BIOHERBICIDAL POTENTIAL OF FUNGAL PATHOGENS OF WATER HYACINTH [*Eichhornia crassipes* (Mart.) Solms]

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Dedicated

to

My

Dearest Appa & Amma

DECLARATION

I hereby declare that this thesis entitled "**Bioherbicidal potential of fungal pathogens of water hyacinth [*Eichhornia crassipes* (Mart.) Solms]**" 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 "**Bioherbicidal potential of fungal pathogens of water hyacinth [*Eichhornia crassipes* (Mart.) Solms]**" is a record of research work done independently by Ms. Ancy M. Salim (2001-11-22) 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
CD	Critical difference
cfu	Colony forming unit
DAS	Days after spraying
et al.	And others
Fig.	Figure
g	Gram
h	Hour
ha	Hectare
m	Metre
ml	Millilitre
sp.	Species
viz.	Namely
WAS	Weeks after spraying
WP	Wettable powder

INTRODUCTION

1. INTRODUCTION

The aquatic weed, water hyacinth [*Eichhornia crassipes* (Mart. Solms: Pontederiaceae] is an erect free floating stoloniferous perennial herb. One of the most successful colonizers in the plant world, the water hyacinth has spread within a hundred years from its homebase, Amazon river basin in Brazil to atleast 50 countries around the world including India. This weed was introduced to India in 1896 as an ornamental pond plant.

Generally, water hyacinth propagates and multiplies vegetatively. Two plants of water hyacinth could multiply to 1,20,000 in 120 days, while 30 off springs could be produced from two parent plants within 23 days (Verma *et al.*, 2003). It now covers more than 20,0000 ha of water surface (Singh, 1999) in 98 out of the 246 districts in India (Harikumar and Madhavan, 2002). It is known to impede navigation, choke waterways and irrigation channels and also provide breeding places for mosquitoes (Nagraj, 1965). The evapotranspiration rate of water hyacinth is recorded to be eight times more than the evaporation from free water surface. Thus the plant appears to derive its Hindi name "Samudrasokh"- which can absorb an ocean (Verma *et al.*, 2003).

Several methods are recommended to manage water hyacinth in lakes, water ways etc. Chemical herbicides, eventhough effective and provide immediate solution, are not advisable for use in waterways due to its harmful effects to the environment as well as to human beings. Looking to the magnitude of health problems, biological control is being popularized to manage this weed. Introduced weeds like water hyacinth are amenable to manage through biocontrol agents. Of the different biocontrol agents, Fungi have received maximum attention (Templeton, 1982).

Bioherbicide based on *Cercospora rodmanii*, a fungal pathogen have been developed for the management of water hyacinth (Charudattan, 1986). As a part of the Department of Science and Technology (DST) project on "Biocontrol of water hyacinth using mycoherbicides" (being conducted at Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram), a number of pathogens have been obtained during the survey, of which extensive studies have been done with *Fusarium pallidoroseum* while many others are yet to be studied in detail.

The present study was conducted to evaluate the bioherbicidal potential of different fungal pathogens of water hyacinth to select the promising ones and to develop a talc based formulation.

The objectives of the present study are:

1. Identification and characterization of fungal pathogens of water hyacinth obtained from the previous survey to species levels.
2. Study on the variation in symptom development by the above pathogens and their cell free extracts on water hyacinth.
3. To select the most promising fungal pathogens by pot culture trial individually and in combination.
4. Detailed study on the host range with common cultivated crops and weeds
5. To standardize the dosage of inoculum required for effective destruction of the weed
6. Develop a talc based formulation
7. Test the formulated product.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

A number of fungal pathogens are found to restrict multiplication and spread of water hyacinth [*Eichhornia crassipes* (Mart.) Solms]. Some of these pathogens could be utilized for its management.

2.1 PATHOGENS OF WATER HYACINTH

During the search for natural enemies of water hyacinth, Nagraj and Ponnappa (1967) isolated *Corticium solani* (in the *Rhizoctonia* phase) and *Myrothecium roridum* from the leaves. In 1970, they reported several fungi to be pathogenic to water hyacinth viz., *Uredo eichhorniae*, *Fusarium equiseti*, *Corticium sasakii*, *Cephalosporium eichhorniae*, *Rhizoctonia solani*, *Cercospora piaropi*, *Marasmiellus inoderma* and *Alternaria eichhorniae*. According to them, *M. inoderma* and *A. eichhorniae* could be used as potential biological control agents of this weed.

Among the 32 fungi tested by Charudattan (1973) on water hyacinth, the most pathogenic ones were *M. roridum*, *R. solani* and *A. eichhorniae*. Charudattan and Conway (1975) suggested the possibility of using the rust disease of water hyacinth caused by *Uromyces eichhorniae* for its control. Conway (1976) reported that *Cercospora rodmanii* was responsible for the decline of water hyacinth in Florida and that the organism was capable of over wintering on dead leaves and becoming established in the following season.

Charudattan *et al.* (1978) worked on the seasonal occurrence of *U. eichhorniae* on water hyacinth in Argentina and found this pathogen to be a promising biocontrol agent of the weed. Out of the 25 fungal and bacterial isolates of water hyacinth in Sudan, only five were found to be

pathogenic *viz.*, *Acremonium zonatum*, *Drechslera spicifera*, *F. equiseti*, *Phoma sorghina* and *Bacillus* sp. (Abdel-Rahim and Tawfig, 1984). In the North Western and Western Provinces of Srilanka, *Penicillium oxalicum*, *Curvularia lunata*, *Fusarium* sp., *Helminthosporium* sp., *M. roridum* and a sterile fungus were the main colonizers of leaf surface of water hyacinth (Balasooriya *et al.*, 1984).

Caunter (1984) made isolations from diseased leaves and leaf stalks of water hyacinth from Penang Island and Kedah and found *Helminthosporium, Myrothecium and Chaetomiella* to be pathogenic. Out of the three fungal pathogens isolated from water hyacinth by Jamil and Narsaiah (1984), *A. eichhorniae* caused more damage than *Cercospora* sp. or *Fusarium solani*. However, *F. solani* showed remarkable selectivity in attacking the older leaves of water hyacinth and was compatible with *Cercospora*. Singh *et al.* (1985) worked on the mycoflora associated with water hyacinth in India. Of the several fungi isolated, *A. eichhorniae*, *Corticium solani*, *Curvularia* sp., *Pestalotia* sp., *M. roridum*, *U. eichhorniae* and *C. piaropi* were potentially pathogenic to the weed. From Andhra Pradesh, Jamil and Rajagopal (1986) reported *Fusarium oxysporum*, *F. semitectum*, *Alternaria* sp., *Curvularia* sp., *Helminthosporium* sp., and a sterile fungus on water hyacinth. According to Galbraith (1987) feeding by *Neochetina eichhorniae* increased infection by *A. zonatum* which resulted in 49 per cent reduction in plant growth.

Aneja *et al.* (1988) and Aneja and Srinivas (1990) reported *Alternaria alternata* and *C. rodmanii* respectively infecting water hyacinth in Haryana.

In a survey at different sites in Egypt, Elwakil *et al.* (1990) could isolate 200 fungi 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 small, punctate leaf spots with ash centers caused by *F. chlamydosporum* on water hyacinth plants. Santhi (1994) isolated *F. equiseti*, *F. pallidoroseum*, *R. solani*, *Colletotrichum gloeosporioides*, *C. lunata* and a sterile fungus from water hyacinth and reported *F. pallidoroseum* and *C. gloeosporioides* as efficient fungi with biocontrol potential.

Shabana *et al.* (1995) reported that among the different isolates of *A. eichhorniae*, isolate five was the most virulent one which caused severe 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* that caused disease on water hyacinth plants.

Naseema et al (2001) found that *F. equiseti* and *F. pallidoroseum* were two effective biocontrol agents of water hyacinth. Praveena (2003) conducted a survey to identify pathogens of water hyacinth and isolated 17 fungi, of which *M. advena* and *F. pallidoroseum* caused more than 50 per cent infection under laboratory condition.

2.2 USE OF CELL FREE METABOLITES IN WEED CONTROL

Secondary metabolites of fungal pathogens have great potential for use as herbicides.

According to Nagraj and Ponnappa (1970), cell free culture filtrates of *A. eichhorniae* developed necrosis within 24 h and complete drying up of leaves of water hyacinth within 48 h. These metabolites were found to

have selective action and had positive effects on water hyacinth and *Monochoria vaginalis*.

Out of the culture filtrates of four pathogenic fungi *viz.*, *F. equiseti*, *Phoma sorghina*, *A. zonatum* and *D. spicifera* tested by Abdel-Rahim and Tawfig (1984), those of *F. equiseti*, *A. zonatum* and *P. sorghina* produced blighting on water hyacinth.

The culture filtrates of *F. moniliforme* caused phytotoxicity symptoms of mild to severe necrosis on Jimson weed (Abbas *et al.*, 1991). Santhi and Naseema (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 and attributed this effect to the production of toxins by the fungi.

The culture filtrates of *Colletotrichum capsicum* isolates inhibited seed germination, seedling wilt and fruit damage of chillies (Mathur, 1995) while those of *Alternaria porri* inhibited root development of onion seedlings and the effect increased with incubation period from 4 to 14 days (Khare and Goswami, 1996).

The culture filtrates of *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides* produced 96.28, 83.00 and 26.28 per cent damage on water hyacinth plants (Susha, 1997). 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*. 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. Praveena (2003) reported that the cell free culture filtrates of *M. advena* (97.75 per cent), *F. pallidoroseum* (48.84), *F. equiseti* (10.88) and *C. gloeosporioides* (42.17 per cent) produced varying degrees of damage on water hyacinth. The toxin produced by *F. pallidoroseum* was identified as fusaric acid.

2.3 HOST RANGE OF PATHOGENS FROM WATER HYACINTH

For the use of pathogens as efficient biocontrol agents detailed host range information is essential to avoid potential hazards to non target plants.

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*. According to Charudattan and Conway (1976), *Mycoleptodiscus terrestris*, a pathogen of water hyacinth was found to infect 10 host plants including eight legumes. In a detailed study on the host range of *C. rodmanii* involving 85 selected plants representing 22 families, Conway and Freeman (1977) found that it was pathogenic only to squash, cucumber and spinach. Rakvidyasastra *et al.* (1978) studied the host range of fungi pathogenic to water hyacinth and reported that *R. solani* was pathogenic to all the test plants viz., Rice, maize, sorghum, cotton, tobacco and *Hibiscus sabdariffa*. *M. roridum* was pathogenic to all the test plants except tobacco. While *A. eichhorniae* infected only *H. sabdariffa*.

F. lateritium which was effective in suppressing the growth of velvet leaf and prickly sida 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. Santhi (1994) conducted host range studies of *C. gloeosporioides*, *F. equiseti*, *F. pallidoroseum* and *F. solani* on six crop plants and six weed plants. *C. gloeosporioides* was found to infect *Capsicum annuum*, *Commelina benghalensis*, *Hydrocotyl asiatica* and *Ludwigia parviflora*. *Fusarium* spp. could not infect the cultivated plants tested whereas they were pathogenic to the weed, *M. vaginalis*.

According to Chetti *et al.* (1999) *C. gloeosporioides* isolated from *Chromolaena odorata* was 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*. 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*. Babu *et al* (2002) studied the host range of *A. alternata* on 29 plant species and found that only water lettuce (*P. stratiotes*) was affected.

Praveena (2003) conducted host range studies 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 nine cultivated and 14 weed plants while *C. gloeosporioides* infected only eight cultivated and 15 weed plants.

2.4 INOCULUM CONCENTRATION REQUIRED FOR WEED CONTROL

The application of inundative doses of inoculum and its proper timing would shorten the lag period for inoculum build up and pathogen distribution (Daniel *et al.*, 1973). Walker (1981) found that spray mixtures *Alternaria macrospora* containing 2.5 to 10 x 10^5 spores / ml gave the best control of spurred anoda (*Anoda cristata).*

Walker and Riley (1982) worked on the biocontrol of sickle pod (*Cassia obtusifolia*) using *Alternaria cassiae.* Maximum weed control was obtained with a spray solution containing more than or equal to 5 x 10^4

conidia per ml applied at cotyledonous to first leaf stage. The factors influencing the biocontrol of velvet leaf and prickly sida with *F. lateritium* was studied by Boyette and Walker (1985). They obtained higher level of control with inoculum concentration of 7.5×10^5 and 1.5×10^6 microconidia per ml. Ridings (1986) obtained effective biocontrol of strangler vine, *Morrenia odorata* in citrus orchard using *Phytophthora palmivora* at eight chlamydospores per cm^2 of the soil. *Fusarium solani* fsp *cucurbitae* effectively controlled Texas gourd when sprayed with a conidial suspension of either microconidia or macroconidia at 3×10^{11} per ml (Weidemann and Templeton, 1988).

Morrrin *et al.* (1989) worked on the efficacy of *Phomopsis convolvulus* for the control of field bind weed (*Convolvulus arvensis*). The fungus reduced the growth and regeneration of the weed under greenhouse conditions. The seedlings at cotyledon stage were severely injured and killed when a spore concentration of 10^8 conidia per ml was used, while at 3 to 5 leaf stage, a concentration of 10^9 conidia per ml was required.

An aqueous suspension of 5×10^6 spores per ml of *Cochliobolus carbonum*, a potential biocontrol agent of *Euphorbia geniculata* caused 98 per cent control of the weed (Lakshmanan *et al.*, 1991). In the field trials conducted by Hildebrand and Jenson (1991) to evaluate the effectiveness of *C. gloeosporioides* as biocontrol agent of St. Johns weed (*Hypericum perforatum*), 72.2 and 83.3 per cent mortality was obtained at 2×10^6 and 8×10^6 spores per ml respectively.

Santhi (1994) determined the quantity of inoculum of *F. equiseti, F. pallidoroseum, F. solani* and *C. gloeosporioides* required for the effective destruction of water hyacinth. According to her, 10^9 spores per ml gave more intensity of infection than 10^3 and 10^6 spores per ml in the case of *Fusarium* spp. and in the case of *C. gloeosporioides* 2×10^9 spores per ml was better than 2×10^3 and 2×10^6 spores per ml. Susha (1997)

crotalariae and *F. udum* f. sp. *crotalariae*. Strobel *et al.* (1990) found that maculosin, a diketo piperazine compound and another phytotoxic tenuzoic acid produced by *A. alternata* infecting spotted knap weed (*Centaurea maculosa*) when used in combination had a synergistic action against the weed. Susha and Naseema (1998) observed that the combined application of *F. pallidoroseum* and *F. equiseti* gave higher intensity of infection than when used alone.

2.6 FORMULATION OF BIOCONTROL FUNGI

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

Walker (1981) described a method for large scale production of granular formulation of *A. macrospora* 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. Walker and Connick (1983) prepared an aqueous mixture of one per cent sodium alginate and homogenized mycelia of *A.cassiae* and *F. lateritium* pelletized by dropwise additions of each mycelial mixture into 0.25 M $CaCl_2$. 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. Quimby (1985) developed a water dispersible spray product, by mixing 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).

Connick *et al.* (1991) developed an oil phase emulsion of *A. 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 water (1: 1 w/w).

A wettable powder formulation of *Fusarium pallidoroseum* with diatomaceous earth was developed by Faizal (1992). The formulation containing 3.5 x 10^6 spores/ml was as effective as aqueous suspension of the spores against aphids. He also found that diatomaceous earth based wettable powder was better than talc based dust formulation. Spores of *Colletotrichum gloeosporioides* 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). Krishnamurthy and Gnanamanickam (1998) conducted studies to develop of an effective formulation of *Pseudomonas putida* with increased shelf life. Of the various combinations tested methyl cellulose : talc in the ratio 1 :4 was found to maintain the viability of the bacterium for 10 months. Shabana (1997) prepared dried mycelium alginate (1 per cent) of *A. eichhorniae* containing 10^{10} propagules/g mixed with 15 per cent vegetable oil, four per cent soybean lecithin and 80 per cent water.

