (90)
	0.19	0.31	75.07 (60.02)	0.15	69.69
	0.05	0.47	62.86 (52.44)	0.17	96.25
	0.01	0.76	39.24 (38.77)	0.25	94.48
5	Glyphosate				
	0.80	0.33	73.78 (59.17)	–	100 (90)
	0.20	0.36	71.58 (57.76)	–	100 (90)
	0.05	0.62	50.60 (45.32)	–	100 (90)
	0.01	0.87	30.42 (33.46)	0.04	99.12
	CD (0.05)		1.25	0.03	

Figures in parentheses indicate arc sine transformation

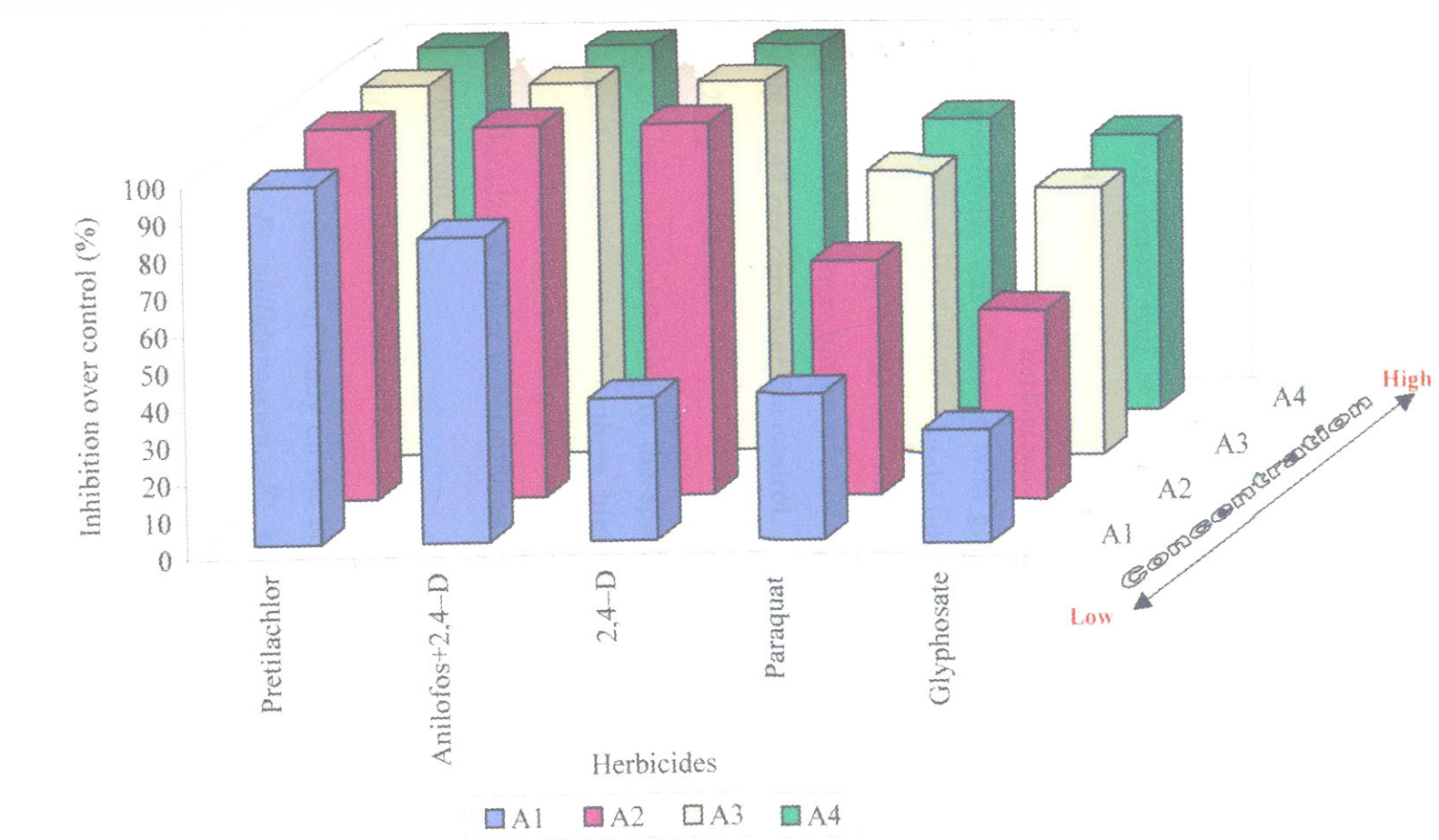


Fig. 21 *In vitro* effect of herbicides on growth of *C. gloeosporioides*

growth, the lowest concentration of 0.02 kg ai/ha could inhibit the growth only by 38.33 per cent.

At the normal field dose of 0.75 kg ai/ha, paraquat caused 78.01 per cent inhibition of the mycelial growth of *C. gloeosporioides*. As the concentration decreased from 0.75 to 0.01 kg ai/ha a corresponding reduction in the inhibition of fungal growth was noticed and the per cent inhibition was almost half at the lowest concentration of 0.01 kg ai/ha (39.24 per cent) compared to the field dose.

Among the five herbicides tried the lowest inhibition of *C. gloeosporioides* was observed with glyphosate. Even at the field dose of 0.80 kg ai/ha it did not completely inhibited the fungus. At one fourth the field dose (0.20 kg ai/ha) the inhibition recorded was 71.58 per cent and at the lowest concentration of 0.01 kg ai/ha the reduction in growth was only 30.42 per cent over the control.

4.5.1.3.2 Spore Count

Cent per cent inhibition in the spore production was noticed at all concentrations of pretilachlor, three higher concentrations of glyphosate and 2,4-D sodium salt and highest concentration of paraquat (Table 36). All the herbicides at lower concentration inhibited the sporulation by more than 90 per cent.

4.5.2 Effect of Lower Concentration of Herbicides on Water Hyacinth

The results of study on the effect of herbicides on growth and sporulation of *F. pallidoroseum, F. equiseti* and *C. gloeosporioides* showed that pretilachlor completely inhibited the growth of these fungi and even the lowest concentrations of the other herbicides were inhibitory. The lowest concentrations of these herbicides when sprayed on water hyacinth plants resulted in the death of plants.

In order to find out lower concentrations of herbicides which are not toxic to test fungi and cause only minor injuries on water hyacinth,

another experiment was conducted on the effect of lower concentrations of herbicides on water hyacinth. In this trial pretilachlor was not included as it completely inhibited both growth and sporulation of all the test fungi.

All the other herbicides were tried at 5 concentrations *viz.*, 200, 100, 50, 30 and 10 ppm. paraquat at concentrations 50 ppm and below caused small brown coloured spreading blotches on older leaves while glyphosate showed slight wilting symptoms. Both anilofos 24 % + 2,4-D 32 % EC and 2,4-D sodium salt exhibited bending of petiole coupled with green discolouration. The intensity of symptom produced by all the herbicides intensified with concentration. All herbicides, above 50ppm (100 and 200 ppm) resulted in wilting or drying up of water hyacinth plants within 3-5 days.

4.5.3 Combined Effect of Biocontrol Fungi and Herbicides on Water Hyacinth

4.5.3.1 Glasshouse Condition

The effect of combined application of biocontrol fungi *viz.*, *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides* and four herbicides (paraquat, glyphosate, anilofos 24 % + 2,4-D 32 % EC, 2,4-D sodium salt) were studied.

4.5.3.1.1 Experiment I – Simultaneous Application of Biocontrol Fungi and Herbicides

Statistical analysis of data on the intensity of infection revealed that there was significant difference between treatments (Table 37 and Fig. 22). anilofos 24 % + 2,4-D 32 % EC at 50 and 30 ppm along with *F. pallidoroseum* resulted in maximum disease intensity (62.18 and 68.86 per cent respectively) (Plate 14) and were statistically on par. The disease intensity, when *F. pallidoroseum* was mixed with other herbicides ranged from 15.41 per cent (paraquat, 50and 10 ppm and 2,4-D sodium salt, 10 ppm) to 37.71 per cent (2,4-D sodium salt, 30 ppm).

Table 37 Effect of simultaneous application of biocontrol fungi and herbicides on water hyacinth

Sl. No.	Treatments (ppm)	Mean disease intensity (%)		
		F. pallidoroseum	*F. equiseti*	*C. gloeosporioides*
1	Paraquat*			
	50	15.41 (4.05)	– (1)	9.07 (3.17)
	30	22.12 (4.81)	– (1)	9.07 (3.17)
	10	15.41 (4.05)	– (1)	1.32 (1.52)
2	Glyphosate			
	50	17.64 (4.32)	8.66 (3.12)	– (1)
	30	22.12 (4.81)	13.33 (3.79)	– (1)
	10	31.03 (5.66)	10.88 (3.44)	– (1)
3	Anilofos 24 % + 2,4-D 32 % EC			
	50	62.18 (7.95)	15.41 (4.05)	24.01 (5.00)
	30	68.86 (8.36)	15.41 (4.05)	31.42 (5.69)
	10	35.49 (6.04)	– (1)	9.07 (3.17)
4	2,4-D			
	50	57.73 (7.66)	8.66 (3.11)	18.44 (4.41)
	30	37.71 (6.22)	- (1)	14.70 (3.96)
	10	15.41 (4.05)	- (1)	12.85 (3.72)
	Control (Fungus alone)	51.06 (7.22)	37.71 (6.22)	48.11 (7.01)
CD (0.05) for treatment		0.72	0.58	0.61

Figures in parentheses indicate $\sqrt{x + 1}$ transformation

*No detectable damage was noted on water hyacinth when herbicides alone were sprayed

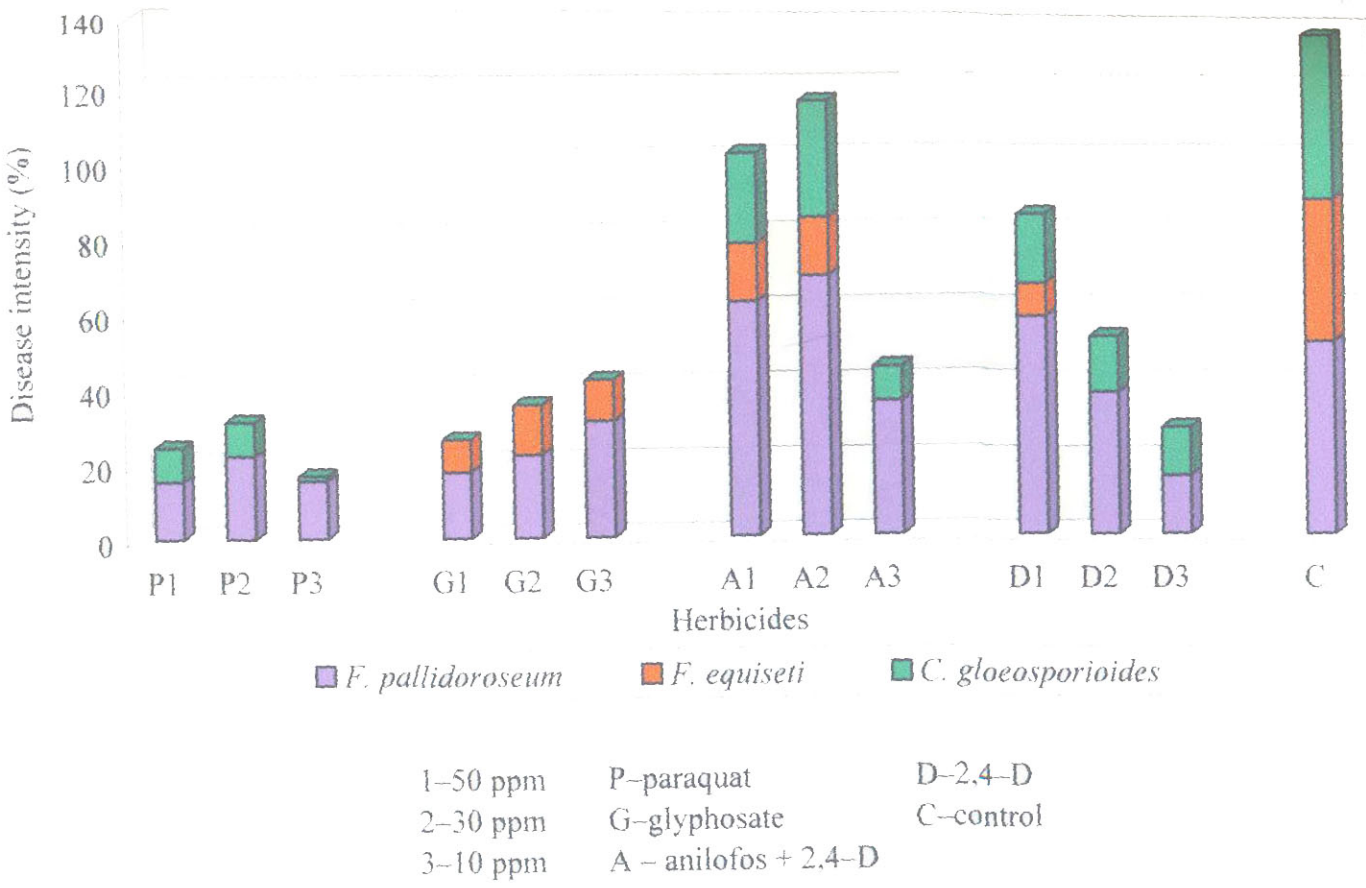


Fig. 22 Effect of simultaneous application of biocontrol fungi and herbicides on water hyacinth

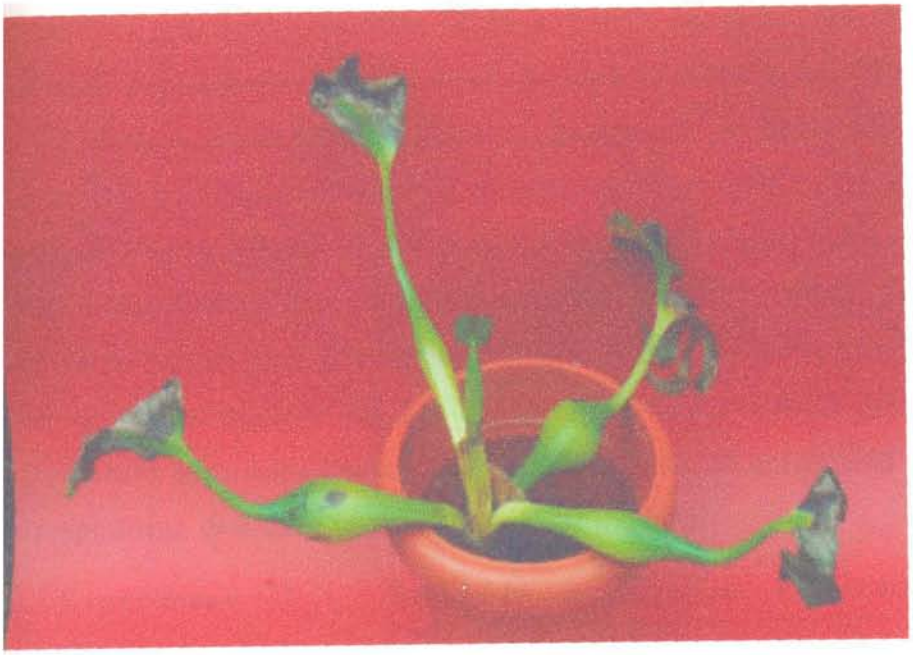


Plate 14. Simultaneous application of *F. pallidoroseum* + [(Anilofos + 2,4–D), 30 ppm] on water hyacinth

The application of *F. equiseti* in combination with herbicides did not have any increase in the intensity of disease then applied alone. *F. equiseti* failed to induce symptom when applied along with paraquat at all concentrations, anilofos 24 % + 2,4-D 32 % EC at 10 ppm and 2,4-D sodium salt at 30 and 10 ppm respectively. In all other treatments the intensity of disease ranged from 8.66 (glyphosate and 2,4-D sodium salt at 50 ppm) to15.41 per cent (anilofos 24 % + 2,4-D 32 % EC 30 and 50 ppm). When *C. gloeosporioides* was applied along with the herbicides, the intensity of disease reduced considerably compared to application of fungus alone. *C. gloeosporioides* with glyphosate at all concentrations on water hyacinth plants did not produce any disease. Highest incidence of disease was noticed when *C. gloeosporioides* was mixed with anilofos 24 % + 2,4-D 32 % EC 50 ppm (24.01 per cent) and 50 ppm (31.41 per cent). These treatments statistically did not differ significantly. Both paraquat and 2,4-D sodium salt, with decrease in concentration of herbicides there was a corresponding decrease in the intensity of disease.

