BIOCONTROL OF WATER HYACINTH USING FUNGAL PATHOGENS

By

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THESIS

SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENT FOR THE DEGREE

MASTER OF SCIENCE IN AGRICULTURE

FACULTY OF AGRICULTURE

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI - THIRUVANANTHAPURAM

1997

DECLARATION

I hereby declare that this thesis entitled "BIOCONTROL OF WATER HYACINTH USING FUNGAL PATHOGENS" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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ACKNOWLEDGEMENT

I wish to place on record my deep sense of gratitude and indebtedness to Dr. A. Naseema, Associate Professor, Department of Plant Pathology and Chairman of the advisory committee for her valuable guidance, healthy criticism and constant encouragement throughout the period of investigation and in the preparation of this thesis.

I am greatly obliged to the members of the advisory committee, Dr. P. Karunakaran, Professor and Head of the Department of Plant Pathology, Dr. (Mrs.) K.R. Sheela, Assistant Professor, Department of Agronomy and Dr. S. Balakrishnan, Professor, Department of Plant Pathology for their pertinent suggestions and criticisms.

My sincere thanks are due to, Dr. M.C.Nair retired Professor and Head of Plant Pathology for his valuable suggestions, constant encouragement and all help rendered during the course of study.

I owe my gratitude to Dr. V.K. Girija and Dr. C. Gokulapalan, Associate Professors, Department of Plant Pathology for the active assistance during the course of research work and for the preparation of thesis.

I am extremely thankful to Sri. Ajith Kumar, Programmer for his active involvement in the computer analysis of the data.

I am very much grateful to the teaching and non-teaching staff of the Department of Plant Pathology for the help rendered to me by each and every one of them.

My friends were of immense help to me especially Anu, Anitha, Arun, Raghi, Sindhu, Asha, Jubi, Blessy, Bindhu, Manoj, Veena chechi to whom I am thankful.

I am grateful to the STED for the fellowship granted to me. Thanks are also due to K. Chandrakumar for typing the manuscript neatly.

I am deeply indebted to my husband and our family members for their encouragement and help which made me possible for the completion of this study.

Above all I thank God Almighty for showering His blessings upon me in completing the thesis successfully.

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INTRODUCTION

1. INTRODUCTION

Water hyacinth [Eichhornia crassipes (Mart) Solms] is one of the most gregariously growing aquatic weeds in the world and it belongs to the family Pontideraceae. In India it is like a devil in the fishery water and paddy fields impounding considerable water area. Besides this, water hyacinth pollutes drinking water, hinders antimosquito operation and serves as alternative host to several insects, pathogens and thus chances of the damage caused by this weed on crop plants are increased. Because of these facts water hyacinth continues to demand constant vigil.

Management of water hyacinth continues to be almost exclusively by mechanical removal and through the use of chemical herbicides. But the trend towards biologicals is strengthened due to harmful chemical residues in food, water, soil and environment from use or misuse of chemicals.

Increased interest has been generated now a days in the use of microoganisms in the biological control of weeds. An ideal material used to control weeds should be easy to produce and store, inexpensive to use, reliable at high infestation and with predictable level of control and safe for use and environment. Many of these characters are exhibited

by plant pathogenic fungi that infect weeds. Among the fungi, Cercospora rodmanii has shown the greatest promise as a biocontrol agent of water hyacinth (Conway, 1976). Santhi Kamath (1994) reported Fusarium equiseti, F. pallidoroseum and Colletotrichum gloeosporioides as good biocontrol agents.

Fungal weed pathogens are difficult to formulate into effective products because, as living organisms, their viability must be preserved throughout processing and storage. Furthermore the pathogens, as packaged in a final product, must be able to control weeds after application under natural conditions.

Based on the foregoing considerations, the aim of the present study is to develop an effective formulation of mycoherbicide for the biocontrol of water hyacinth. The various steps undertaken in the study are:

1) Host range studies of the pathogens of water hyacinth with common cultivated crops and other weeds.

2) Standardization of dosage of inoculum required for effective destruction of water hyacinth.

3) Use of cell free metabolites produced by the pathogenic fungi.

4) Field evaluation of the pathogens singly and in combination on water hyancith.

5) Field evaluation of the metabolites singly and in combination on water hyacinth.

6) Standardization of carrier materials for the storage and field application of the pathogens and it's metabolites.

7) Standardization of method of field application of pathogens and cell free metabolites for biological control of water hyacinth.

8) Characterisation of the toxin.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Over the past several years, new pathogens and microbial phytotoxin from pathogens and other microorganisms have been discovered which can be added to the arsenal of biological weed control weapons.

2.1 Reports of pathogens on water hyacinth

The use of plant pathogens as biocontrol agents of aquatic plants has been considered recently as a major area of research. Prior to 1970's virtually no work has been conducted in this specialised area.

Nagaraj and Ponnappa (1970) reported that water hyacinth was known to be infected by several plant pathogens including Uredo eichhorniae Gronz Fragand Cif, Fusarium equiseti (Corda) Sacc., Corticium sesakii, Cephalosporium eichhorniae, Rhizoctonia solani Kuhn., Cercospora piaropi Tharp, Marasmiellus inderma and Alternaria eichhorniae Sp. Nov. Of the several fungi so far recorded as pathogens of water hyacinth, Marasmiellus inderma and A. eichhorniae appear to hold some promise as possible agents of biological control.

During a survey conducted by Freeman and Zetler (1971) in the canal zone of Panama, to obtain fungal

pathogens from Eichhornia azurea Kunth, observed a blight caused by Rhizoctonia solani Kuhn. It caused severe blight of immersed portion of plants which frequently resulted in the death of entire plant. The sclerotia of the fungus remained viable for about nine months in the lake water without loss of virulence. Freeman and Charudattan (1974) observed purplish black spot with a tan centre in the distal portion of the leaf blade of water hyacinth and identified the pathogen as Cercospora piaropi.

In 1975, Charudattan and Conway reported Uredo eichhorniae Gronz Frag and Cif causing rust on water hyacinth. This isolate was different from the previously reported C. piaropi and similar to Cercospora rodmanii (Conway). Rakvidyasastra and Visaranthanond (1975) isolated thirteen fungi from water hyacinth and among them Alternaria eichhorniae, Myrothecium roridum and Rhizoctonia solani Kuhn were found more pathogenic. A species of Cercospora was isolated from declining water hyacinth in Rodman Reservoir in Florida by Conway (1976).

Mycoleptodiscus terrestris Gerdemann a root and crown rot organism on several legumes was reported for the first time on water hyacinth (Charudattan and Conway, 1976). Martin and Freeman (1978) reported that Acremonium zonatum Sawada Gams could be used as a biocontrol agent of water hyacinth. It was also observed that plants inoculated with

the fungus A. zonatum responded differently to infection depending on the stage of development of the plant.

Balasooriya et al. (1984) worked on the fungi associated with water hyacinth in North West and Western provinces of Srilanka and found Penicillium oxalicum Currie and Thorn, Curvularia lunata (Wakker) Boaedjin, Fusarium spp., Myrothecium roridum and a sterile fungus on the leaf. Caunter (1984) made isolations from diseased leaves and leaf stalks of Eichhornia crassipes from Penang islands and Kedah. Pathogenicity tests revealed that species of Helminthosporium, Myrothecium roridum and Chatomella spp. were pathogenic to water hyacinth.

Jamil et al. (1984) found that of the three fungi pathogenic to water hyacinth , Alternaria eichhorniae caused more damage than Cercospora sp. or Fusarium solani (Mart) Sacc. Fusarium solani showed remarkable selectivity in attacking older leaves. In a study conducted to obtain biocontrol organism for the troublesome weed Eichhornia crassipes, Phoma sorghina (Sacc.) Bernia Dorenbosch var. kesteren was isolated by Rahim (1984). In another study conducted by Rahim and Tawfig (1984), many bacteria and fungi were isolated from diseased plant parts of water hyacinth. Out of the 25 fungal and bacterial isolates only five were found

pathogenic, viz., Acremonium zonatum (Sawada) Gams, Drechslera spicifera (Bain) Nicof, Fusarium equiseti (Corda) Sacc, Phoma sorghina and Bacillus sp.

Rahim and Tawfig (1985) observed zonal leaf spot of water hyacinth in different parts of the world and found to cause considerable damage to the weed. The causal organisms were identified as Acremonium zonatum and Phoma sorghina (Sacc.). Singh et al. (1985) worked on the mycoflora associated with water hyacinth in India at diffent stages of the plant throughout the year. It was found that the fungal flora was more during the rainy season than in the hot summer. Of the various fungi isolated, Alternaria eichhorniae, Corticium solani Prill and Delacr , Curvularia sp., Pestalotia sp., Myrothecium roridum and Cercospora piaropi were found potentially pathogenic.

A survey on the mycoflora of water hyacinth in Andhra Pradesh was conducted by Jamil and Rajagopal (1986). They reported Fusarium oxysporum Schlet, Fusarium semitectum Berk and Rav, Alternaria sp., Curvularia sp., Helminthosporium sp., and a sterile fungus. Rahim and Tawfig (1986) isolated Drechslera spicifera (Bain) Varx causing leaf spot of Eichhornia crassipes.

Aneja and Srinivas (1990) conducted surveys in Haryana and observed a leaf spot disease on water hyacinth

at Kurukshetra. The affected leaves had small, punctate to circular dark coloured spots with necrosis of leaf tip and chlorosis of lamina and petiole. Fungus isolate obtained from infected water hyacinth was identified as Cercospora rodmanii. Aneja et. al. (1990) observed heavy infection of water hyacinth population in different ponds in India. Plants showing small punctate leaf spots with an ash coloured centre becoming eliptical to irregular shape structures yielded Fusarium chlamydosporum Wollen and Reinking. Plants showing leaf spot leading to compact zonation starting from tip of leaf and spreading backwards yielded Epicoccum nigrum Link. Plants showing small brown coloured leaf spots, forming lesions of irregular shapes on the leaves and petioles yielded Phoma sorghina.

Santhi Kamath (1994) conducted an experiment to screen the fungal pathogens for biocontrol of water hyacinth, at College of Agriculture, Vellayani and isolated Fusarium equiseti (Corda) Sacc. Fusarium pallidoroseum (Cook) Sacc, Fusarium solani(Mart) Sacc, Rhizoctonia solani, Colletotrichum gloeosporioides (Penzig) Penzig and Sacc, Curvularia lunata (Wakker) Boedjin and a sterile fungus. It was also observed that F. equiseti, F. pallidoroseum, and C. gloeosporioides could be used as biocontrol agents of water hyacinth.

2.2 Host range of *Colletotrichum* spp. and *Fusarium* spp.

Detailed host range information is required to avoid potential hazards associated with introduction into an area where pathogens did not occur previously or to accomodate changes in cropping pattern.

Colletotrichum spp.

Butler (1951) reported that in summer rainfall regions of New South Wales, a disease caused by the fungus *Colletotrichum xanthii* on Bathurst Burr, one of the most serious weeds in pasture lands in these areas was observed.

Daniel *et al.* (1973) tested 30 plant species and 46 cultivars of economic and wild plants and found only the target weed *Aeschynomene virginica* L. and a related weed *A. indica* L. to be susceptible to *Colletotrichum gloeosporioides* f. sp. *aeschynomene*. Boyette *et al.* (1979) identified a pathogenic fungus *C. gloeosporioides* (Penzig) Penzig and Sacc. f. sp. *jussiaeae* var. *glabrescens* from *Jussiaeae* (*Ludwigia*) *decurrens* Walt. The fungus was not pathogenic on *Jussiaeae repens* var. *glabrescens*, rice, soybean and cotton. Karunakaran *et al.* (1980) found that the clove pathogen *C. gloeosporioides* survived on the weed clerodendron. Kirkpatrick *et al.* (1982) reported *Colletotrichum malvarum* A. (Braun and Casp.) on prickly sida, causing heavy destruction of the weed. In 1985 Anderson

and Walker reported a disease caused by Colletotrichum coccodes (Walr.) Hughes on Solanum ptycanthum Dunn.

Smith (1986) reported the biocontrol of Northern joint vetch (Aeschynomene virginica) in rice fields using C. gloeosporioides f. sp. aeschynomene. Trijillo et al. (1986) worked on biocontrol of Clidemia hirta. (L). D. Don using C. gloeosporioides in Hawaii. Host range studies indicated appressoria formation on leaves of all the eleven ornamental species of the family Melastomataceae.

Cardina et al. (1988) reported Colletotrichum truncatum (Schw) Andrus and Moore on Desmodium tortuosum (SW) Dc. Mc Rea and Auld (1988) found that Colletotrichum orbiculare Berk. could be used for controlling spiny cockle burr (Xanthium spinosum L.). Mortenson (1988) formulated the mycoherbicide Biomal using the pathogen C. gloeosporioides f. sp. malvae against round leaf mallow (Malva pusilla Sm.).

Te Beest (1988) tested 77 plant species and 43 genera in ten families in connection with the host range studies of C. gloeosporioides f sp. aeschynomene and found five genera in the subfamily Pappilionaceae were susceptible including 23 out of the 26 pea cultivars tested.

Chang et al. (1989b) evaluated endemic foliar fungi for the biological control of Johnson's grass (Sorghum halepense (L.) Pers) and found Colletotrichum graminicola Ces. as potent

pathogen. Wymore and Watson (1989) conducted extensive testing for the commercial development of Colletotrichum coccodes against velvet leaf. Host range of several isolates of Colletotrichum orbiculare Berk from Xanthium spinosum L. were conducted by Weidmann (1991) and found them to have difference in host range. Several isolates infected economic hosts, such as safflower.

Santhi Kamath (1994) conducted host range studies of Colletotrichum gloeosporioides in six crop plants, viz., Amaranthus, chilli, cowpea, cucumber, rice and tomato and six weed plants, viz., Commelina benghalensis L., Fimbristylis miliaceae L., Hydrocotyl asiatica Urban, Monochoria vaginalis Prest, Panicum repens L. and Ludwigia parviflora L. Of these the fungus infected chilli, C. benghalensis, H. asiatica and L. parviflora.

Fusarium spp.

Rai and Bridgmon (1971) isolated Fusarium roseum Snyder and Hans from canada thistle. Andrews and Hecht (1981) observed Fusarium sporotrichoides Sherb on the aquatic weed Myriophyllum spicatum L. and found it to be pathogenic. Walker (1981 b) identified a broad spectrum mycoherbicide of Fusarium lateritium Nees. ex. Fr. capable of controlling more than one weed, viz., spurred anoda, prickly sida and velvet leaf.

Nagalingam (1983) reported that Fusarium semitectum Berk and Rav was safe to plants like chilli, cabbage,

brinjal and tobacco. Boyette and Walker (1984) observed that F. lateritium was effective in suppressing the growth of velvet leaf and prickly sida and it did not infect corn, soybean and cotton. Rahim and Tawfig (1984) reported that the host range of F. equiseti pathogenic to water hyacinth included the following crop plants, viz., Allium cepa L, Beta vulgaris L., Chenopodium amaranticolor Coste and Reyn, Hordeum vulgare L. Cyperus rotundus L., Hibiscus esculentus L. and Zea mays L. Boyette et al. (1984) reported that texas gourd Cucurbita texana could be controlled by Fusarium solani f. sp. cucurbitae. Fusarium lateritium could also infect Ambroscia trifida L. (giant rag weed).