Smitha (2000) prepared a talc based formulation of *Trichoderma longibrachiatum* by mixing the powdered fungal mat with sterilized talc @ 10 per cent w/w and 1 per cent carboxy methyl cellulose. Vidhyasekaran and Muthumilan (1995) developed a talc based formulation of *Pseudomonas fluorescens* by mixing the bacterial growth with sterilized talc @ 400 ml / kg talc along with 5 g of the sticker carboxy methyl cellulose.

Praveena (2003) developed a wettable powder formulation of *F. pallidoroseum* containing 40 per cent fungus, 2 per cent each of talc, sucrose and glycerol and 54 per cent talc. Application of this @ 5 g and 10 g /100 ml recorded 82.22 and 97.78 per cent disease intensity on water hyacinth plants respectively.

2.6.1. Shelf Life of Formulated Biocontrol Fungi

There are various reports on the shelf life of the bioherbicide formulations. Walker and Connick (1983) reported that sodium alginate-clay formulation of *A. cassiae* could be stored at 4°C for 6-8 months. Mortensen (1988) found that spores of *C.gloeosporioides* in wettable powder can survive upto two years without considerable loss of viability and efficacy. *F. pallidoroseum* spores retained 75 per cent viability for four days and thereafter a marked decrease was noticed in the virulence of the fungus in water, talc and diatomaceous earth formations (Faizal, 1992). Nakkeeran *et al.* (1997) reported that in the talc based formulation of *Trichoderma*, from an initial population of 31 x 10^7 cfu per gram, there was a decline in the population to 13 x 10^7 cfu per gram in 75 days when stored at 20- 30^0C.

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. 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). Praveena (2003) observed that the viability of spores in the dust formulations of *F. pallidoroseum* was reduced drastically when stored at room temperature. Dust formulation containing sucrose retained viability for one month. She also observed that wettable powder formulation of *F. pallidoroseum* when stored under refrigerated conditions retained viability of the spores for a period of four months. But under room temperature, viability of spores reduced drastically.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Laboratory and pot culture experiments were carried out at the College of Agriculture, Vellayani, Thiruvananthapuram, Kerala to evaluate the bioherbicidal potential of different fungal pathogens of water hyacinth and to develop a talc based formulation of the effective biocontrol fungus.

3.1 IDENTIFICATION AND CHARACTERIZATION OF FUNGAL PATHOGENS

Cultural and morphological characters of the fungal pathogens of water hyacinth, obtained during the survey carried out as part of the DST project on "Biocontrol of water hyacinth using mycoherbicides" in the department, were studied. Four to ten day old cultures of the fungi grown on appropriate media were used for the study (Appendix I). Of the 14 fungi used for the study, 11 were already identified and the remaining three were provisionally identified and was confirmed by sending to Agharkar Research Institute, Pune.

Table 1 Fungi used for the study

Sl. No.	Fungus	Media
1	*Alternaria eichhorniae*	Modified potato dextrose agar
2	*Colletotrichum gloeosporioides*	Potato dextrose agar
3	*Curvularia lunata*	"
4	*Fusarium equiseti*	Potato sucrose agar
5	*F. moniliforme*	"
6	*F. oxysporum* (Isolate 1)	"
7	*F. oxysporum* (Isolate 2)	"
8	*F. pallidoroseum* (Isolate 1)	"
9	*F. pallidoroseum* (Isolate 2)	"
10	*F. pallidoroseum* (Isolate 3)	"
11	*Myrothecium advena*	Potato dextrose agar
12	Unidentified 1	"
13	Unidentified 2	"
14	Unidentified 3	"

conidia per ml applied at cotyledonous to first leaf stage. The factors influencing the biocontrol of velvet leaf and prickly sida with *F. lateritium* was studied by Boyette and Walker (1985). They obtained higher level of control with inoculum concentration of 7.5×10^5 and 1.5×10^6 microconidia per ml. Ridings (1986) obtained effective biocontrol of strangler vine, *Morrenia odorata* in citrus orchard using *Phytophthora palmivora* at eight chlamydospores per cm^2 of the soil. *Fusarium solani* fsp *cucurbitae* effectively controlled Texas gourd when sprayed with a conidial suspension of either microconidia or macroconidia at 3×10^{11} per ml (Weidemann and Templeton, 1988).

Morrrin *et al.* (1989) worked on the efficacy of *Phomopsis convolvulus* for the control of field bind weed (*Convolvulus arvensis*). The fungus reduced the growth and regeneration of the weed under greenhouse conditions. The seedlings at cotyledon stage were severely injured and killed when a spore concentration of 10^8 conidia per ml was used, while at 3 to 5 leaf stage, a concentration of 10^9 conidia per ml was required.

An aqueous suspension of 5×10^6 spores per ml of *Cochliobolus carbonum*, a potential biocontrol agent of *Euphorbia geniculata* caused 98 per cent control of the weed (Lakshmanan *et al.*, 1991). In the field trials conducted by Hildebrand and Jenson (1991) to evaluate the effectiveness of *C. gloeosporioides* as biocontrol agent of St. Johns weed (*Hypericum perforatum*), 72.2 and 83.3 per cent mortality was obtained at 2×10^6 and 8×10^6 spores per ml respectively.

Santhi (1994) determined the quantity of inoculum of *F. equiseti*, *F. pallidoroseum*, *F. solani* and *C. gloeosporioides* required for the effective destruction of water hyacinth. According to her, 10^9 spores per ml gave more intensity of infection than 10^3 and 10^6 spores per ml in the case of *Fusarium* spp. and in the case of *C. gloeosporioides* 2×10^9 spores per ml was better than 2×10^3 and 2×10^6 spores per ml. Susha (1997)

standardized the dosage of inoculum required for the effective destruction of water hyacinth. For *F. pallidoroseum*, *F. equiseti* and *C. gloeosporioides*, 10^{11} spores per ml was the most effective spore concentration resulting in 74.30, 98.01 and 89.70 per cent intensity of infection respectively. Naseema *et al* (2001) reported *F. equiseti* and *F. pallidoroseum* to be effective biocontrol agents of water hyacinth at 10^{11} spores per ml.

2.5 USE OF PATHOGENS IN COMBINATION FOR WEED CONTROL

Biological control efficiency can be improved by combining more than one pathogens.

Boyette *et al.* (1979) worked on the control of winged water prim rose (*Jussiaeae decurrens*) and Northern joint vetch (A*eschynomene virginica*) with fungal pathogens and found that mixture of *C. gloeosporioides* f.sp. *jussiaeae* and *C. gloeosporioides* f.sp. *aeschynomene* each at a concentration of one to two million spores per ml was effective.

Jamil and Narsaiah (1984) found that *A. eichhorniae*, *Cercospora* sp. and *F. solani* were pathogenic to water hyacinth. It was also observed that *F. solani* had remarkable selectivity in attacking older leaves and so it can be used as a co pathogen with *Cercospora* sp. Studies on the combined effect of *A. macrospora* and *F. lateritium* on spurred anoda conducted by Crawly *et al.* (1985) revealed that highest level of plant death occurred when *A. macrospora* was applied five days before the application of *F. lateritium*. This interaction was potentially useful to increase the effectiveness of the two pathogens as mycoherbicides

Charudattan (1986a) demonstrated the combined use of two mycoherbicides for controlling weeds. He found that showy crotalaria was controlled by the combined application of *Colletotrichum dematium* f.sp.

3.1.1 Cultural and Morphological Characters

The cultural characters such as type, texture and colour of mycelium and colouration of media were studied. Also the morpholgical characters of the fungal pathogens such as size, colour and shape of conidia and conidiophore and presence of chlamydospores were studied using slide culture technique described by Riddel (1950).

3.2 SYMPTOM DEVELOPMENT BY THE PATHOGENIC FUNGI ON WATER HYACINTH

Variation in the symptoms produced by the fungi were studied by artificially inoculating them on water hyacinth plants.

3.2.1 Pathogenicity

The fungi were tested for their pathogenicity on water hyacinth under *in vitro* condition. Water hyacinth plants collected from the water ways were allowed to establish in pots (1 litre capacity) filled with mud and water and were inoculated with respective fungi. The leaves and petioles were given pinpricks before inoculation. Seven day old culture bits of the fungi grown on PDA were placed on the injured portion and covered with small bits of moist cotton wool. The entire plant was covered with polythene cover to maintain sufficient humidity. Control plants were maintained by applying cotton wool soaked with sterile water alone on the punctured leaves.

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

3.3 SELECTION OF MOST PROMISING FUNGAL PATHOGENS

Pot culture trial was conducted to select the most promising fungal pathogens of water hyacinth.

3.3.1 Effect of Individual Fungal Pathogens

Fungi listed under 3.1 were used in this experiment. The fungi were grown in sterilized Czapek's broth (Appendix I). Each of the 250 ml flasks containing 100 ml broth were inoculated with 5 mm culture disc cut from actively growing seven day old culture of the fungi and incubated for seven days at room temperature (28 ± 4°C). After incubation, the fungal mat was thoroughly mixed with broth and it was allowed to settle. The supernatant liquid with the spores was decanted and used for spraying on water hyacinth plants maintained in pots (1 litre capacity) using an atomizer @ 10ml/plant. Leaves and petioles were given pinpricks before spraying. Three replications were maintained for each treatment. Water hyacinth plants sprayed with sterile water and seven day old uninoculated Czapek's solution served as control. Intensity of infection was recorded using the score chart (Praveena, 2003), given below (Plate 1).

0	No symptom
1	Symptom development around the pinpricked area only
2	Upto 10 per cent leaf area showing yellowing / browning symptom
3	11 - 25 leaf area 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

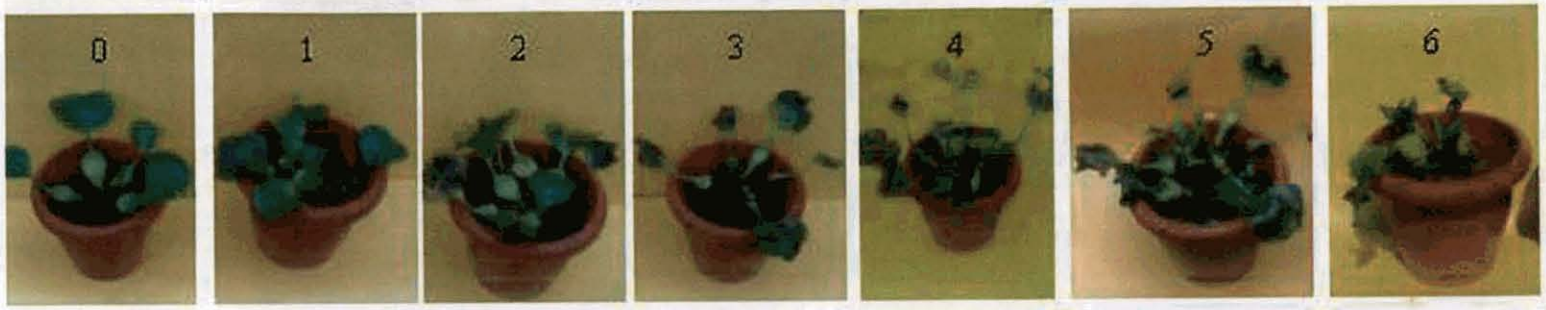


Plate 1. Score chart for extent of damage

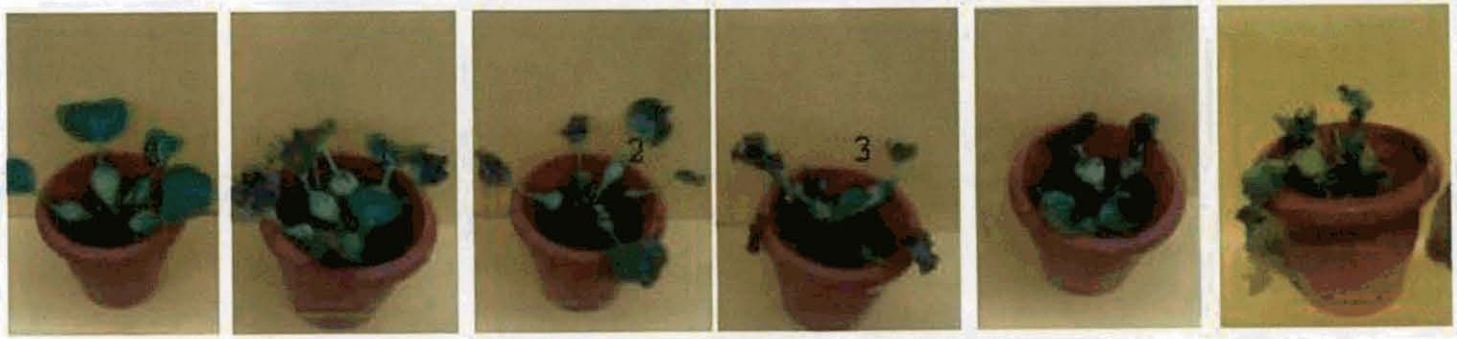


Plate 2. Score chart for cell free metabolites

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

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

3.3.2 Effect of Cell Free Metabolites of the Pathogenic Fungi

The effect of cell free metabolites of the fungi listed under 3.1 on water hyacinth plants were studied.

3.3.2.1 Preparation of Cell Free Metabolites

The fungi were grown in 250 ml conical flasks containing 100 ml Czapek's broth, sterilized in autoclave at 121°C for 20 minutes (1.1 kg cm^{-2} pressure). Each of the flasks were inoculated with 5 mm culture disc cut from actively growing seven day culture of each of the fungi and incubated for 15 days at room temperature (28 ± 4°C). The fungal growth was then filtered through Whatman No.1 filter paper. The filtrate collected was made cell free by passing it through 0.45 µm Sartorius membrane filter in a sterilized filter assembly by applying pressure.

3.3.2.2 Testing the Efficacy of Cell Free Metabolites on Water Hyacinth

Water hyacinth plants maintained in small plots were sprayed with cell free metabolite of each of the fungi @ 10 ml/ plant using an atomiser. Three replications were maintained for each treatment. Water hyacinth plants sprayed with sterile water and 15 day old uninoculated Czapek's solution sprayed served as control. Intensity of damage was recorded seven days after spraying by using the score chart (Praveena, 2003), as follows (Plate 2).

0	No scorching
1	Upto 10 per cent leaf area showing scorching
2	11-25 per cent leaf area showing scorching
3	26-50 per cent of leaf area including bulbous portion showing scorching
4	50-75 per cent of leaf area showing scorching with shrinkage of bulbous portion
5	Complete decay of the plant

Intensity of damage was calculated as described under 3.1.3.

3.3.3 Host Range Studies

Host range studies of the most promising fungal pathogens of water hyacinth *viz.*, *A. eichhorniae*, *F. oxysporum* and *F. moniliforme* were carried out on common weeds and cultivated plants in and around water ways infested with the weed. *F. pallidoroseum* and *M. advena*, eventhough recorded high intensity of damage, were not included in the present study as work on these fungi were already undertaken in the Department.

Each fungus was inoculated on 30 species of cultivated plants and 31 species of weeds (Table 2). Vegetables, pulses, oil seed crops and weeds were grown in plastic cups (200 g capacity). While detached branches of tree crops were maintained in nutrient solution (Appendix I). These were inoculated with seven day old cultures of the respective fungi by giving pinpricks on leaves and stem. The inoculated plants were covered with polythene cover to maintain sufficient humidity. The experiment was replicated thrice. Control plants were maintained by applying cotton wool soaked with sterile water on the punctured parts.