4.5.3.1.2 Experiment II – Herbicide Application Followed by Biocontrol Fungi

Statistical analysis of the disease intensity produced by herbicide application followed by fungus recorded significant difference between treatments (Table 38 and Fig. 23). None of treatments recorded higher intensity of disease than the control. However maximum disease intensity of 55.55 per cent was recorded by the treatment, anilofos 24 % + 2,4-D 32 % EC 10 ppm followed by *F. pallidoroseum* which was statistically on par with *F. pallidoroseum* alone (51.06 per cent). When different concentrations of herbicides and *F. pallidoroseum* was applied, disease intensity in glyphosate and anilofos 24 % + 2,4-D 32 % EC ranged from 35.49 to 55.51 per cent while with paraquat and 2,4-D sodium salt it was 0 to 17.64 per cent.

Table 38 Effect of herbicide application followed by biocontrol fungi on water hyacinth

Sl. No.	Treatments (ppm)	Mean disease intensity (%)		
		F. pallidoroseum	*F. equiseti*	*C. gloeosporioides*
1	Paraquat			
	50	10.88 (3.45)	– (1)	3.17 (2.04)
	30	3.75 (2.18)	– (1)	9.07 (3.17)
	10	– (1)	– (1)	14.7 (3.96)
2	Glyphosate			
	50	35.49 (6.04)	13.83 (3.79)	3.17 (2.04)
	30	42.17 (6.57)	10.88 (3.45)	5.56 (2.56)
	10	44.39 (6.74)	10.88 (3.45)	9.07 (3.17)
3	Anilofos 24 % + 2,4-D 32 % EC			
	50	35.49 (6.04)	10.88 (3.45)	11.11 (3.48)
	30	37.71 (6.22)	– (1)	16.67 (4.20)
	10	55.51 (7.52)	– (1)	20.29 (4.61)
4	2,4-D			
	50	15.41 (4.05)	– (1)	7.22 (2.87)
	30	15.41 (4.05)	– (1)	9.07 (3.17)
	10	17.64 (4.32)	– (1)	9.07 (3.17)
	Control (Fungus alone)	51.06 (7.22)	37.71 (6.22)	48.11 (7.01)
CD (0.05) for treatments		0.86	0.5	0.75

Figures in parentheses indicate $\sqrt{x + 1}$ transformation

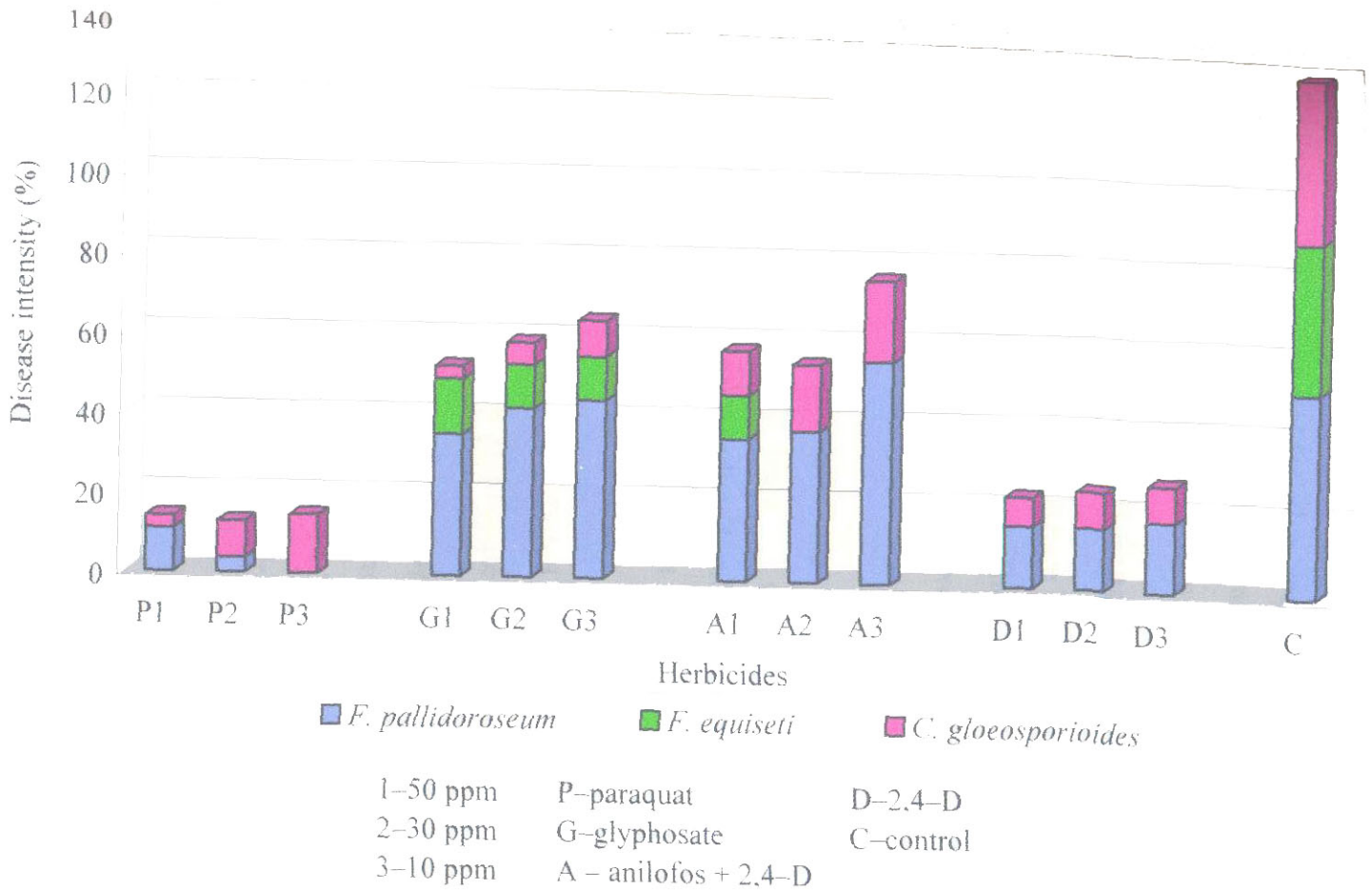


Fig. 23 Effect of herbicide application followed by biocontrol fungi on water hyacinth

F. equiseti recorded lower disease intensity in all treatments with herbicides when compared to application of fungus alone. paraquat and 2,4-D sodium salt at all concentrations did not produce any symptom.

Similarly *C. gloeosporioides* also recorded lower disease intensity and it ranged from 3.17 per cent (paraquat and glyphosate, 50 ppm) to 20.29 (anilofos 24 % + 2,4-D 32 % EC, 10 ppm)

4.5.3.1.3 Experiment III – Biocontrol Fungi Followed by Herbicides

The results of the treatments, where biocontrol fungi followed by herbicide application was almost similar to that observed in the experiment of herbicide application followed by biocontrol fungi (Table 39 and Fig. 24).

F. pallidoroseum did not cause any disease with 30 and 50 ppm paraquat. While all other herbicides at all concentrations and paraquat at lowest concentration (10 ppm) induced more than 20 per cent disease. The maximum disease intensity of 48.84 per cent was recorded with anilofos 24 % + 2,4-D 32 % EC at 50 ppm.

In this experiment *F. equiseti* failed to induce any disease at all concentrations tested except with glyphosate at 50 ppm (3.75 per cent).

Application of paraquat and *C. gloeosporioides* reduced the intensity of disease to less than 10 per cent at all concentrations while with anilofos 24 % + 2,4-D 32 % EC it ranged from 14.7 per cent (50 ppm) to 24 per cent (10 ppm). *C. gloeosporioides* and 2,4-D sodium salt (10 ppm) failed to induce any disease symptom.

4.5.3.2 Trough Condition

Most effective treatments obtained from the glasshouse trial were tried under trough condition. The treatments tested were simultaneous application of *F. pallidoroseum* along with anilofos 24 % + 2,4-D 32 % EC at 50 and 30 ppm and 2,4-D sodium salt at 50 ppm (Table 40).

Table 39 Effect of biocontrol fungi followed by herbicides on water hyacinth

Sl. No.	Treatments (ppm)	Mean disease intensity (%)		
		F. pallidoroseum	*F. equiseti*	*C. gloeosporioides*
1	Paraquat			
	50	– (1)	– (1)	5.56 (2.56)
	30	– (1)	– (1)	5.56 (2.56)
	10	22.12 (4.81)	– (1)	9.07 (3.17)
2	Glyphosate			
	50	41.98 (6.56)	3.75 (2.18)	9.07 (3.17)
	30	26.67 (5.26)	– (1)	11.11 (3.48)
	10	20.00 (4.58)	– (1)	11.11 (3.48)
3	Anilofos 24 % + 2,4-D 32 % EC			
	50	48.84 (7.06)	– (1)	14.70 (3.96)
	30	33.32 (5.86)	– (1)	20.29 (4.61)
	10	37.71 (6.22)	– (1)	24.00 (5.00)
4	2,4-D			
	50	22.12 (4.81)	– (1)	18.44 (4.41)
	30	28.81 (4.56)	– (1)	14.70 (3.96)
	10	37.71 (6.22)	– (1)	– (1)
	Control (Fungus alone)	51.06 (7.22)	37.71 (6.22)	48.11 (7.01)
CD (0.05) for treatment		0.50	0.50	0.53

Figures in parentheses indicate $\sqrt{x + 1}$ transformation

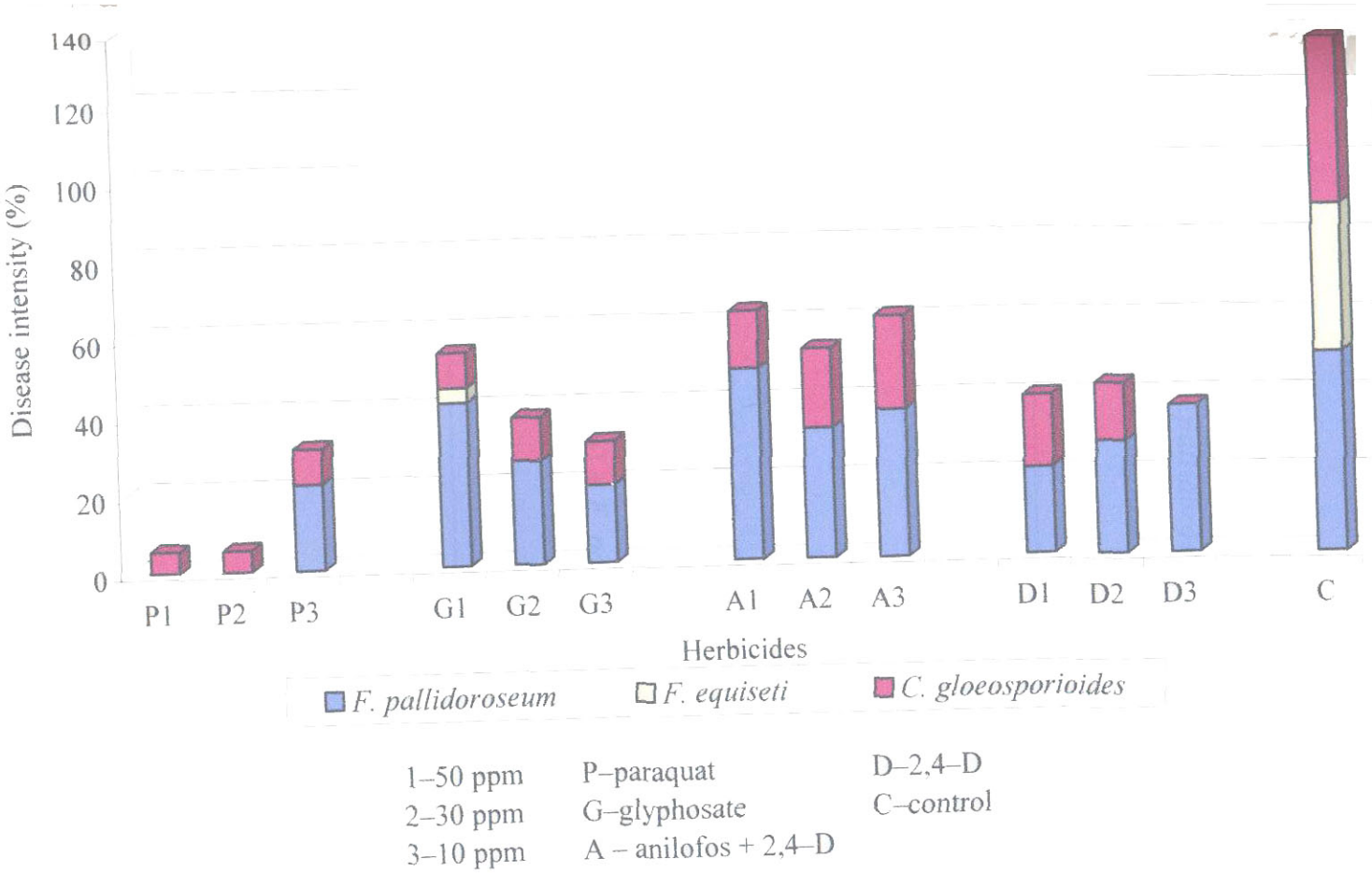


Fig. 24 Effect of biocontrol fungi followed by herbicides on water hyacinth

Table 40 Combined effect of *F. pallidoroseum* and herbicides on water hyacinth under trough condition

Sl. No.	Treatment	Mean per cent disease intensity
1	Anilofos 24 % + 2,4-D 32 % EC (30 ppm) + *F. pallidoroseum*	26.67
2	Anilofos 24 % + 2,4-D 32 % EC (50 ppm) + *F. pallidoroseum*	11.33
3	2,4-D sodium salt (50 ppm) + *F. pallidoroseum*	13.33
4	Control (*F. pallidoroseum* alone)	50.44

The intensity of damage produced on water hyacinth by the treatments were comparatively low when compared to application of *F. pallidoroseum* alone (50.49 per cent). Maximum intensity of damage (26.67 per cent) was recorded with application of *F. pallidoroseum* and anilofos 24 % + 2,4-D 32 % EC (30 ppm).

4.6 EFFECT OF DIFFERENT OILS ON WATER HYACINTH

Among the different oils tested CNSL at different concentrations produced maximum damage to water hyacinth. CNSL at all concentrations *viz.*, 1, 2 and 3 per cent produced brown spots on both young and old leaves and also on the petiole. At three per cent concentration drying up of the older leaves at the tip were observed.

Marotty oil at all concentrations produced scorching in the form of large patches on older leaves. Castor and neem oil produced pin head sized black spots on the older leaves only. In the case of coconut oil no injury was observed on leaves and petiole.

4.7 FORMULATION OF *F. PALLIDOROSEUM*

For formulating only *F. pallidoroseum* was used as it was effective against water hyacinth and also it had a narrow host range among the cultivated plants. *M. advena* eventhough highly effective was not used as it was pathogenic to many of the cultivated plants.

4.7.1 Compatibility of Formulation Ingredients with *F. pallidoroseum*

4.7.1.1 Mycelial Growth

Mean dry mycelial weight of *F. pallidoroseum* in media incorporated with Tween-80, Glycerol and CNSL at 2.0 per cent and above were almost similar to the control (Table 41). The maximum dry weight was noticed with Tween-80 at 2.0 per cent (0.89 g). The growth of the fungus was less in media incorporated with lower concentrations of all the ingredients used for formulation.