Studies by Hareendranath (1989) on the safety aspect of Fusarium pallidoroseum (Cook) Sacc., showed that it was not pathogenic to rice, bhindi, chilli and tomato.

Santhi Kamath (1994) conducted host range studies of F. equiseti, F. solani and F. pallidoroseum in six crop plants. It was observed that Fusarium spp. could not infect the cultivated plants tested whereas, it was pathogenic to Monochoria vaginalis Prest. Koning et al. (1995) tested seed samples of six South African soybean cultivars and reported that 65 per cent of the fungal isolates were identified as Fusarium equiseti (Corda) Sacc.

2.3 Inoculum Concentration Required For Weed Control

The application of inundative dose of inoculum and its proper timing would shorten the lag period for inoculum build up and pathogen distribution (Daniel et al. 1973). Walker and Riley (1982) worked on the biocontrol of sickle pod using Alternaria cassiae Jurair and Khan and maximum weed control was obtained with a spray solution containing more than or equal to $5x10^4$ conidia per ml applied at cotyledon to first leaf stage. The factors influencing the biocontrol of velvet leaf and prickly sida with Fusarium lateritium Nees.ex.fr. was studied by Boyette and Walker (1985a) and found that higher level of control was obtained for both weeds with inoculum concentration of $7.5x10^5$ and $1.5x10^6$ microconidia per ml.

Ridings (1986) suggested biocontrol of strangler vine, Morrenia oderata L. in citrus orchard using Phytophthora palmivora (Butl.). Effective vine killing was obtained at eight chlamydospores per cm^2 of the soil. Weidmann and Templeton (1988) reported that Fusarium solani f.sp, cucurbitae effectively controlled texas gourd when applied as pre-plants incorporated conidial suspension of either microconidia or macro conidia at $3x10^{11}$/ml.

Chang et al. (1989a) observed more than 90 per cent leaf injury to Johnson's grass by Exserohilum turcicum (Pass). Leonard and Sugg at $2x10^5$ conidia per ml.

Morin et al. (1989) worked on the efficacy of Phomopsis convolvulus Ormeno for the control of field bind weed (Convolvulus arvensis J.). 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, whereas, 3 to 5 leaf stage weeds were controlled with 10^9 conidia per ml.

Lekshmanan et al. (1991) reported Cochliobolus carbonum Nelson and Haasis as a potential biocontrol agent for Euphorbia geniculata L. and 98 per cent control of the weed was obtained by spraying with an aqeous suspension of $5x10^6$ spores per ml of the pathogen.

In the field trails conducted by Hildebrand and Jenson (1991) to evaluate the effectiveness of Colletotrichum gloeosporioides as biocontrol agent of St.Johns weed Hypericum perforatum L, • 72.2 and 83.3 per cent mortality was obtained at $2x10^6$ and $8x10^6$ spores per ml respectively.

Santhi Kamath (1994) conducted studies to determine the quantity of inoculum of the pathogens viz. Fusarium equiseti, F. pallidoroseum, F. solani and C. gloeosporioides required for the effective destruction of water hyacinth. It was

observed that $1x10^9$ spores per ml was effective than $1x10^3$ and $1x10^6$ spores per ml in the case of Fusarium spp. and in the case of C.gloeosporioides 2 x 10^9 spores per ml was effective than $2x10^3$ and $2x10^6$ spores per ml.

2.4 Use of cell free metabolites in weed control

Research on the potential of secondary metabolites from microorganisms for use as herbicides has met with practical success in the development of herbicides.

In 1967 Davis isolated Fusaric acid from Fusarium infected plants which caused wilt. Maity and Samaddar (1977) isolated a toxic metabolite from fourteen day old culture filtrate of Alternaria eichhorniae. This was heat stable and partially purified toxin showed some degree of host specificity. At lower concentration it reproduced typical blight symptoms on water hyacinth leaves.

Stevens et al. (1979) made an attempt to isolate and characterise the phytotoxic substance produced by Alternaria sp.. However, it was observed that the major metabolic constituent bostrycin of A. eichhorniae (81 per cent) showed no herbicidal activity towards E. crassipes.

Kalidas (1981) found that the toxin isolated from Helminthosporium sp., Alternaria sp. and Fusarium sp. induced wilting in seedlings of Xanthium strumarium L. and Striga sp. but not in parthenium.

A compound called maculosin was isolated from Alternaria alternata (Fr. Keissler) infecting spotted knap weed by Dinoor and Eshed (1984). Nineteen other grasses and broad leaved plants tested were not injured by maculosin, even at concentration of 1 mμ. Robenson et al. (1984) reported an unusual phytotoxin altechin, obtained from liquid culture of Alternaria eichhnorniae a pathogen of water hyacinth. Altechin is a double hydrated form of 4, 9 dihydroxy perylene 3,10 quinone.

Howell and Stiponovic (1984) demonstrated the potential of necrogenic toxin producing fungus Gliocladium virens Miller Giddens and Foster, as mycoherbicide on various weeds. De Frank (1985) reported the isolation and screening of phytoalexins as herbicides from actinomycetes.

The herbicidal activity of Gliocladium virens, a soil borne fungus was reported by Jones and Hancock (1990). They could isolate a steroidal phytotoxin, viridiol which caused severe necrosis of roots. Strobel et al. (1990) isolated and identified a diketopiperazine compound, maculosin from a strain of Alternaria alternata (Fr.) Keissler infecting spotted knapweed (Centoaurea maculosa Lam). This toxin was phytotoxic and host specific at 10^{-3} and 10^{-5} concentration. Another phytotoxin tenuzoic acid was also produced by Alternaria alternata.

A major phytotoxin fumonisin B1, was isolated from Fusarium moniliforme. Sheld that killed 95 per cent of the Jimson weed plants. The toxin caused soft rot diffusing along leaf veins (Abbas et al., 1991). Stierle et al (1991) isolated cyperine a phytotoxin from Aschochyta cypericola, pathogen of Cyperus rotundus.

Santhi Kamath (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 plants as these contained toxin.

Abbas et al. (1995) reported that Fumonisins were first isolated and identified from Fusarium moniliforme J. sheld. Later, six other species were reported to produce Fumonisins. Identity of Fumonisins was confirmed by thin layer chromatography. Culture filtrate of these Fuminosin producing isolates exihibited phytotoxicity from mild to severe necrosis and mortality. Abbas and Ocamb (1995) examined 14 isolates of F. polyphialielidicum and all were found to produce Fumonisin B1, when grown on rice.

Bilgrami (1995) studied the synthesis of zearalenone, deoxy nivalenol and T-2 toxins by the isolates of Fusarium spp. under varied environmental conditions including temperature, grain moisture and incubation period. Increase in moisture content and incubation period positively influenced toxin production.

Phytotoxic polypeptide was identified by Jin et al. (1996) in culture filtrate of Fusarium solani, the causal agent of soybean sudden death syndrome.The toxicity of the culture filtrate and fractions obtained during purification was bioassayed by measuring browning of soybean calli.

2.5 Use of pathogens in combination for weed control

Boyette et al. (1979) worked on the control of winged water primrose and Northern joint vetch with fungal pathogens. The feasibility of control of the two weeds by combined application of two mycoherbicide was tried. Mixture of C. gloeosporioides f. sp. jussiaeae and C. gloeosporioides f.sp aeschynomene at a concentration of one to two million spores per ml of each fungus was effective against Jussiaeae decurrens and Aeschynomene virginica L.

Jamil et al. (1984) found that Alternaria eichhorinae Cercospora sp. and Fusarium solani (Mart) Sacc were pathogenic to water hyacinth. It was also observed that Fusarium solani had remarkable selectivity in attacking older leaves and so it can be used as a copathogen with Cercospora. sp.

Studies on the combined effect of Alternaria macrospora Zimm and Fusarium lateritium on spurred anoda were conducted by Crawly et al. (1985) and observed that highest level of plant death occurred when A. macrospora was applied

five days before F. lateritium. This interaction was potentially useful to increase the effectiveness of the two pathogens as mycoherbicides.

Charudattan (1986) demonstrated the combined use of two mycoherbicides for controlling weeds. He found that showy crotalaria was controlled by the combined application of Colletotrichum dematium Fr. Grove f.sp.crotalariae and Fusarium udum f.sp. crotalariae.

Strobel et al. (1990) found that maculosin, a diketo piperazine compound and another phytotoxin tenuzoic acid produced by Alternaria alternata(Fr.) Keissler infected spotted knap weed (Centaurea maculosa Lam). when used in combination had a synergistic action against the weed.

2.6 Carrier materials for the storage and field application of fungi.

Ease and cheapness of production and application are the principal characteristics of a desirable biocontrol agent (Butcher, 1958).

Backmann and Rodrigus (1975) reported diatomaceous earth as a suitable inert material for the formulation of mycofungicide with Trichoderma harzianum. Villacorta (1976) developed a technique for mass culturing of Metarrhizium anisopliae in granular form. Various substrates were tried for

large scale production of Fusarium oxysporum f. sp. cannabis Snyd. a pathogen of Cannabis sativa.L. Large scale inoculum production was achieved on a mixture of barley straw with glycine succinate, sodium nitrate solution, Alfalfa straw, cotton seed meal or soybean meal. Chlamydospores produced in glycine succinate, sodium nitrate, barley straw substrate retained their disease potential for over six months at room temperature (28±4°C) (Hildebrand and Mc Cain, 1978).

Beevi (1979) reported that for mass production of Fusarium moniliforme var. subglutinans, sorghum and bajra grains appeared to be the most suitable as they produced maximum spores with higher virulence. The best combination for all materials was found to be 30 g of material in 25 ml of water.

Kuruvila and Jacob (1981) showed that green gram, wheat or sorghum could be used as substrates for easy mass production of the fungus Fusarium oxysporum. Nagalingam (1983) observed broken maize grain plus black gram husk or red gram husk at 4:1(w/w) as a suitable medium for the mass production of Fusarium semitectum.

Walker and Connick (1983) used sodium alginate for pelletalised formulation of mycoherbicides using Alternaria alternata, Fusarium lateritium, Colletotrichum malvarum, Alternaria macrospora and Phyllosticta sp.

A method to encapsulate microorganisms was developed by Fravel et al. (1985). Aqeous solution containing 1 per cent alginate and 10 per cent pyrax were mixed in a blender. Solutions were ammended singly with either ascospores or conidia of Penicillium oxalicum or Trichoderma viride. Initial population ranged from 10^7 to 10^8 propagules per ml of alginate suspension. These populations declined during the test period. Losses were 10 to 100 fold after four weeks.

The survival of Talaromyces flavus in alginate pellets was studied by Papavizas et al. (1987). Conidia of Talaromyces flavus in alginate bran pellets stored for 15 weeks survived better at 5 and 15°C than at 25°C. Ascospore viability declined moderately during the first three weeks with no further decline from third to fifteen week. Raghavendran et al. (1987) mass cultured Fusarium subglutinans on moist sterile sorghum grains and the fungus sporulated abundantly by three weeks.

Hareendranath (1989) reported broken maize grains as a suitable medium for the mass multiplication of F. pallidoroseum followed by tapioca chips and jack seeds as they produced maximum number of spores. Jones and Hancock (1990) conducted a study on the use of Gliocladium virens Miller, Giddens and Foster in pre-emergence weed control. G. virens was cultured with sucrose and ammonium nitrate.

This gave 90 per cent control and those emerged were severely stunted.

Faizal (1992) reported that for mass culture of F. pallidoroseum, wheat bran and rice branwere good substrates for its growth sporulation and virulence. A method for preserving Puccinia abrupta Diet and Holw var. parthenicola Jackson and Pormalee was developed by Holder and Smith (1992). Here the dry harvested spores of the pathogen was cooled and stored at 19 ^{o}C. These spores remained viable for 32 days following thawing and were able to cause normal infection, thus enhancing its use as mycoherbicide.

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

Pyrophyllite clay, milled chitin, corn cobs, fish meal, neem cake, peanut hulls, soyfibre, and wheat bran were used by Fravel et al. (1995) to make alginate prill formulation of Talaromyces flavus. Jeyarajan and Ramakrishnan (1995) developed a talc based formulation of oil seeds and pulses using fermented grown biomass in molasses yeast medium. It maintained adequate population even after four months of

storage at ambient temperature in sealed transparent alkathene bags.

Mehta et al. (1995) reported the production of biomass containing the most effective conidia, condiophore or mycelium of Trichoderma harzianum by solid state fermentation using rice bran and wheat bran. Pelleted formulationswere made in various concentration with sodium alginate and inert filler like lignite. They also formulated chlamydospore inoculant in molasses corn strip liquor fermentation.

Preparations of Trichoderma harzianum, T. viride, T .koningi and Gliocladium virens had been developed in Pantnagar in wheat bran - sawdust medium. This preparation was relatively less expensive. (Sharma and Basondrai, 1996).

2.7 Methods of application of Mycoherbicides

The method of production of mycoherbicides determine the method of application. The simplest mycoherbicide delivery system is the fungus contained in and sprayed in water.

Mycoleptodiscus terrestris a root and crown rot organism on several legumes, was reported by Charudattan and Conway (1976) for the first time to cause a necrotic leaf spot on water hyacinth. In green house tests, a suspension of conidia and mycelia of the organism was sprayed on aerial part and this caused spots on the leaves.

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

Procedure for granulation of mycelial inoculum of Cercospora kikuchi Mats and Tomy was developed by Boyette and Walker (1985b). This granular formulation contained mixture of sodium alginate, kaoline, clay and mycelium in a 0.25 M calcium chloride solution. TeBeest and Templeton (1985) reported that the commercial product 'Collego' of C.gloeosporioides f. sp.aeschynomene against Northern jointvetch, is a wettable formulation of dried conidia.

De Vine marketted by Abbot Laboratories, is the first registered mycoherbicide. This is a liquid formulaton of chlamydospores of Phytophthora palmivora (Kenney, 1986).