Table 2 Plants tested for host range

Sl. No.	Family	Scientific *name*	Common name / vernacular name
1	Acanthaceae	*Justicia diffusa* Wild	Justicia
2		*J. prostrata* Gamble N. Comb	Justicia
3	Amaranthaceae	*Amaranthus viridis* (Linn.) Notrysag	Slender Amaranthus
4		*A. incolor* L.	Amaranthus
5		*Gomphrena decumbens*	Balippovu
6		*Alternanthera sissilis*	Alligator weed
7	Anacardiaceae	*Mangifera indica* L.	Mango
8		*Anacardium occidentale* L.	Cashew
9	Araceae	*Colocasia esculenta*	Taro
10		*Amorphophallus companulatus*	Elephant foot yam
11		*Anthurium andreanum* L.	Anthurium
12		*Pistia stratiotes*	Water lettuce, muttapayal
13	Asclepidiaceae	*Hemidesmus indicus* R. Br	Naruneendi
14	Asteraceae	*Tridax procumbens* L.	Odiyan
15		*Vernonia cineria* L.	Poovam kurunnu
16		*Synedrella nodiflora* L.	Venppacha
17		*Chromolaena odoratum* (L.) King and Robinson	Communist pachha
18		*Emilia sonchifolia* (L.) DC	Muyal chevian
19		*Eclipta alba* (L.) Hassk	Kayonni
20		*Ageratum conyzoides*	Goat weed/ Appa Knoxia
21		*Knoxia* sp	Knoxia
22	Boraginaceae	*Heliotropium indicum*	Venppacha
23	Capparidaceae	*Cleome viscose* L.	Wild mustard, Kattu Kaduku
24	Caricaceae	*Carica papaya*	Papaya
25	Commelinaceae	*Commelina benghalensis* L.	Tropical spider wort
26		*C. jacobi*	Vazhappadathy
27	Convolvulaceae	*Ipomoea batatus* (L.) Lam	Sweet potato
28	Cucurbitaceae	*Momordica charantia* L.	Bittergourd
29		*Cucumis sativus* L.	Cucumber
30		*Trichosanthes anguina* L.	Snake gourd
31	Cyperaceae	*Bulbostylis barbata*	Sooryan
32	Dioscoriaceae	*Dioscorea alata*	Yam
33	Euphorbiaceae	*Phyllanthus niruri* (L.) Hoof F.	Keezharnelli
34		*Ephorbia geniculata* L.	Paloorippacha
35		*E. hirta* L.	Tharavu
36		*Manihot esculenta* L.	Tapioca
37		*Hevea brasilensis*	Rubber
38	Labiatae	*Hyptis suaveolens* Port.	Nattapoochedi
39	Lamiaceae	*Leucas aspera* spring	Thumba

Table 2 Continued

40	Lauraceae	*Cinnamomum zeylanicum*	Cinnamom
41		*Jasminum sambac*	Jasmine
42	Leguminosae	*Clitorea ternacea* L.	Sankhupushpam
43		*Vigna unguiculata* Savi	Cowpea
44		*Phaseolus mungo* L.	Black gram
45	Malvaceae	*Abelmoschus esculentus* L. Mench	Bhindi
46		*Sida acuta* Burm	Vellakurumthotti
47		*Marselia quadrifolia*	TinyPepper wort
48	Moraceae	*Artocarpus integrifolia* L.	Jack
49	Musaceae	*Musa* sp	Banana
50	Myrtaceae	*Eugenia caryophyllus* L.	Clove
51		*Psidium guajava* L.	Guava
52	Myristicaceae	*Myristica fragrans* L.	Nutmeg
53	Nymphiaceae	*Nymphaea nouchali* Burm F.	Water lily
54	Onagraceae	*Ludwigia parviflora*	Roxb
55	Orchidaceae	*Dendrobium* sp.	Orchid
56	Oxalidaceae	*Oxalis corniculata* L.	Puliyarila
57	Palmae	*Cocos nucifera* L.	Coconut
58		*Areca catechu* L.	Arecanut
59	Piperaceae	*Piper nigrum* L.	Pepper
60		*Piper betle* L.	Betel vine
61		*Pepperomia* sp.	Kolumashi
62	Poaceae	*Oryza sativa* L.	Rice
63		*Sorghum vulgare* Pers.	Sorghum
64		*Saccharum officinarum L.*	Sugarcane
65		*Cynodon dactylon (L.) Pers.*	Bermuda grass
66		*Echinochloa colonum* Beaur	Jumela rice, Kavada
67	Pontederaceae	*Monochoria vaginalis* Prest	Pickerel weed, Neerthamara
68	Portulacaceae	*Portulaca oleraceae*	Indian purselane/ karichera
69	Rubiaceae	*Oldenlandia umbellata* L.	Nongunam
70		*Coffea arabica* L.	Coffee
71	Scrophulariaceae	*Scoparia dulcis* L.	Kallurukki
72	Umbelliferae	*Centella asiatica* Urban	Pennywort/ Kudangal
73	Solanaceae	*Solanum melongena* L.	Brinjal
74		*Capsicum annuum* L.	Chilli
75		*Lycopersicon esculentum*	Tomato
76	Zingiberaceae	*Zingiber officinale* L.	Ginger

Based on these studies, the most potent pathogen with narrow host range was selected.

3.4 COMBINED EFFECT OF PROMISING FUNGAL PATHOGENS

The most promising fungal pathogen (3.2), *A. eichhorniae* was used in combination with *F. pallidoroseum*, the already identified efficient biocontrol fungi of water hyacinth, to find out the combined effect of these two biocontrol agents. *F. oxysporum* and *F. moniliforme* were not included in this study as they had wide host range (section 3.5).

3.4.1 Using Fungi

Equal quantities of spore suspensions (3.3.1) of *F. pallidoroseum* and *A. eichhorniae* were mixed and applied on water hyacinth plants.

Treatments

1. *A. eichhorniae* alone

2. *F. pallidoroseum* alone

3. *F. pallidoroseum* + *A. eichhorniae*

Three replications of each treatment were maintained. Sterile water and 15-day-old uninoculated Czapek's solution sprayed water hyacinth plants served as control. Intensity of infection and Disease Index were calculated (3.3.1).

3.4.2 Using Cell Free Metabolite

The cell free metabolites of *A. eichhorniae* and *F. pallidoroseum* were prepared as described under 3.3.2.1. Equal volumes of cell free metabolite of each fungi were mixed and used for the experiment.

Treatments

1. *A. eichhorniae* alone

2. *F. pallidoroseum* alone

3. *F. pallidoroseum* + *A. eichhorniae*

Three replications of each treatment were maintained. Sterile water and 15 day old uninoculated Czapek's solution sprayed water hyacinth plants served as control. Intensity of damage was recorded seven days after spraying by using the score chart described under 3.3.2.2. Index for intensity of damage was assessed using the formula given under section 3.3.1.

3.5 STANDARDISATION OF DOSAGE OF INOCULUM

This experiment was conducted to standardise the dosage of inoculum of *A. eichhorniae*, the most promising fungal pathogen of water hyacinth. The spore concentrations at five levels viz., 10^4, 10^5, 10^6, 10^7 and 10^8 spores per ml were tested. The spore suspensions at the required concentrations were prepared in sterile water and sprayed @10 ml/plant on water hyacinth plants maintained in pots (1 litre capacity) using an atomizer. Plants sprayed with sterile water served as control. The sprayed plants were covered with polythene cover to maintain sufficient humidity. Three replications were maintained for each concentration. Intensity of infection was recorded after six days. Disease index was calculated as described in 3.3.1.

3.6 EVALUATION OF THE EFFECTIVE DOSAGE OF INOCULUM

Pot culture trial was carried out to evaluate the effective dosage of *A. eichhorniae* and *F. pallidoroseum* individually and in combination on water hyacinth. Spore concentration that gave maximum intensity of

infection *viz.*, 10^7 spores/ ml in the case of *A. eichhorniae* and 10^{11} spores/ ml (from previous studies) in the case of *F. pallidoroseum* were used for the study. Their combination was prepared by mixing equal volumes of spore suspensions of each fungi.

The spore suspensions were sprayed @ 10 ml/ plant on water hyacinth plants maintained in pots (1 litre capacity). Plants sprayed with sterile water served as control. The plants were covered with polythene cover to maintain sufficient humidity. Three replications were maintained for each treatment. Intensity of infection was recorded after six days and disease index was calculated as described in 3.3.1.

3.7 DEVELOPMENT OF A TALC BASED FORMULATION

A talc based combination product of the fungi (*F. pallidoroseum* + *A. eichhorniae*) was prepared, as these two together recorded maximum intensity of infection in 3.6.

Stock culture of *F. pallidoroseum* and *A. eichhorniae* were maintained on potato dextrose agar. Discs of 5 mm diameter from seven day old culture of *F. pallidoroseum* + *A. eichhorniae* were inoculated into 100 ml sterilized Czapek's broth in 250 ml conical flasks. 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 filter paper. Fungal mats thus obtained were pressed between layers of sterile coarse filter paper to remove excess moisture and thoroughly ground using a blender. This was mixed with 10 per cent talc and 1.00 per cent Carboxy methyl cellulose.

3.7.1 Evaluation of the Formulated Product on Water Hyacinth

The formulated product was sprayed on water hyacinth plants @ 10 ml/ plant at four different concentrations viz. 1, 3, 5 and 10 per cent.

Three replications were maintained for each treatment. Water hyacinth plants sprayed with sterile water served as control. The plants were covered with polythene cover to maintain sufficient humidity. Intensity of infection was recorded on six and ten DAS and disease index was calculated as described in 3.3.1.

3.7.2 Shelf Life of the Formulated Product

In order to find out the shelf life of the formulated product, it was kept in sterilized polythene covers at room temperature (28 ± 4^{0}C). Viability of the 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. STATISTICAL ANALYSIS

The data obtained during the study were analysed statistically by applying the techniques of analysis of variance (Panse and Sukhatme, 1967). Angular transformation was used for transforming the values.

RESULTS

4. RESULTS

4.1 IDENTIFICATION AND CHARACTERIZATION OF FUNGAL PATHOGENS OF WATER HYACINTH

Fungi which were not identified in the earlier study (DST project on 'Biocontrol of water hyacinth using mycoherbicides') were provisionally identified and confirmed by Agharkar Research Institute, Pune. The cultural and morphological characters of each of the pathogenic fungi are as follows.

***Alternaria eichhorniae* Nagraj and Ponnappa ITCC No. 5349**

It produced velvetty mycelial growth with brownish white mycelium. In culture, the fungus produced intense red pigment which darkened with age (Plate3A). Conidiophores were simple, at times branched bearing conidia which were round to ovate, provided with 3-6 longitudinal septa and 1-4 transverse septa and long cylindrical beak which tapered gradually. The conidia were 32-64.5 x 9.84-13.1 μm in size(Plate4A).

***Colletotrichum gloeosporioides* (Penz.) Penz and Sacc.**

It produced abundant aerial mycelium which was white at first and later changed to greyish white(Plate3C). Hyphae were branched and septate. Fungus produced large number of acervuli in culture, which were globose, dark brown to black coloured. Conidiophores were non septate and hyaline. Conidia were single celled, hyaline, straight with blunt ends, oil globule in the centre and 12.2 x 16.9 x 3-8 μm in size(Plate4B).

***Curvularia lunata* (Wak.) Boedjin IMI No. 357146**

The fungus produced black velvetty mycelium. Conidiophores were branched, septate and dark brown coloured (Plate3D). Conidia were three celled, with the middle cell slightly curved and 18.72 – 29.1 x 9.1 x 13.9 μm in size (Plate4C).

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

It produced abundant aerial mycelium, which was wooly and white, later becoming cream coloured(Plate3F). Hyphae branched and septate. Macroconidia and microconidia abundant. Macroconidia were larger in size, long and hyaline with round tips and 34-60 x 3-5 μm in size. Microconidia were hyaline, small oval and 5-13 μm x 3-4.5 μm in size (Plate 4E).

***F. moniliforme* Sheld**

Aerial mycelium was white initially, turning rosy peach colour later. Powdery growth was also seen towards the centre of the colony (Plate3G). Conidiophores were simple and hyaline. Chlamydospores were present. Macroconidia were somewhat straight, tapering towards either ends, hyaline, 3-5 septate and 3.6-12.2 x 1.8-3.1 μm size (Plate 4 F).

***F. oxysporum* Schlet. Isolate 1**

It produced whitish puffy mycelium with no colouration from below (Plate3H). Hyphae were branched and septate. Conidiophores were simple. Few chlamydospores were present. Macroconidia were subcylindrical with narrow tip, round base, hyaline, 3-4 septate and 19.4-41 x 2.5-4.1 μm in size. Microconidia were oval, hyaline and 4.0-6.54 x 2.0-3.1 μm size (Plate 4G).

***F. oxysporum* Isolate 2**

The fungus produced white mycelium first, which later turned light pinkish on upper side and light pink below (Plate 3F). Hyphae and conidiophores were hyaline. Macroconidia were seen in large numbers which were hyaline and cylindrical to falcate, 4-6 septate and 18.5-48 x 2.5-4.3 μm size. Microconidia were single celled, oval, hyaline, 6.24-13.2 x 2-3.1 μm in size (Plate 4H).

***F. pallidoroseum* (Cooke.) Sacc. Isolate 1**

Aerial mycelium of the fungi was dull white initially later deep yellow at the centre of colony from below (Plate3J). Conidiophores were branched. Falcate macroconidia which gradually tapered at one end but bluntly rounded at the apex were present which were 3-4 septate and 15.2-37.63 x 2.3-4.1 μm in size. Microconidia hyaline, single celled, oval and 4.13-14.5 x 1.6-7.2 μm in size (Plate 4 I).

***F. pallidoroseum* Isolate 2**

It produced white vigorously growing aerial mycelium which later changed to dark purplish peach on the lower side. Powdery growth was seen towards the centre of the colony (Plate 3K). Conidiophores branched. Macroconidia were falcate, tapering towards one end and blunt at the other end, hyaline, 3-4 septate and 12.2-39.1 x 2.5-4.5 μm size. Microconidia single celled, small, hyaline, oval and 3.5-6.8 x 1.6-3.54 μm size (Plate 4 J).

***F. pallidoroseum* Isolate 3 IMI No. 357140**

Cultures at first white later turning light purple colour below(Plate3L). Hyphae branched and septate. Conidiophores branched, non-septate and hyaline. Macroconidia slightly curved, hyaline, 3-4 septate and 25.4-40.1 x 3.2-5 μm size. Microconidia hyaline, oval, single celled and 4.9-8.3 μm x 1.6-3.1 μm in size (Plate 4K).