Table 41 Effect of formulation ingredients and CNSL on the growth of *F. pallidoroseum*

Sl. No.	Formulation ingredients	Mean dry mycelial weight (g)			Mean
		Concentration (%)			
		1	2	3	
1	Tween 80	0.35	0.82	0.79	0.66
2	Paraffin liquid	0.35	0.38	0.57	0.43
3	Glycerol	0.54	0.70	0.81	0.69
4	Sodium alginate	0.34	0.42	0.44	0.40
5	Cashew nut shell liquid	0.33	0.75	0.80	0.63
	Control				0.70
	Mean	0.38	0.62	0.69	

4.7.1.2 Spore Count

Compared to control (2.9 x 10^6 spores) the ingredients used for formulating the *F. pallidoroseum* inhibited the sporulation of the fungus when it was incorporated in Czapek's broth. However, the CNSL had a stimulatory effect on sporulation (Table 42). As the concentration of CNSL increased from 1-3 per cent there was a corresponding increase in the spore count from 57.54 to 75.86 x 10^5 spores/ml. Among the other formulating ingredients maximum spore count was recorded with two per cent paraffin liquid and minimum with Tween-80 at one per cent.

4.7.2 Different Formulations

4.7.2.1 Dust

4.7.2.1.1 Effect of Dust Formulation on Water Hyacinth

To study the effect of dust formulation on water hyacinth plants an experiment was set up under glasshouse condition. The formulations prepared at different proportions were dusted on healthy water hyacinth plants grown in pots.

Maximum intensity of infection was recorded by dusting Product A (20 per cent) followed by B and C, each recorded 10.89 per cent damage (Table 43 and Fig. 25).

4.7.2.1.2 Effect of Dust Formulation on Mite Infected and Oil Injured Water Hyacinth

The effect of dust formulations *viz.*, A, B and C on mite infected and oil injured water hyacinth plants were studied under glasshouse condition.

Statistical analysis of mean per cent intensity of disease revealed that there was significant difference between the treatments tested (Table 44 and Fig. 26).

Maximum intensity of damage (68.89 per cent) was recorded by the treatment T_{12} (dusting product A on plants sprayed with 2.00 per cent CNSL) (Plate 15). This was followed by T_{13} (dusting product B on plants

Table 42 Effect of formulation ingredients and CNSL on the sporulation of *F. pallidoroseum*

Sl. No.	Formulation ingredients	Mean spore count / ml x 10^5			Mean
		Concentration (%)			
		1	2	3	
1	Tween 80	5.01	72.44	12.30	29.92
2	Paraffin liquid	6.17	12.59	7.59	8.78
3	Glycerol	11.22	7.41	7.41	8.68
4	Sodium alginate	3.50	4.89	4.89	4.43
5	Cashew nut shell liquid	57.54	61.66	75.86	65.02
	Control				29.00
	Mean	16.69	31.80	21.61	

Table 43 Effect of dust formulation on water hyacinth under glasshouse condition

Treatments	Mean disease intensity (%)
T_1 – A	20
T_2 – B	10.89
T_3 – C	10.89
T_4 – D	6.67
T_5 – E	2.22
T_6 – F	2.22
T_7 – Control (Talc alone)	–

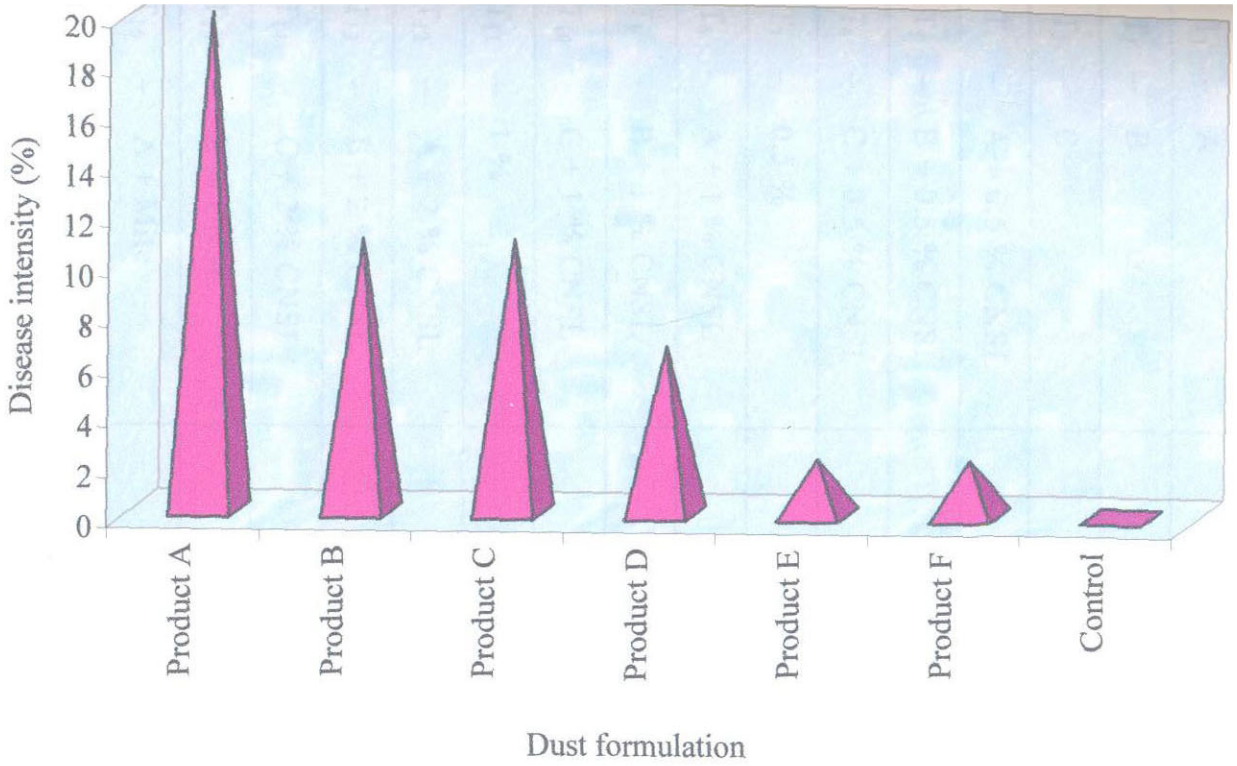


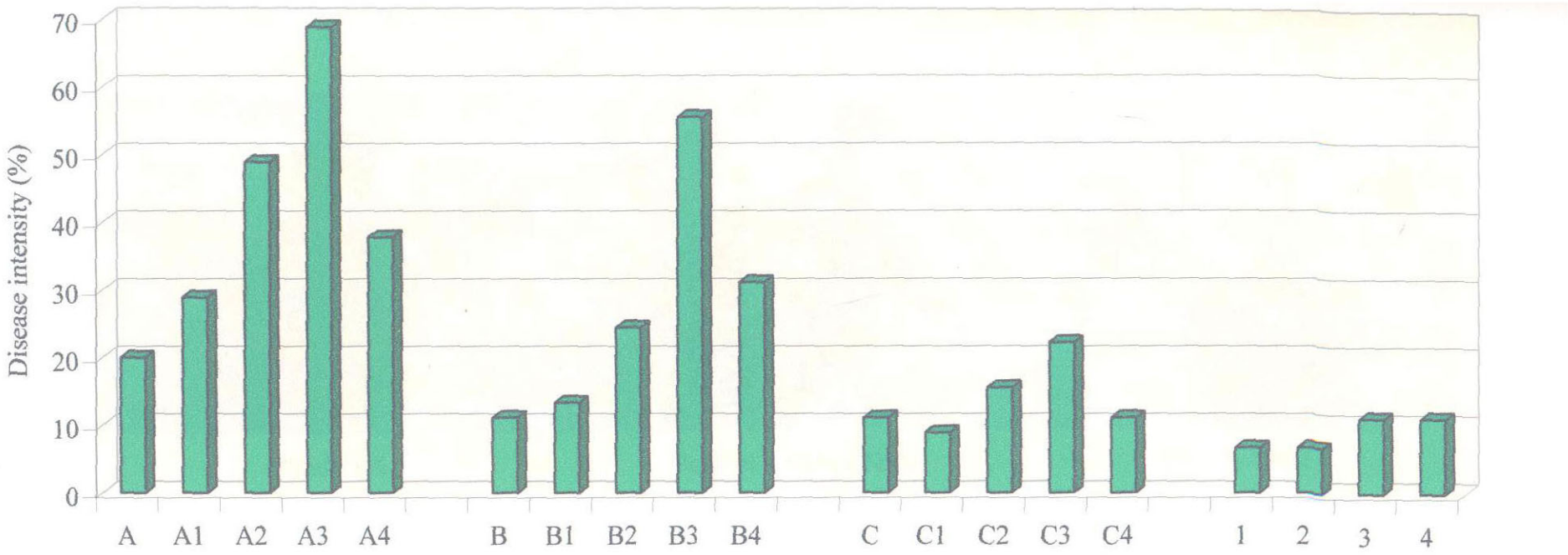
Fig. 25 Effect of dust formulation on water hyacinth under glasshouse condition

Table 44 Effect of dust formulations on oil injured and mite infected water hyacinth under glasshouse condition

Treatments	Mean disease intensity (%)
T_1 – A	20 (26.57)
T_2 – B	11.11 (19.26)
T_3 – C	11.11 (19.26)
T_4 – A + 0.5 % CNSL	28.89 (32.48)
T_5 – B + 0.5 % CNSL	13.33 (21.41)
T_6 – C + 0.5 % CNSL	8.88 (17.11)
T_7 – 0.5 %	6.66 (14.96)
T_8 – A + 1 % CNSL	48.89 (44.36)
T_9 – B + 1 % CNSL	24.45 (29.58)
T_{10} – C + 1 % CNSL	15.55 (23.13)
T_{11} – 1 %	6.66 (14.96)
T_{12} – A + 2 % CNSL	68.89 (56.13)
T_{13} – B + 2 % CNSL	55.55 (48.20)
T_{14} – C + 2 % CNSL	22.22 (28.08)
T_{15} – 2 % CNSL	11.11 (19.26)
T_{16} – A + Mite	37.78 (37.91)
T_{17} – B + Mite	31.11 (33.87)
T_{18} – C + Mite	11.11 (19.26)
T_{19} – Mite	11.11 (19.26)
T_{20} – Talc	–

CD (0.05) = 4.30

Figures in parentheses indicate arc sine transformation



A, B, C – Dust formulations

1 – 0.5 % CNSL, 2 – 1 % CNSL, 3 – 2 % CNSL, 4 – Mite

Fig. 26 Effect of dust formulation on oil injured and mite infected water hyacinth under glasshouse condition

Product A | Product A+2 % CNSL | 2 % CNSL

Plate 15. Effect of dust formulation on water hyacinth

sprayed with 2.0 per cent CNSL) which recorded 55.55 and 48.89 per cent intensity of infection in T_8 (dusting product A on plants with 1.0 per cent CNSL) which were statistically on par.

In treatments where 0.5 per cent CNSL was sprayed, T_4 (dusting of product A) recorded maximum damage of 28.89 per cent.

In treatments where dusting the formulation on mite injured plants, treatment T_{16} (dusting product A) recorded maximum damage of 37.78 per cent which was on par with T_{17} (dusting product B), which gave 31.11 per cent damage.

4.7.2.1.3 Shelf Life of Dust

The results of cfu/g sample of dust formulation stored at room temperature is presented in Table 45 and Fig. 27. Statistical analysis of the cfu/g revealed that there was significant difference between the treatments and period of storage.

It was observed that the viability of spores in the dust formulations were reduced drastically when stored at room temperature. Of the dust formulations prepared, the spores in the formulation A, B and C retained their viability upto 6 WAS.

Formulation A, which gave the maximum intensity of infection and a viable count of 5×10^6 cfu/g on the day of preparation declined gradually and reached 2.22×10^3 cfu/g, 6 WAS.

Similar trend was recorded in the case of product B also. On the initial day of storage it recorded 3.49×10^6 cfu/g and which reduced to 1.49×10^3 cfu/g, 6 WAS.

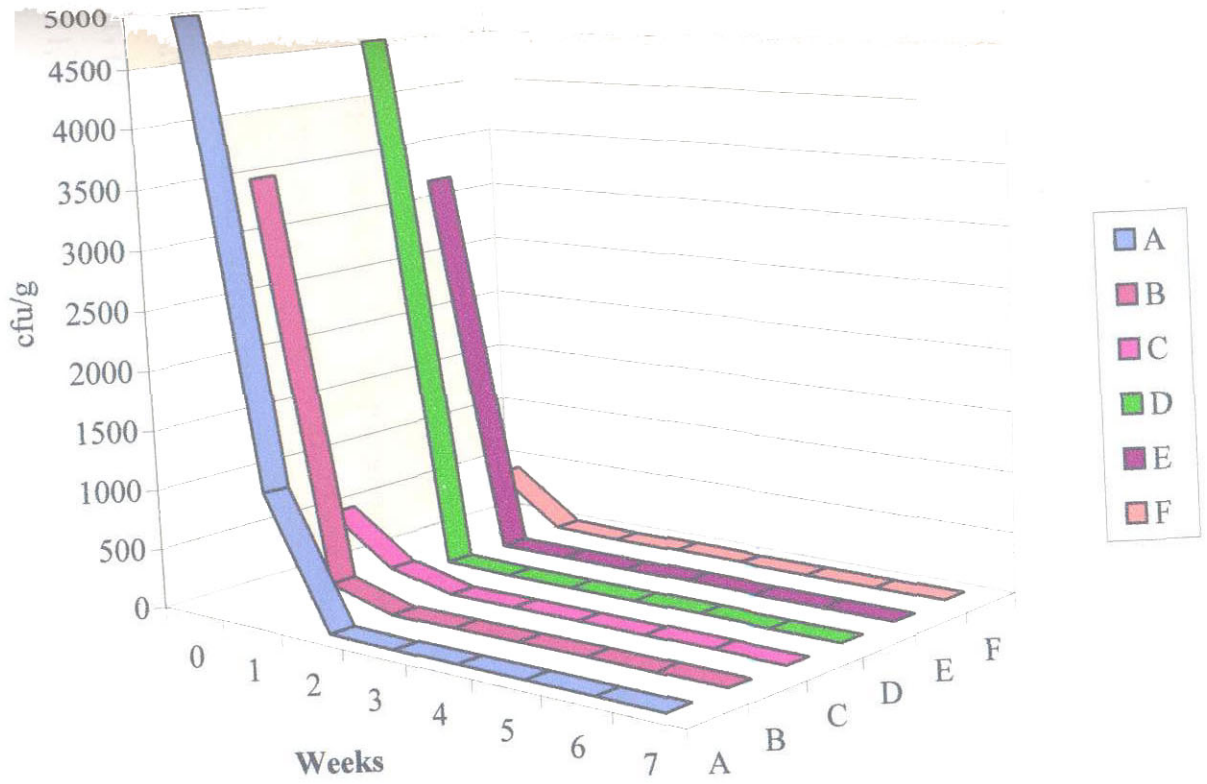
Eventhough the product C retained viability for six weeks from the initial count of 5.09×10^5 cfu/g sample, it reduced to 1.06×10^3 cfu/g by 6 WAS and reached zero count by 7 WAS.

Table 45 Shelf life of dust formulations

Sl. No.	Dust formulation	Mean cfu/g of formulation at weekly intervals x 10^3								Mean
		0	1	2	3	4	5	6	7	
1	A	4995.79 (3.70)	1065.70 (3.02)	13.28 (1.15)	3.23 (0.63)	3.10 (0.61)	2.99 (0.60)	2.22 (0.51)	–	760.9
2	B	3485.06 (3.54)	155.79 (2.20)	3.10 (0.61)	2.60 (0.41)	2.93 (0.47)	2.20 (0.51)	1.49 (0.40)	–	456.65
3	C	509.98 (2.71)	128.98 (2.11)	3.10 (0.61)	3.23 (0.63)	2.20 (0.34)	1.53 (0.40)	1.06 (0.31)	–	81.26
4	D	4566.49 (3.41)	17.03 (1.26)	3.10 (0.61)	1.10 (0.32)	–	–	–	–	573.47
5	E	3198.70 (3.34)	3.23 (0.63)	1.10 (0.32)	–	–	–	–	–	400.38
6	F	423.18 (2.63)	15.58 (1.22)	–	–	–	–	–	–	54.85
	Mean	2863.2	231.05	2.96	1.69	1.03	1.12	0.81		

Figures in parentheses indicate log (x + 1) transformation

CD (0.05) for dust formulations = 0.02
" " period of storage = 0.02
" " interaction = 0.04



cfu/g – colony forming unit per gram

Fig. 27 Shelf life of dust formulation

In product D and E, the initial viable count of 4.57 x 10^6 and 3.20 x 10^6 cfu/g, reduced drastically to 3.1 x 10^3 and 1.1 x 10^3 cfu/g respectively by 2 WAS. In formulation F which had 4.23 x 10^5 cfu/g on the day of preparation was drastically reduced to 1.56 x 10^4 cfu/g by 1 WAS and thereafter no viable fungal colonies were recorded.