Mortenson(1988) reported that round leaved mallow (Malva pusilla Sm) and velvet (Abutilon theophrasti Medik) were found to be killed within 17 to 20 days when inoculated with spore suspension of C. gloeosporioides f.sp. malvae. Morris(1989) sprinkled a dried formulation of C. gloeosporioides in wheat bran on young Hakea serica (Schrad) seeds and seedlings and observed the death of seedlings from the stem tips.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

An experiment was conducted at College of Agriculture, Vellayani to develop an effective formulation of a mycoherbicide for the biocontrol of water hyacinth. The materials and methods used for this experiment are summarised below

3.1 Study on the host range of fungal pathogens of water hyacinth

Detailed study on the host range of the already identified fungal pathogens of water hyacinth viz., Colletotrichum gloeosporioides, Fusarium equiseti, and F. pallidoroseum on common weeds and cultivated plants in and around waterways infested with water hyacinth was carried out. This was done by pot culture experiment for weeds, vegetables, pulses and oil seeds and by growing plants in nutrient solution for other crops. Each fungus was inoculated on 30 species of cultivated plants and the 41 species of selected weeds. For this seven-day old cultures of the respective fungi were used for inoculating on leaves after giving pin pricks. Inoculated areas were covered with moistened cotton wool and covered with polythene bags to maintain humidity. Control plants were also maintained by applying sterile water on the

punctured leaves. Three replications were maintained for each fungus. The plants used for host range studies were

I. Crop Plants

Common name		Scientific name
a. Vegetables		
1. Amaranthus	-	Amaranthus tricolour Linn
2. Bittergourd	-	Momordica charantia Linn
3. Bhindi	-	Abelmoschus esculentus (L.) Moench
4. Brinjal	-	Solanum melongena Linn
5. Chilli	-	Capsicum annuum Linn
6. Cowpea	-	Vigna unguiculata Savi
7. Cucumber	-	Cucumis sativus Linn
8. Snakegourd	-	Trichosanthes anguina Linn
b. Pulses and Oil Seeds		
9. Black gram	-	Phaseolus mungo Linn
10. Green gram	-	Phaseolus aureus Roxh
11. Ground nut	-	Arachis hypogaea Linn
12. Sesamum	-	Sesamum indicum Linn
c. Field crops		
13. Ragi	-	Eleusine coracana Gaertn
14. Rice	-	Oryza sativa Linn
15. Sorghum	-	Sorghum vulgare Pers
16. Sugar cane	-	Saccharum officinarum Linn
17. Sweet potato	-	Ipomoea batatus (L.) Lam

d. Fodder

18. Guinea grass - Panicum maximum Jseq
19. Napier grass - Pennisetum purpureum Schum

e. Cover crop

20. Butterfly pea - Centrocema pubescens Benth Centro

f. Plantation crops

21. Arecanut - Areca catechu Linn
22. Coconut - Cocos nucifera Linn

g. Spices

23. Pepper - Piper nigrum Linn
24. Nutmeg - Myristica fragrans Houtt

h. Fruit and Forest crops

25. Acacia - Acacia arabica Lam
26. Jack - Artocarpus integrifolia Linn
27. Mango - Mangifera indica Linn
28. Teak - Tectona grandis Linn

i. Ornamentals

29. Lotus - Nelumbo nucifera Gaertn
30. Water lilly - Nymphaea nouchali Burm. F

II. Weed Plants

	Common name		Scientific name
1.	Amaranthus	-	Amaranthus viridis (Linn) Notrysag
2.	Appa	-	Ageratum conyzoides Linn.
3.	Asystacia	-	Asystacia coromandelina T.And
4.	Axonopus	-	Axonopus sp.
5.	Balippoovu	-	Aerva lanata (Linn) Juss
6.	Bermuda grass	-	Cynodon dactylon (L) Pers
7.	Erukku	-	Calotropis gigantea R.Br
8.	Eragrostis	-	Eragrostis tenella Beauv
9.	Eupatorium	-	Eupatorium odoratum HB K.
10.	Fowl foot grass	-	Eleusine indica (L) Gaertn
11.	Justicia	-	Justicia diffusa Willd
12.	Justicia	-	J. prostrata Gamble N. Comb
13.	Kannuneerthulli	-	Alloteropis cimicina (L.) Staff
14.	Kattukaduku	-	Cleome viscosa Linn
15.	Kavada	-	Echinochloa colonum Beauv
16.	Keezhar nelli	-	Phyllanthus niruri Linn Hoof. F
17.	Kudangal	-	Hydrocotyl asiatica Urban
18.	Lantana	-	Lantana camara Linn
19.	Naruneendi	-	Hemidesmus indicus R Br.
20.	Natta poochedi	-	Hyptis suaveolens Poit
21.	Neerthamara	-	Monochoria vaginalis Prest
22.	Nonganam pullu	-	Oldenlandia umbellata Linn
23.	Odiyan	-	Tridax procumbens Linn

24.	Palpullu	-	Panicum distachyum syn. Brachiaria ramosa (L) Stapf
25.	Paloori pacha	-	Euphorbia geniculata Linn
26.	Peruvalam	-	Clerodendron infortunatum Linn
27.	Poovamkurunnu	-	Vernonia cinaria Linn
28.	Puliyarila	-	Oxalis corniculata Linn
29.	Sankhupushpam	-	Clitoria ternatea Linn
30.	Scoparia	-	Scoparia dulcis Linn
31.	Sooryan	-	Bulbostylis barbata
32.	Thakara	-	Cassia occidentalis Linn
33.	Tharavu	-	Euphorbia hirta Linn
34.	Thazhuthama	-	Boerhaavia diffusa Linn
35.	Thumba	-	Leucas aspera Spreng
36.	Torpedo grass	-	Panicum repens Linn
37.	Vazhappadathy	-	Commelina benghalensis Linn
38.	Vazhappadathy	-	C. jacobi Fischer
39.	Vellakurunthotti	-	Sida acuta Burm
40.	Venappacha	-	Synedrella nodiflora Linn
41.	Venpacha	-	Heliotropium indicum Linn

3.2 Standardisation of dosage of inoculum required for effective destruction of the weed

An experiment was conducted in the laboratory to standardise the dosage of inoculum required for effective destruction of the weed. The spore concentrations were

fixed at three levels for each of the selected fungi. The dosage fixed are 1 x 10^9, 1 x 10^{10} and 1 x 10^{11} spores per ml for the three fungi, viz., F. equiseti, F. pallidoroseum and C. gloeosporioides. The spore suspensions with the specified spore counts were prepared in sterile water. These were sprayed on the healthy water hyacinth plants. The inoculated plants were covered with polythene bags to maintain high humidity. Three replications were maintained for each fungus for each concentration. Control plants were kept for each fungus. Intensity of infection was measured by using score charts (Plate 1 and 2).

Score chart for each pathogen.

C. gloeosporioides

0 - No Symptom

1 - Small spots covering less than 1 per cent leaf area.

2 - Small spots covering 1 to 10 per cent leaf area.

3 - Lesions big not coalesing covering 11 to 25 per cent leaf area.

4 - Lesions coalesing covering 26 to 50 per cent leaf area.

5 - Blighting covering 51 to 75 per cent leaf area.

6 - Blighting covering more than 75 per cent leaf area.

F. equiseti and F. pallidoroseum

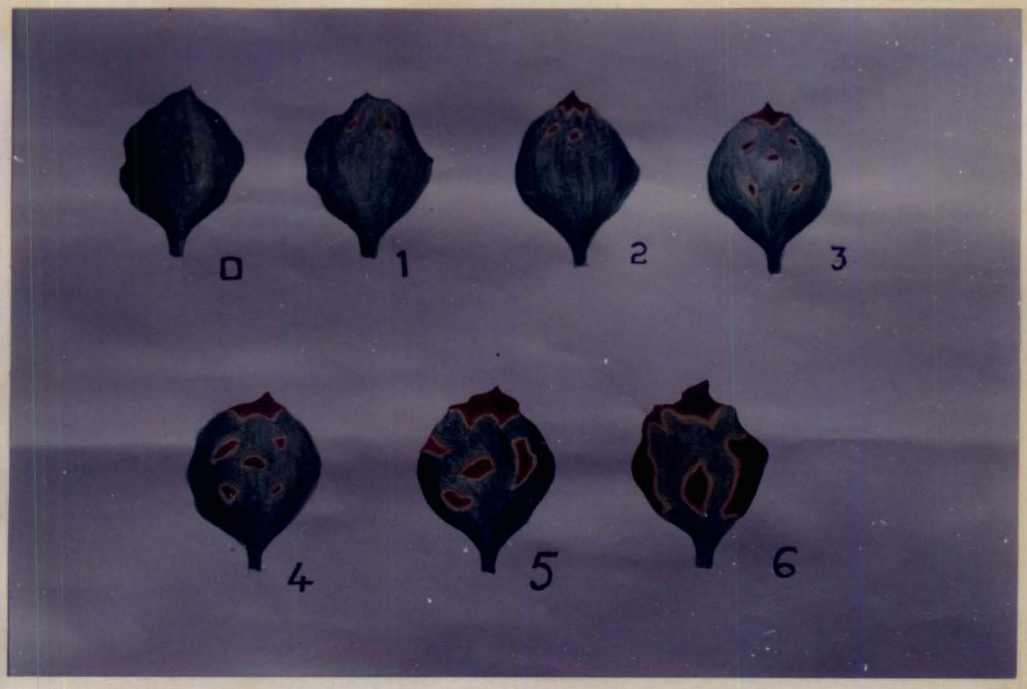
0 - No Symptom

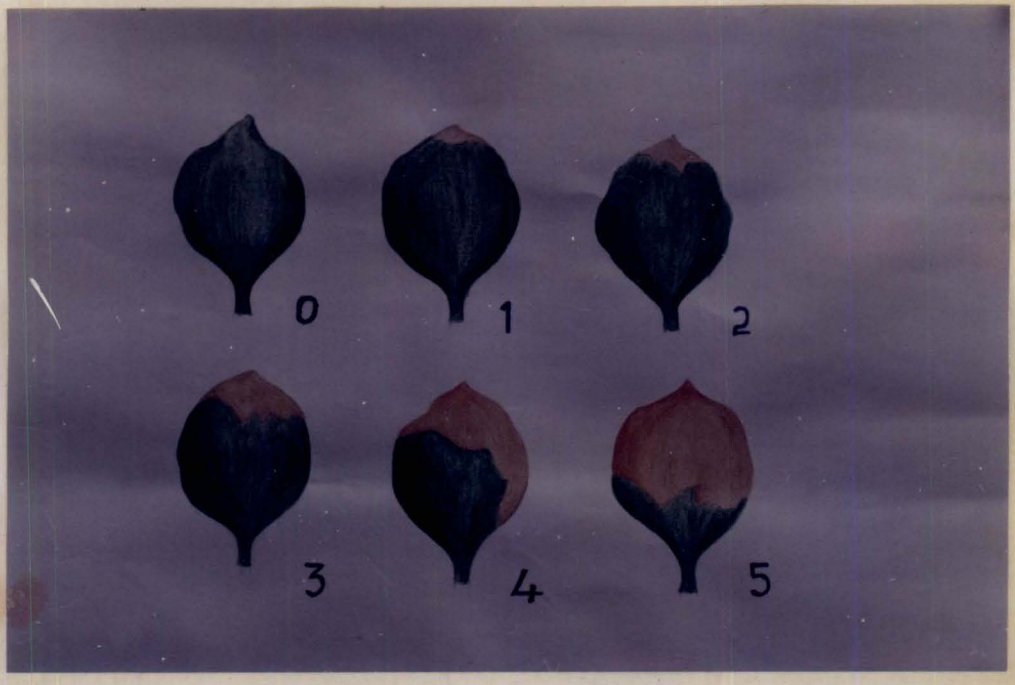
1 - Blighting from the tip covering less than 1 per cent leaf area.

2 - Blighting covering 25 per cent leaf area.

Plate 1 Score chart for Colletorichum gloeosporioides

Plate 2 Score chart for Fusarium spp.


0
1
2
3
4
5
6


0
1
2
3
4
5

3 - Blighting covering 26 to 50 per cent leaf area.

4 - Blighting covering 51 to 75 per cent leaf area.

5 - Blighting covering more than 75 per cent leaf area.

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

$$D.I = \frac{\text{Sum of the grades of each leaf}}{\text{No. of leaves assessed x maximum grade used}} \times 100$$

3.3 Use of cell free metabolites produced by the pathogenic fungi in the destruction of water hyacinth

Toxins produced by Fusarium species were extracted as per the procedure described by Abbas et al. (1991). Fresh cultures of Fusarium spp. were grown on Potato Dextrose Agar in sterile petri dishes. From 7 to 10 day old cultures, 5 mm discs were cut and inoculated on autoclaved rice. After 14 days of growth this inoculum of fungus infested rice was dried at room temperature (28 +/- 4°C) for five days. The inoculum was ground into fine powder. Five g of inoculum was added to 50 ml of distilled water, stirred for 1 to 2 minutes and sieved through double layer of cheese cloth to remove large particles and [illegible]. This filtrate was applied on healthy water hyacinth plants. Suitable control was also maintained. The intensity of damage was measured by using score charts.

Toxin production of C. gloeosporioides was studied in Czapek (Dox) broth. For this Czapek (Dox) broth was prepared

and dispensed at the rate of 50 ml in 250 ml conical flask and sterilized by autoclaving at 1.02 kg/m^2 for 15 min. The medium was inoculated with mycelial disc of five mm diameter from five-day-old cultures on Potato Dextrose Agar medium. The inoculated flasks were incubated at room temperature. After 14 days of growth the cultures were filtered and filtrate tested for toxic activity. The filtrate was applied on healthy water hyacinth plants. Suitable control plants were kept. The extent of damage caused by the fungi was expressed as per cent damage which was measured using score chart.

3.4 Field evaluation of the pathogen singly and in combination on water hyacinth

Water hyacinth plants were allowed to establish in troughs of size 0.75 x 0.75 m^2. Spore suspension of the above pathogens were prepared with uniform spore concentration of $1x10^{11}$ spores per ml which is found to be more effective in controlling the weed. Their combinations were also prepared by mixing equal volumes of spore suspensions. The different treatments tried were:-

1) F. equiseti
2) F. pallidoroseum
3) C. gloeosporioides
4) F. equiseti + F. pallidoroseum
5) F. equiseti + C. gloeosporioides

6) F. pallidoroseum + C. gloeosporioides

7) F. equiseti + F. pallidoroseum + C. gloeosporioides

8) Control [Distilled water]

Each treatment was applied on separate troughs and the intensity of infection was measured using the score chart. For combined infection score chart for Fusarium spp. was used since the symptoms were similar to those caused by Fusarium spp.

3.5 Field evaluation of the metabolites singly and in combination on water hyacinth

Metabolites of the pathogenic fungi were prepared as described under 3.3. Their combinations were prepared by mixing equal volume of the filtrates. Then the metabolites singly and their combinations were applied on healthy water hyacinth. Plants grown in troughs of uniform size 0.75 x 0.75 m^2 by simulating the field conditions. The intensity of damage was measured, using the score chart. For measuring the damage caused by the metabolite of more than one fungi, the score chart for Fusarium spp. was used.