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

The fungus had off white mycelium initially which changed dark green when spore masses formed on the surface of mycelial mat. The fungus produced sporodochia in cultures, which aggregated to form globose mass of conidia.. The spore masses were at first green in colour later becoming dark (Plate3M). The conidiophores were fasiculate,

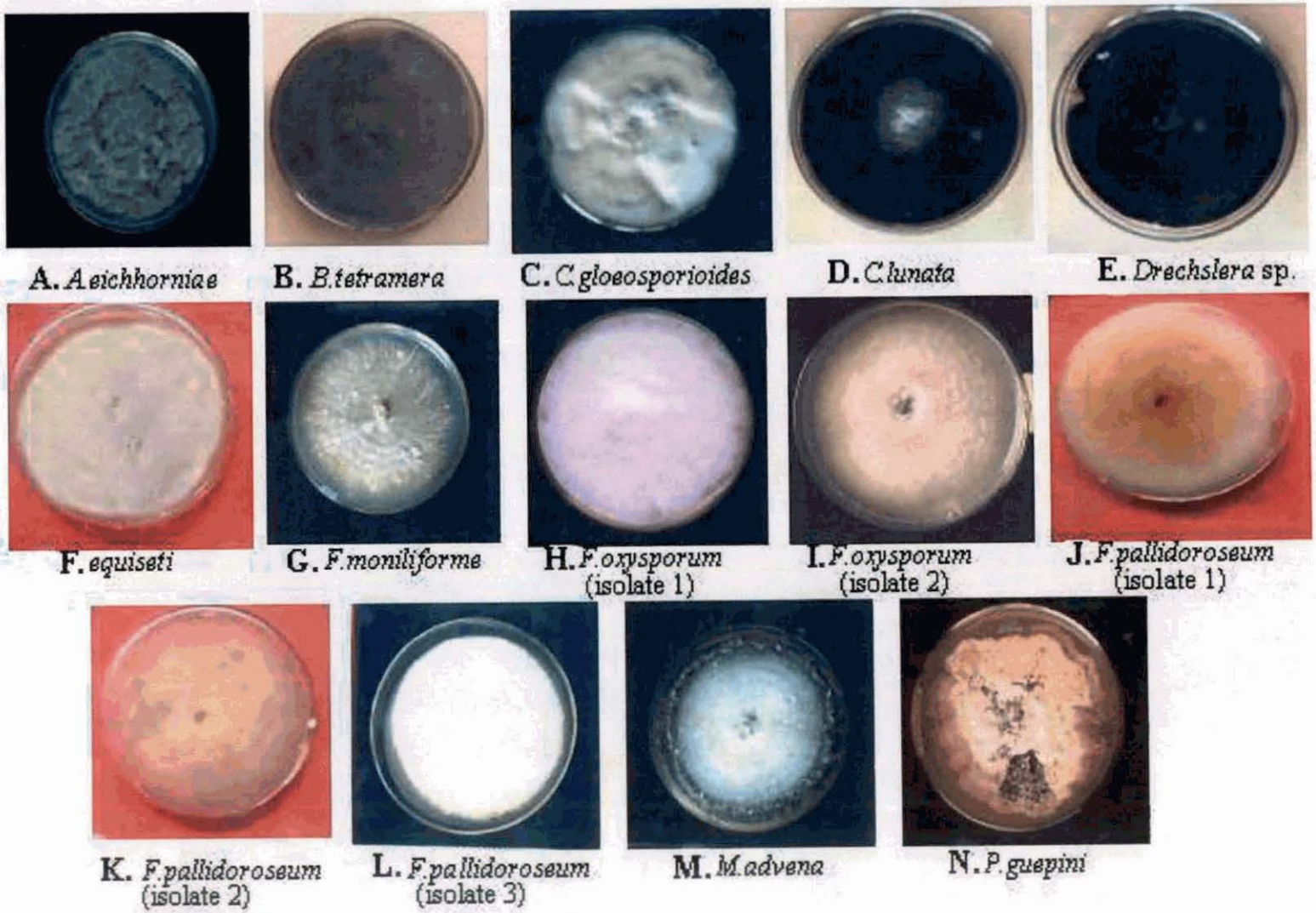
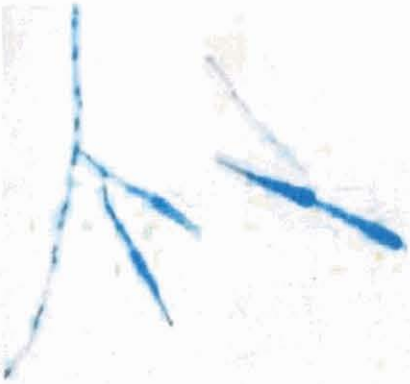
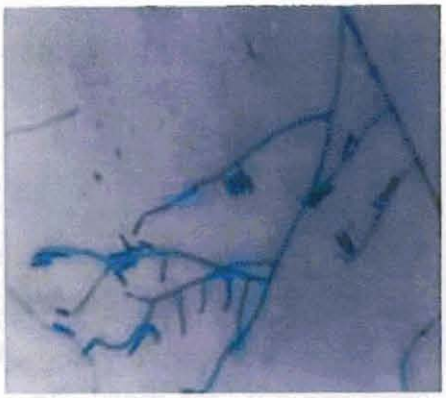


Plate 3. Growth of different fungi on culture media



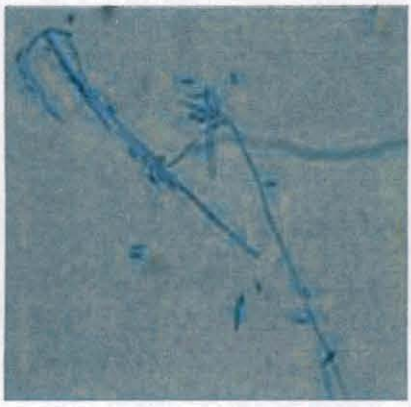
A. *Aeichhorniae*



B. *C.gloeosporioides*

C. *C.lunata*

D. *Drechslera* sp.



E. *F.equiseti*

F. *F.moniliforme*

Plate 4. Microphotographs of different fungi

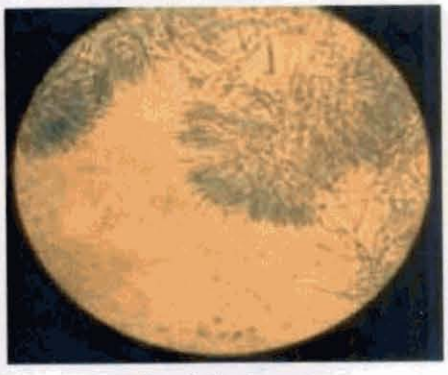
G. *F.oxysporum* (isolate 1)

H. *F.oxysporum* (isolate 2)

I. *F.pallidoroseum* (isolate 1)

J. *F. pallidoroseum* (isolate 2)

K. *F.pallidoroseum* (isolate 3)



L. *M.advena*

M. *P.guepinii*

Plate 4 continued

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 (Plate 4L).

Unidentified 1

The fungus produced brownish velvetty mycelium. Conidiophores were brown and septate. Conidia were straight or slightly curved, brown, 8-12 septate and 31-98.2 x 11.2 x 25.3 μm size. This was identified and confirmed as *Bipolaris tetramera* (Mc Kinney) Shoemaker (Plates 3B and 4M).

Unidentified 2

It produced black velvetty mycelium. Hyphae were branched, septate and brown. Conidiophores were simple, septate, Conidia were straight, brown, 8-10 transverse septa and 46.8-93.42 x 12.21-21.1 μm in size. This fungus was identified and confirmed as *Drechslera* state of *Cochliobolus sativus* (Ito and Kuribayashi) Dreschler ex Dastur (Plates 3E and 4D).

Unidentified 3

It produced white mycelium which turned pale yellow as it became older with distinct zonations. Hyphae were branched, septate and brown. Black erumpent conidiomata was present. Conidia were straight, 5 celled with middle three cells coloured and the apical two cells were hyaline. Abundant conidia and 18.4-25 x 5.2-8.5 μm size. The identification of this fungus was confirmed as *Pestalotiopsis guepinii* (Desm.) Stey (Plates 3N and 4M).

4.2 PATHOGENICITY

The variations in the nature of symptoms produced by the pathogenic fungi on water hyacinth were studied by testing their pathogenicity (Table 3, Plates 5A-E). It was observed that the time taken for the symptom

development varied with the pathogen. Based on the time taken for the symptom development, the pathogens were grouped into three categories.

Group I : Symptom developed in less than five days

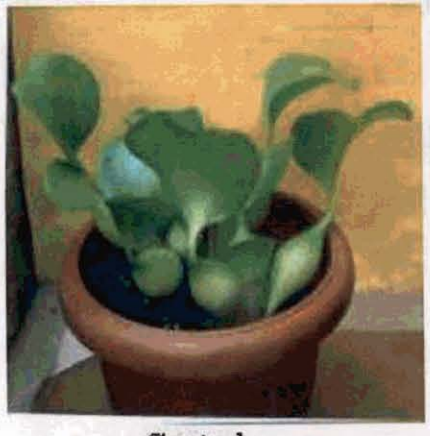
Group II : Symptom developed in five to seven days

Group III : Symptom developed in more than seven days.

M. advena and *F. pallidoroseum* (isolate 3) belonged to group I. The symptom produced by these fungi was in the form of small brown spots which coalesced to form large lesions and resulted in complete drying up of the plant within a week. Group II included the pathogens, *A. eichhorniae, B. tetramera, F. moniliforme, F. oxysporum* (isolates 1 and 2), and *F. pallidoroseum* (Isolate 1 and 2). In general, the fungi coming under this group developed symptoms on the leaves as well as on stalk of the host plant. These fungi produced brown spots with yellow halo on the leaf lamina only. Those fungi which are classified under group III *viz., C. gloeosporioides, C. lunata, Drechslera* sp., *F. equiseti* and *P. guepinii* took eight to ten days to develop symptoms. Of these *F. equiseti* and *P. guepinii* produced symptom on leaf lamina only. *B. tetramera, C. lunata* and *P. guepinii* produced small brown spots which remained without further spread. Though *C. gloeosporioides,* took more time for symptom development (eight to ten days) and produced small dark brown spots on the leaf lamina initially, these later developed into large lesions on the leaf and the petiole.

4.3 SELECTION OF MOST PROMISING FUNGAL PATHOGENS

An experiment was conducted to select the most promising fungal pathogens of water hyacinth by analysing the intensity of infection produced by them. On statistical analysis, significant difference was noticed among the fungi. The extent of damage ranged from 9.94 to 58.80



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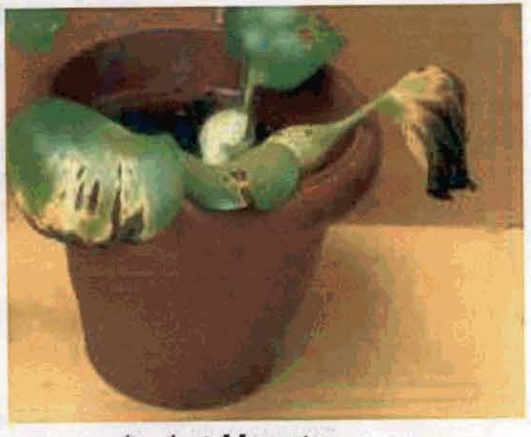


A. *Aeichhorniae*

B. *F.moniliforme*

C. *F.oxysporum*
(isolate 2)

D. *F.pallidoroseum*
(isolate 3)

E. *M.advena*

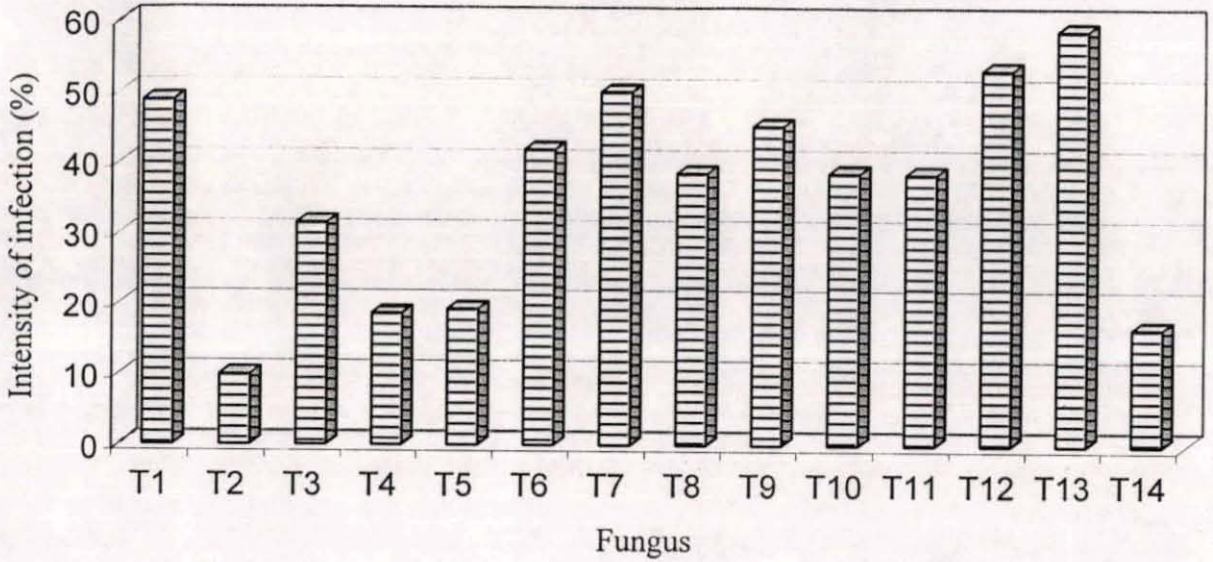
Plate 5. Symptoms produced by the fungi

Table 3 Variation in symptom development on water hyacinth by pathogenic fungi

Sl. No.	Pathogenic fungi	Time taken for symptom development (days)	Group	Description of symptoms	
				Part/s affected	Symptoms
1	*Alternaria eichhorniae*	5-6	II	Leaf and petiole	Initially small brown spots with yellow halo developed on the leaf lamina, which coalesced to form large spots, later resulted in blighting. Small brown spots were seen on petiole also
2	*Bipolaris tetramera*	6-7	II	Leaf	Small isolated light brown spots appeared and remained without further spreading
3	*Colletotrichum gloeosporioides*	8-10	III	Leaf and petiole	Initiated as dark brown spots on the leaves later enlarged and coalesced to from large patches. Brown spots were seen on petiole also
4	*Curvularia lunata*	9-10	III	Leaf and petiole	Brownish black pin head sized spots developed.
5	*Drechslera* sp.	8	III	Leaf	Light brown irregular spots appeared and these spots remained as such.
6	*Fusarium equiseti*	10	III	Leaf	Appeared as small brown spots with prominent yellow halo, later the spots enlarged to form large brown lesions.
7	*F. moniliforme*	6-7	II	Leaf and petiole	Brown spots developed which later enlarged to form big lesions
8	*F. oxysporum* Isolate 1	5-6	II	Leaf and petiole	Small brown spots with yellow halo appeared, which later enlarged to form large irregular lesions.

Table 3 continued

9	*F. oxysporum* Isolate 2	5-7	II	Leaf and petiole	Brown spots enlarged to form dark brown lesions with prominent yellow halo.
10	*F. pallidoroseum* Isolate 1	5-7	II	Leaf and petiole	Brown spots with faint yellow halo developed, later coalesced to form large brown lesions
11	*F. pallidoroseum* Isolate 2	5-7	II	Leaf and petiole	Brown spots enlarged to form large lesions with yellow halo.
12	*F. pallidoroseum* Isolate 3	4-5	I	Leaf and petiole	Small brown spots with distinct yellow halo developed. Later enlarged to form large brown irregular lesions spreading from the tip downwards, resulted in blighting and drying.
13	*Myrothecium advena*	3	I	Leaf and petiole	Water soaked spots developed initially on leaves that later enlarged and coalesced to form dull greyish water soaked lesions. On petioles, spots appeared which later formed lesions and finally resulted in the drying up of the whole plant
14	*Pestalotiopsis guepinii*	8-10	III	Leaf	Isolated small pinhead sized brown spots were seen.



T1 *Alternaria eichhorniae*
T2 *Bipolaris tetramera*
T3 *Colletotrichum gloeosporioides*
T4 *Curvularia lunata*
T5 *Drechslera sp.*
T6 *Fusarium equiseti*
T7 *F. moniliforme*
T8 *F. oxysporum I* solate 1
T9 *F. oxysporum I* solate 2
T10 *F. pallidoroseum I* solate 1
T11 *F. pallidoroseum* Isolate 2
T12 *F. pallidoroseum* Isolate 3
T13 *Myrothecium advena*
T14 *Pestalotiopsis guepinii*

Fig. 1 Extent of damage produced by pathogenic fungi on water hyacinth

Table 4 Extent of damage produced by pathogenic fungi on water hyacinth

Sl. No.	Fungus	*Intensity of infection (%)
1	*Alternaria eichhorniae*	48.89 (44.35)
2	*Bipolaris tetramera*	9.94 (18.37)
3	*Colletotrichum gloeosporioides*	31.56 (34.16)
4	*Curvularia lunata*	18.66 (25.58)
5	*Drechslera* sp..	19.26 (26.04)
6	*Fusarium equiseti*	41.99 (40.37)
7	*F. moniliforme*	50.14 (49.08)
8	*F. oxysporum* Isolate 1	38.66 (38.43)
9	*F. oxysporum* Isolate 2	45.33 (42.30)
10	*F. pallidoroseum* Isolate 1	38.69 (38.45)
11	*F. pallidoroseum* Isolate 2	38.53 (38.35)
12	*F. pallidoroseum* Isolate 3	53.27 (46.85)
13	*Myrothecium advena*	58.80 (50.05)
14	*Pestalotiopsis guepinii*	16.67 (24.08)

CD (0.05) : 1.96

Figures in parentheses indicate transformed values
*Average of three replications

per cent. Maximum extent of damage was recorded by *M. advena* (58.80 per cent), followed by *F. pallidoroseum* (isolate 3) (53.27 per cent), *F. moniliforme* (50.14 per cent), *A. eichhorniae* (48.89 per cent) and *F. oxysporum* (isolate 2) (45.33 per cent) (Table 4 and Fig. 1). *M. advena* was significantly better than other fungi. *F. moniliforme* was on par with *F. pallidoroseum* isolate 3 and *A. eichhorniae*. Similarly, *F. oxysporum* (isolate 1) and isolate 1 and 2 of *F. pallidoroseum* were on par. Least intensity of infection was recorded by *B. tetramera* (9.94 per cent).