4.7.2.2 Wettable Powder

4.7.2.2.1 Effect of Wettable Powder Formulation on Water Hyacinth

An experiment was set up under glass house condition to study the effect of 40 per cent WP formulation of *F. pallidoroseum* (Plate 16) on water hyacinth plants and the results are presented in Table 46 and Fig. 28.

Statistical analysis of per cent intensity of disease produced by wettable powder formulation revealed that there was significant difference between the treatments tested.

Maximum intensity of damage of 97.78 per cent was recorded in T_{28} (application of WP @ 10g/100 ml on 2.0 per cent CNSL sprayed plants) (Plate 17) followed by T_{27} (application of WP @ 5 g/100 ml on 2.0 per cent CNSL sprayed plants) gave 82.22 per cent.

In treatments where wettable powder was applied alone, maximum intensity of 48.89 per cent was produced by T_4 (@ 10 g/100 ml) followed by T_7 (@ 25 g/100 ml), T_6 (@ 20 g/100ml) and T_5 (@ 15 g/100 ml) being 39.11, 37.78 and 37.78 per cent respectively and were statistically on par. Least damage of 4.44 per cent was recorded in treatments T_1 (@ 1 g/100 ml) and T_2 (@3 g/100 ml).

When WP formulations was applied on 0.5 per cent CNSL sprayed plants, maximum damage of 56.89 per cent was recorded in T_{12} (WP @ 10 g/100 ml) which was significantly superior to treatments where wettable powder was applied alone. Treatments T_{11} (WP @ 5 g/ 100 ml), T_{14} (@ 20 g/100 ml) and T_{15} (WP @ 25 g/100 ml) induced more than 40 per cent

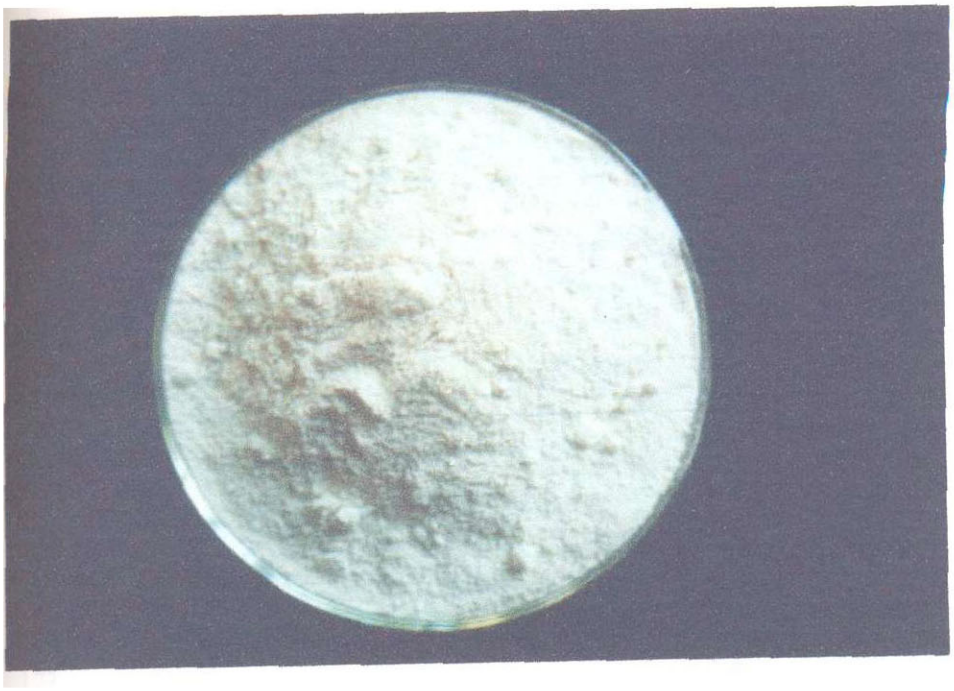


Plate 16. Wettable powder formulation

Table 46 Effect of wettable powder formulation on water hyacinth under glasshouse condition

Treatments	Mean disease intensity (%)	Treatments	Mean disease intensity (%)
T_1 – WP @ 1 g/100 ml	4.44(10.53)	T_{18} – $T_{16} + T_2$	26.67(31.09)
T_2 – WP @ 3 g/100 ml	4.44(10.53)	T_{19} – $T_{16} + T_3$	64.45(53.42)
T_3 – WP @ 5 g/100 ml	31.11(38.87)	T_{20} – $T_{16} + T_4$	71.11(57.52)
T_4 – WP @ 10 g/100 ml	48.89(44.36)	T_{21} – $T_{16} + T_5$	46.67(43.09)
T_5 – WP @ 15 g/100 ml	37.78(37.87)	T_{22} – $T_{16} + T_6$	46.67(43.09)
T_6 – WP @ 20 g/100 ml	37.78(37.87)	T_{23} – $T_{16} + T_7$	44.45(41.8)
T_7 – WP @ 25 g/100 ml	39.11(38.86)	T_{24} – 2 % CNSL	15.55(23.13)*
T_8 – 0.5 % CNSL	6.67(14.96)*	T_{25} – $T_{24} + T_1$	28.89(32.48)
T_9 – $T_8 + T_1$	24.45(29.58)	T_{26} – $T_{24} + T_2$	35.55(36.58)
T_{10} – $T_8 + T_2$	24.45(29.58)	T_{27} – $T_{24} + T_3$	82.22(65.15)
T_{11} – $T_8 + T_3$	40.00(39.23)	T_{28} – $T_{24} + T_4$	97.78(83.91)
T_{12} – $T_8 + T_4$	56.89(48.96)	T_{29} – $T_{24} + T_5$	73.33(58.91)
T_{13} – $T_8 + T_5$	37.78(37.91)	T_{30} – $T_{24} + T_6$	68.89(56.13)
T_{14} – $T_8 + T_6$	42.22(40.51)	T_{31} – $T_{24} + T_7$	66.67(54.74)
T_{15} – $T_8 + T_7$	42.22(40.51)	T_{32} – Talc spray	– (1.65)
T_{16} – 1 % CNSL	6.67(14.97)*	T_{33} – water + glycerol + Teepol	– (1.65)
T_{17} – $T_{16} + T_1$	24.45(29.58)	T_{34} – water	– (1.65)

Figures in parentheses indicate arc sine transformation
CD (0.05) = 5.15
*Per cent scorching

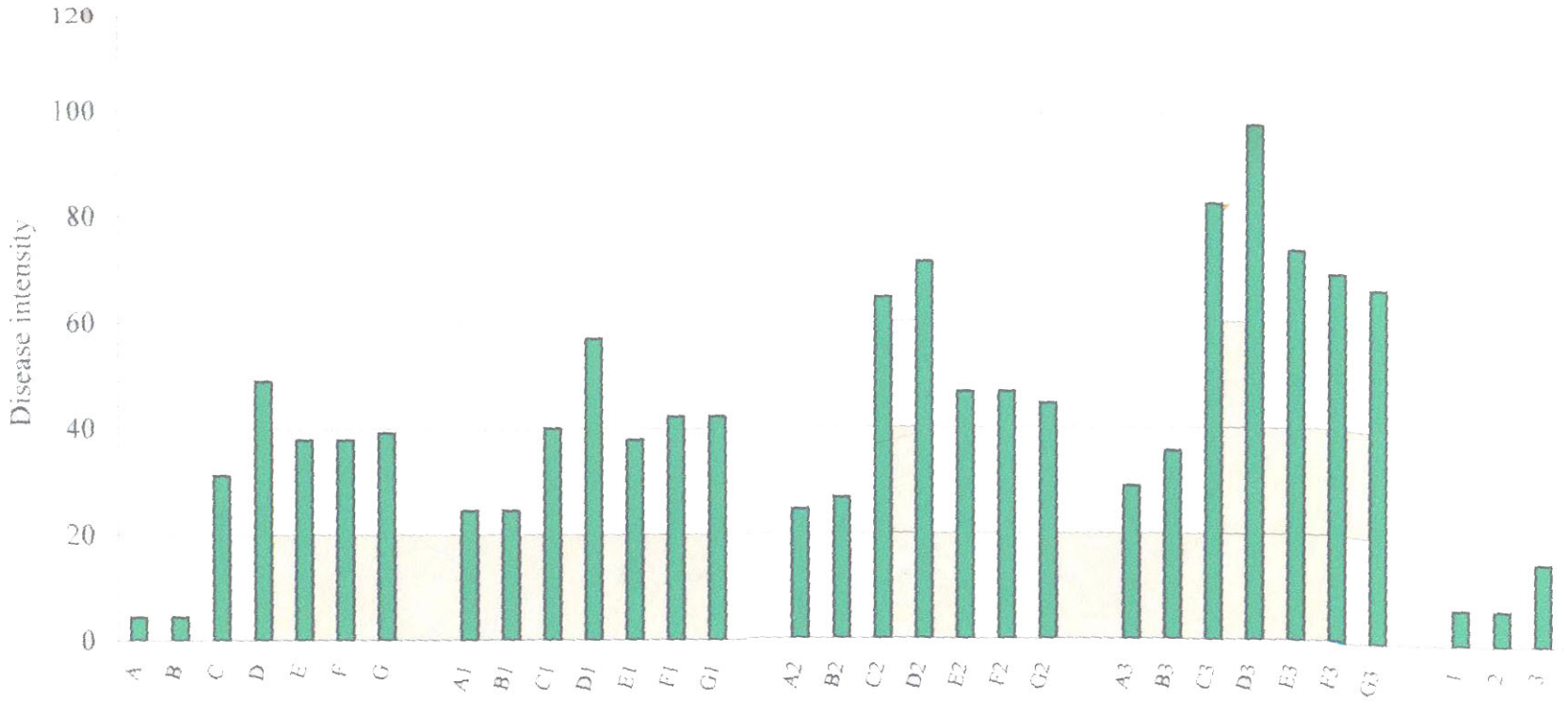


Fig. 28 Effect of wettable powder formulation on water hyacinth under glasshouse condition

Plate 17. Effect of wettable powder formulation on water hyacinth

disease intensity. Least damage of 24.45 per cent was produced in treatments T_9 (WP @ 1 g/ 100 ml) and T_{10} (WP @ 3 g/100 ml).

Spraying of CNSL at 1.0 per cent alone caused 6.66 per cent scorching on water hyacinth. In treatments involving application of WP on 1 per cent CNSL injured plants recorded a maximum damage of 71.11 per cent in T_{20} (WP @ 10 g/100 ml) followed by T_{19} (WP @ 5g/100 ml) being 64.45 per cent which were statistically on par, whereas, WP alone T_4 (@ 10g/100 ml) and T_3 (@ 5 g/100 ml) recorded only 48.89 and 31.11 per cent damage respectively. Least damage of 24.45 per cent was recorded in T_{17} (WP @ 1 g/100 ml) which was on par with 26.67 per cent in T_{18} (WP @ 3 g/100 ml).

Application of WP on 2.0 per cent CNSL sprayed plants (15.15 per cent scorching) recorded maximum mean per cent disease intensity. Treatment T_{28} (WP @ 10 g/100 ml) gave 97.78 per cent intensity of damage followed by T_{27} (WP @ 5 g/100 ml) which recorded 82.22 per cent intensity of damage. Least damage of 28.89 per cent was observed in T_{25} (WP @ 1 g/100 ml).

4.7.2.2.2 Shelf Life of Wettable Powder

Under Room Temperature

There was a reduction in the viability of spore from an initial count of 8.07×10^8 cfu/g to 6.39×10^7 cfu/g within 1 WAS and it reduced drastically to 7.0×10^4 cfu/g by 5 WAS (Table 47 and Fig. 29).

Refrigerated condition

The wettable powder formulation when stored under refrigerated condition, the spores retained its viability upto a period of four months (Table 48). As the storage period increased, there was a gradual reduction in the cfu/g of wettable powder (Fig. 30).

Wettable powder formulation recorded 8.07×10^8 cfu/g on the day of preparation which reduced to 6.47×10^7 cfu/g, 1 WAS and remained

Table 47 Shelf life of wettable powder stored at room temperature

Weeks after storage	Mean cfu /g x 10^5
0	8066.62 (3.91)
1	639.04 (2.81)
2	609.99 (2.79)
3	85.66 (1.94)
4	26.81 (1.44)
5	0.70 (0.23)
6	– –

Figures in parentheses indicate log (x + 1) transformation
CD (0.05) for period of storage – 0.05

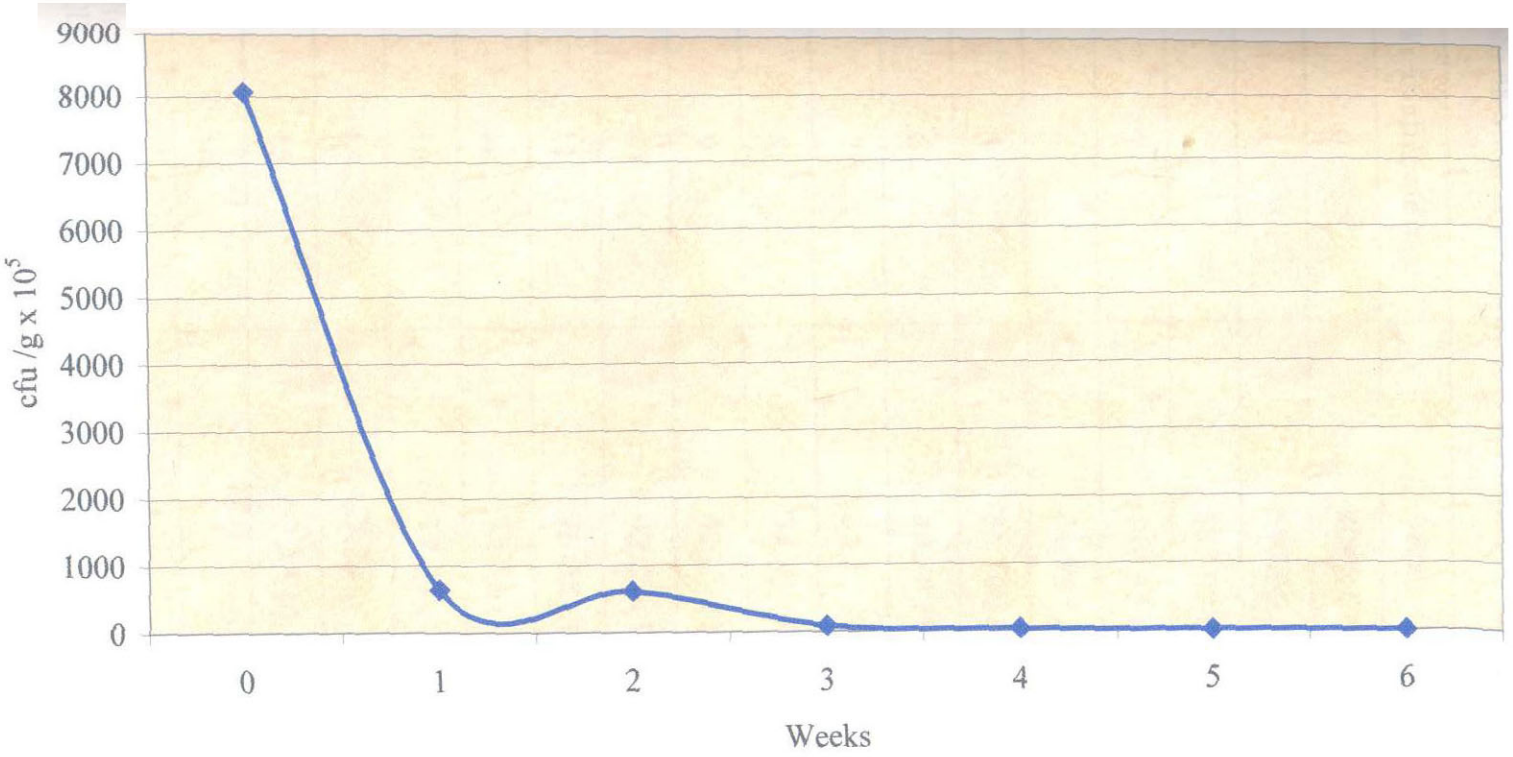


Fig. 29 Shelf life of wettable powder formulation under room temperature

'able 48 Shelf life of wettable formulation under refrigerated condition

Weeks after storage	Mean cfu /g x 10^5
0	8066.62 (3.90)
1	647.05 (2.81)
2	685.02 (2.84)
3	440.35 (2.64)
4	84.98 (1.93)
5	86.10 (1.94)
6	80.78 (1.91)
7	81.58 (1.92)
8	74.43 (1.88)
9	41.79 (1.63)
10	42.23 (1.64)
11	13.68 (1.17)
12	12.84 (1.14)
13	12.89 (1.14)
14	7.26 (0.97)
15	7.43 (0.93)
16	– –