The different treatments were:-

1) Metabolite of F. equiseti

2) Metabolite of F. pallidoroseum

3) Metabolite of C. gloeosporioides

4) Metabolites of F. equiseti + F. pallidoroseum

5) Metabolites of F. equiseti + C. gloeosporioides

6) Metabolites of F. pallidoroseum + C. gloeosporioides

7) Metabolites of F. equiseti + F. pallidoroseum + C. gloeosporioides

8) Control [Distilled water & Czapek's (Dox) broth]

3.6 Standardisation of carrier materials for the storage of the fungi

In order to find out suitable carrier material for storage and field application of the pathogen, trials were conducted with the following meterials:-

1) Coir pith
2) Guinea grass straw powder
3) Rice bran
4) Rice straw powder
5) Sand-maize medium
6) Vermiculite
7) Water hyacinth leaf powder
8) Wheat bran

In the case of wheat bran and rice bran, 25 g. of the material was taken in 250 ml conical flask and 30 ml of water was added to moisten them. But for coir pith and vermiculite 10 g of the material was taken in conical flask and 30 ml of a solution [20 g sucrose, 2 g KNO_3 and 1 l distilled water] containing carbon and nitrogen source was added to enrich the material. Rice straw, guinea grass straw and water hyacinth

leaves were dried and ground into powder and 25 g of them were taken in conical flask and enough water was added to moisten them. Sand-maize medium was prepared by mixing equal weights of sand and corn flour and little water was added to moisten. Fifteen gram of this carrier material was also taken in conical flasks. All the materials in the conical flasks were sterilised at 15 lb/in² pressure for 20 minutes for two successive days. Five mm discs from seven-day-old cultures of the test pathogens on Potato Dextrose Agar medium were taken and inoculated on the carrier materials. These were incubated at room temparature (28 +/- 4°C).

3.6.1 Effect of carrier materials on the sporulation of the fungi

The spore count and viability of the spores were tested at weekly intervals. For taking spore count, a loopful of the inoculum was taken and a spore suspension made in 5 ml of water. From this one drop was taken and placed on haemocytometer and stained using cotton blue lactophenol. Number of spores in one microscopic field was counted under low power of the microscope. For large number haemocytometer was used.

3.6.2 Effect of carrier material on spore viability of the fungi

For testing the viability of spores, five ml of water was taken in a test tube and sterilised. Into these tubes, one loopful of the inoculum from each carrier material was

added. One drop of this spore suspension was placed on glass slide and kept in moist chamber to allow the spores to germinate. Germination count was taken after 48 hours.

3.6.3 Storage life of metabolites produced by the pathogenic fungi

For testing the storage life of metabolites, healthy water hyacinth plants were applied with filtrates on the day of preparation, one day, three days, Five days and Seven days after preparation and also by the filtrate stored at 5°C (refrigerator). Three replications were maintained for each treatments. Suitable control plants were also kept. Intensity of infection was measured using score chart.

3.6.4 Extent of damage caused by fungi grown on different carrier materials

A laboratory experiment was conducted to evaluate the performance of the fungal pathogen in different carrier materials. For this, healthy plants were inoculated with each fungal pathogen in different carrier materials viz. coir pith, guinea grass straw, rice bran, rice straw, water hyacinth leaf and wheat bran. Three replications and suitable control were maintained for each carrier material . Using score chart the intensity of infection on the plants was measured.

3.6.7 Standardisation of method of application of pathogens for biocontrol of water hyacinth

Experiments in laboratory were conducted to standardize the method of application of pathogen for biocontrol of water hyacinth. Five methods of applications were tested.

1. Dusting the inoculum, uniformly @ 5g/plant
2. Placing bits of inoculum on leaves and leaf axil.
3. Spore suspension in water.

   For this five g of the inoculum was taken in 100 ml of water. This suspension was filtered and filtrate was used for spraying.
4. Dust formulation using diatomaceous earth as inert material.

   The concentrated spores were mixed with diatomaceous earth powder. It was standardized to contain $1x10^{11}$ spores per g of formulation.
5. Wettable powder formulation using diatomaceous earth.

   It was prepared by mixing dust formulation with equal quantity water containing 0.1% teepol (Wetting agent)

3.6.8 Characterisation of the toxin

Isolation of fusaric acid from culture filtrates of Fusarium spp. was done according to the method of Mahadevan et al. (1986). Fusarium spp. were grown for 10 days in 50 ml of

Czapek's liquid medium in 250 ml flask. Culture filtrates were collected and centrifuged at 2000 g for 20 min. Clear supernatent was collected. P^H was adjusted to 4 by adding 2 N HCl and 100 ml of the filtrate was extracted repeatedly with equal volume of ethyl acetate four times in a separating funnel allowing 15 minutes for each extraction. All the ethyl acetate extracts were combined and evaporated to near dryness on a hot water bath. The residue was resuspended in 1 to 2 ml of ethanol. This was spotted on Whatman No.1 filter paper. Chromatogram was developed descendingly for 10 to 12 hr in secondary butanol: formic acid: water (75:15:10 v/v). The paper was dried for 14-16 hr under a hood and sprayed with bromophenol blue. Toxin was identified based on the colour developed on the paper.

3.7 Statistical analysis

The data obtained during the study were analysed statisticaly by applying the techniques of analysis of variance (Panse and Sukhatme, 1967). Angular transformation was used for transforming the values except that in table 8, 9 and 10.

RESULTS

4. RESULTS

The results of the study conducted for the biological control of water hyacinth using fungal pathogens are presented.

4.1 Study on the host range of fungal pathogens of water hyacinth

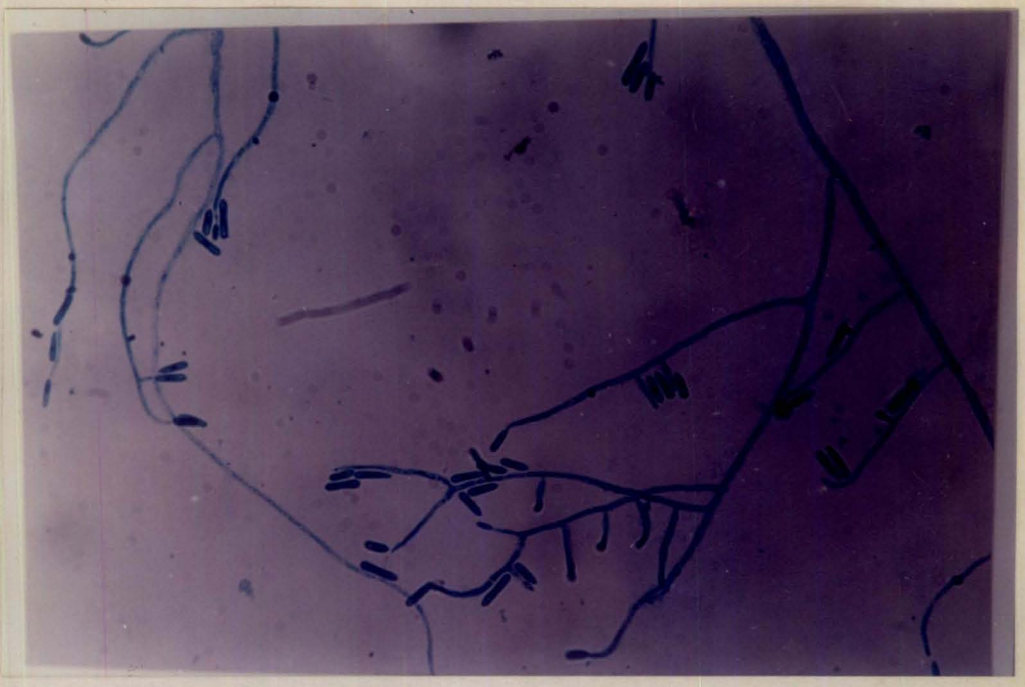
Detailed study was conducted on the host range of the already identified fungal pathogens of water hyacinth, viz., Colletotrichum gloeosporioides (Penz.) Penz. and Sacc., Fusarium equiseti (Corda) Sacc. and F. pallidoroseum (Cooke) Sacc (Plate 3, 4, 5) on 30 cultivated plants including vegetables, pulses and oil seeds, field crops, fruits and forest crops and ornamental plants and 41 common weed plants in and around water ways infested with water hyacinth. The susceptibility of the plants to the pathogens tested are given in Table 1.

Of the 30 cultivated plants tested C. gloeosporioides was found to be pathogenic to three vegetable crops, viz., amaranthus, bhindi and chilli (Plate 6, 7). It could also infect mango. All the other crop plants tested were not infected by the fungus.

Among the various crop plants tested, F. equiseti produced symptoms on amaranthus only while others were not susceptible to F. equiseti.

Plate 3 Conidia and conidiophores of C. gloeosporioides

Plate 4 Conidia and conidiophores of Fusarium equiseti



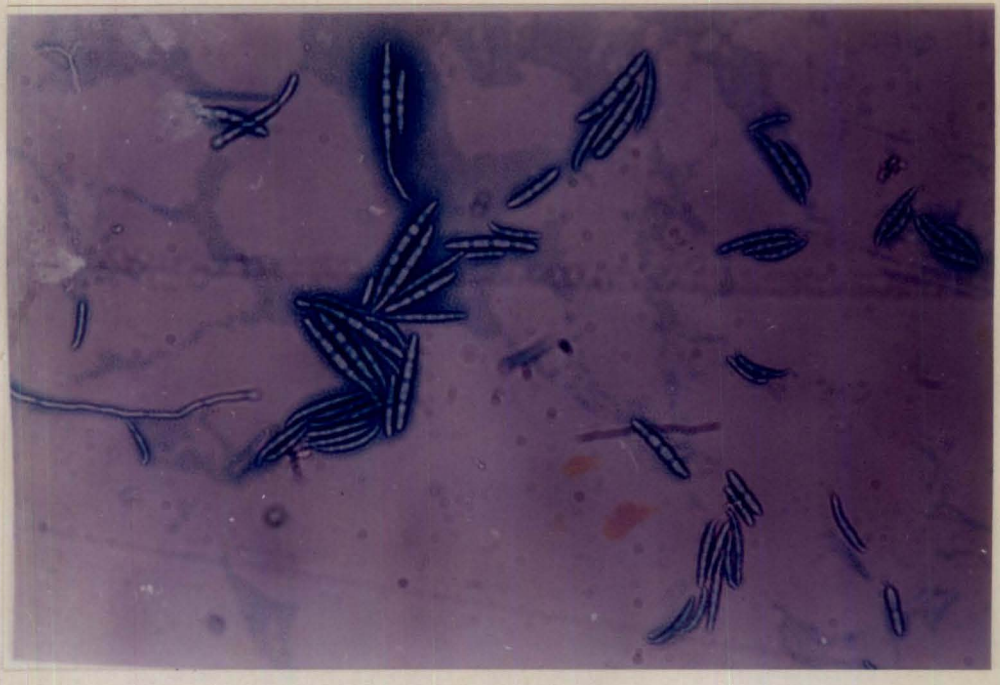
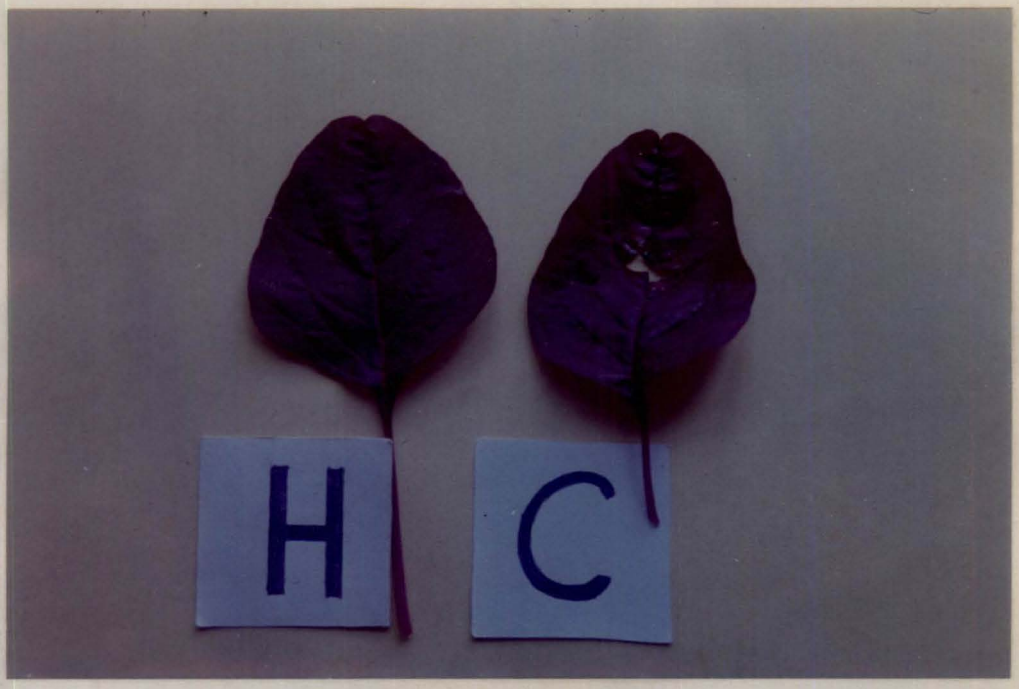
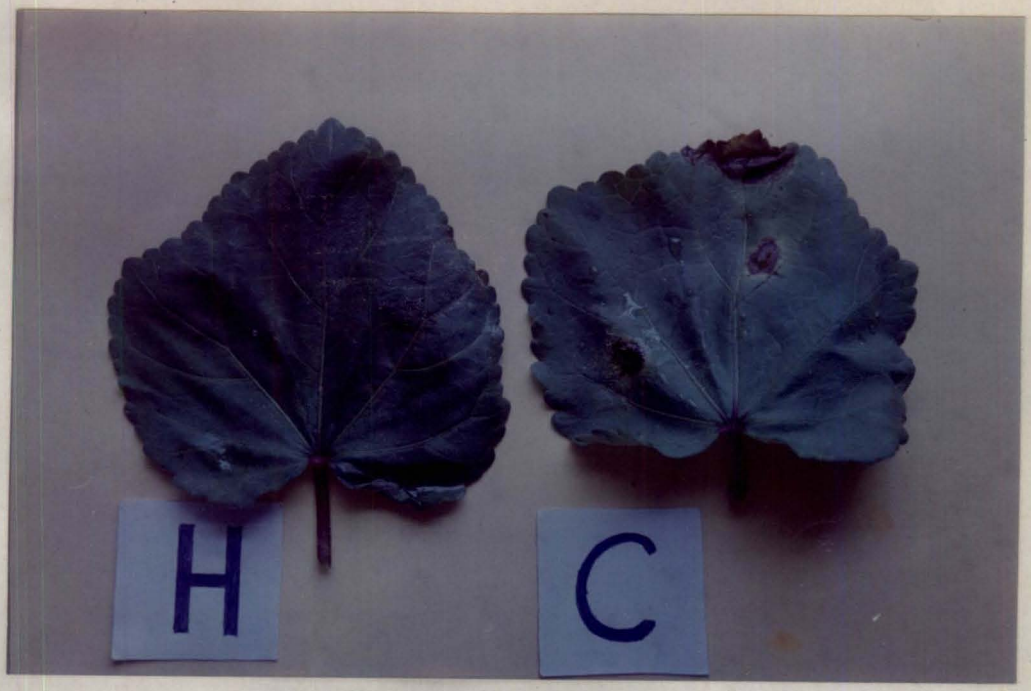


Plate 5 Conidia and conidiophores of *Fusarium pallidoroseum*

Plate 6 Symptoms produced by C. gloeosporioides on Amaranthus
H - Healthy leaf C - Infected leaf

Plate 7 Symptoms produced by C. gloeosporioides on Bhindi
H - Healthy leaf C - Infected leaf


H
C


H
C

Of the various crop plants tested against F. pallidoroseum, it could produce symptoms on napier grass only.