4.3.1 Effect of Cell Free Metabolites of the Pathogenic Fungi

The cell free metabolite of each of the pathogenic fungi was extracted and its effect on water hyacinth plants was studied by recording the nature of symptoms produced (Table 5, Plates 6A-E). Based on the time taken for the symptom development by the cell free metabolites of the pathogenic fungi, they were grouped into three categories.

Group I : The cell free metabolite of this category of fungi produced symptoms in three days. *M. advena* comes under this group which produced brown patches on the plant surface in two days resulting in the scorching of the entire plant.

Group II : The cell free metabolites of these fungi developed symptoms within four to five days. This included *A. eichhorniae*, *F. moniliforme*, *F. oxysporum* (isolate 1 and 2), *F. pallidoroseum* (isolate 2 and 3) and *Drechslera* sp. This group of fungi developed symptoms on leaf lamina or leaf lamina and petiole as scorching coupled with yellowing and finally drying up of the leaves and petiole.

Group III : Fungi coming under this group developed symptoms within six to seven days. This included *B. tetramera, C. gloeosporioides, C. lunata, F. pallidoroseum* (isolate 1), *F. equiseti* and *P. guepinii.*

Control

A. *Aeichhorniae*

B. *F.moniliforme*

C. *F.oxysporum* (isolate 2)

D. *F.pallidoroseum* (isolate 3)

E. *M.advena*

Plate 6. Symptoms produced by the cell free metabolites of fungi

Table 5 Effect of cell free metabolites of pathogenic fungi on water hyacinth

Sl. No.	Pathogenic fungi	Time taken for symptom development (days)	Group	Description of symptoms	
				Part/s affected	Symptoms
1	*Alternaria eichhorniae*	4	II	Leaf and petiole	Small necrotic spots developed on leaf, which later enlarged and became brown irregular lesions with yellow halo and resulted in scorching
2	*Bipolaris tetramera*	6	III	Leaf	Minute necrotic spots developed on the leaves only
3	*Colletotrichum gloeosporioides*	6	III	Leaf	Chlorotic spots developed and finally resulted in scorching
4	*Curvularia lunata*	7	III	Leaf	Necrotic spots developed and resulted in yellowing of leaves
5	*Drechslera* sp.	4	II	Leaf	Small black necrotic spots seen. No spreading of spots
6	*Fusarium equiseti*	6	III	Leaf	Minute irregular spots appeared and remained without further spread
7	*F. moniliforme*	4	II	Leaf and petiole	Brown patches appeared on the leaves and petiole and later coalesced, scorching and yellowing seen
8	*F. oxysporum* Isolate 1	5	II	Leaf	Initially scorching of younger leaves developed, later yellowing was seen
9	*F. oxysporum* Isolate 2	4	II	Leaf and petiole	Initially scorching seen on older leaves. Elongated brown spots with yellow halo produced. Yellowing seen on stalk also

Table 5 Continued

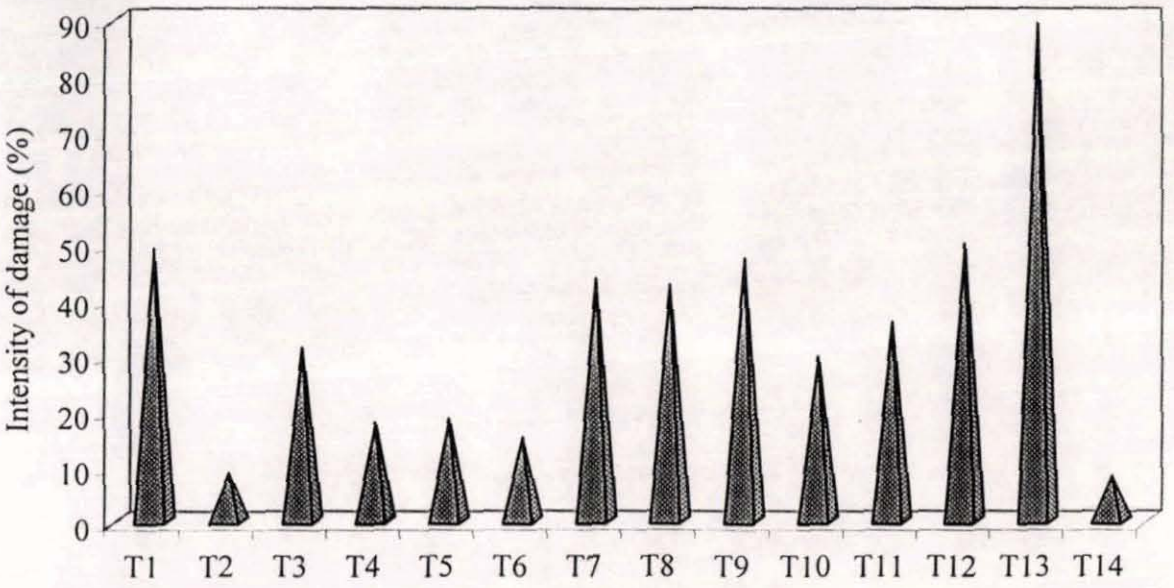
10	*F. pallidoroseum* Isolate 1	6	III	Leaf and petiole	Scorching started on younger leaves followed by yellowing of leaves and stalk
11	*F. pallidoroseum* Isolate 2	5	II	Leaf	Scorching of leaves followed by yellowing and drying
12	*F. pallidoroseum* Isolate 3	4	II	Leaf and petiole	Scorching coupled with yellowing of older leaves. Gradually leaves were dried, mild symptom also observed on the petiole
13	*Myrothecium advena*	2	I	Leaf and petiole	Small brown patches developed on leaves and covered the entire leaf within a week. Scorching of leaves and petioles was observed
14	*Pestalotiopsis guepinii*	7	III	Leaf	Yellowing of leaves seen

Table 6 Extent of damage produced by cell free metabolites of pathogenic fungi on water hyacinth

Sl. No.	Fungus	*Intensity of damage (%)
1	*Alternaria eichhorniae*	48.80 (44.29)
2	*Bipolaris tetramera*	8.59 (17.03)
3	*Colletotrichum gloeosporioides*	31.09 (33.87)
4	*Curvularia lunata*	17.59 (24.79)
5	*Drechslera* sp.	18.30 (25.31)
6	*Fusarium equiseti*	15.00 (22.78)
7	*F. moniliforme*	43.40 (41.19)
8	*F. oxysporum* Isolate 1	42.52 (40.67)
9	*F. oxysporum* Isolate 2	47.20 (43.19)
10	*F. pallidoroseum* Isolate 1	29.40 (32.82)
11	*F. pallidoroseum* Isolate 2	35.70 (36.67)
12	*F. pallidoroseum* Isolate 3	49.80 (44.86)
13	*Myrothecium advena*	89.00 (70.60)
14	*Pestalotiopsis guepinii*	8.03 (16.46)

CD (0.05) : 1.12

Figures in parentheses indicate transformed values
*Average of three replications



T1 *Alternaria eichhorniae*
T2 *Bipolaris tetramera*
T3 *Colletotrichum gloeosporioides*
T4 *Curvularia lunata*
T5 *Drechslera sp.*
T6 *Fusarium equiseti*
T7 *F. moniliforme*
T8 *F. oxysporum I* solate 1
T9 *F. oxysporum I* solate 2
T10 *F. pallidoroseum I* solate 1
T11 *F. pallidoroseum* Isolate 2
T12 *F. pallidoroseum* Isolate 3
T13 *Myrothecium advena*
T14 *Pestalotiopsis guepinii*

Fig. 2 Extent of damage produced by the cell free metabolites of pathogenic fungi on water hyacinth

Of these *F. pallidoroseum* (isolate 1) alone developed symptoms on the leaves as well as petiole. The fungi of this group produced yellowing or necrotic spots resulted in scorching. In the case of *B. tetramera* and *F. equiseti* these necrotic spots failed to spread further.

4.3.2 Testing the Efficacy of Cell Free Metabolites

The efficacy of the cell free metabolite of various pathogenic fungi was evaluated by calculating the extent of damage produced by each of them. The statistical analysis of the extent of damage caused by the culture filtrate of the pathogenic fungi revealed that there was significant difference among the fungi (Table 6 and Fig. 2).

From the extent of damage developed by cell free metabolites of pathogenic fungi on water hyacinth, it was observed that maximum intensity of damage *i.e.*, 89.0 per cent, was produced by the filtrate of *M. advena.* This was followed by *F. pallidoroseum* (isolate 3), *A. eichhorniae, F. oxysporum* (isolate 2) and *F. moniliforme* being 49.80, 48.80, 47.20 and 43.40 per cent respectively. *M. advena* was significantly different from other fungi. The extent of damage caused by the cell free metabolites of *F. pallidoroseum* (isolate 3) and *A. eichhorniae* were on par. *F. moniliforme* and *F. oxysporum* (isolate 1), C. *lunata* and *Drechslera* sp., and *B. tetramera* and *P. guepinii* were on par. Least damage was recorded by the filtrate of *P. guepinii* being 8.03 per cent.

4.3.3 Host Range Studies

Detailed study on the host range of the three promising fungal pathogens of water hyacinth *viz.*, *A. eichhorniae*, *F. moniliforme* and *F. oxysporum* were carried out on common cultivated plants and weed plants seen near the water ways of Kerala.. This included 37 cultivated plants and 39 weed plants coming under 38 families (Table 7). The results of the study revealed that plants coming under Anacardiaceae, Asclepidiaceae,

Table 7 Host range studies of fungal pathogens of water hyacinth

Sl. No.	Family	Scientific *name*	Common name / vernacular name	Susceptibility (+/–) to		
				A. e	F. m	F. o
1	Acanthaceae	*Justicia diffusa* Wild	Justicia	–	+	–
2		*J. prostrata* Gamble N. Comb	Justicia	–	+	–
3	Amaranthaceae	*Amaranthus viridis* (Linn.) Notrysag	Slender Amaranthus	–	–	–
4		*A. incolor* L.	Amaranthus	–	+	–
5		*Gomphrena decumbens*	Balippovu	+	–	–
6		*Alternanthera sissilis*	Alligator weed	–	–	+
7	Anacardiaceae	*Mangifera indica* L.	Mango	–	–	–
8		*Anacardium occidentale* L.	Cashew	–	–	–
9	Araceae	*Colocasia esculenta*	Taro	–	+	–
10		*Amorphophallus companulatus*	Elephant foot yam	–	–	–
11		*Anthurium andreanum* L.	Anthurium	–	–	–
12		*Pistia stratiotes*	Water lettuce, muttapayal	–	–	–
13	Asclepidiaceae	*Hemidesmus indicus* R. Br	Naruneendi	–	–	–
14	Asteraceae	*Tridax procumbens* L.	Odiyan	–	+	–
15		*Vernonia cineria* L.	Poovam kurunnu	–	+	–

Table 7 Continued

Sl. No.	Family	Scientific *name*	Common name / vernacular name	Susceptibility (+/–) to		
				A. e	F. m	F. o
16		*Synedrella nodiflora* L.	Venppachha	–	–	–
17		*Chromolaena odoratum* (L.) King and Robinson	Communist pachha	–	–	–
18		*Emilia sonchifolia* (L.) DC	Muyal chevian	–	+	+
19		*Eclipta alba* (L.) Hassk	Kayonni	–	–	+
20		*Ageratum conyzoides*	Goat weed/ Appa Knoxia	–	–	–
21		*Knoxia* sp	Knoxia	–	–	–
22	Boraginaceae	*Heliotropium indicum*	Venppacha	–	–	–
23	Capparidaceae	*Cleome viscose* L.	Wild mustard, Kattu Kaduku	–	–	–
24	Caricaceae	*Carica papaya*	Papaya	–	+	–
25	Commelinaceae	*Commelina benghalensis* L.	Tropical spider wort	–	+	+
26		*C. jacobi*	Vazhappadathy	–	+	+
27	Convolvulaceae	*Ipomoea batatus* (L.) Lam	Sweet potato	–	–	–
28	Cucurbitaceae	*Momordica charantia* L.	Bittergourd	–	–	–
29		*Cucumis sativus* L.	Cucumber	–	–	–
30		*Trichosanthes anguina* L.	Snake gourd	–	–	–

Table 7 Continued

Sl. No.	Family	Scientific *name*	Common name / vernacular name	Susceptibility (+/–) to		
				A. e	F. m	F. o
31	Cyperaceae	*Bulbostylis barbata*	Sooryan	–	–	–
32	Dioscoriaceae	*Dioscorea alata*	Yam	–	–	–
33	Euphorbiaceae	*Phyllanthus niruri* (L.) Hoof F.	Keezharnelli	–	–	–
34		*Ephorbia geniculata* L.	Paloorippacha	–	–	–
35		*E. hirta* L.	Tharavu	–	–	–
36		*Manihot esculenta* L.	Tapioca	–	–	+
37		*Hevea brasilensis*	Rubber	–	–	–
38	Labiatae	*Hyptis suaveolens* Port.	Nattapoochedi	–	–	–
39	Lamiaceae	*Leucas aspera* spring	Thumba	–	–	–
40	Lauraceae	*Cinnamomum zeylanicum*	Cinnamom	–	–	–
41		*Jasminum sambac*	Jasmine	–	–	–
42	Leguminosae	*Clitorea ternacea* L.	Sankhupushpam	–	–	–
43		*Vigna unguiculata* Savi	Cowpea	–	–	+
44		*Phaseolus mungo* L.	Black gram	–	–	–
45	Malvaceae	*Abelmoschus esculentus* L. Mench	Bhindi	–	–	–
46		*Sida acuta* Burm	Vellakurumthotti	–	–	–

Table 7 Continued

Sl. No.	Family	Scientific *name*	Common name / vernacular name	Susceptibility (+/–) to		
				A. e	F. m	F. o
47		*Marselia quadrifolia*	TinyPepper wort	+	–	–
48	Moraceae	*Artocarpus integrifolia* L.	Jack	–	–	–
49	Musaceae	*Musa* sp	Banana	–	–	–
50	Myrtaceae	*Eugenia caryophyllus* L.	Clove	–	–	–
51		*Psidium guajava* L.	Guava	–	–	+
52	Myristicaceae	*Myristica fragrans* L.	Nutmeg	–	–	–
53	Nymphiaceae	*Nymphaea nouchali* Burm F.	Water lily	–	–	–
54	Onagraceae	*Ludwigia parviflora*	Roxb	–	–	–
55	Orchidaceae	*Dendrobium* sp.	Orchid	–	–	+
56	Oxalidaceae	*Oxalis corniculata* L.	Puliyarila	–	–	–
57	Palmae	*Cocos nucifera* L.	Coconut	–	–	–
58		*Areca catechu* L.	Arecanut	–	–	–
59	Piperaceae	*Piper nigrum* L.	Pepper	–	–	+
60		*Piper betle* L.	Betel vine	–	–	–
61		*Pepperomia* sp.	Kolumashi	+	+	+

Table 7 Continued

Sl. No.	Family	Scientific *name*	Common name / vernacular name	Susceptibility (+/–) to		
				A. e	F. m	F. o
62	Poaceae	*Oryza sativa* L.	Rice	–	–	+
63		*Sorghum vulgare* Pers.	Sorghum	–	–	–
64		*Saccharum officinarum L.*	Sugarcane	–	–	–
65		*Cynodon dactylon (L.) Pers.*	Bermuda grass	–	–	+
66		*Echinochloa colonum* Beaur	Jumela rice, Kavada	–	+	–
67	Pontederaceae	*Monochoria vaginalis* Prest	Pickerel weed, Neerthamara	+	+	+
68	Portulacaceae	*Portulaca oleraceae*	Indian purselane/ karichera	–	–	–
69	Rubiaceae	*Oldenlandia umbellata* L.	Nongunam	–	–	+
70		*Coffea arabica* L.	Coffee	–	–	+
71	Scrophulariaceae	*Scoparia dulcis* L.	Kallurukki	–	–	–
72	Umbelliferae	*Centella asiatica* Urban	Pennywort/ Kudangal	–	+	–
73	Solanaceae	*Solanum melongena* L.	Brinjal	–	–	–
74		*Capsicum annuum* L.	Chilli	–	+	–
75		*Lycopersicon esculentum*	Tomato	–	+	–
76	Zingiberaceae	*Zingiber officinale* L.	Ginger	–	–	–

A.e. : *A. eichhorniae*, F.m. : *F. moniliforme*, F.o. : *F. oxysporum*,

A. *G.decumbens* B. *M.quadrifolia* C. *M.vaginalis*

Plate 7. Symptoms of *Aeichhorniae* on weed hosts

A. *C.asiatica* B. *E.colonum* C. Tomato

Plate 8. Symptoms of *F.moniliforme* on various hosts

A. Rice B. Orchid C. *C.dactylon*

Plate 9. Symptoms of *F.oxysporum* on various hosts

Boraginaceae, Capparadaceae, Convonvulaceae, Cucurbitaceae, Cyperaceae, Dioscoriaceae, Labiatae, Lamiaceae, Lauraceae, Musaceae, Moraceae, Myristicaceae, Nymphiaceae, Onagraceae, Oxalidaceae, Palmae, Portulacaceae, Scrophulariaceae and Zingiberaceae were free from infection by all the three fungi tested. The nature of symptoms produced by each of these pathogens on the susceptible plants are presented in Tables 8, 9 and 10. All the three fungi were found to infect *Monochoria vaginalis* which belongs to Pontederiaceae–the same family of water hyacinth.