F gures in parentheses indicate log (x + 1) transformation
C D (0.05) for storage period – 0.05

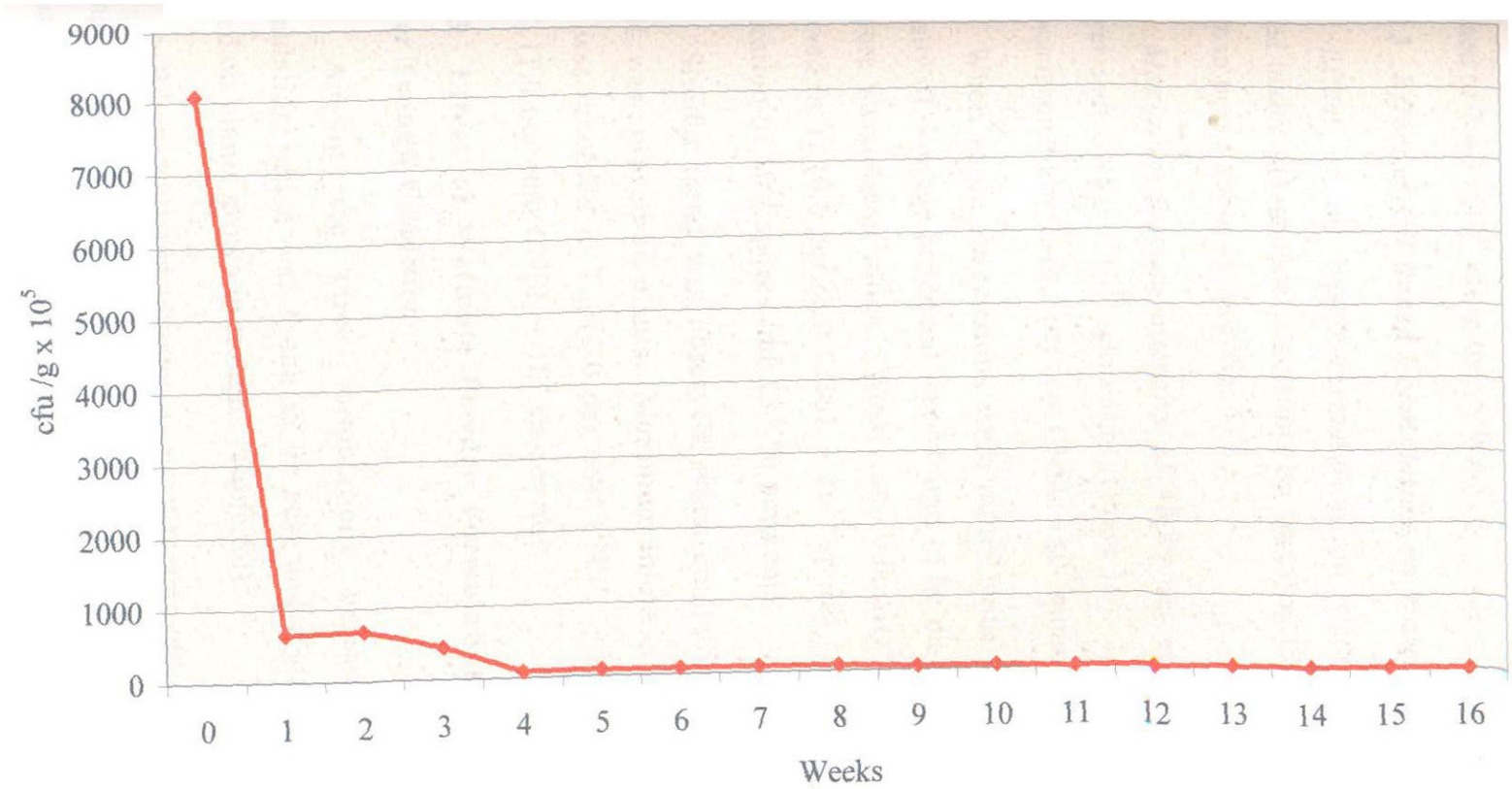


Fig. 30. Shelf life of wettable powder formulation under refrigerated condition

static and decreased to 8.50 x 10^6 cfu/g by 4 WAS. Thereafter the viable count remained static for a period of one month and then gradually declined to 7.43 x 10^6 cfu/g by 15 WAS.

4.7.2.3 Effect of Oil Based Formulation on Water Hyacinth

Effect of oil based formulation on water hyacinth plants was studied under glasshouse conditions by spraying the formulation on plants grown in pots (Table 49 and Fig. 31).

Maximum disease intensity of 48.89 per cent was recorded in T_{14} (2.0 per cent CNSL + 10^{11} spores/ml) (Plate 18). Least damage of 6.66 per cent was recorded by T_4 (0.5 per cent CNSL + 10^7 spores/ml).

When spore suspensions were mixed with 0.5 per cent CNSL, the intensity of damage produced were found to be on par with the application of spore suspension alone. Maximum intensity of 24.37 per cent was observed in T_6 (0.5 per cent CNSL + 10^{11} spores / ml) and was on par with application of 10^{11} spores /ml (31.06 per cent).

Similar trend was observed, when emulsion containing 1 per cent CNSL was sprayed on plants. Maximum intensity of damage of 28.84 per cent was recorded in T_{10} (1.0 per cent CNSL + 10^{11} spores/ml) followed by T_9 (1.0 per cent CNSL + 10^9 spores /ml).

4.7.3 Effect of Wettable Powder Formulation on Water Hyacinth under Trough Condition

Among the three formulations prepared, wettable powder formulation which was found to be best under glasshouse condition was tested on plants grown in troughs (Table 50).

When wettable powder alone was sprayed, mean disease intensity ranged from 32 – 46.67 per cent (Fig. 32). Similarly when CNSL alone was sprayed intensity of scorching ranged from 20 – 27.33 per cent. However when the formulation was applied on CNSL sprayed plants a

Table 49 Effect of oil based formulation on water hyacinth under glasshouse condition

	Treatments	Mean disease intensity (%)
T_1	10^7 spores / ml	17.66 (24.84)
T_2	10^9 spores / ml	24.37 (29.57)
T_3	10^{11} spores / ml	31.06 (33.36)
T_4	10^7 + 0.5 % CNSL	6.66 (14.95)
T_5	10^9 + 0.5 % CNSL	17.66 (23.12)
T_6	10^{11} + 0.5 % CNSL	24.37 (29.57)
T_7	0.5 % CNSL	6.66 (14.95)*
T_8	10^7 + 1 % CNSL	15.43 (23.12)
T_9	10^9 + 1 % CNSL	22.14 (28.06)
T_{10}	10^{11} + 1 % CNSL	28.84 (32.47)
T_{11}	1 % CNSL	6.66 (14.95)*
T_{12}	10^7 + 2 % CNSL	15.43 (23.12)
T_{13}	10^9 + 2 % CNSL	24.37 (29.57)
T_{14}	10^{11} + 2 % CNSL	48.89 (44.35)
T_{15}	2 % CNSL	10.88 (19.25)*
T_{16}	Water + glycerol + teepol	4.44 (2.54)*
T_{17}	Water spray	–

Figures in parentheses indicate are sine transformation
CD (0.05) for treatments – 3.37
*Per cent scorching

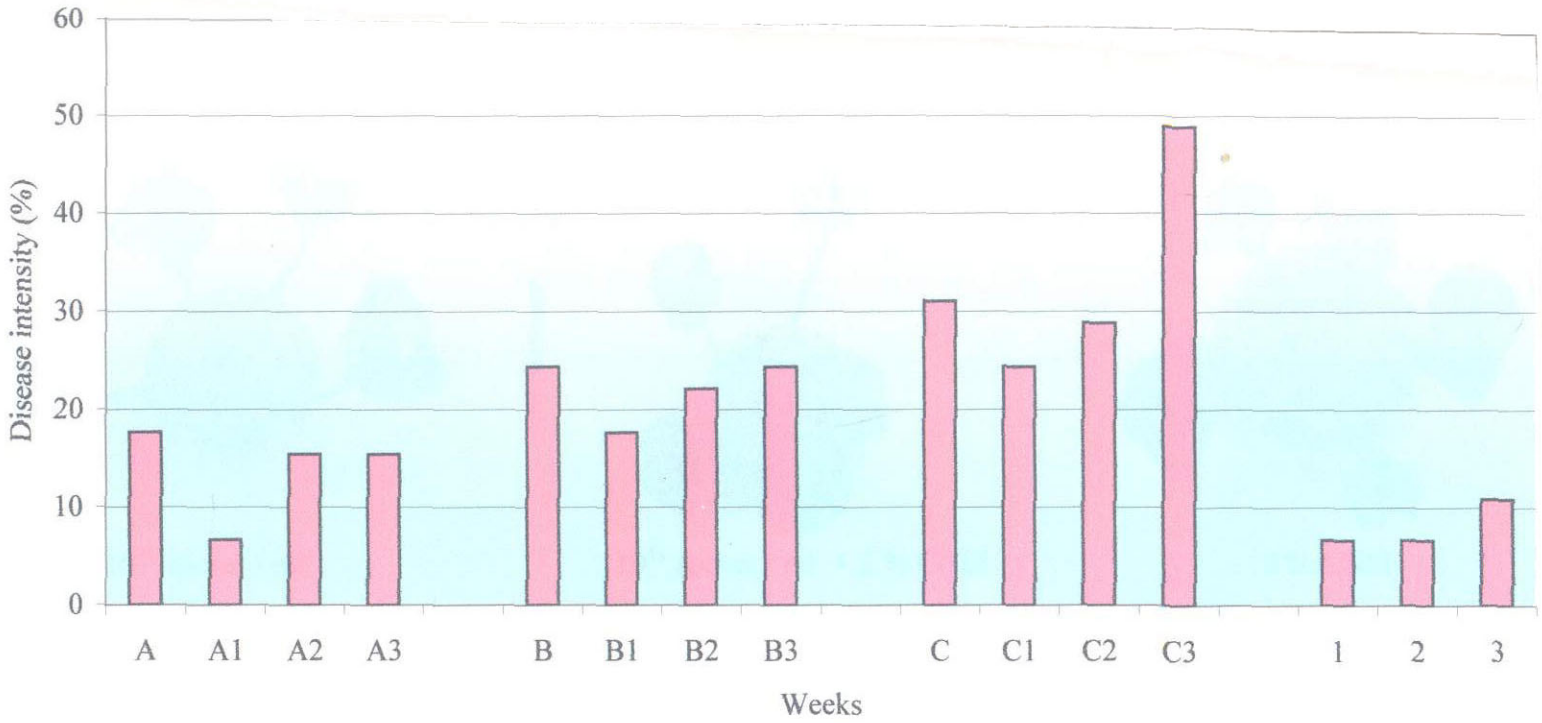
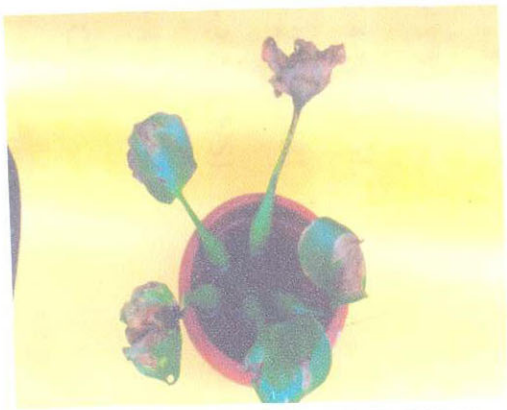
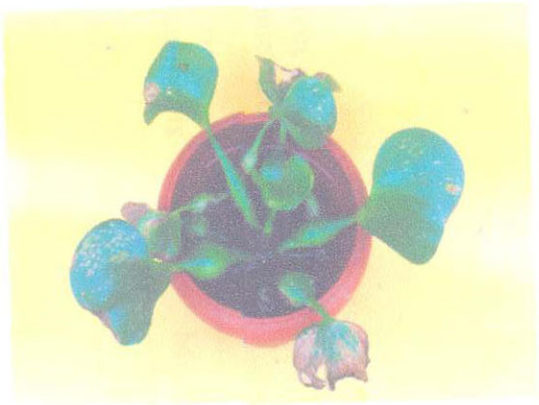


Fig. 31 Effect of oil based formulation on water hyacinth under glasshouse condition

10^{11} spores / ml



10^{11} spores / ml + 2 % CNSL



2 % CNSL

Plate 18. Effect of oil based formulation on water hyacinth

Table 50 Effect of wettable powder formulation on water hyacinth under trough condition

	Treatments	Mean disease intensity (%)
T_1	WP @ 5 g / 100 ml	32 (34.44)
T_2	WP @ 10 g / 100 ml	46.67 (41.94)
T_3	T_1 + 2 % CNSL	43.33 (41.17)
T_4	T_1 + 3 % CNSL	65.67 (54.13)
T_5	T_1 + 4 % CNSL	97.33 (81.82)
T_6	T_2 + 2 % CNSL	57.33 (49.22)
T_7	T_2 + 3 % CNSL	89.33 (71.06)
T_8	T_2 + 4 % CNSL	98.67 (84.03)
T_9	2 % CNSL	20.00 (26.57)*
T_{10}	3 % CNSL	23.33 (29.32)*
T_{11}	4 % CNSL	27.33 (31.52)*

Figures in parentheses indicate arc sine transformation
CD (0.05) – 4.00
*Per cent scorching