The symptoms produced on various crop plants by the pathogens were as follows.

Pathogen	Host	Symptom	No.of days for the appearance of symptom
C. gloeosporioides	Amaranthus	Dull straw coloured irregular spots on the leaf lamina	4-6
	Bhindi	Large brown irregular lesion on the leaf lamina	5-7
	Chilli	Small brown specks which enlarged into large spots. As the spots become old central necrotic area falls off leaving shot hole on the infected area	7-10
	Mango	Small dark brown spots on leaf lamina	7-8
F. equiseti	Amaranthus	Dull green coloured irregular lesions on leaf blade which enlarged rapidly to cause drying up of whole leaf	4-6
F. Pallidoroseum	Napier grass	Straw coloured lesions on leaf lamina. These lesions enlarged gradually resulting in the blighting of the leaf	6-8

Plate 8 Symptoms produced by *C. gloeosporioides* on *Euphorbia hirta*
C - Healthy leaf C - Infected leaf

Among the 41 weed plants tested C. gloeosporioides produced symptoms on Euphorbia hirta, Hydrocotyl asiatica and Phyllanthus niruri (Plate 8). None of the other species of weed plants was infected by the pathogen.

Of the various weed plants tested, F. equiseti could infect only few plants viz., Amaranthus viridis, Commelina benghalensis, C. jacobi and Monochoria vaginalis. The other plants were not susceptible to the fungus.

F. pallidoroseum could produce symptoms on 14 plants out of the 41 weed plants tested. The plants that were susceptible to F. pallidoroseum are Axonopus sp., Boerhaavia diffusa, Calotropis gigantea, Cassia occidentalis, Commelina benghalensis, C. jacobi, Echinochloa colonum, Euphorbia hirta, Justicia diffusa, J. prostrata, Monochoria vaginalis, Oldenlandia umbellata, Phyllanthus niruri and Scoparia dulcis (Plate 9, 10, 11, 12, 13, 14).

The symptoms produced on each of the weed plants infected by the pathogenic fungi are described below:


C
H

Plate 9 Symptoms produced by F. pallidoroseum on Axonopus sp.
F_3 - Infected leaf H - Healthy leaf

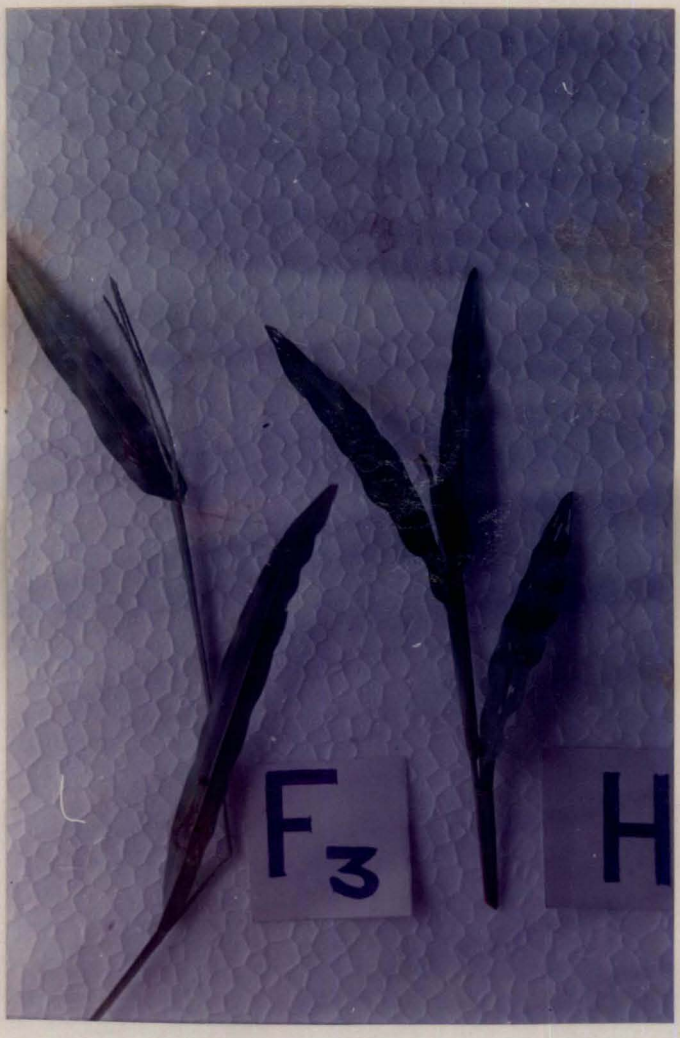

F3
H

Plate 10 Symptoms produced by *F. pallidoroseum* on *Boerhaavia diffusa*
F_3 - Infected leaf H - Healthy leaf

Plate 11 Symptoms produced by *F. pallidoroseum* on *Calotropis gigantea*
F_3 - Infected leaf H - Healthy leaf


F3
H

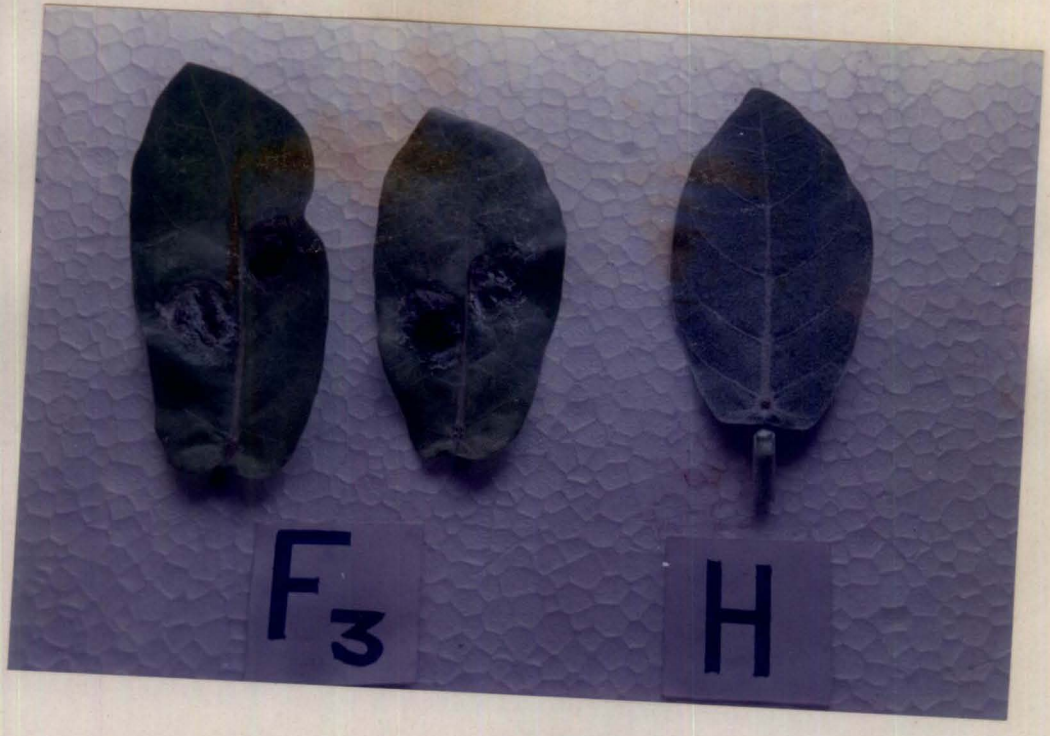

F3
H

Plate 12 Symptoms produced by *F. pallidoroseum* on *Commelina benghalensis*
F_3 - Infected leaf H - Healthy leaf

Plate 13 Symptoms produced by *F. pallidoroseum* on *C. jacobi*
F_3 - Infected leaf H - Healthy leaf


F1
H

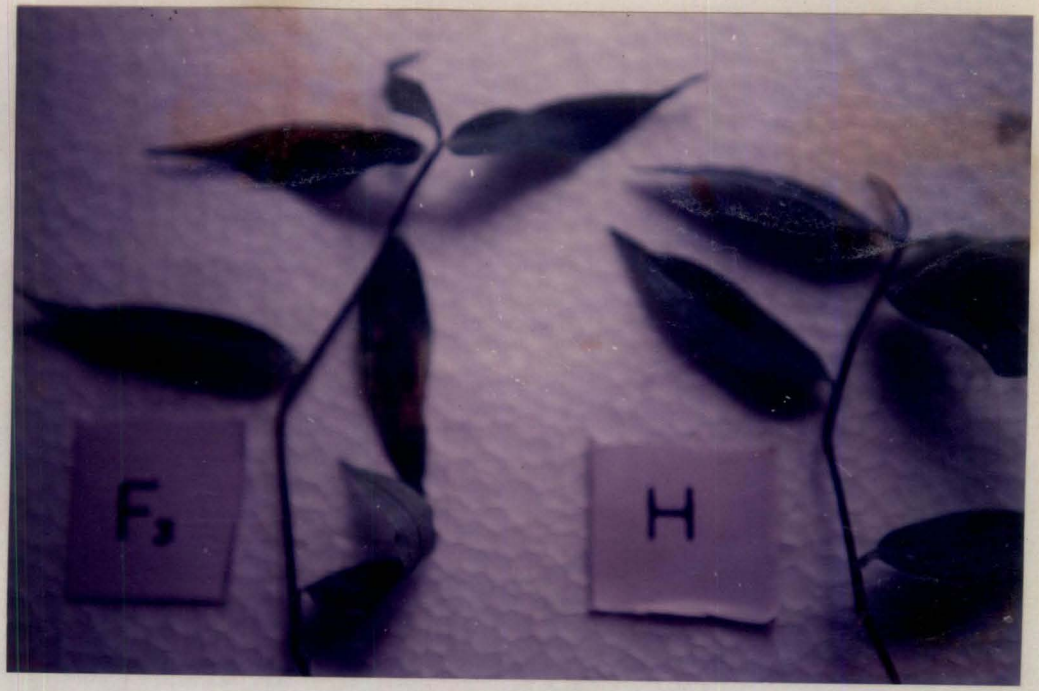

F1
H

Plate 14 Symptoms produced by F. pallidoroseum on Euphorbia hirta
F_3 - Infected leaf H - Healthy leaf


H
F3

Pathogen	Host	Symptom	No.of days for the appearance of symptom
C. gloeosporioides	Euphorbia hirta	Yellowing of the infected part, which spread to the whole leaf resulting in drying and shedding of the leaves.	5-7
	Hydrocotyl asiatica	Small brown spots on the leaf lamina with a distinct yellow halo	7
	Phyllanthus niruri	Small light brown lesions on the infected part, which gradually enlarged resulting in the drying up of the leaf.	5-7
Fusarium equiseti	Amaranthus viridis	Straw to dull green coloured irregular lesions on the infected leaves.	5-7
	Commelina benghalensis	Light brown lesions with an yellow margin on leaf lamina. Later these lesions enlarged resulted in the rotting of the leaves.	4-6
	C. jacobi	Light brown lesions, which gradually resulted in the rotting of the infected leaves.	4-6
	Monochoria vaginalis	Blighting of the leaves starting from tip downwards.	5-7
F.pallidoroseum	Axonopus sp	Light brown lesions on the lamina, which later enlarged.	5-7
	Boerhaavia diffusa	Brown spots with prominent yellow halo on leaf lamina	4-6
	Calotropis gigantea	Circular brown spots surrounded by yellow halo on the leaf lamina.	3-5
	Cassia occidentalis	The leaves become light brown and rot. The leaflets shed rapidly.	3-5
	Commelina banghalensis	Rotting of the leaf. Brown areas surrounded by distinct yellow halo. Infection spreads resulting in the rotting of the leaf.	4-5

Pathogen	Host	Symptom	No.of days for the appearance of symptom
	C. jacobi	Yellowing followed by rotting of the leaves.	4-5
	Echinochloa colonum	Light brown coloured irregular spots surrounded by dark brown margin.	5-6
	Euphorbia hirta	Brown spots on the infected area, general yellowing of the infected leaves was also observed.	5-7
	Justicia diffusa	The infected leaves become brown and the leaves shed easily	3-5
	J. prostrata	The leaves became brown in colour and shed rapidly.	3-5
	Monochoria vaginalis	Small brown spots surrounded by yellow halo developed on the leaf lamina. In advanced stage, individual spots coalesced causing blighting of the whole leaves.	4-6
	Oldenlandia umbellata	Light brown coloured irregular spots on leaf lamina after inoculation. Gradually these spots enlarged resulting in blighting of the leaves.	4-5
	Phyllanthus niruri	Water soaked light brown lesions are produced on leaf lamina resulting in the falling off of leaflets.	3-5
	Scoparia dulcis	Light to dark brown coloured irregular spots which later enlarged and resulted in blighting of leaves.	4-5

Table 1 Host range of fungal pathogens of water hyacinth

Host	C	F1	F2
Crop plants			
a. Vegetables			
1. Amaranthus	+	+	-
2. Bhindi	+	-	-
3. Bitter gourd	-	-	-
4. Brinjal	-	-	-
5. Chilli	+	-	-
6. Cowpea	-	-	-
7. Cucumber	-	-	-
8. Snake gourd	-	-	-
b. Pulses and Oilseeds			
9. Black gram	-	-	-
10. Green gram	-	-	-
11. Ground nut	-	-	-
12. Sesamum	-	-	-
c. Field crops			
13. Ragi	-	-	-
14. Rice	-	-	-
15. Sorghum	-	-	-
16. Sugarcane	-	-	-
17. Sweet potato	-	-	-
d. Fodder			
18. Guinea grass	-	-	-
19. Napier grass	-	-	+

Host	C	F1	F2
e. Cover crop.			
20. Butterfly pea	-	-	-
f. Plantation crops			
21. Arecanut	-	-	-
22. Coconut	-	-	-
g. Spices			
23. Pepper	-	-	-
24. Nutmeg	-	-	-
h. Fruits & Forest crops			
25. Acacia	-	-	-
26. Jack	-	-	-
27. Mango	+	-	-
28. Teak	-	-	-
i. Ornamentals			
29. Lotus	-	-	-
30. Water lilly	-	-	-
Weed Plants			
1. Aerva lanata	-	-	-
2. Ageratum conyzoides	-	-	-
3. Amaranthus viridis	-	+	-
4. Asystacia coromandelina	-	-	-
5. Axonopus sp.	-	-	+
6. Boerhaavia diffusa	-	-	+
7. Brachiaria ramosa	-	-	-
8. Bulbostylis barbata	-	-	-

Table 1 contd.