4.3.3.1 *A. eichhorniae*

Of the 37 cultivated plants tested, *A. eichhorniae* did not infect any of them. However, it was found to be pathogenic on three weed plants *viz.*, *Gomphrena decumbens, M. vaginalis* and *Marselia quadrifolia* out of the 39 weed plants tested. In general, the fungus produced small brown necrotic spots and yellowing of the leaves of the above weed plants (Table 8, Plate7 A, B and C).

4.3.3.2 *F. moniliforme*

F. moniliforme infected five out of the 37 cultivated plants and 11 out of the 39 weed plants tested. The cultivated plants found to be susceptible to *F. moniliforme* were amaranthus, chilli, papaya, taro and tomato. The weed plants that developed symptoms of *F. moniliforme* were *Centella asiatica, Commelina benghalensis, C. jacobi, Echinochloa colonum, Emilia sonchifolia, Justicia diffusa, J. prostrata, M. vaginalis, Pepperomia* sp., *Tridax procumbens* and *Vernonia cineria*. The type of symptom produced by the pathogen on the different host plants are described in Table 9 (Plate 8 A, B and C).

4.3.3.3 *F. oxysporum*

Out of the 37 cultivated plants and 39 weed plants tested, *F. oxysporum* was pathogenic to seven cultivated plants and nine weed plants. The

Table 8 Symptoms produced by *A. eichhorniae* on susceptible weed plants

Plants Weeds	Time taken for symptom development (days)	Symptoms
Gomphrena decumbens	7–9	Yellowing followed by drying up of infected leaves
Marselia quadrifolia	6–7	Initially brown slots with yellow halo, later resulted in complete drying up of the leaves.
Monochoria vaginalis	3–4	Small brown spots developed on leaves, which enlarged to form lesions with yellow halo. Brown lesions with yellow halo developed on the stalk also.

Table 9 Symptoms produced by *F. moniliforme* on various host plants

Host plants	Time taken for symptom development (days)	Symptoms
Cultivated plants		
Amaranthus, chilli, taro	6–9	Brown lesions on leaves, later enlarged resulted in blighting.
Papaya, Tomato	5–7	Initially brown spots on leaves, which gradually caused yellowing and drying up of leaves.
Weed plants		
Centella asiatica	6–8	Brown lesions on the infected part which gradually enlarged and resulted in blighting of the leaves
Echinochloa colonum	7–9	Brown spots with yellow halo developed on the leaves, later resulted in complete drying up of the leaves
Commelina benghalensis, C. jacobi, Emilia sonchifolia, justicia diffusa, J. prostrata, Monochoria vaginalis, Pepperomia sp, *Tridax procumbens, Vernonia cineria*	7–10	Initially as brown spots, later leaves showed blighting and drying.

Table 10 Symptoms produced by *F. oxysporum* on various host plants

Host plants	Time taken for symptom development (days)	Symptoms
Cultivated plants		
Coffee, Pepper	6–8	Brown lesions on the leaves, which gradually spread and resulted in defoliation.
Cowpea, Rice, Tapioca	5–7	Small brown spots with yellow halo appeared on leaves, later blighting of the leaves
Guava	5–7	Dark brown spots developed on the leaves with no further spread
Orchid	6–7	Initially as small brown spots with whitish centre, which later enlarged to form large brown lesions with prominent yellow halo.
Cynodon dactylon	7–9	Light brown irregular spots with yellow halo enlarged and resulted in drying of the leaves.
Alternanthera sissilis, Commelina benghalensis, C. Jacobi, Pepperomia sp, *Monochoria vaginalis, Oldenlandia* umbellata	5–9	Small brown lesions on the infected part which gradually enlarged and resulted in drying up of the leaves
Eclipta alba, Emilia sonchifolia	7–9	Small brown spots on the leaf with distinct yellow halo

susceptible cultivated plants included coffee, cowpea, guava, orchid, pepper, rice and tapioca (Table 10 and Plate 9 A, B and C). The weed plants viz., *A. sissilis*, *C. benghalensis*, *C. jacobi*, *Cynodon dactylon*, *Eclipta alba*, *E. sonchifolia*, *M. vaginalis*, *Oldenlandia umbellata* and *Pepperomia* sp. were also found to be infected by the fungus.

4.4 COMBINED EFFECT OF MOST PROMISING FUNGAL PATHOGENS

4.4.1 Using fungi

An experiment was conducted to study the efficacy of *A. eichhorniae* and *F. pallidoroseum* individually and in combination on water hyacinth (Table 11 and Fig. 3). It was observed that combined application of the fungi gave 58.60 per cent intensity of infection whereas individual application of *A. eichhorniae* and *F. pallidoroseum* recorded 50.4 and 54.81 per cent intensity of infection respectively (Plate 10).

4.4.2 Using cell free metabolites

In the case of cell free metabolite, maximum intensity of damage of 55.44 per cent was recorded by the combined application of the metabolites of *A. eichhorniae* and *F. pallidoroseum* (Table 11 and Fig. 3). The time taken for the development of infection on water hyacinth was less when compared with the application of the pathogens individually. When applied individually, cell free metabolite of *A. eichhorniae* and *F. pallidoroseum* recorded 49.6 and 52.3 per cent intensity of damage respectively (Plate 10).

4.5 STANDARDISATION OF DOSAGE OF INOCULUM REQUIRED FOR THE EFFECTIVE DESTRUCTION

An experiment was conducted to standardise the spore concentration of *A. eichhorniae* required for the effective destruction of the weed (Table 12 and Fig. 4). Of the different doses of inoculum tested *viz.*, 10^4, 10^5, 10^6, 10^7 and 10^8 spores per ml, maximum intensity of infection of 63.01 per cent

Table 11 Extent of damage caused by *F. pallidoroseum* and *A. eichhorniae* individually and in combination

Sl. No.	Treatment	*Intensity of infection by spore suspension (%)	*Intensity of damage by cell free metabolite (%)
1	*A. eichhorniae*	50.40	49.60
2	*F. pallidoroseum*	54.81	52.30
3	*A. eichhorniae* + *F. pallidoroseum*	58.60	55.44

* Average of three replications

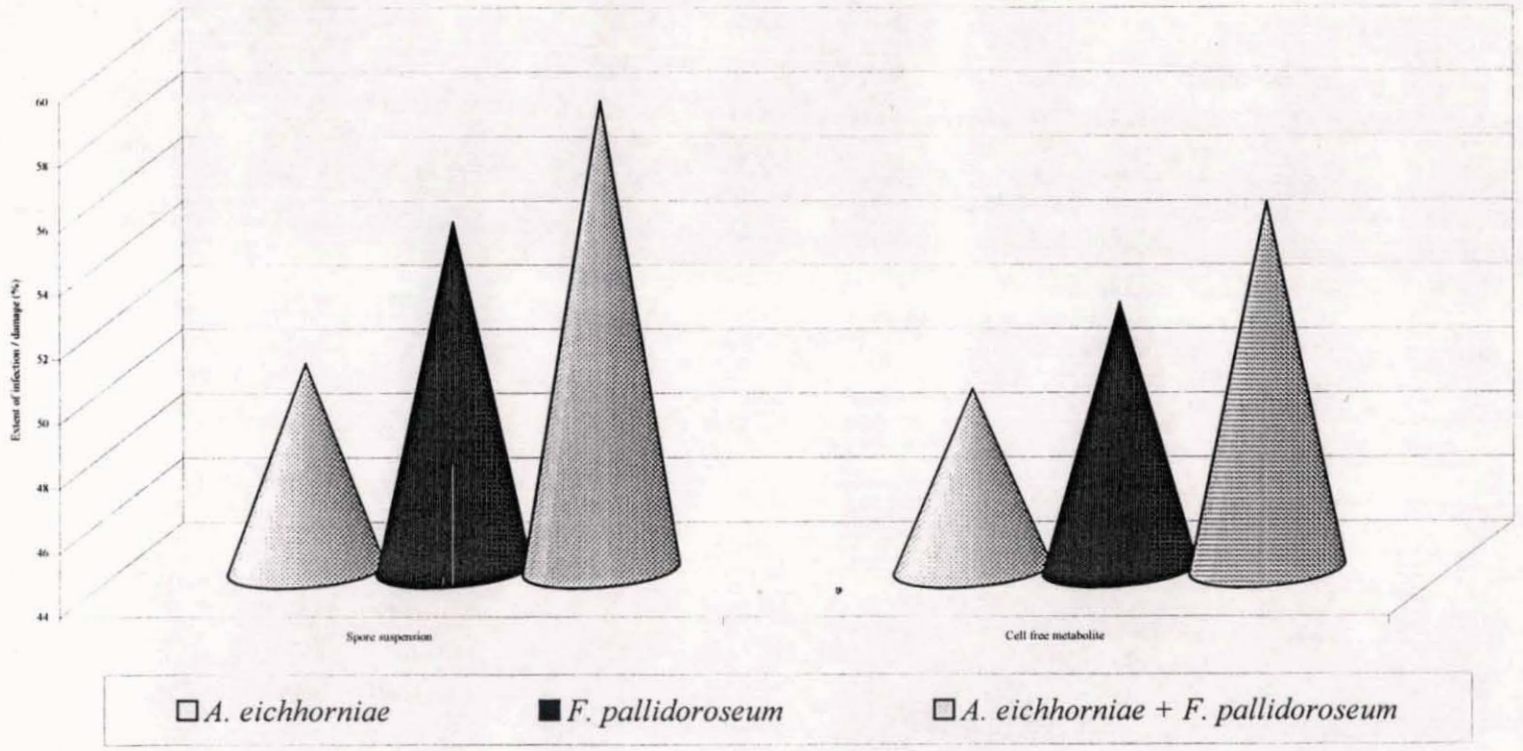


Fig. 3 Extent of damage caused by *F. pallidoroseum* and *A. eichhorniae* individually and in combination

Table 12. Extent of damage caused by different doses of inoculum of *A. eichhorniae*

Sl. No.	Doses of inoculum (spores/ ml)	* Intensity of infection (%)
1	10^4	35.64 (37.44)
2	10^5	39.22 (38.76)
3	10^6	58.22 (49.71)
4	10^7	62.83 (52.41)
5	10^8	63.01 (52.52)

CD (0.05) : 0.58
Figures in parentheses indicate transfored values
* Average of three replications

Table 13. Effect of effective dose of inoculum of the most promising fungi

Sl. No.	Treatments	Spore concentration	*Intensity of infection (%)
1	*A. eichhorniae*	10^7 spores/ ml	64.10
2	*F. pallidoroseum*	10^{11} spores/ ml	85.63
3	*A. eichhorniae* + *F. pallidoroseum*	10^7 spores/ ml + 10^{11} spores/ml	89.84

* Average of three replications

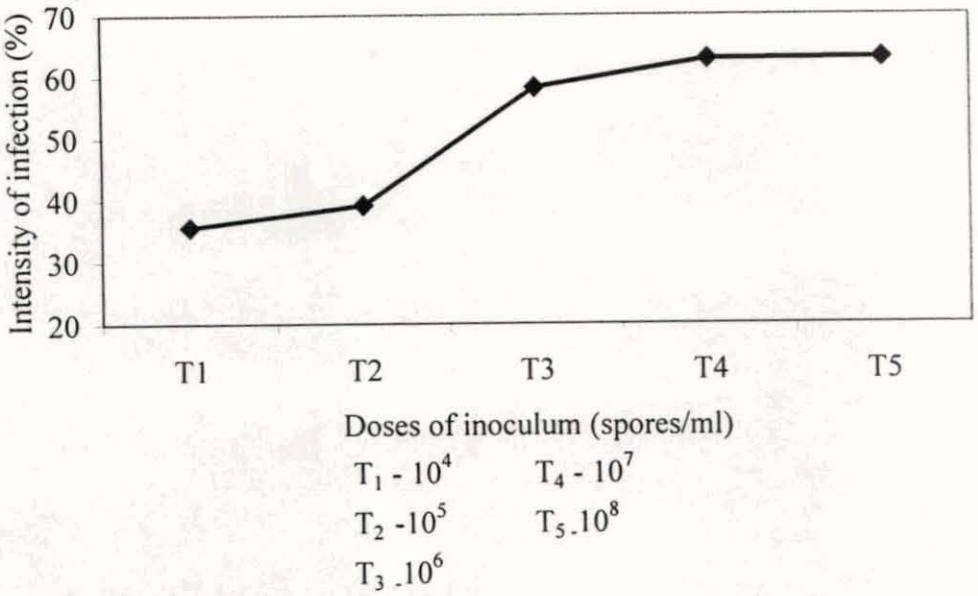
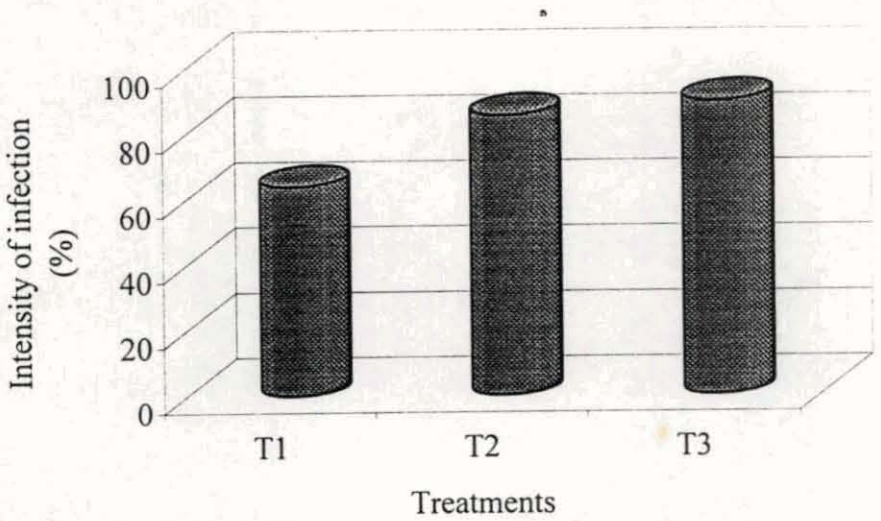


Fig. 4 Extent of damage caused by different doses of inoculum of *A. eichhorniae*



T1 - *A. eichhorniae* (10^7 spores / ml),
T2 - *F. pallidoroseum,* (10^{11} spores / ml),
T3 - *A. eichhorniae* + *F. pallidoroseum* (10^7 spores / ml + 10^{11} spores / ml)

Fig. 5 Effect of effective dose of inoculum of the most pormising fungi on water hyacinth

A. Control B. Fungi C. Cell free metabolites

Plate 10. Combined effect of *A eicohhorniae* and *F.pallidoroseum*

A. 10^7 spores per ml of *A eichhorniae*

B. 10^{11} spores per ml of *F.pallidoroseum*

C. 10^7 spores per ml of of *A eichhorniae* + 10^{11} spores per ml of *F.pallidoroseum*

Plate 11. Effect of effective dose of *A eicohhorniae* and *F.pallidoroseum*

was recorded in the case of 10^8 spores per ml, followed by 10^7 spores per ml being 62.83 per cent, which were on par.