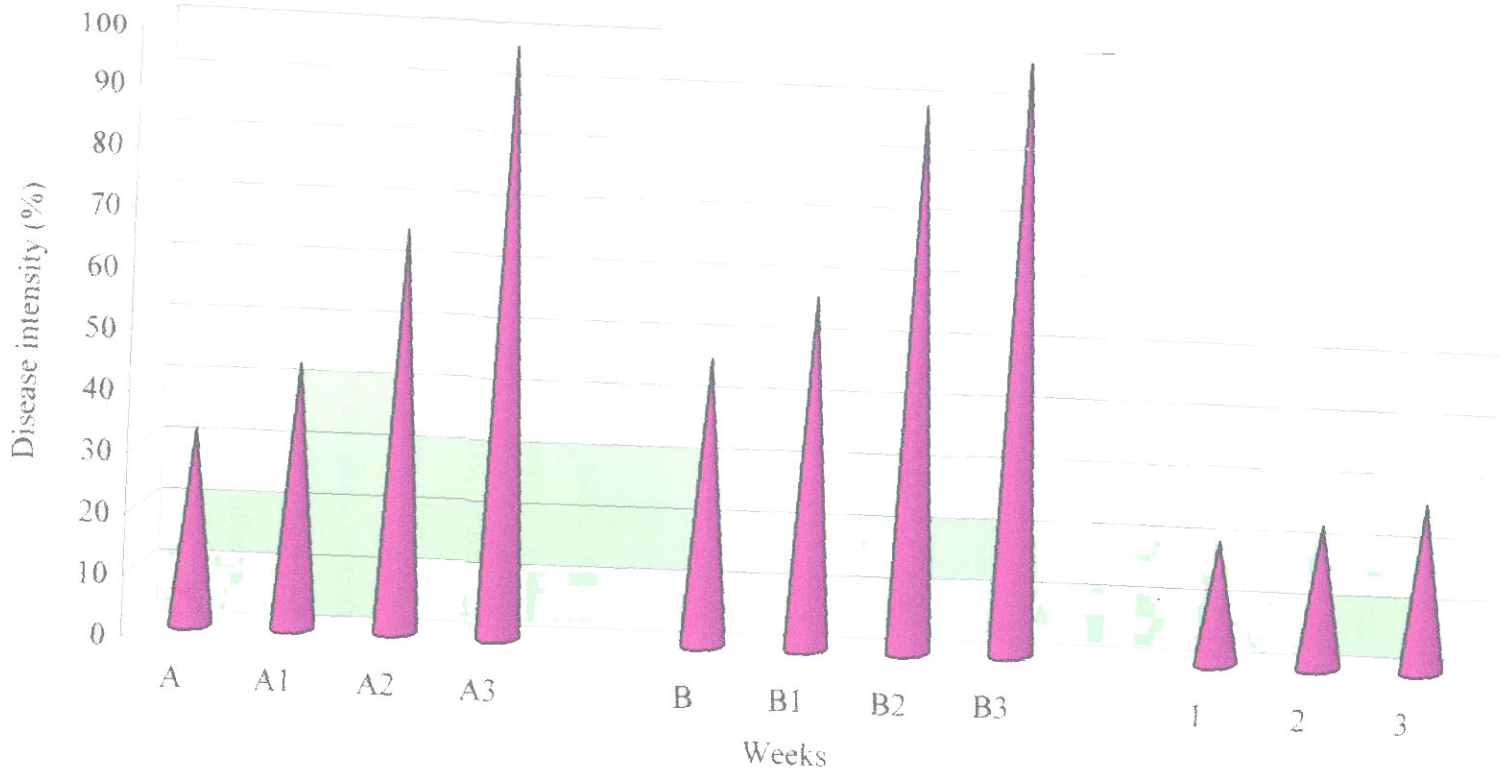


Fig. 32 Effect of wettable powder formulation on water hyacinth under trough condition

marked increase in disease intensity was observed in T_8 (98.67 per cent) and T_5 (97.33 per cent) and were statistically on par (Plate 19).

4.7.4 Field Trial

The water hyacinth plants exhibited typical blighting symptom on the fourth day of observation. The disease intensity from treatment plots ranged from 38.92 to 50.04 per cent (Table 51). The disease gradually spread from the leaves to the swollen petiole and the plants started sinking to the bottom of the lake. Seven days after application of treatment, the disease intensity ranged from 83.40 to 94.52 (mean – 91.43 per cent) (Plate 20). The plants from the control plots remained healthy and produced new healthy leaves.

4.8 EFFECT OF METABOLITES OF *F.PALLIDOROSEUM*, FORMULATED PRODUCT AND CNSL ON AQUATIC FAUNA AND FLORA

4.8.1 Metabolites

Fauna

Solutions of metabolites were sprayed on plants grown troughs where fishes, snails, frogs and water skaters were maintained. No change in the movement of fishes, snails, water skaters and frogs were observed in the initial hours and later stages also.

No lesion development on skin of frogs and fishes and no change in the colour of shells of snails were observed. Population of aquatic fauna tested remained constant before and after application of metabolites.

Flora

Metabolites when applied to aquatic flora *viz.*, Pistia, Lemna, Hydrilla, Salvinia and water lily had no toxicity symptoms.

4.8.2 Wettable Powder

No toxicity symptoms were observed on the flora and fauna tested.

Plate 19. Effect of wettable powder on water hyacinth (trough condition)

Table 51 Effect of wettable powder formulation on water hyacinth under field conditions

Plot No.	Mean disease intensity (%)	
	4 DAS	7 DAS
1 [CNSL (5%) + wettable powder 5 g/100 ml]	44.48	94.52
2	50.04	94.52
3	38.92	94.52
4	44.48	83.40
5	44.48	94.52
6	50.04	94.52
7	50.04	94.52
8	38.92	88.96
9	38.92	83.40
10 (Control)	-	-
11 (Control)	-	-
12 (Control)	-	-
13 (Control)	-	-
14 (Control)	-	-

A. 4 days after spraying

B. Close up

C. 7 days after spraying

Plate 20. Effect of wettable powder on water hyacinth (field condition)

4.8.3 CNSL (4.0 per cent)

CNSL at 4.0 per cent concentration did not produce any toxicity symptoms on fauna tested. On the leaves of Pistia, water lily and Lemna brown spots were observed.

4.8.4 Wettable Powder Application after CNSL Spraying

The formulated product (WP @ 10 g/100 ml) and CNSL (4.0 per cent) was sprayed sequentially on water hyacinth plants grown in troughs.

Fauna

Fifteen minutes after application of the product and oil, a change in the movement of fishes *viz.*, *Etroplus* sp. and *Puntius* spp. were observed. These fishes were found to swim near the surface layer of water for 1 hour. After that it reverted back to its normal behaviour towards interior layers. Fishes belonging to genus *Aplocheilus* spp. did not show any change in movement.

Snails were observed to move towards the edges of trough. They remained there for 2 h, later on they moved back to water hyacinth plants. No change in movement of water skaters and frogs were noticed.

Fishes and frogs were observed for any lesion development on the surface of skin. No change in colour or lesions on the skin were observed. Similarly in snails no discolouration on shells or rotting of flesh were observed.

Mortality rate was taken by observing initial and final population. No reduction in the population of fishes, snails, water skaters and frogs were recorded.

Flora

Yellowing of older leaves were observed on Pistia plants after the application of formulated product and oil. These leaves later became water soaked and dried up. But new leaves were produced and were free

from symptoms. On Lemna small brown spots were observed, but there was no further spread. On water lily brown spots with yellow halo were observed. Salvinia and Hydrilla did not show any damage on the leaves.

DISCUSSION

5. DISCUSSION

Water hyacinth (*Eichhornia crassipes*) and Salvinia (*Salvinia molesta* D. Mitch.) are the two water weeds which are seen in vast areas of Southern Kerala. The problem of Salvinia was managed successfully by biological means using the weevil (*Cyrtobagus salviniae* Calder and Sands). This is a success story of biological control of weeds in Kerala. In the present study attempts were made to manage water hyacinth by integrating biocontrol fungi, herbicides and oils. Attempts were also made to formulate biocontrol fungi for field use.

Preliminary studies conducted on the biological control of water hyacinth (Santhi, 1994; Susha, 1997) resulted in the isolation and purification of three potentially useful biocontrol agents *viz.*, *Fusarium pallidoroseum, F. equiseti* and *C. gloeosporioides*. In order to find out more number of pathogens associated with water hyacinth and also to use them in managing water hyacinth a survey was conducted in four southern districts of Kerala, where problem of this weed is severe.

The survey yielded 18 different fungi from water hyacinth of which *Curvularia lunata, Colletotrichum gloeosporioides, Fusarium pallidoroseum, F. moniliforme, F. oxysporum* and *Myrothecium advena* were frequently observed in all the areas.

Among the fungi *M. advena* isolated from Thiruvananthapuram, Alappuzha and Kottayam district was a new report on water hyacinth. The symptoms were observed mainly on leaves and rarely on stem, initially as roundish oil soaked spots turning brown towards the later stages. Individual spots enlarged with rounded side facing the petiole and tapering to a narrow point in the direction of the laminar tip. On the upper surface distinct concentric zonations appeared which present a target board appearance. The fruiting bodies of the fungus were noticed on the upper surface of the leaves along the concentric rings.

Apart from water hyacinth the *M. advena* has been reported only from coffee (Nagraj and George, 1958)

Among the different fungi isolated, *M. advena* and *C. lunata* were found to be present in highest frequency, irrespective of season and location. Of the different species of *Fusarium, F. pallidoroseum* was found to occur in maximum frequency and present throughout the year. Other species of *Fusarium viz., F. oxysporum* and *F. moniliforme* were mainly present during rainy season.

It was also observed that *Pestalotia* sp. and *Helminthosporium* sp were present during rainy season whereas *Alternaria eichhorniae* and a sterile fungus were present throughout the year. Work conducted by Jamil and Rajagopal (1986) revealed that species of *Fusarium, Alternaria and Helminthosporium* appeared in the winter season.

In the present study *C. gloeosporioides* was present on water hyacinth during summer and rainy season. Butler (1951) reported that anthracnose caused by *C. xanthii* on Bathurst Burr was considerably reduced by periods of hot, dry weather. Also the disease declined when monthly rainfall was less, and low mean maximum temperature favoured disease development.

In this study, the presence of *Fusarium* spp throughout the period of observation led to the conclusion that this pathogen can survive in the off season and make its presence during the rainy season, when the host plants have a thick vegetative growth. Also this fungus has the capacity to thrive in moist condition for a long time.

The frequency of occurrence of fungi on plants varied with season. However, unlike in other parts of India, in Kerala well defined seasons are not seen. The major seasons in Kerala are rainy and summer season. Hence the variations on the population of varying groups of organisms on plant surface is not as pronounced as in other parts of the country.

Unlike other crop plants water hyacinth always grows as a thick carpet where there is not much difference in the microclimatic during dry and rainy season. Further the high humidity and temperature available in that niche encourage general growth of fungi.

However the presence of the waxy cuticle prevents infection of this weed by many of the common pathogens. Only those pathogens which can cross this barrier can induce disease on water hyacinth. This is one of the reason why eventhough conditions are favourable for the development of fungi less number of fungi are seen compared to other plants.

Correlation studies indicated a significant positive correlation between occurrence of fungi and minimum temperature, total rainfall, number of rainy days and relative humidity and a negative correlation with maximum temperature.

It was observed that maximum temperature ranged from 28-35°C and minimum temperature (19-24°C) in different districts surveyed. The temperature in places where water hyacinth was growing also varied from 19-35°C. Most of the genera isolated from water hyacinth preferred a temperature between 15-37°C (Bilgrami and Verma, 1978).

In order to prove the pathogenicity of the fungi isolated from water hyacinth plants, they were artificially inoculated on test plants. Out of the 21 fungi tested (including three isolates from previous studies), 17 were found to be pathogenic.

The pathogenic nature of fungi isolated were proved by earlier workers. *C. lunata* (Abdel-Rahim and Tawfig, 1984), *Colletotrichum gloeosporioides* (Santhi and Naseema, 1995), *F. equiseti* (Agharkar and Banerjee, 1932), *F. moniliforme* (Abdel-Rahim and Tawfig, 1984), *F. oxysporum* (Jamil and Rajagopal, 1986), *F. pallidoroseum* (Jamil and Rajagopal, 1986), *Pestalotia* sp. (Singh *et al.*, 1985), *Alternaria eichhorniae* (Nagraj and Ponnappa, 1970), *Helminthosporium* sp.

(Balasooriya *et al.*, 1984), *Rhizoctonia solani* (Rakvidyasastra and Visarathanonth, 1975) and a sterile fungus was reported by Jamil and Rajaopal (1986).

The fungi isolated differed in their ability to cause infection in water hyacinth. The maximum disease intensity of 61.11 per cent was recorded with *M. advena* and 53.44 per cent with *F. pallidoroseum* collected during previous survey and maintained in the department.

Santhi (1994) reported that 51.10 per cent intensity of infection by *F. semitectum* (Syn. *F. pallidoroseum*) on water hyacinth plants, whereas, *F. pallidoroseum* obtained from the present study recorded a maximum of 45.09 per cent intensity of infection.

The symptoms observed in the field under natural conditions and those observed on artificial inoculation differed slightly with *M. advena*. Under natural conditions the fungus produces brown dry spots which slowly enlarges and result in blighting of leaves. But on artificial inoculation water soaked dull greenish lesions appeared on leaves and petiole which spread within a short time resulting in rotting of the plant. Similar variations have been recorded for other pathogens also.

Morphological characters of those fungi which caused more than 40 per cent disease intensity upon artificial inoculation were only studied in detail. The isolates of *Fusarium* obtained from the present survey were identified as *F. pallidoroseum, F. moniliforme* and *F. oxysporum*. Similarly based on morphological, cultural and artificial inoculation studies, the *Alternaria* and *Myrothecium* isolates were identified as *A. eichhorniae* and *M. advena* respectively.

The determination of host range is an important component in developing a plant pathogen into a bioherbicide. The safety to non target economic and wild plants must be assessed before experimental release

and commercial use. A pathogen causing sufficient damage and has reasonably narrow host range could be used safely.

The host range studies were conducted only with four promising fungi *viz.*, *M. advena, F. pallidoroseum, F. equiseti* and *C. gloeosporioides*. Water hyacinth being a plant which is very common in Kerala, while studying the host range of the pathogens, crop plants and weeds commonly seen in this agroecological system were taken as test plants. Thus 53 species of cultivated plants and 54 species of weeds coming under 47 families were screened against the four pathogens.

Among the four pathogens *M. advena* was found to have a wide host range covering 27 cultivated plants and 45 weed plants. Among them, included some of the economically important crop plants of Kerala *viz.*, rice, papaya, tapioca, clove, pepper, betelvine etc. Paddy fields which may have direct link with the water hyacinth growing belt may get infected if this pathogen is used as a biocontrol agent for managing the weed.

Similarly weeds that are seen around the paddy fields, coconut gardens and homestead are also found infected by this highly virulent pathogen of water hyacinth. Thus the broad host range and the similarity of the ecosystem of the host plants of *M. advena* limits its usefulness as a potential biocontrol agent of water hyacinth. Observations similar to this was made by Caunter and Lee (1996). They found that a related pathogen (*M. roridum*) of water hyacinth, eventhough a potent organism for biological control of water hyacinth, it could not be used for the management of this weed because of its wide host range.

The intensity of damage on water hyacinth by *F. pallidoroseum* was only next to *M. advena*. Its host range was less compared to *M. advena*. Among the cultivated plants tested, amaranthus, tomato, banana, cashew, colocasia and papaya were susceptible. Even in these host plants the *F. pallidoroseum* could initiate infection only when the host surface was

injured. This indicates that this is a weak pathogen unlike *M. advena* where it could induce the disease even without the injury.

Even among the weeds *F. pallidoroseum* was pathogenic mostly to terrestrial flora rather than those seen near water ways and paddy fields. The only exception being *Monochoria vaginalis*.

The host range of *F. pallidoroseum* isolated from insect pests (cowpea aphid) was conducted by Hareendranath (1989) and Rejirani (2001). They reported that this fungus is not pathogenic to rice or common crop plants. However the fungus isolated from aphid could not infect tomato, amaranthus and banana eventhough the strain isolated from water hyacinth was pathogenic to these crop plants. This difference may be due to strain variation observed in pathogenic microorganisms.

The host range of *F. equiseti* was almost similar to that of *F. pallidoroseum*. It could infect only eight cultivated and 15 weed plants tested. The host range of *F. equiseti* was studied by Abdel-Rahim and Tawfig (1984) and Naseema and Balakrishnan (1999) and they reported a narrow host range for the pathogen.

C. gloeosporioides is a ubiquitous pathogen in the humid tropics. This fungus has been reported on a wide variety of crop plants grown in Kerala and in other sub tropics (Vasudeva, 1960; Peethambaran and Wilson, 1970).

However, isolates of *C. gloeosporioides* are highly variable within the species, consisting of different strains (Karunakaran, 1981). *C. gloeosporioides* isolated from water hyacinth have a narrow host range and it could infect only eight cultivated and 15 weed plants. The narrow host range of *C. gloeosporioides* isolated from water hyacinth was also reported earlier by Susha (1997).

According to Chetti *et al.* (1999) *C. gloeosporioides* isolated from *Chromolaena odorata* is host specific and safe to be used as a

mycoherbicide. They found that none of the field crops *viz.*, rice, wheat, sunflower, groundnut, cowpea, greengram, soybean and cotton and plantation crops *viz.*, coconut, arecanut, pepper, betelvine, cocoa, coffee and cardamom were susceptible to *C. gloeosporioides* isolate of *C. odorata*.