Host	C	F1	F2
9. Calotropis gigantea	-	-	+
10. Cassia occidentalis	-	+	-
11. Cleome viscosa	-	-	-
12. Clerodendron infortunatum	-	-	-
13. Commelina benghalensis	+	+	-
14. C. jacobi	+	+	-
15. Clitoria ternatea	-	-	-
16. Cynadon dactylon	-	-	-
17. Eragrostis tenella	-	-	-
18. Echinochloa colonum	-	+	-
19. Eleusine indica	-	-	-
20. Eupatorium odoratum	-	-	-
21. Euphorbia geniculata	-	-	-
22. E. hirta	-	+	+
23. Heliotropium indicum	-	-	-
24. Hemidesmus indicus	-	-	-
25. Hydrocotyl asiatica	-	-	+
26. Hyptis suaveolens	-	-	-
27. Justicia diffusa	-	+	-
28. J. prostrata	-	+	-
29. Lantana camara	-	-	-
30. Leucas aspera	-	-	-
31. Monochoria vaginalis	-	+	+
32. Oldenlandia umbellata	-	+	-

Table 1 contd.

Host	C	F1	F2
33. *Oxalis corniculata*	-	-	-
34. *Panicum distachyum*	-	-	-
35. *P. repens*	-	-	-
36. *Phyllanthus niruri*	+	-	+
37. *Sida acuta*	-	-	-
38. *Scoparia dulcis*	-	-	+
39. *Synedrella nodiflora*	-	-	-
40. *Tridax procumbens*	-	-	-
41. *Vernonia cineria*	-	-	-

C - *C. gloeosporioides*

F1 - *F. equiseti*

F2 - *F. pallidoroseum*

\+ - Susceptible

\- - Not susceptible

4.2 Standardization of dosage of inoculum required for effective destruction of the weed

An experiment under laboratory condition was conducted to standardise the dosage of inoculum required for effective destruction of the weed. Three levels of spore concentrations were fixed viz., $1x10^9$, $1x10^{10}$ and $1x10^{11}$ spores per ml for each of the fungi tested, viz., C.gloeosporioides, F. equiseti and F. pallidoroseum. Observations on the intensity of infection produced at different spore concentrations by the fungi tested were recorded by using the score charts given under 3.2. The results are presented in Table 2, Fig.1.

On statistical analysis of the intensity of infection caused by different spore concentrations of fungi, there was significant difference was observed among the treatments.

In the case of all the three pathogenic fungi, there was significant difference between the three concentrations tested, viz., $1x10^9$, $1x10^{10}$ and $1x10^{11}$ spores per ml. Third concentration ie., $1x10^{11}$ spores per ml was most effective causing a damage of 74.63, 89.70 and 98.01 per cent for C. gloeosporioides, F. equiseti and F. pallidoroseum respectively. This was followed by the concentration of $1x10^{10}$ spores per ml (Plate 15, 16, 17).

Table 2 Extent of damage caused by different doses of inoculum of the pathogenic fungi

Dose of inoculum (Spores/ml)	* Mean per cent infection caused by					
	Colletotrichum gloeosporioides		*Fusarium equiseti*		*F. pallidoroseum*	
$1x10^9$	46.66	(43.07)	62.67	(52.32)	57.0	(49.00)
$1x10^{10}$	72.23	(58.18)	70.68	(57.19)	88.34	(70.00)
$1x10^{11}$	74.63	(59.73)	89.70	(71.25)	98.01	(83.94)
CD (0.05)	1.43		2.65		6.42	

* Average of three replications
Figures in parantheses indicate transformed values

Fig. 1 Extent of damage caused by different dose of inoculum of the pathogenic fungi.

Plate 15 Symptoms produced by C. gloeosporioides on water hyacinth at different concentration 1 - $1x10^{11}$ spores per ml, 2 - $1x10^{10}$, 3 - $1x10^{9}$ spores per ml

H - Healthy plant

Plate 16 Symptoms produced by F. equiseti on water hyacinth at different concentrations
1 - $1x10^{11}$ spores per ml,
2 - $1x10^{10}$ spores per ml,
3 - $1x10^{9}$ spores per ml.
H - Healthy plant


B
C
1
2
3
6 1 '96


B
E
1
2
3
6 1 '96

Plate 17 Symptoms produced by F. pallidoroseum at different concentration
1 - $1x10^{11}$ spores per ml
2 - $1x10^{10}$ spores per ml
3 - $1x10^{9}$ spores per ml
H - Healthy plant


B
F5
1
2
3

4.3 Use of cell free metabolites produced by the pathogenic fungi in the control of water hyacinth

Culture filtrates of each of the pathogenic fungi were collected and applied on healthy water hyacinth plants. Statistical analysis of the inensity of damage produced by the cell free metabolites of each of the pathogenic fungi, viz., C. gloeosporioides, F .equiseti and F. pallidoroseum was carried out and it was observed that there was significant difference between the treatments (Table 3). Maximum per cent intensity of damage of 96.28 was produced by the metabolite of F. pallidoroseum. This was followed by F. equiseti and C. gloeosporioides and intensity of damage being 83.00 and 26.28 per cent respectively.

4.4 Field evaluation of the pathogen singly and in combination on water hyacinth

Spore suspension of $1x10^{11}$ spores/ml of each of the fungi and their combination were sprayed on water hyacinth plants. Observations on the extent of damage caused by each of the fungi and their combinations were statistically analysed (Table 4). In vitro studies revealed that combined application of F. equiseti and F. pallidoroseum gave maximum intensity of infection of 96.45 per cent followed by F. pallidoroseum alone being 78.36 per cent. Least damage of 19.63 per cent was caused by the application of C. gloeosporioides singly.

Table 3 Extent of damage caused by cell free metabolites of the pathogenic fungi on water hyacinth

Metabolites of	* Mean per cent damage
C. gloeosporioides	26.28 (30.83)
F. equiseti	83.00 (65.68)
F. Pallidoroseum	96.28 (81.02)

Figures in parantheses indicate transformed values.
CD (0.05) = 9.96
*Average of three replications

Table 4 Extent of damage caused by pathogens singly and in combination on water hyacinth under *in vitro* conditions.

Treatments	* Mean per cent infection
C	19.63 (26.29)
F1	58.33 (49.78)
F2	78.36 (62.25)
C + F1	69.68 (56.57)
C + F2	59.34 (50.36)
F1 + F2	96.45 (79.11)
C + F1 + F2	64.34 (53.31)

Figures in parantheses indicate transformed values
CD (0.05) - 2.72
C - *Colletotrichum gloeosporioides*
F1 - *Fusarium equiseti*
F2 - *F. palli doroseum*
* Average of three replications

Plate 18 Symptoms produced by F. equiseti on water hyacinth
H - Healthy plant, F_1 - Infected plant

Plate 19 Symptoms produced by F. pallidoroseum on water hyacinth
H - Healthy plant F_3 Infected plant


H
F1


H
F3

Plate 20 Symptoms produced by the combination of *C. gloeosporioides* and *F. equiseti* on water hyacinth
H - Healthy plant, F_1+C - Infected plant

Plate 21 Symptoms produced by the combination of *C. gloeosporioides* and *F. pallidoroseum* on water hyacinth
H - Healthy plant, F_3+C - Infected plant


H
F1+C


H

Plate 22 Symptoms produced by the combination of *F. equiseti* and *F. pallidoroseum* on water hyacinth

H - Healthy plant, F_1+F_3 - Infected plant

Plate 23 Symptoms produced by the combination of *C. gloeosporioides*, *F. equiseti* and *F. pallidoroseum* on water hyacinth
H - Healthy plant, F_1+F_3+C - Infected plant


H
F2+F3

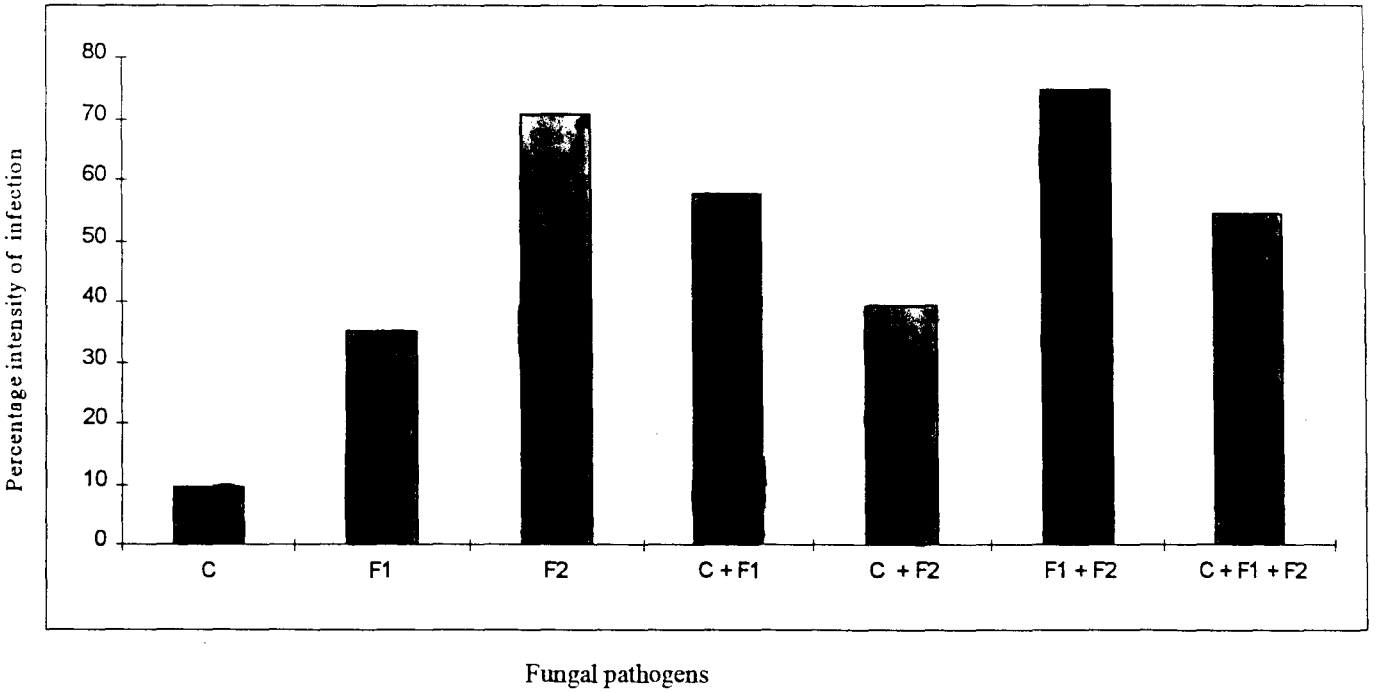

H
F2+F3C

Table 5 Extent of damage caused by pathogens singly and in combination on water hycinth under field conditions.

Treatments	* Mean per cent infection
C	9.56 (18.00)
F1	34.93 (36.21)
F2	70.67 (57.19)
C + F1	57.67 (49.39)
C + F2	39.33 (38.82)
F1 + F2	74.82 (59.86)
C + F1 + F2	54.33 (47.47)

Figures in parantheses indicate transformed values
CD (0.05) - 4.06
C - Colletotrichum gloeosporioides
F1 - Fusarium equiseti
F2 - F. pallidoroseum
* Average of three replications

Fig. 2 Extent of damage caused by Pathogens singly and in combination on water hyacinth under field conditions.



C - *Colletotrichum gloeosporioides*
F1 - *Fusarium equiseti*
F2 - *F. pallidoroseum*

The per cent intensity of infection produced by other treatments were as follows, F. equiseti and C. gloeosporioides (69.68), F. equiseti, F. pallidoroseum and C. gloeosporioides (64.34) F. pallidoroseum and C. gloeosporioides (59.34) and F. equiseti alone (58.33) (Plate 18, 19, 20, 21, 22, 23).

The same trend was observed in the field also (Table 5, Fig. 2). On statistical analysis of the intensity of infection produced by the pathogens singly and in combination, it was found that there was significant difference between the [illegible] treatments tested. The combined application of F. pallidoroseum and F. equiseti caused maximum infection of 74.82 per cent followed by F. pallidoroseum alone of 70.67 per cent. F. equiseti in combination with C. gloeosporioides gave 57.67 per cent infection and F. equiseti in combination with F. pallidoroseum and C. gloeosporioides gave 54.33 per cent infection. Least damage of 9.56 per cent was caused by C. gloeosporioides alone.

4.5 Field evaluation of metabolites singly and in combination on water hyacinth

Metabolites of the pathogens individually and in combination were applied on healthy water hyacinth plants in laboratory and field conditions. Observations were taken based on the score chart prepared. Stastitical analysis of the per

Table 6 Extent of damage caused by metabolite of pathogens singly and in combination on water hyacinth under *in vitro* condition.

Treatments	* Mean percent damage
C	26.28 (30.83)
F1	83.07 (65.67)
F2	97.58 (81.02)
C + F1	32.32 (34.63)
C + F2	64.03 (53.13)
F1 + F2	87.48 (69.25)
C + F1 + F2	95.47 (77.68)

Figures in parantheses indicate transformed values
CD (0.05) - 7. 3
C - Metabolite of *Colletotrichum gloeosporioides*
F1 - Metabolite of *Fusarium equiseti*
F2 - Metabolite of *F. pallidoroseum*
* Average value of three replications

Plate 24 Damage caused by the metabolites of the pathogenic fungi on water hyacinth singly and in combination

H - Healthy leaf, C - C. *gloeosporioides*
F_1 - F. *equiseti*, F_3 - F. *pallidoroseum*

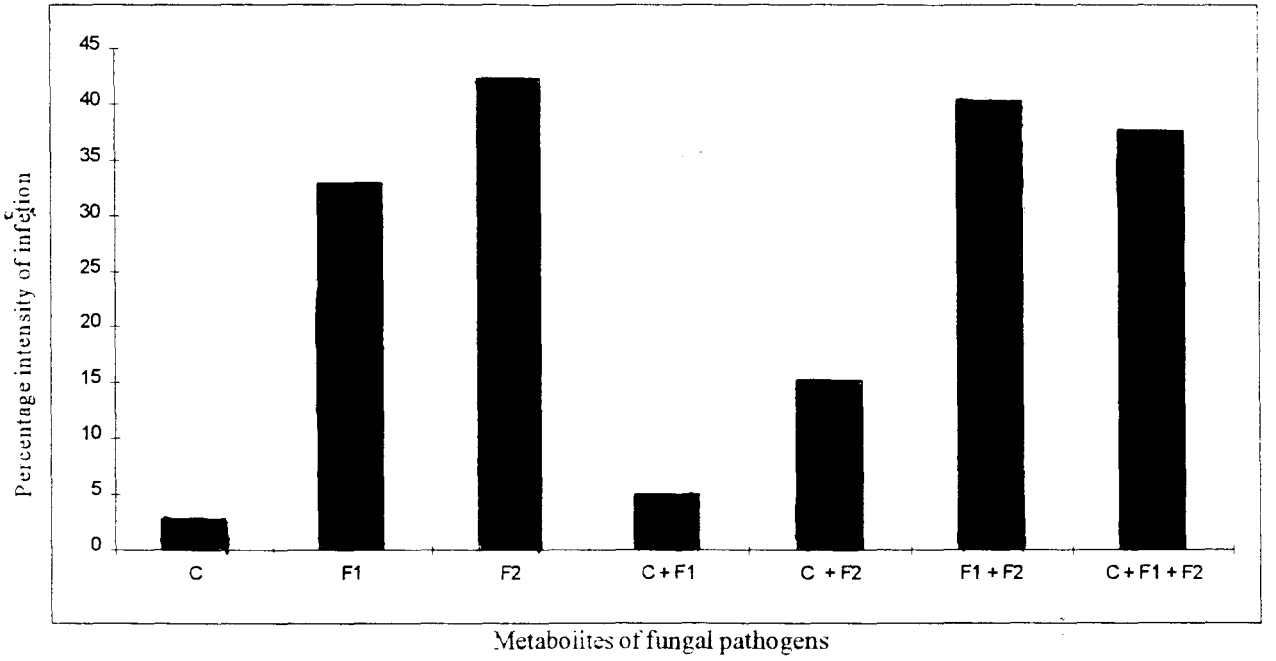

H
F3
F1+F3C
F1
F3C
F1F3
F1C
C

Table 7 Extent of damage caused by metabolite of pathogens singly and in combination on water hyacinth under field condition.