4.6 EVALUATION OF THE EFFECTIVE DOSAGE OF INOCULUM

The most effective spore concentration of *A. eichhorniae* that caused maximum destruction of water hyacinth obtained from the previous experiment(10^7 spores per ml) was compared with *F. pallidoroseum* (10^{11} spores per ml). The effective dosage of the pathogen was evaluated individually and in combination with the effective dosage of *F. pallidoroseum* and the intensity of infection was recorded (Table 13 and Fig. 5). By spraying *A. eichhorniae* at 10^7 spores per ml alone recorded 64.1 per cent intensity of infection. Similarly when *F. pallidoroseum* at 10^{11} spores per ml was tested, 85.63 per cent intensity of infection was recorded. However, when *A. eichhorniae* and *F. pallidoroseum* each at 10^7 and 10^{11} spores per ml respectively was sprayed together, it recorded an intensity of infection of 89.84 per cent (Plate 11). It was also observed that when the pathogens were sprayed individually, 7-10 days were required for development of symptom whereas it took only 5-6 days when these were sprayed together.

4.7 DEVELOPMENT OF TALC BASED FORMULATION

From experiment 4.6, it was observed that the maximum intensity of infection was in the treatment, combined application of *A. eichhorniae* and *F. pallidoroseum*. A talc based combination product of these two fungi was prepared by mixing equal quantity (w/w) of the mycelial mats of *A. eichhorniae* and *F. pallidoroseum* with 50 per cent moisture content, 10 per cent talc and one per cent carboxy methyl cellulose (Plate 12).

4.7.1 Evaluation of the Formulated Product on Water Hyacinth

The formulated product was evaluated by spraying on water hyacinth plants maintained in pots (1 litre capacity) at different concentrations *viz.*,

Table 14 Extent of damage caused by the formulated product

Sl. No.	Concentration (%)	*Per cent intensity of infection	
		6 DAS	10 DAS
1	1	36.20	47.40
2	3	47.20	72.30
3	5	55.00	81.20
4	10	69.80	93.20

* Average of three replications

Table 15 Shelf life of the formulated product stored at room temperature

Sl. No.	Weeks after storage (WAS)	*Mean cfu/g x 10^4
1	0	762 (27.6)
2	1	574 (23.99)
3	2	498 (22.34)
4	3	94 (9.76)
5	4	38 (6.18)

* Average of three replications CD : 0.18

Figures in parenthesis indicate transformed values

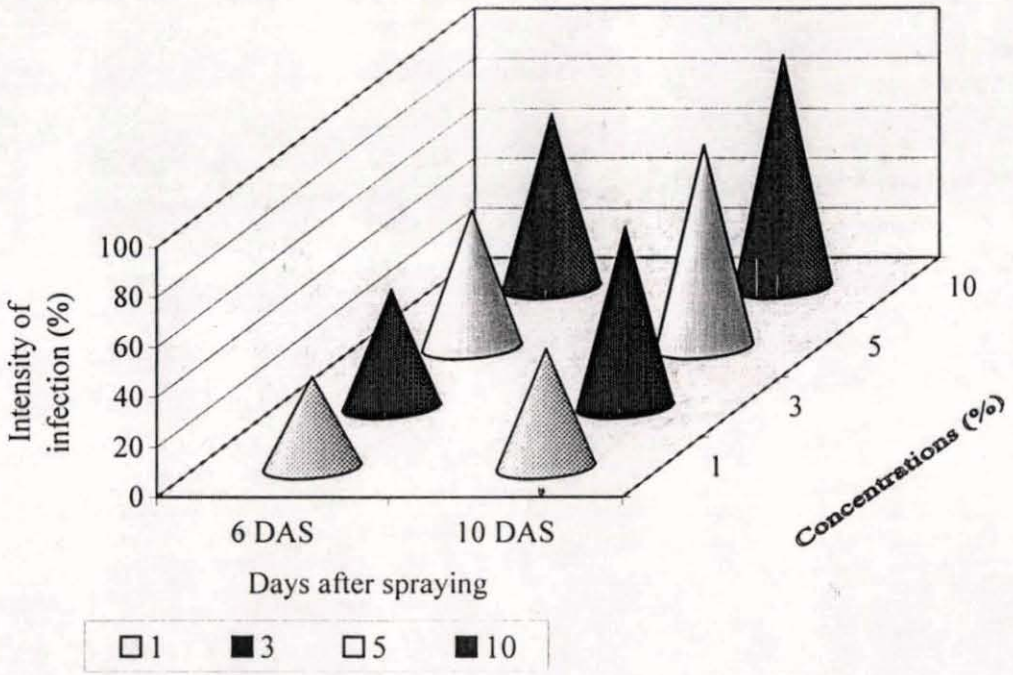


Fig. 6 Extent of damage caused by the formulated product on water hyacinth

Plate 12. Formulated product

Plate 13. Effect of formulated product (10 %) on water hyacinth

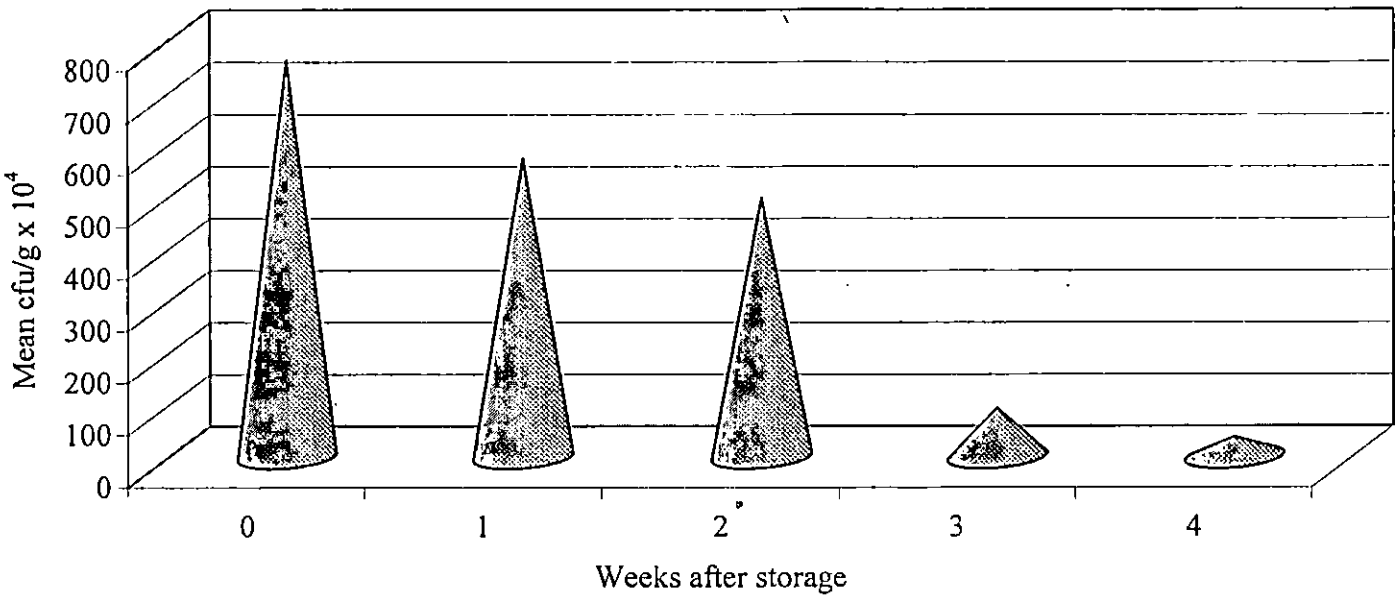


Fig. 7 Effect of storage period on spore viability of fungi at room temperature

1.0, 3.0, 5.0 and 10.0 per cent. The intensity of infection was recorded at 6 and 10 days after spraying (DAS) (Table 14 and Fig. 6).

Maximum intensity of infection of 93.20 per cent was recorded 10 DAS by 10 per cent formulated product (Plate 13). As the concentration of the formulated product decreased, a corresponding decrease in the intensity of infection was also recorded. Thus spraying with 1 per cent formulated product caused only 47.40 per cent intensity of infection 10 DAS. A similar pattern was also observed when the intensity of infection was recorded 6 DAS. It was observed that 10 per cent of the product recorded 69.80 per cent intensity of infection at 6 DAS.

4.7.2 Shelf life of the Formulated Product

The formulated product was kept in sterilized polythene covers at room temperature (28 ± 4°C) and the viability of the spores was tested at weekly intervals by estimating the number of colony forming units in one gram (cfu/g) sample, by serial dilution technique.

The viability of the spores decreased as the storage time increased. In the initial period, there was a viability of 762 x 10^4 cfu/g and there was gradual reduction in the viability to 498 x 10^4 cfu/ g 2 WAS. Thereafter, a sudden decline in the viability was noticed and it reached to 38 x 10^4 cfu/g at the end of the observation i.e. 4 WAS (Table 15 and Fig. 7).

DISCUSSION

5. DISCUSSION

Water hyacinth [*Eichhornia crassipes* (Mart.) Solms] is a serious aquatic weed in Kerala. Use of fungal plant pathogens for the biocontrol of weeds is being practiced in some countries like USA, Sudan, China etc. In the present investigation, the possibility of utilizing fungal pathogens of water hyacinth obtained from a survey carried out as a part of the DST project (Biocontrol of water hyacinth using mycoherbicides) for management of the weed was attempted. During the survey, 18 fungi were isolated, of which 14 were found to be pathogenic on water hyacinth *viz.*, *Alternaria eichhorniae, Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, F. moniliforme, F. oxysporum* (2 isolates), *F. pallidoroseum* (3 isolates), *Myrothecium advena*, Unidentified 1, 2 and 3. These 14 pathogenic fungi were utilized for the present study.

The morphological and cultural characters of all the pathogenic fungi were studied by growing them in the respective media and by preparing slide cultures. All the 14 fungi except three were already identified.

Those fungi which were not identified earlier, were provisionally identified and was confirmed by sending them to Agharkar Research Institute, Pune. The fungi identified are *Bipolaris tetramera, Drechslera* state of *Cochliobolus sativus* and *Pestalotiopsis guepinii.* These are new records on water hyacinth.

The pathogenic nature of these fungi were proved by inoculating them on the test plants. Different type of symptoms were produced by the fungi.

The pathogenic nature of *A. eichhorniae* (Nagraj and Ponnappa, 1970), *C. gloeosporioides* (Santhi and Naseema, 1995), *C. lunata* (Abdel-Rahim and Tawfig, 1984), *F. equiseti* (Agharkar and Banerjee, 1932), *F. moniliforme* (Abdel-Rahim and Tawfig, 1984), *F. oxzysporum* (Jamil and Rajagopal, 1986), *F. pallidoroseum* (Jamil and Rajagopal, 1986), *M. advena* (Praveena & Naseema, 2003) and were confirmed by earlier workers.

Pathogenicity tests on water hyacinth using *A. eichhorniae* produced symptoms similar to those reported by Nagraj and Ponnappa (1970). *Fusarium* spp. produced brown spots with yellow halo both on leaf lamina as well as on petiole which resulted in yellowing of the lamina. Infection was more pronounced on older leaves. Praveena (2003) also reported similar observations.

In the case of *C. gloeosporioides*, symptom was limited to older leaves as small dark brown spots with yellow halo on the leaf lamina only. Later these spots enlarged and adjacent spots coalesced to form large patches. *Curvularia lunata* produced brownish black coloured pin head sized spots on leaf lamina and petiole. Similar observations were made by Santhi (1994).

The fungi were grouped into three categories based on the time taken for the development of symptom. *M. advena* and *F. pallidoroseum* (isolate 3) were categorised under group I, as they developed symptoms on host plants within five days in the form of large lesions leading to complete drying up of the plants. *A. eichhorniae, B. tetramera, F. moniliforme, F. oxysporum* (Isolates 1 and 2) and *F. pallidoroseum* (Isolates 1 and 2) were grouped as those fungi which developed symptoms within seven days. These fungi except *B. tetramera* had the peculiarity of developing symptoms on leaves as well as on stalk with a prominent yellow halo on the leaf. In the later stages, the infection led to blighting

and drying up of the plants. Fungi viz., *C. gloeosporioides*, *C. lunata*, *Drechslera* sp., *F. equiseti* and *P. guepinii* were classified as those fungi which took more than seven days to develop symtpoms. Symptoms of these fungi except *C. gloeosporioides* were limited to the leaf lamina only. Any amount of symptom on the leaves of water hyacinth will not result in the death of the plant as it can regenerate from the parts of the plant submerged in water. A fungus can be considered as an effective biocontrol agent of water hyacinth only if it can infect both the foliage and the swollen stalk. Once the swollen stalk is infected, the plants will sink to the bottom of water thereby suppressing its regeneration. Water hyacinth multiplies at a very fast rate and can double its biomass in ten days (Singh, 1999). Therefore, fungus, which is effective in killing the weed in less than ten days, can be selected as an effective biocontrol agent. The fungi belonging to group III with retarded symptom development is less appealing as biocontrol agent. On the contrary, fungi coming under groups I and II seem more potent with quick symptom production on both leaves and swollen basal parts.

An attempt was made to select the most promising fungal pathogens of water hyacinth from the pathogenic fungi based on the extent of damage produced on the host plant. The pathogenic fungi varied in their extent of infection caused to water hyacinth. The extent of damage ranged from 9.94 to 58.80. The maximum disease intensity of 58.80 per cent was recorded by *M. advena*, followed by *F. pallidoroseum* being 53.27 per cent. The extent of damage of *A. eichhorniae* was comparable to that of *F. moniliforme*.

Variation in the extent of damage by the fungal pathogens has been reported by several workers. Praveena (2003) also reported that out of the 18 fungi tested, *M. advena* produced maximum disease intensity of 61.11 per cent followed by *F. pallidoroseum* (53.44 per cent). Santhi (1994) reported 51.10 per cent intensity of infection of *F. semitectum* (Syn.

F. pallidoroseum) on water hyacinth plants. *A. eichhorniae* produced 48.89 per cent intensity of infection.

The cell free metabolites of various fungi showed significant difference in the intensity of damage on water hyacinth. Maximum intensity of damage of 89.0 per cent was produced by *M. advena* followed by *F. pallidoroseum* (Isolate 3) (49.80 per cent). *A. eichhorniae*, *F. oxysporum* (isolate 2) and *F. moniliforme* produced 48.80, 47.20 and 43.20 per cent intensity of damage respectively.

On grouping the pathogens based on the extent of damage caused by their cell free metabolites, *M. advena* comes under group I, which took only two days to develop symptoms. It was also noticed that *M. advena* caused quick induction of disease, taking only three days to develop symptom. Previous studies revealed that *M. advena* infects a number of cultivated and weed plants (Praveena, 2003). Since the cell free metabolites of *M. advena* could produce symptom within a very short period and also high intensity of damage, it is probable that it may contain a potent toxin. So there is high possibility of exploiting this toxin for the management of this weed.

On grouping the pathogens based on the extent of damage developed by their cell free metabolites, *A. eichhorniae*, *F. moniliforme*, *F. oxysporum* (Isolate 1 and 2), *F. pallidoroseum* (isolate 2 and 3) and *Drechslera* sp. come under group II in that cell free metabolites of these fungi produced symptoms within four to five days. Like the pathogens, cell free metabolites of these pathogens has also got the ability to produce symptom on leaf as well as on stalk region. The cell free metabolites of these fungi coming under group II were more efficient than the fungi in that it caused drying up of the plant within five days. The variation in the symptom produced by these fungi may be due to the difference in the nature and type of toxin produced by the various isolates of the fungi.