Symptom development in many of the diseases are associated with the toxins produced by the pathogen. The culture filtrates of fungi may reproduce the symptoms as those incited by the fungi. This indicates the presence of toxic substances in the filtrate and its role in disease development.

In order to find out whether the four pathogens associated with water hyacinth produce toxin, a study was conducted to isolate and purify the toxic components from them.

The cell free culture filtrate of *M. advena* when applied on water hyacinth could induce scorching and produce 97.75 per cent damage. The cell free metabolites of *F. pallidoroseum* (48.84 per cent), *F. equiseti* (10.88 per cent) and *C. gloeosporioides* (42.17 per cent) also produced symptoms on water hyacinth. The intensity of damage was less compared to *M. advena*. This clearly indicates that the pathogenicity of these organisms are related with the toxin produced by them.

Several workers had reported the phytotoxicity of culture filtrates of pathogenic fungi such as *Alternaria eichhorniae* (Nagraj and Ponnappa, 1970) and *F. pallidoroseum, F. equiseti* and *C. gloeosporioides* on water hyacinth (Santhi, 1994 and Susha, 1997) and *Phoma herbarum* on *Lantana camara* (Pandey *et al.*, 2002).

In some fungi the host range of the pathogen and toxin produced by them are similar while in others it may show differences. Cell free filtrates of *F. equiseti* and *C. gloeosporioides* did not produce any symptom on crop and weed plants which were originally infected by the pathogen under artificial inoculation. While metabolite from *F. pallidoroseum*

developed symptoms only on one weed plant (*Monochoria vaginalis*) which belonged to the same family of water hyacinth. The culture filtrates of *M. advena* had a wide host range and could produce symptoms on eight cultivated and nine weed plants.

The host range of *F. pallidoroseum* was narrow both when artificially inoculated with cultures or when cell free extracts were sprayed. The toxin produced by *F. pallidoroseum* was also specific towards water hyacinth and a weed plant (*Monochoria vaginalis*) from the same family. This further points out to the specificity of this organism, making it an ideal candidate for biological control of water hyacinth.

Symptom producing ability of the filtrates decreased with dilution. Eventhough *M. advena* toxin was the most potent in inducing the symptom in concentrated form, it failed to develop symptom at lowest concentration of one per cent on water hyacinth. While *F. pallidoroseum* which was not as potent as *M. advena* toxin could produce symptom even at the lowest concentration of one per cent.

C. gloeosporioides toxin also behaved like *M. advena* while *F. equiseti* toxin was the least effective and lost its ability to produce symptoms when it was diluted to 50 per cent. This study indicates that even a very small concentration of toxin produced by *F. pallidoroseum* can induce symptoms on water hyacinth thereby indirectly indicating the host specificity of the pathogen.

The effect of dilution on reducing the symptom development was reported by several workers. Vidhyasekaran (1977) observed that the toxin produced by *Helminthosporium nodulosum* was highly active upto 1:10 dilution but further dilution reduced its activity markedly. According to Maji and Prasanta (1986) dilution of culture filtrate of *Rhynchosporium oryzae* recorded appreciable degree of phytotoxicity upto 40 per cent concentration. On further dilution, phytotoxic effect was greatly reduced.

The toxins of *M. advena* and *F. pallidoroseum* are highly thermostable in nature and could withstand even autoclaving. *C. gloeosporioides* toxin was inactivated on autoclaving while in *F. equiseti* the toxin was inactivated when temperature was increased to 50°C.

The ability to withstand high temperature shows that the toxin is a stable product and it will be able to withstand the alternating temperature fluctuations to which the pathogen is exposed.

Pandey *et al.* (2002) conducted thermal sensitivity test of cell free culture filtrate of *Phoma sorghina* at 121°C for 15 minutes and found that the toxin produced by the fungus was temperature tolerant and thermostable.

In the present investigation, experiment was conducted to study the effect of different pH on the cell free metabolites. The efficacy of metabolites of fungi was affected by changes in pH.

Metabolites of all the four fungi tested preferred a neutral pH range. Extreme acidic and alkaline condition, reduced the activity of cell free metabolites. But *F. pallidoroseum* metabolites recorded only 13.67 per cent inhibition over control at an alkaline pH of 9.0. Whereas *C. gloeosporioides* metabolites at acidic pH of 3.0 showed only 15.84 per cent inhibition over control.

According to Vidhyasekaran (1977) the toxin produced by *Helminthosporium nodulosum* was stable at pH 3-7, but unstable at alkaline range.

Attempts were made to isolate the toxic principle from the cell free, metabolites of four fungi. From the culture filtrate of *F. pallidoroseum* needle shaped white crystals were isolated. These crystals when spotted on paper chromatograph, exhibited bluish pink coloured spots with Rf value of 0.15 which is an indication of fusaric acid. Paterson and Rutherford (1991) working with *F. oxysporum* obtained a similar dark blue

coloured spot with an rf value 0.16. They identified that to be fusaric acid. The toxin was further purified and spotted on thin layer chromatograph, where again bluish pink colour developed under iodine vapours indicating the presence of pure toxin.

For structural confirmation of the toxic principle, it was subjected to NMR spectral analysis. The presence of the peak observed towards the down field of the spectra at δ 9 is an indication of a pyridine ring and the peak obtained towards the upfield of the spectrum at δ 0.9 as a triplet is an indicative of a methyl group coupled to a two proton methylene group. Similarly the peak observed at δ 1-1.5 in the form of a multiplet indicated that the methylene groups are attached to other dissimilar groups resulting in different couplings.

Analysis of the integration curve revealed that triplet obtained at δ 1.2 – 1.5 contained double the number of protons than when compared to the first triplet at δ 0.9. So it can be presumed that the first peak being methyl and the second one contains six protons $(CH_3)_2$. These two peaks indicate the butyl side chain of fusric acid.

Multiplets obtained at δ 7.7-7.8 and 4.4 – 4.6 could be due to three protons of pyridine ring with different interactions *viz.*, –COOH and butyl group at second and sixth position. The integration curve of these peaks indicated the presence of three protons.

The peak indicative of –COOH group was not evident in the spectrum presumably due to the shielding effect of π electrons present in the pyridine ring which otherwise would have appeared at δ 11-12. So by NMR spectral analysis of pure crystalline toxin, confirmed the presence of fusaric acid.

Eventhough a brownish precipitate was obtained from the cultural filtrates of *F. equiseti* it did not develop any coloured spot in paper chromatograph and further purification was not successful. The fungus

F. equiseti on artificial inoculation on water hyacinth also produced symptoms of less intensity compared to *F. pallidoroseum* may be due to the absence or lower quantity of toxin produced by the fungus. David (1969) observed that symptom development by *F. oxysporum* is proportional to the toxin produced.

Toxic principle could not be extracted from *C. gloeosporioides*. On extraction of cultural filtrates of *M. advena* dull brownish oily precipitate was obtained. However, it did not develop any well defined spots on chromatograph paper.

The presence of fusaric acid in Fusaria associated with water hyacinth was indicated by Susha (1997) and Naseema *et al.* (2001b).

In 1969 David, also isolated the phytotoxin fusaric acid from the plants infected with *Fusarium* spp. Other compounds such as moniliformin (Cutler, 1986), fumonisins (Abbas *et al.*, 1991), zearalenone, deoxynivalenol and T-2 mycotoxins (Bilgrami *et al.*, 1995), polypeptide (Jin *et al.*, 1996) and 9, 10-dehydro fusaric acid (Zonno *et al.*, 1996) were isolated from *Fusarium* spp.

In order to confirm the role of fusaric acid in symptom development, the purified toxin of *F. pallidoroseum* was applied on water hyacinth. Typical symptoms associated with the disease was obtained on water hyacinth. This confirms the role of fusaric acid in disease development.

Singh and Saxena (1997) observed that fusaric acid produced by *Fusarium solani* and *F. equiseti* produced typical symptoms of wilting, early yellowing and defoliation in cauliflower indicating a positive role of toxin in disease development.

Role of fusaric acid in disease development indicates the possibility of using fusarial toxins for management of water hyacinth. The work conducted by Abbas *et al.* (1995) indicated the use of phytotoxins of *Fusarium* spp. against weeds.

In order to use the biocontrol agents for the management of water hyacinth it is necessary to develop methods for production of large amounts of the fungal inoculum. As water hyacinth is seen in localised area it is better to mass multiply the biocontrol agents using locally available material for its growth.

In the present study 10 commonly available solid substrates were used for mass multiplying *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides*. Among the solid substrates tested, *F. pallidoroseum* and *F. equiseti* grew and sporulated well in rice bran and rice bran + coirpith. Coconut oil cake and gingelly oil cake were the most favoured substrates for *C. gloeosporioides*.

The present study with respect to growth and sporulation of *Fusarium* spp., is in agreement with that of Faizal (1992) who reported wheat bran and rice bran as good substrates for growth, sporulation and virulence of *F. pallidoroseum*. Work done by Santhi (1994) and Susha (1997) concluded that wheat bran and rice bran were good materials for *Fusarium* spp. and *C. gloeosporioides* isolates from water hyacinth.

Among 14 liquid substrates tried groundnut oil cake extract gave maximum mycelial weight of *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides*. Sporulation and spore viability was less in groundnut oil cake extract compared to other substrates.

Out of the liquid substrates, coconut water and jaggery water supported maximum sporulation and spore viability of *Fusarium* spp. Coconut water was reported to be a good substrate for maximum sporulation of biocontrol fungi by many authors (Ibrahim and Low, 1993; Manisekarame and Letchoumanane, 1996; Rejirani, 2001).

Whenever a substrate is selected for mass multiplication two things are to be taken into account *viz.*, ability of the organism to grow in the substrate and ability to withstand minimum viable propaules (10^6) for a

longer period of time. In most of the biocontrol agents a minimum population of 10^6 cfu/g of substrate is required for effective management.

In the present study, rice bran and coirpith + rice bran retained *Fusarium* spp. for a period 10-12 weeks, thereafter it declined. Among the liquid substrates, jaggery water and coconut water recorded 10^6 cfu of *Fusarium* spp. for a period of 17-19 days.

The results of the study indicated that rice bran, coconut water and jaggery water could be used for the mass multiplication of *F. pallidoroseum*. Rice bran and coconut water are two locally available substrates in Kerala. Also coconut water a by product of the copra industry which is currently wasted. This is could be used for large scale production of *F. pallidoroseum*.

Integration of herbicides at lower doses with biocontrol fungi could impose an additional stress to the weed making it more susceptible to infection by the biocontrol agent resulting in better weed management.

In vitro studies on the effect of five herbicides *viz.*, paraquat, glyphosate, anilofos 24 % + 2,4-D 32 % EC, 2,4-D sodium salt and pretilachlor on the growth and sporulation of *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides* revealed that except pretilachlor all the other herbicides supported growth of fungi at lower concentrations. Herbicides that was not inhibitory to the growth and sporulation of the test fungi at 50, 30 and 10 ppm concentrations weakened the water hyacinth plants.

When biocontrol fungi and herbicides were simultaneously applied maximum disease intensity was recorded by the application of anilofos 24 % + 2,4-D 32 % EC 30 and 50 ppm with *F. pallidoroseum* (68.86 and 62.18 per cent respectively). Sequential application of herbicides and biocontrol fungi recorded a decrease in disease intensity than applying the fungi alone.

The results of the study indicate that many of the commonly used herbicides are not effective when used in combination with the biocontrol

agents. However, anilofos 24 % + 2,4-D 32 % EC which is a combination product of 2,4-D sodium salt could be mixed with *F. pallidoroseum* to enhance its efficacy.

It was already reported that cell enlargement could be induced by low levels of 2,4-D sodium salt which is indirectly due to increased activity of autolytic enzymes that is responsible for cell wall loosening. Herbicides also affects the integrity of leaf surface by acting on the thick waxy cuticle.

In the present experiment 2,4-D sodium salt and its combination product (anilofos 24 % + 2,4-D 32 % EC) was used at still lower concentrations. Thus, the ill effect of 2,4-D sodium salt on the fungus was reduced considerably and at the same time it might have weakened the growth of water hyacinth thereby increasing the ability of *F. pallidoroseum* to infect the weed.

Charudattan (1986) reported that spraying water hyacinth with the mycoherbicide *Cercospora rodmanii* and sublethal rates of 2,4-D sodium salt (154 ppm) resulted in improved weed control compared to using both alone.

Liu and Lozano (1987) observed that sequential application of propanil (1 kg ai/ha) followed by the mycoherbicide Collego was not satisfactory in managing jointvetch and water primrose in rice fields resulting in poor yield.

Most effective treatment in integrating biocontrol fungi and herbicides when tried under trough condition, recorded only 26.67 per cent disease intensity in the treatment simultaneous application of *F. pallidoroseum* and anilofos 24 % + 2,4-D 32 % EC (30 ppm).

Oils are used to formulate biocontrol fungi as it enhance disease development.

Egley and Boyette (1995) reported that the water unrefined maize oil emulsion of *Colletotrichum truncatum* improved conidial germination and did not effect appressoria formation. The emulsion enhanced mycoherbicide efficacy by increasing conidial germination and protecting the conidia during dew free period.

Among the different oils sprayed on water hyacinth plants, CNSL at different concentrations produced brown spots on leaf lamina.

For formulating only *F. pallidoroseum* was used as it was effective against water hyacinth and also it had a narrow host range among the cultivated plants. *M. advena* eventhough highly effective was not used as it was pathogenic to many of the cultivated plants.

None of the formulation ingredients *viz.*, CNSL, Tween-80, glycerol, paraffin liquid and sodium alginate inhibited the growth and sporulation of *F. pallidoroseum*. Based on the results, ingredients *viz.*, CNSL, Glycerol and Tween-80 were used for formulating the fungus.

The formulation ingredients tested, differed in their ability to promote growth and sporulation. This may be due to the differences in the pH and nutritional composition of the compounds tested (Burges, 1998). Moisture on plant surface and relative humidity play key role in the infection and sporulation processes of foliar fungal pathogens. Adjuvants like glycerol and Tween-80 can reduce the moisture requirement of spores.

Based on the high germination, good spore production and normal mycelial development of *C. gloeosporioides* f. sp. *malvae* by Tween 20, starch, Bioveg and sucrose, Grant *et al.* (1990a) reported that these adjuvants have the potential to enhance the development of the fungus on plants and reduce dew period requirements.

Of the different dust formulations prepared, product A containing 10 per cent mycelium gave maximum disease of 20 per cent on water hyacinth plants. An increase in the intensity of disease was observed

when dust formulations were applied on plants previously sprayed with CNSL. Maximum intensity of damage (68.89 per cent) was recorded by dusting product A on two per cent CNSL sprayed plants.

Oils play a role in modifying the integrity of thick waxy leaf surface of water hyacinth plants and thereby facilitating fungal penetration and establishment. Oils also reduce the free moisture requirement of spores for germination.

The increase in infection by the dust formulation may be due to enhanced penetration of fungus through the injury produced by the CNSL on the surface of plant. CNSL is a byproduct of the cashew processing industry and is available in large quantities in Kerala. This material being of plant origin may not cause pollution problems in water ways at the concentration tired.

It was observed that the viability of spores in the dust formulations were reduced drastically when stored at room temperature. Dust formulations containing sucrose retained viability for one month. This may be due to the capacity of sucrose to retain moisture.

Accordig to Faizal (1992) *F. pallidoroseum* spores retained 75 per cent viability till 4 DAS and thereafter a significant decrease was noticed in the virulence of the fungus in water, talc and diatomaceous earth formulations. The present study and studies conducted by other workers clearly indicate that dust formulation is not suitable for *F. pallidoroseum* as the spore viability of the fungus could not be retained in this formulation for a longer period of time and the intensity of infection produced is less.

Wettable power formulation recorded maximum intensity of disease. Application of wettable powder @ 5 g and 10 g/100 ml on two per cent CNSL sprayed plants showed 82.22 and 97.78 per cent disease intensity respectively.

According to Butt *et al.* (2001) wettable powder comprising of a hydrophilic carrier material with hydrophobic spores was the most efficient formulation of fungal control agents. Wettable powder formulation of *Cercospora rodmanii* was found to be successful for the management of *Eichhornia crassipes* (Te Beest, 1991).