Treatments	* Mean per cent damage	
C	2.89	(9.78)
F1	32.98	(35.03)
F2	42.33	(40.57)
C + F1	4.98	(12.90)
C + F2	15.20	(22.93)
F1 + F2	40.33	(39.41)
C + F1 + F2	37.66	(37.84)

Figures in parantheses indicate transformed values
CD (0.05) - 9.79
C - Colletotrichum gloeosporioides
F1 - Fusarium equiseti
F2 - F. pallidoroseum
* Average of three replications

Fig. 3 Extent of damage caused by metabolites of Pathogen singly and in combination on water hyacinth plants under field conditions.



C - *Colletotrichum gloeosporioides*
F1 - *Fusarium equiseti*
F2 - *F. pallidoroseum*

cent infection under laboratory conditions by the treatments was carried out (Table 6, Plate 24). It was found that maximum damage was caused by F. pallidoroseum alone (97.58 per cent) and the combination of metabolites of three fungi viz. C. gloeosporioides, F. equiseti and F. pallidoroseum (95.47 per cent) followed by F. pallidoroseum and F. equiseti (87.48 per cent) and F. equiseti alone (83.07 per cent). This was followed by F. pallidoroseum and C .gloeosporioides (64.03 per cent), F. equiseti and C. gloeosporioides (32.32 per cent). Least damage was produced by C. gloeosporioides (26.28 per cent).

Statistical analysis of the infection percentage under field condition revealed that metabolites of F. pallidoroseum gave maximum damage of 42.33 per cent followed by combination of F. equiseti and F. pallidoroseum (40.33 per cent). (Table 7, Fig. 3). This was followed by the combination of the metabolite of the three fungi F. equiseti, F. pallidoroseum and C. gloeosporioides , the infection percentage being 37.66 per cent. Least damage was caused by metabolites of F. equiseti and C. gloeosporioides in combination (4.98 per cent) and C. gloeosporioides alone (2.89 per cent).

4.6 Standardisation of carrier materials for the storage of the fungi

An experiment was conducted to find out suitable carrier material for the mass multiplication and storage of

pathogen. The different carrier meterials tested are coir pith, guinea grass straw, rice bran, rice straw, sand-maize medium, vermiculite, water hyacinth leaf and wheat bran. The spore count was taken at 10 days interval starting from 10 days after inoculation for a period of 90 days. Visible mycelial growth of the fungi was observed in rice bran, wheat bran, water hyacinth leaf and rice straw three days after inoculation. In guinea grass straw and coir pith, mycelial growth could be observed five days after inoculation. In sand-maize medium and vermiculite neither mycelial growth nor sporulation of the fungi could be observed through out the period of observation (90 days).

4.6.1 Effect of carrier materials on the sporulation of fungi

4.6.1.1 Colletotrichum gloeosporioides

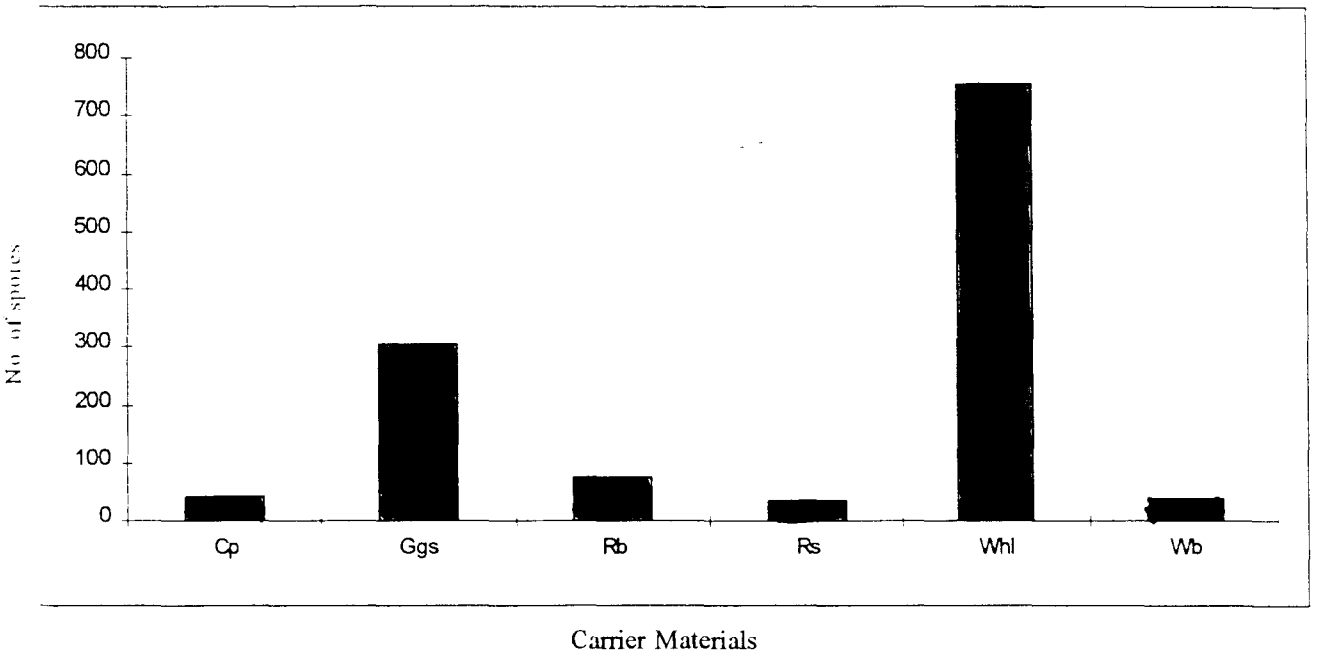
Statistical analysis of the spore count of C. gloeosporioides on different carrier materials revealed that there was significant difference between the effects of carrier materials and periods of storage on sporulation (Table 8, Fig. 4). Maximum spore count was observed on water hyacinth leaf (753.85), followed by guinea grass straw with a spore count of 303.04. The spore count of rice bran was 76.41. Coir pith, wheat bran and rice straw were on par with each other and had a spore count of 39.74, 36.22 and 33.33 respectively. Among

Table 8 Effect of different carrier materials on sporulation of *Colletotrichum gloeosporioides*

Carrier material	* Mean spore count per microscopic field at 10 days intervals									
	1	2	3	4	5	6	7	8	9	Mean
Coir pith	76.33	101.67	50.00	37.00	31.33	26.33	18.33	9.67	7.00	39.74
Guinea grass straw	205.33	263.33	1003.33	596.67	438.33	157.67	51.00	9.33	2.33	303.04
Rice bran	206.33	271.67	88.33	36.00	24.07	19.33	18.33	14.33	8.67	76.41
Rice straw	76.07	101.00	53.33	31.67	22.67	10.33	4.33	0.00	0.00	33.33
Sand-maize medium	-	-	-	-	-	-	-	-	-	-
Vermiculite	-	-	-	-	-	-	-	-	-	-
Water hyacinth leaf	1976.67	2245.00	1146.67	818.33	458.33	101.67	26.67	9.67	1.07	753.85
Wheat bran	74.67	102.00	50.67	31.33	24.33	18.33	10.33	5.33	1.00	36.22
Mean	436.00	514.11	400.06	285.50	166.61	55.61	21.50	8.06	3.44	

CD (0.05) for carrier material - 8.6
CD (0.05) for period of storage - 15.35
CD (0.05) for interaction - 37.61
* Average of three replications.

Fig. 4 Effect of different carrier materialson sporulation of *C. gloeosporioides*



Cp - Coir pith
Ggs - Guinea grass straw
Rb - Rice bran
Rs - Rice straw
Whl - Water hyacinth leaf
Wb - Wheat bran

the different periods of storage, maximum spore count was observed on twentieth day (514.11). Then the spore count decreased gradually to 3.44 on ninetieth day.

In the case of coir pith, the initial spore count was 76.33 which increased to 101.67 on twentieth day. On thirtieth day the spore count was only 50.00 and there after reduction was only gradual and reached 7.00 on ninetieth day.

The initial spore count on guinea grass straw was 205.33 and on twentieth day it was 263.33. The maximum spore count of 1003.33 was observed on thirtieth day. The spore count decreased drastically and finally reached 2.33 on ninetieth day. The fungus also produced large number of fruiting body i.e. acervuli on the carrier material from twenty fifth day after inoculation.

The average spore count on rice bran was only 76.41 and the initial spore count was 206.33. The maximum spore count was observed on twentieth day (271.67). After this the spore count decreased and reached 8.67 on ninetieth day.

Rice straw yeilded an average spore count of 33.33. The initial spore count was 76.67 and maximum spore count was observed on twentieth day (101.00) and it reached zero on eightieth day and remained like that on ninetieth day also.

Water hyacinth leaf had average spore count of 753.85 of 753.85. Here the initial spore count was 1976.67 and reached 2245 on twentieth day. From twentieth day onwards a decreasing trend in spore count was observed. The fungus produced acervuli on the water hyacinth leaf powder fifteen days after inoculation.

When wheat bran was used as carrier material the average spore count was 36.22. The initial spore count of 74.67 reached 1.07 by the ninetieth day. The maximum spore count of 102.00 was observed on twentieth day.

Statistical analysis of the spore counts on different carrier materials at different periods of storage revealed that maximum spore count was observed on water hyacinth leaf on twentieth day (2245.00) followed by the same carrier material on tenth day (1976.67) and thirtieth day (1146.67). The spore count on guinea grass straw on thirtieth day of storage was 1003.33. Spore count was zero on rice straw from eightieth day of observation.

4.6.1.2 Fusarium equiseti

On statistical analysis of the spore count of F. equiseti on various carrier materials revealed that there was significant difference among the effects of carrier materials and the periods of storage on sporulation (Table 9, Fig. 5).

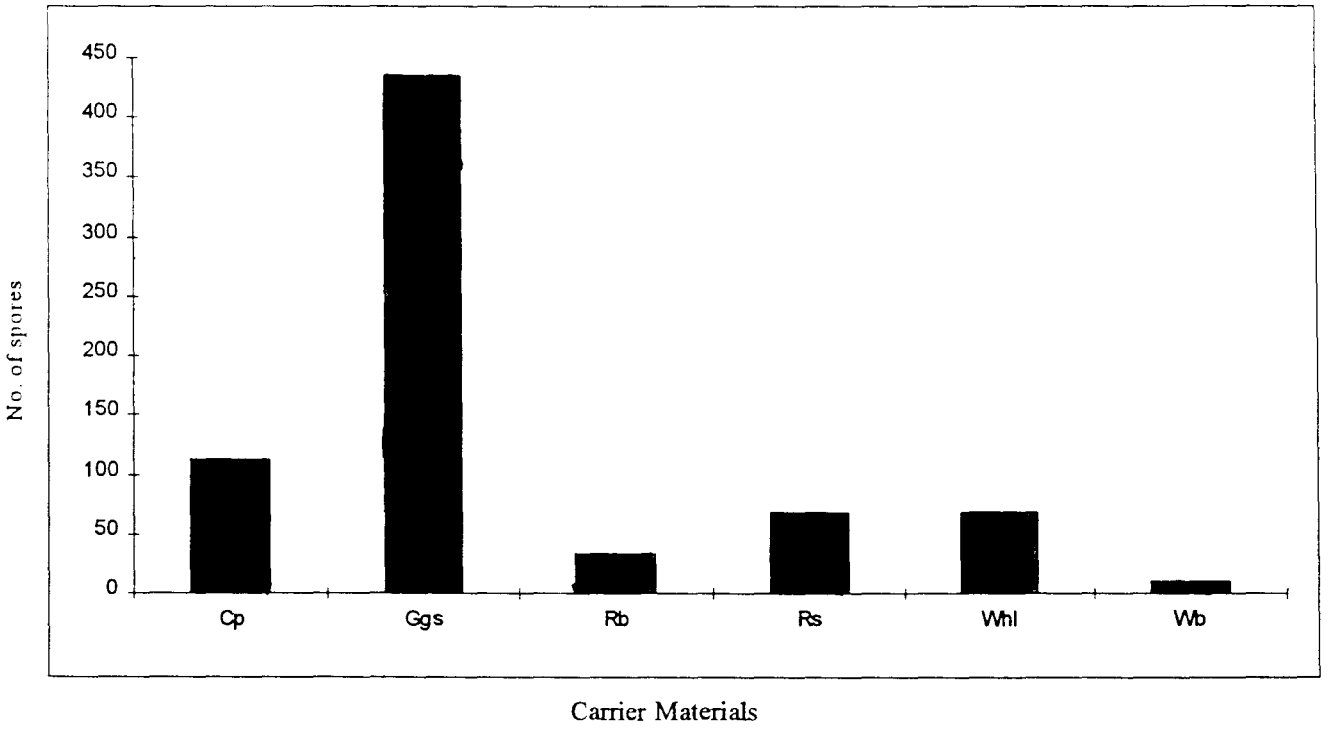
Table 9 Effect of different carrier materials on sporulation of *Fusarium equiseti*

Carrier material	* Mean spore count per microscopic field at 10 days intervals									
	1	2	3	4	5	6	7	8	9	Mean
Coir pith	80.33	84.00	244.00	272.33	193.33	67.00	29.00	26.00	9.67	111.74
Guinea grass straw	82.33	1270.00	1098.33	1059.33	371.67	28.67	3.33	0.67	0.00	44.93
Rice bran	9.33	14.00	89.33	105.67	31.00	20.00	13.67	13.00	4.33	33.37
Rice straw	37.67	57.33	194.00	221.67	75.33	17.00	1.00	0.00	0.00	67.11
Sand-maize medium	-	-	-	-	-	-	-	-	-	-
Vermiculite	-	-	-	-	-	-	-	-	-	-
Water hyacinth leaf	76.33	81.00	217.33	208.33	221.00	10.33	2.00	0.00	0.00	68.48
Wheat bran	18.33	20.00	14.67	14.67	10.33	6.67	1.67	0.00	0.00	9.59
Mean	50.72	254.39	309.61	313.67	117.11	24.94	8.44	6.61	2.83	

CD (0.05) for carrier material - 18.12
CD (0.05) for period of storage - 10.49
CD (0.05) for interaction - 25.7

* Average of three replications.