Hoagland (1990) reported that members of the genus *Fusarium* produced a range of phytotoxic compound that are chemically diverse. Abbas *et al.* (1991) reported that culture filtrate of *F. moniliforme* exhibited phytotoxicity symptoms of mild to severe necrosis on jimson weed. Cutler (1986) isolated and characterized a mycotoxin with herbicidal activity synthesized by *F. moniliforme*.

Cell free metabolites of *A. eichhorniae* produced small necrotic spots with prominent yellow halo on the leaves. According to Nagraj and Ponnappa (1970), cell free metabolite of *A. eichhorniae* showed necrosis on water hyacinth plants within 24 h and in the next 24 h the leaves scorched and dried up. Whereas in the present study, it took four days for the symptom development. Small necrotic spots developed and resulted in scorching and drying. Extent of damage of 48.80 per cent was produced by its cell free metabolite. The pathogen also took almost same time for developing symptom as the cell free metabolite. Regarding the extent of damage, it was comparable with *F. moniliforme*. Brown patches followed by scorching and yellowing was developed by the cell free metabolites of *F. moniliforme*. Cell free metabolites of *F. pallidoroseum* produced scorching coupled with yellowing of leaves. *F. equiseti* produced minute irregular spots on the leaves. Santhi and Naseema (1994) observed that the culture filtrate of *F. equiseti, F. pallidoroseum* and *F. solani* produced symptoms on water hyacinth as these contained toxin.

Pandey *et al.* (2002) reported that the phytotoxicity of cell free culture filtrate of various fungal isolates varied significantly. Stevens *et al.* (1979) isolated and characterised a phytotoxic substance bostrycin from *A. eichhorniae*, which showed no herbicidal activity towards water hyacinth. Dimoor and Eshed (1984) isolated maculosin from *A. alternata*. Maity and Samaddar (1977) isolated a stable toxic metabolite from fourteen day old culture filtrate of *A. eichhorniae*. *A. eichhorniae* also

produced a phytotoxin alteichin which caused necrotic lesions (Robenson *et al.*, 1984).

Safety to non-target plants is important in the development of a plant pathogen into a bioherbicide. *F. pallidoroseum* was already selected as an efficient biocontrol agent (Naseema *et al.*, 2001). The best method for finding the host range is to test the potential biocontrol agent against those plants which are commonly seen in the locality of the target weed (Weidemann, 1991). In the present investigation, the plants were selected based on this criteria. Hence the host range of the efficient pathogens *viz.*, *A. eichhorniae*, *F. moniliforme* and *F. oxysporum* was studied in detail on 37 cultivated plants and 39 weed plants seen in and around the water ways infested with water hyacinth.

A. eichhorniae could not infect any of the cultivated plants tested, whereas it was found to infect three weeds *viz.*, *Gomphrena decumbens Marselia quadrifolia* and *Monochoria vaginalis,* out of the 39 weed plants tested. Studies on host range of *A. eichhorniae* conducted by Nagraj and Ponnappa (1970) revealed that out of the 42 genera of plants, it was pathogenic only to *M. vaginalis*. The narrow host range of *A. eichhorniae* was also reported by Rakvidyasastra *et al.* (1978) and Shabana *et al.* (1995). *F. moniliforme* was found to infect five out of 37 cultivated plants and 11 out of 39 weed plants. It produced symptoms on important cultivated crops like amaranthus, chilli, papaya, taro and tomato.

Host range study of *F. oxysporum* revealed that it could infect seven out of 37 cultivated and nine out of 39 weed plants. Boyette *et al.* (1993) observed that *F. oxysporum*, a potential mycoherbicide for senna, sickle pod and hemp sesbania was not pathogenic to cucumber, squash, corn, johnson grass and rice. Whereas, in this study, the isolate of *F. oxysporum* from water hyacinth was found to infect many important cultivated crops of Kerala like rice, tapioca, pepper, orchid etc. It may be

due to the strain variation of the pathogenic microorganisms. All the three fungi tested for host range studies in the present investigation did not infect water lily (Family: Nymphiaceae), an ornamental pond plant.

Since *F. moniliforme* and *F. oxysporum* had wide host range including common cultivated crops, they could not be selected as efficient biocontrol fungi whereas *A. eichhorniae* with narrow host range caused infection only on three weed plants out of the several plants tested. So *A. eichhorniae* was selected as an efficient biocontrol agent against water hyacinth.

The effect of pathogens individually and in combination in causing destruction on water hyacinth plants was studied. The combined application of *A. eichhorniae* and *F. pallidoroseum* gave more intensity of infection than when used alone. The time taken for the development of infection on water hyacinth was less when compared to the application of the pathogens individually. The toxin produced by *F. pallidoroseum* might have weakened the plant and made the host more vulnerable to the infection by *A. eichhorniae*. Studies conducted by Susha and Naseema (1998) on the effect of pathogens singly and in combination on water hyacinth plants revealed that the combined application of *F. pallidoroseum* and *F. equiseti* gave maximum disease intensity than when sprayed alone. There are reports on the use of more than one pathogen together for getting effective weed control (Jamil and Narsaiah, 1984; Charudattan, 1986a).

From the previous studies, the concentration of *F. pallidoroseum* used was 10^{11} spores per ml for getting maximum intensity of infection. In the present study, as the concentration of *A. eichhorniae* was increased from 10^4 to 10^8, there was a corresponding increase in the intensity of infection. However the effect of 10^7 and 10^8 were statistically on par. So 10^7 was fixed as the standard dose for the effective destruction of the

weed. At 10^7 spores per ml concentration, the intensity of infection was 62.83 per cent, which was less than that, recorded by *F. pallidoroseum* at 10^{11} spores per ml. The concentration of *A. eichhorniae* could not be increased more than 10^8 unlike *F. pallidoroseum* due to the difficulty that occur while spraying the spore suspension. It is assumed that due to multiple germpores, *A. eichhorniae* can cause more intensity of infection than *F. pallidoroseum*, even if the spore concentration is less.

Work carried out by Santhi (1994) revealed that 10^9 spores per ml of *Fusarium* spp. and 2 x 10^9 spores per ml of *C. gloeosporides* were required for the effective destruction of water hyacinth. Whereas Susha (1997) found that a higher dose of inoculum of 10^{11} spores per ml of *C. gloeosporides* and *Fusarium* sp. gave more intensity of infection on water hyacinth. Similar trials were carried out by others (Walker, 1981; Walker and Riley, 1982; Boyette and Walker, 1985; Morrin *et al.*, 1989; Hildebrand and Jenson, 1991; Lakshmanan *et al.*, 1991).

A talc based combination product of the selected pathogens *viz.*, *A. eichhorniae* and *F. pallidoroseum* was prepared by mixing their mycelial mats with 10 per cent talc and one per cent carboxy methyl cellulose. A talc based wettable powder formulation of biocontrol fungi is effective in retaining the spore viability and for getting maximum efficiency under field conditions (Burges, 1998). There are reports on the talc based formulations of biocontrol agents (Walker and Connick, 1983; Krishnamurthy and Gnanamanickam, 1998; Smitha, 2000; Praveena, 2003).

The formulated product at 10 and 5 per cent caused maximum intensity of infection of 93.20 per cent and 81.20 per cent at 10 DAS. The formulated product retained viability of spores upto two weeks under room temperature. After this period, a sudden decline in the spore viability was observed. Earlier reports also indicated a negative

correlation between the spore viability and storage period (Nagesh *et al.*, 2001, Praveena, 2003, Faizal, 1992). Faizal (1992) noticed that in the wettable powder formulation of *F. pallidoroseum*, the spores retained 75 per cent viability for four days and thereafter there is a marked decrease in the virulence of the fungus. Praveena (2003) reported that in the wettable powder formulation of *F. pallidoroseum*, from the initial spore count of 8.07×10^8 cfu/g, it was decreased to 6.39×10^7 cfu/g within 1 WAS and it reduced drastically to 7.0×10^4 cfu/g by 5 WAS.

According to TeBeest and Templeton (1985), the organism used to control weeds should be easy to culture, yield infective mycelia or spores, have a good viability and shelf life, yield infections and disease cycle over a range of environmental conditions, inexpensive to use, reliable at a high and predictable level of control and safe for the user and the environment.

A. eichhorniae satisfies many of these criteria and hence its bioherbicidal potential singly or in combination with other efficient biocontrol agents like *F. pallidoroseum* could be exploited for large scale field application under different agroecological conditions.

SUMMARY

6. SUMMARY

Bioherbicidal potential of fungal pathogens of water hyacinth was evaluated to develop a talc based formulation of the efficient ones.

Out of the 14 pathogenic fungi included in the study, 11 were already identified and the remaining three were provisionally identified and was confirmed by sending to Agharkar Research Institute, Pune. These three fungi *viz.*, *Bipolaris tetramera*, *Drechslera* sp. and *Pestalotiopsis guepinii* were new records on water hyacinth.. The cultural and morphological characters of the fungi were studied by growing them in the respective media and by slide culture technique. Variation in symptom development of these pathogenic fungi observed by artificial inoculation studies. Those fungi which produced infection on both leaves and stalk include *Alternaria eichhorniae*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Drechslera* sp., *Fusarium moniliforme*, *F. oxysporum* (isolates 1 and 2), *F. pallidoroseum* (isolates 2 and 3) and *Myrothecium advena*. Out of these, only *A. eichhorniae*, *F. moniliforme*, *F. oxysporum* (isolates 1 and 2), *F. pallidoroseum* (isolates 1, 2 and 3) and *M. advena* developed symptoms within seven days.

The intensity of infection of the pathogenic fungi was studied to select promising fungal pathogens of water hyacinth. Only five fungi viz., *A. eichhorniae*, *F. moniliforme*, *F. oxysporum* (isolate 2), *F. pallidoroseum* (isolate 3) and *M. advena* gave more than 45 per cent intensity of infection.

On evaluation of cell free metabolites of pathogenic fungi on water hyacinth, *M. advena* developed symptoms in two days and resulted in scorching of the entire plant. *A. eichhorniae*, *F. moniliforme*, *F. oxysporum* (isolates 1 and 2), *F. pallidoroseum* (isolates 2 and 3) and

Drechslera sp. developed symptoms on leaf lamina and petiole within four to five days. The cell free metabolites of *A. eichhorniae*, *F. oxysporum* (isolate 2), *F. pallidoroseum* (isolate 3) and *M. advena* recorded more than 45 per cent intensity of damage on water hyacinth plants.

Detailed host range of the three promising fungal pathogens of water hyacinth *viz.*, *A. eichhorniae*, *F. moniliforme* and *F. oxysporum* were carried out on common cultivated and weed plants seen near waterways of Kerala. Of the 37 cultivated plants tested, *A. eichhorniae* did not infect any of them whereas it was found pathogenic on three weed plants *viz.*, *Gomphrena decumdens*, *Marselia quadrifolia* and *Monochoria vaginalis*. *F. moniliforme* infected five out of the 37 cultivated plants and 11 out of the 39 weed plants tested. *F. oxysporum* was pathogenic to seven cultivated and nine weed plants out of the 37 cultivated and 39 weed plants tested. All the three pathogenic fungi was found to infect *M. vaginalis* which belongs to the same family of water hyacinth *viz.* Pontederiaceae.

The efficacy of *A. eichhorniae* and *F. pallidoroseum* and their metabolites was tested individually and in combination on water hyacinth. The combined application of pathogens as well as their cell free metabolites gave higher disease intensity of 58.60 and 55.44 per cent respectively than applying alone. Also the time taken for the development of infection on water hyacinth was less in their combined application in both cases when compared with the individual application.

The dosage of inoculum required for the effective destruction of the weed was fixed as 10^7 spores per ml. This effective dosage of *A. eichhorniae* when evaluated in combination with 10^{11} spores per ml of *F. pallidoroseum*, recorded 89.84 per cent intensity of infection.

A talc based combination product of *A. eichhorniae* and *F. pallidoroseum* was prepared and evaluated at different concentrations

on water hyacinth plants. Ten DAS, maximum intensity of infection of 93.20 per cent was recorded by spraying 10 per cent of the formulated product. In the formulated product, the pathogens could retain the viability (498 x 10^4 cfu/g) upto 15 days under room temperature.

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7. REFERENCES

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*Original not seen

cultivated and 11 weed plants. *F. oxysporum* was found to be pathogenic to seven cultivated and nine weed plants.

Combined application of *A. eichhorniae* and *F. pallidoroseum* recorded higher intensity of infection than when sprayed alone. *A. eichhorniae* was found to give effective destruction of the weed at 10^7 spores per ml concentration. *A. eichhorniae* at 10^7 spores per ml and *F. pallidoroseum* at 10^{11} spores per ml together recorded more intensity of infection of 89.84 per cent than when sprayed alone.

Talc based combination product of *A. eichhorniae* and *F. pallidoroseum* when sprayed at 10.00 per cent concentration recorded an intensity of infection of 93.20 per cent, 10 days after spraying. The formulated product could retain the viability of spores of the pathogens at room temperature for 15 days.

APPENDIX

APPENDIX – I

1. Potato Dextrose Agar (PDA)

Potato	-	200 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water	-	1 litre

2. Potato Sucrose Agar (PSA)

Potato water	-	500 ml
Sucrose	-	20 g
Agar	-	20 g
Distilled water	-	500 ml

3. Modified Potato Dextrose Agar

Potato	-	500 g
Dextrose	-	10 g
NaCl	-	1 g
Agar	-	20 g
Distilled water	-	1 litre

4. Czapek's Solution

Sucrose	-	30 g
$NaNO_3$	-	2 g
K_2HPO_4	-	1 g
$MgSO_4.\ 7H_2O$	-	0.5 g
KCl	-	0.5 g
$FeSO_4$	-	0.01 g
Distilled water	-	1 litre

5. Nutrient Solution (Knop's Solution)

$CaNO_3$	-	0.8 g
KNO_3	-	0.2 g
KH_2PO_4	-	0.2 g
$MgSO_4$. 7 H_2O	-	0.2 g
Ferrous sulphate	-	trace
Water	-	1 litre

6. Martin's Rose Bengal Agar

Dextrose	-	10 g
Pepton	-	5 g
KH_2PO_4	-	1 g
$MgSO_4$	-	0.5 g
Rose Bengal	-	33 mg/l
Distilled water	-	1 litre
Agar	-	20 g
Streptomycin	-	30 mg

BIOHERBICIDAL POTENTIAL OF FUNGAL PATHOGENS OF WATER HYACINTH [*Eichhornia crassipes* (Mart.) Solms]

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Abstract of the
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ABSTRACT

The study entitled "Bioherbicidal potential of fungal pathogens of water hyacinth [*Eichhornia crassipes* (Mart.) Solms]", was conducted at College of Agriculture, Vellayani, Thiruvananthapuram during 2001-2003.

Fourteen pathogenic fungi of water hyacinth obtained from the survey conducted as a part of the DST project on "Biocontrol of water hyacinth using mycoherbicides" were used for the study. Fungi which were not identified earlier were characterized and identified based on the cultural and morphological characters and was confirmed by sending to Agharkar Research Institute, Pune. These three fungi viz., *Bipolaris tetramera*, *Drechslera* sp. and *Pestalotiopsis guepinii*, were new records on water hyacinth.

The fungi varied in the symptom development on water hyacinth with respect to nature of symptoms, parts affected and the time taken for the symptom development. The intensity of infection by the fungi varied from 9.94 to 58.80 per cent. Only five fungi viz., *A.eichhorniae*, *F. moniliforme*, *F. oxysporum* (isolate 2), *F. pallidoroseum* (isolate 3) and *M. advena* gave more than 45 per cent intensity of infection.

Cell free metabolites of the various fungi also varied in the symptom development. Intensity of damage varied from 8.03 to 89.00 per cent. Maximum was recorded by *M. advena* followed by *F. pallidoroseum* (49.80 per cent), *A. eichhorniae* (48.80 per cent) and *F. oxysporum* (47.20 per cent).

Host range of *A. eichhorniae*, *F. moniliforme* and *F. oxysporum* was studied in detail. *A. eichhorniae* infected only three weed plants out of the 37 cultivated and 39 weed plants tested. *F. moniliforme* infected five