Wettable powder when stored under refrigerated condition retained viability of spores for a period of four months. But under room temperature, viability of spores reduced drastically 5 WAS.

The result of the present experiment is contradictory to Sunitha (1997) who reported that the viability of *F. pallidoroseum* formulation could be retained for 10 months, under refrigeration when stored as wettable powder using diatomaceous earth as carrier material. Two factors *viz.*, strain variation of the fungus and carrier material used may be attributed to the variation in the viability between the observations made by Sunitha (1997) and in the present study.

Oil based formulation of *F. pallidoroseum* (10^{11} spores/ml) and CNSL (2 per cent) recorded only 48.89 per cent disease intensity. According to Boyette *et al.* (1993b) application of *Colletotrichum truncatum* in an invert emulsion formulation gave 100 per cent control of hemp sesbania.

Among the three formulations prepared, wettable powder formulation was found to be the best under glasshouse condition. The effect of wettable powder formulation was enhanced when applied on plants sprayed with CNSL (2 per cent).

Under trough conditions application of wettable powder @ 5 g and 10 g/100 ml on CNSL (4 per cent) sprayed plants recorded 97.33 and 98.67 per cent disease respectively.

Under field condition since the water hyacinth plants were much more vigorous and growing in a vast floating ecosystem, five per cent

concentration of CNSL was used for pretreating the plants. Intensity of damage ranged from 38.92 to 50.04 on fourth day and 83.40 to 94.52 on seventh day of observation respectively. The plants started sinking to the bottom of the lake indicating effectiveness of the treatment.

The metabolites and wettable powder formulation of *F. pallidoroseum* did not produce toxicity on the aquatic flora and fauna tested. Spraying of CNSL (4 per cent) did not show any toxicity to aquatic fauna. But it produced phytotoxicity on the leaves of Pistia, water lily and Lemna as isolated brown spots.

Sequential application of CNSL (4 per cent) and wettable powder (@ 5g/100 ml) showed slight change in the movement of fishes and snails during initial hours of observation. But later it reverted back normal behaviour. CNSL formed a thin film over the water surface which might have reduced the O_2 availability to the fishes. Even with slight disturbance this film breaks and creates condition favourable for the fishes. Phytotoxicity symptoms were observed on aquatic flora *viz.*, water lily, Pistia and Lemna. But plants were not killed and the new leaf produced were free of such symptoms. There was no damage on Salvinia and Hydrilla.

Deepa *et al.* (2000) observed that *Fusarium moniliforme* and *F. udum* caused mycoses and high mortality in fresh water fishes. According to Abbas and Duke (1997) *F. moniliforme* produces a toxin moniliformin having toxicity to animals, so not suitable as a herbicide. But *F. pallidoroseum* produces the toxin fusaric acid which has got no mammalian toxicity. This toxin is reported to be the most potent toxin that can be developed into a herbicide (Abbas *et al.*, 1991).

The results of the present study indicate that *F. pallidoroseum* is an effective biocontrol agent of water hyacinth. The efficacy of *F. pallidoroseum* could be further enhanced by pretreating the plants with CNSL at lower concentrations which is a product of plant origin easily available in

Kerala. *F. pallidoroseum* is not harmful to commonly cultivated plants, weeds or to the fauna found in the water ways. This biocontrol agent could be easily mass produced on locally available substrates and could be stored for a reasonable period of time without loosing its viability. Thus all these factors make *F. pallidoroseum* an ideal fungus for management of water hyacinth plants.

SUMMARY

6. SUMMARY

The objective of the study was to explore the feasibility of using fungal pathogens as mycoherbicides and formulating it for field application.

A survey was conducted for one year (February 2000 - February 2001) at quarterly intervals in four southern districts of Kerala to isolate fungi infecting water hyacinth.

Twelve different fungi were found associated with water hyacinth in Thiruvananthapuram district. The water ways in Kollam district was less frequently infested with water hyacinth. Maximum number of pathogenic fungal genera (12 nos.) were isolated from water hyacinth in Alappuzha district. Population of fungi in Kottayam district was similar to those observed in Thiruvananthapuram except that *Rhizoctonia* solani was recorded from the dstrict.

Myrothecium advena was the most frequently isolated fungal pathogen from water hyacinth followed by *Fusarium pallidoroseum.* Both fungi were present on the weed irrespective of seasons.

A positive correlation was recorded between the occurrence of fungi and weather parameters *viz.*, minimum temperature, relative humidity, total rainfall and number of rainy days and a negative correlation with maximum temperature.

Eighteen fungi isolated during the survey and three isolates obtained from the Department of Plant Pathology, College of Agriculture, Vellayani *viz.*, *F. pallidoroseum*, *F. equiseti* and *Colletotrichum gloeosporioides* were tested for its pathogenicity on water hyacinth. Out of the 21 fungi tested, 17 were found to be pathogenic.

M. advena was the most virulent fungus isolated. The extent of damage by the pathogenic fungi on water hyacinth ranged from 16.67 (*Curvularia lunata* and sterile fungus) to 61.11 per cent (*M. advena*).

Only two fungi *viz.*, *M. advena* and *F. pallidoroseum* (53.44 per cent) caused more than 50 per cent infection.

Morphological characters of those fungi which caused more than 40 per cent disease intensity were studied. The isolates of *Fusarium* obtained from the present survey were identified as *F. pallidoroseum*, *F. moniliforme* and *F. oxysporum*. The *Alternaria* and *Myrothecium* isolates were identified as *A. eichhorniae* and *M. advena* respectively.

M. advena was a new report on water hyacinth.

The host range studies were conducted using *M. advena*, *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides* on 53 species of cultivated plants and 54 species of weeds coming under 47 families.

M. advena was found to have a wide host range covering 27 cultivated plants and 45 weed plants. The host range of *F. pallidoroseum* was limited to six cultivated plants *viz.*, Amaranthus, tomato, banana, cashew, colocasia and papaya and 20 weed plants. *F. equiseti* produced symptoms on eight cultivated and 15 weed plants while *C. gloeosporioides* infected only eight cultivated and 15 weed plants.

The cell free culture filtrate of *M. advena* (97.75 per cent), *F. pallidoroseum* (48.84 per cent), *F. equiseti* (10.88 per cent) and *C. gloeosporioides* (42.17 per cent) produced varying degrees of damage on water hyacinth.

Cell free filtrates of *F. equiseti* and *C. gloeosporioides* did not produce any symptom on crop and weed plants tested. Metabolites from *F. pallidoroseum* developed symptoms only on one weed plant (*Monochoria vaginalis*) and *M. advena* produced symptoms on eight cultivated and nine weed plants.

Symptom producing ability of the toxin decreased with dilution. *F. pallidoroseum* produced symptoms even at the lowest concentration of one per cent. The cell free metabolites of *M. advena* and *F. pallidoroseum*

are highly thermostable on nature and could withstand even autoclaving. *C. gloeosporioides* toxin was inactivated on autoclaving while *F. equiseti* toxin was inactivated when temperature was increased to 50°C.

Based on paper chromatography, thin layer chromatography and NMR spectral peak analysis the toxin produced by *F. pallidoroseum* was identified as fusaric acid.

Eventhough a brownish precipitate was obtained from the culture filtrates of *M. advena* and *F. equiseti*, it did not develop any coloured spot in paper chromatograph. Toxic principle could not be extracted from *C. gloeosporioides*.

Purified toxin of *F. pallidoroseum* developed typical symptoms of fungal infection within 48 h after application on water hyacinth and the plants blighted within 10 days.

In the present study, ten commonly available solid substrates were used for mass multiplying *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides*. Among the solid substrates, *F. pallidoroseum* and *F. equiseti* grew and sporulated well in rice bran and rice bran + coirpith. Coconut oil cake and gingelly oil cake were the most favoured substrates for *C. gloeosporioides*.

Out of the 14 liquid substrates tried groundnut oil cake extract gave maximum mycelial weight of *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides*. *F. pallidoroseum* recorded a maximum mean viable spore count in jaggery water (5.57×10^7 cfu/ml). But for *F. equiseti* maximum mean viable colonies was observed in coconut water (4.18×10^7 cfu/ml). Coconut oil cake extract recorded maximum average viable spores of *C. gloeosporioides* (5.08×10^5 cfu/ml).

Paraquat, glyphosate, anilofos 24 % + 2,4-D 32 % EC and 2,4-D sodium salt supported growth and sporulation of *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides* and only pretilachlor inhibited the

growth. Herbicides at lower concentrations of 50, 30 and 10 ppm weakened water hyacinth plants.

When biocontrol fungi and herbicides were simultaneously applied maximum disease intensity of 68.86 per cent was recorded with anilofos 24 % + 2,4-D 32 % EC at 30 ppm concentration and *F. pallidoroseum*. But in trough condition the same treatment recorded only 26.67 per cent disease intensity. Sequential application of herbicides and biocontrol fungi recorded a decrease in disease intensity.

Among the five oils sprayed on water hyacinth plants, CNSL at concentration more than 0.5 per cent produced brown spots on leaf lamina.

F. pallidoroseum was only used for formulating as it was effective and also had a narrow host range among the cultivated plants.

Of the different dust formulations prepared, product containing 10 per cent mycelium gave the maximum disease of 20 per cent on water hyacinth plants. Dusting of this product on plants sprayed with two per cent CNSL recorded a maximum disease intensity (68.89 per cent).

Viability of spores in dust formulations reduced drastically when stored at room temperature. Dust formulations containing sucrose retained viability for one month.

Application of wettable powder @ 5 g and 10 g/100 ml on 2.0 per cent CNSL sprayed plants recorded 82.22 and 97.78 per cent disease intensity on water hyacinth plants.

Wettable powder formulation when stored under refrigerated condition retained viability of spores for four months. But under room temperature viability of spores lasted only for five weeks.

Oil based formulation of *F. pallidoroseum* (10^{11} spores/ml) and CNSL (2.0 per cent) caused only 48.89 per cent disease intensity.

Among the three formulations prepared, wettable powder formulation was found to be the best under glasshouse and trough conditions. Under trough conditions application of wettable powder @ 5 g and 10 g/100 ml on 4.0 per cent CNSL sprayed plants recorded 97.33 and 98.67 per cent disease respectively.

Field application using CNSL (5.0 per cent) and *F. pallidoroseum* as wettable powder formulation (@ 5 g / 100 ml) recorded a mean per cent damage of 91.43 on water hyacinth plants.

The metabolites and wettable powder formulation of *F. pallidoroseum* did not produce toxicity on the aquatic flora and fauna tested. CNSL at 4.0 per cent did not cause any toxicity to aquatic fauna. But it produced brown isolated spots on the leaves of Pistia, water lily and Lemna. Sequential application of CNSL (4.0 per cent) and wettable powder showed slight change in the movement of fishes and snails during initial hours of observation, while later it reverted back to normal behaviour. On aquatic flora *viz.*, water lily, Pistia and Lemna brown isolated spots were observed.

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*Original not seen

MANAGEMENT OF WATER HYACINTH [*Eichhornia crassipes* (Mart.) Solms] USING FUNGAL PATHOGENS

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8. ABSTRACT

The study entitled "Management of water hyacinth using fungal pathogens" was conducted in College of Agriculture, Vellayani, Thiruvananthapuram during 1999-2002 period.

A survey was carried out for a period of one year at quarterly intervals in four southern districts of Kerala to isolate fungi infecting water hyacinth.

Myrothecium advena was the most commonly seen fungus followed by *Fusarium pallidoroseum* irrespective of the season.

A positive correlation between the occurrence of fungi and maximum temperature, relative humidity, total rainfall and number of rainy days and negative correlation with maximum temperature was observed.

Out of the 21 fungi tested for pathogenicity, 17 were found to be pathogenic on water hyacinth. Only *M. advena* (61.11%) and *F. pallidoroseum* (53.44 per cent) caused more than 50% infection.

The Fusaria, *Alternaria* and *Myrothecium* isolated from water hyacinth were identified as *F. oxysporum*, *F. pallidoroseum*, *F. moniliformae*, *A. eichhorniae* and *M. advena*, of which *M. advena* was a new report on water hyacinth.

Host range of *M. advena, F. pallidoroseum, F. equiseti* and *C. gloeosporioides* were studied. *M. advena* infected 27 cultivated and 45 weed plants, while *F. pallidoroseum* was limited to six cultivated and 20 weed plants.

Cell free metabolites of *M. advena* produced maximum damage of 97.75 per cent on water hyacinth followed by metabolites of *F. pallidoroseum* (48.84 per cent). The cell free culture filtrate of *M. advena* could produce

symptoms on eight cultivated and nine weed plants, while that of *F. pallidoroseum* developed symptoms only on one weed plant (*Monochoria vaginalis*). Toxicity of cell free metabolites was affected by dilution, temperature and pH.

Based on paper chromatography, thin layer chromatography and NMR spectral peak analysis the toxin produced by *F. pallidoroseum* was identified as fusaric acid. Application of purified toxin on water hyacinth plants produced typical symptoms resembling fungal infection.

Among the ten solid substrates tested *F. pallidoroseum* and *F. equiseti* grew and sporluated well in rice bran and rice bran + coirpith. Coconut oil cake and gingelly oil cake were the most favoured substrates of *C. gloeosporioides*. Of the 14 liquid substrates listed maximum average viable colonies of *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides* were recorded in jaggery water, coconut water and coconut oil cake extract respectively.

Under glasshouse condition simultaneous application of anilofos 24 % + 2,4-D 32 % EC (30 ppm) with *F. pallidoroseum* recorded 68.86 per cent disease intensity while under trough condition, this combination gave only 26.67 per cent disease intensity.

Dust formulation of *F. pallidoroseum* resulted in 20 per cent disease while sequential application of 2.0 per cent CNSL and dust increased disease intensity to 68.89 per cent. Spores in dust formulation remained viable for one month at room temperature.

Wettable powder of *F. pallidoroseum* was formulated using mycelium, talc, tween-80, glycerol and sucrose. This @ 5 g and 10 g/100 ml on 2.0 per cent CNSL sprayed plants recorded 82.22 and 97.78 per cent disease intensity on water hyacinth plants. While under trough condition application of wettable powder @ 5 g and 10 g/100 ml on 4.0 per cent CNSL sprayed plants recorded 97.33 and 98.67 per cent disease.

Viability of spores in wettable powder formulation lasted for five weeks under room temperature and for four months under refrigerated condition.

Oil based formulation of *F. pallidoroseum* (10^{11} spores/ml) and CNSL (2.0 per cent) recorded only 48.89 per cent disease intensity.

Field application using CNSL (5.0 per cent) and *F. pallidoroseum* as WP formulation (@ 5 g/100 ml) recorded a mean per cent damage of 91.43 on water hyacinth plants.

Safety test of formulated product and metabolites of *F. pallidoroseum* revealed that they are safe on commonly seen aquatic flora and fauna.

APPENDICES

APPENDIX – I

Nutrient solution (Knops Solution)

Calcium nitrate	–	0.8 g
KNO_3	–	0.2 g
$KH_2 PO_4$	–	0.2 g
$MgSO_4 7H_2O$	–	0.2 g
$FeSO_4$	–	Trace
Distilled water	–	1 L

Richard's solution

Sucrose	–	50 g
KNO_3	–	10 g
$KH_2 PO_4$	–	5 g
$MgSO_4 7H_2O$	–	2.5 g
$FeCl_3$	–	0.02 g
Distilled water	–	1 L
pH	–	7.2

Martin's rose bengal agar medium

Dextrose	–	10 g
Peptone	–	5 g
$KH_2 PO_4$	–	1 g
$MgSO_4. 7H_2O$	–	0.5 g
Rose Bengal	–	33 mg/L
Distilled water	–	1 L
Streptomycin	–	30 mg
Agar	–	20 g

Czapek's (Dox) Broth

Sucrose	–	30 g
$NaNO_3$	–	2 g
$K_2 HPO_4$	–	1 g
$MgSO_4 . 7H_2O$	–	0.5
KCl	–	0.5 g
$Fe SO_4$	–	0.01 g
Distilled water	–	1 L