Fig. 5 Effect of different carrier material on sporulation of *F. equiseti*



Cp - Coir pith
Ggs - Guinea grass straw
Rb - Rice bran
Rs - Rice straw
Whl - Water hyacinth leaf
Wb - Wheat bran

Among the different carrier materials tested, guinea grass straw gave maximum spore count of 434.93 followed by coir pith with 111.74. The average spore count in water hyacinth leaf, rice straw and rice bran being 68.48, 67.11 and 33.37 respectively. The spore count was the least in wheat bran (9.59).

In coirpith an initial spore count of 80.33 was observed after 10 days and it increased to 272.33 after 40 days. From 40th day onwards decreasing trend was observed and reached 9.67 by ninety days of storage with an average spore count of 111.74.

In guinea grass straw, the initial spore count was 82.33 and it suddenly increased to 1270 on twentieth day of observation and gradually declined to zero on ninetieth day of storage with an average spore count of 434.93.

The initial spore count of rice bran was only 9.33 and maximum spore count of 105.67 was reached on forty days of storage. On ninetieth day of storage spore count was 4.33 and the average spore count was 33.37.

In rice straw the initial spore count was 37.67 and increased gradually and reached a maximum of 221.67 on fourtieth day and decreased drastically and reached zero on eightieth day and remained as zero on ninetieth day also.

The initial spore count was 76.33 in water hyacinth leaf and reached a maximum of 217.33 on thirtieth day and afterwards it declined and reached zero on eightieth day.

Wheat bran yielded an average spore count of 9.59 which was the lowest, among the carrier materials tested. Initial spore count was 18.33 and on the twentieth day it increased to 20.00 and afterwards decreased and became zero in eightieth day. The spore count remained zero in ninetieth day also.

On statistical analysis of the spore count on different carrier materials at 10 days interval it was observed that the spore count was maximum in guinea grass straw on thirtieth day (1270.00) followed by the same carrier material on fourtieth day (1059.33). Fifty days of storage, guinea grass straw yielded a spore count of 371.67. Coir pith on fourtieth day produced 272.33 spores per microscopic field. The spore count reached zero in guinea grass on ninetieth day and in rice bran, water hyacinth leaf and wheat bran on eightieth day.

4.6.1.3 *Fusarium pallidoroseum*

Statistical analysis of spore count of *F. pallidoroseum* revealed that there was signifiant difference between the effects of carrier materials tested and the time of storage on sporulation (Table 10, Fig. 6). The spore count

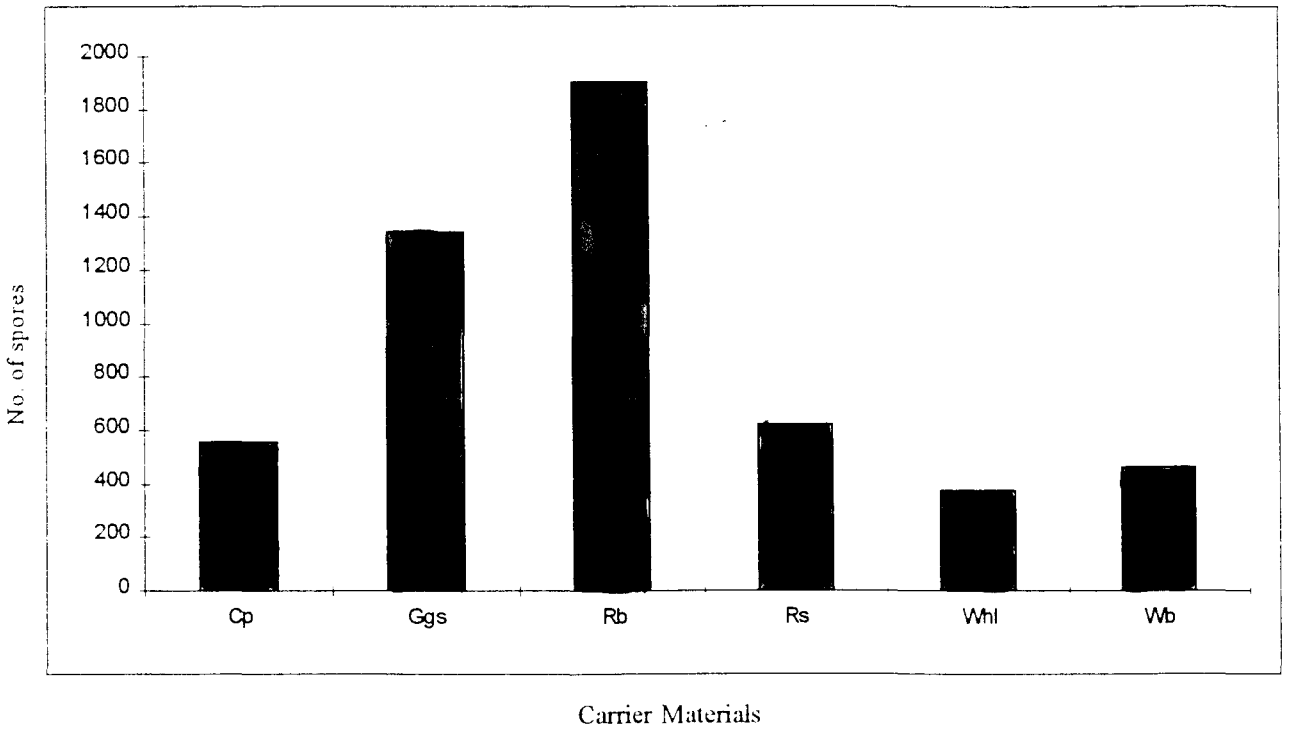
Table 10 Effect of different carrier materials on sporulation of *Fusariuam pallidoroseum*

Carrier material	* Mean spore count per microscopic field at 10 days interval									
	1	2	3	4	5	6	7	8	9	Mean
Coir pith	491.67	612.00	661.67	658.67	597.33	541.67	502.33	499.33	453.67	557.59
Guinea grass straw	1490.00	1700.00	1803.33	1738.33	1751.67	1526.67	1030.00	511.07	511.67	1340.37
Rice bran	2493.33	2750.00	2075.00	1876.67	1746.67	1591.67	1590.00	1526.67	1500.00	1905.55
Rice straw	583.33	751.67	751.67	681.67	598.33	596.67	558.33	535.67	501.67	617.67
Sand-maize medium	-	-	-	-	-	-	-	-	-	-
Vermiculite	-	-	-	-	-	-	-	-	-	-
Water hyacinth leaf	514.33	576.00	653.33	448.33	406.00	304.00	254.00	156.00	96.67	378.74
Wheat bran	511.67	691.67	712.67	465.00	404.33	401.67	357.67	346.67	285.00	464.04
Mean	1014.06	1180.22	1109.61	978.11	917.39	827.06	715.39	596.00	558.11	

CD (0.05) for carrier material - 22.65
CD (0.05) for period of storage - 23.13
CD (0.05) for interaction - 56.66

* Average of three replications.

Fig. 6 Effect of different carrier material on sporulation of *F. pallidoroseum*



Cp - Coir pith
Ggs - Guinea grass straw
Rb - Rice bran
Rs - Rice straw
Whl - Water hyacinth leaf
Wb - Wheat bran

was maximum for rice bran (1905.55) followed by guinea grass straw (1340.37). The least count was observed in water hyacinth leaf with an average of 464.04. During different periods of storage spore count increased till twentieth day (1180.22), then decreased gradually and reached 558.11 on ninetieth day.

In coir pith, the initial spore count was 491.67 and it reached 661.67 on thirtieth day. After that the spore count decreased and reached 453.67 on ninetieth day.

In the case of guinea grass straw the initial spore count was 1490.00 which increased to 1803.33 on thirtieth day and then declined. In guinea grass straw the count was comparatively higher through out the period of storage.

The average spore count in rice bran was 1905.55 and was the highest among the carrier materials tested. The initial count was 2493.33 and reached 2750.00 on twentieth day. Then it gradually decreased to 1500.00 on ninetieth day of storage.

Rice straw yeilded an average spore count of 617.67 with a maximum count of 751.67 on twentieth day and it remained static for ten days and then it declined and reached 501.67 on ninetieth day.

The average spore count in water hyacinth leaf was 378.74 which was the least among the carrier materials tested.

In water hyacinth leaf the spore count was less throughout the storage period.

In the case of wheat bran initial spore count was 511.67 and maximum count of 712.67 was reached on thirtieth day and count on ninetieth day of storage was 285.00.

Statistical analysis of the data showed that maximum spore count was in rice bran on twentieth day (2750.00), tenth day (2493.33), thirtieth day (2075.00) and on fourtieth day (1876.67). The least spore count was observed on water hyacinth leaf (254.00) on seventieth day, (156.00) on eightieth day and (96.67) on ninetieth day.

4.6.2 Effect of carrier materials on the spore viability of the fungi

Effect of carrier materials on the vaiability of spores of fungi was tested. Number of spores germinated per microscopic field was counted and the percentage viability was calculated.

4.6.2.1 *Colletotrichum gloeosporioides*

Statistical analysis of the data showed that there was significant difference between the carrier materials used (Table 11, Fig. 7). The percentage of spore viability was

Table 11 Effect of different carrier materials on the spore viability of *Colletotrichum gloeosporioides*

Carrier material	* Mean percent viable spores at 10 days interval									
	1	2	3	4	5	6	7	8	9	Mean
Coir pith	98.00 (85.26)	97.66 (82.54)	83.66 (66.25)	71.00 (57.57)	36.00 (36.80)	30.66 (33.60)	20.66 (26.96)	10.66 (18.94)	9.60 (18.07)	50.99 (47.33)
Guinea grass straw	82.33 (65.14)	55.66 (47.67)	46.00 (42.69)	41.33 (39.99)	11.66 (19.94)	6.66 (14.89)	4.33 (11.93)	0 (0.00)	0 (0.00)	29.85 (29.91)
Rice bran	95.00 (79.53)	86.33 (68.30)	82.00 (64.89)	77.00 (61.34)	39.66 (39.01)	26.00 (30.64)	21.33 (27.49)	11.66 (19.82)	8.00 (16.34)	49.66 (45.26)
Rice straw	99.00 (86.66)	97.33 (82.29)	89.33 (71.12)	50.00 (44.98)	30.66 (33.60)	20.33 (26.78)	7.66 (15.92)	1.33 (5.24)	0 (0.00)	43.96 (40.73)
Water hyacinth leaf	81.00 (64.30)	72.66 (58.47)	50.66 (45.36)	24.66 (29.63)	19.66 (26.30)	14.33 (22.23)	10.00 (18.37)	3.33 (10.34)	0 (0.00)	30.70 (30.56)
Wheat bran	97.00 (81.83)	86.33 (68.30)	81.33 (64.38)	77.00 (61.34)	40.33 (39.41)	24.66 (29.76)	14.33 (22.10)	5.00 (12.74)	0.66 (2.71)	47.40 (42.51)
Mean	92.06 (77.12)	82.83 (67.93)	72.16 (59.11)	56.83 (49.14)	31.16 (33.65)	21.27 (27.16)	13.44 (20.96)	6.05 (13.17)	3.04 (6.19)	

Figures in parantheses indicate transformed values.

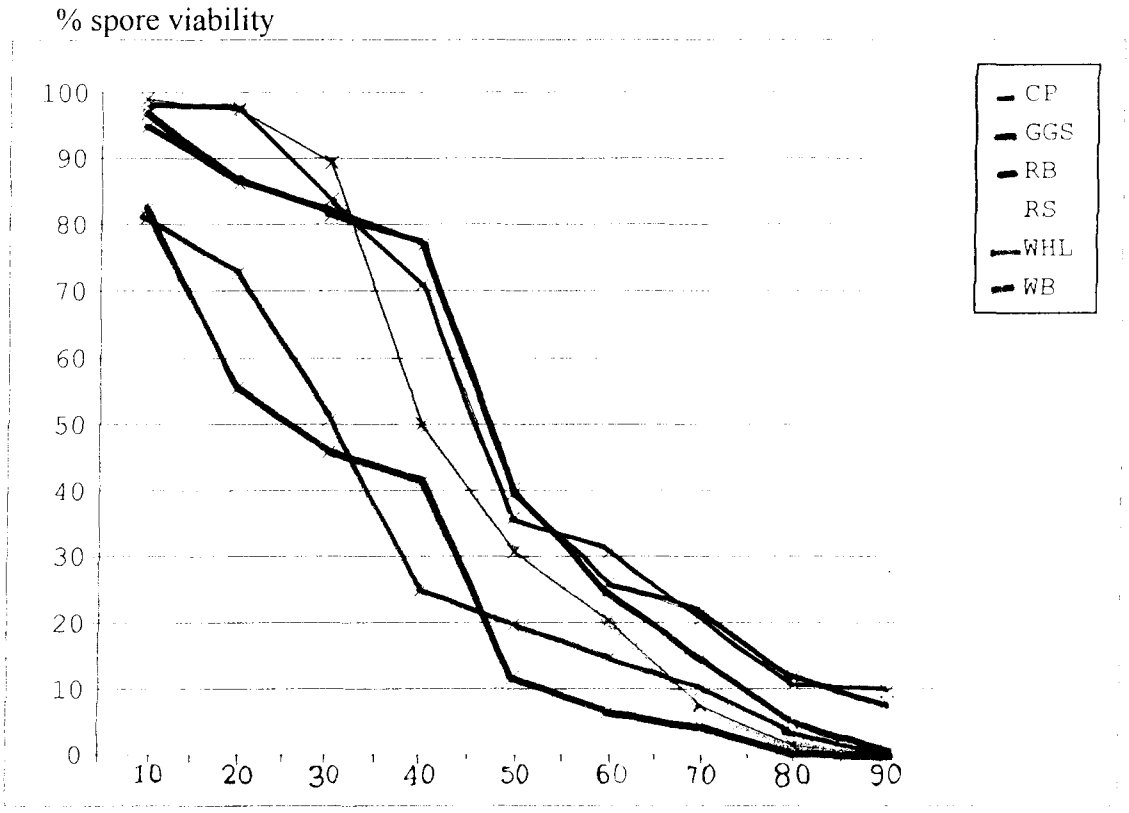
* Average of three replication

CD (0.05) for carrier material - 2.39

CD (0.05) for period of storage - 2.24

CD (0.05) for interaction - 5.50

Fig-7 Effect of different carrier materials on the spore viability of *C. gloeosporioides*



CP- Coir Pith
GGS- Guinea Grass Straw
RB- Rice Bran
RS- Rice Straw
WHL- Water Hyacinth Leaf
WB- Wheat Bran

maximum for coir pith (50.99) and rice bran (49.60) and were statistically on par. This was followed by wheat bran (47.40) and rice straw (43.96). Spore viability was minimum in water hyacinth leaf (30.7) and guinea grass straw (29.85). It was observed that in general the spore germination was maximum on the tenth day (92.00 per cent) and declined gradually and reached 3.04 per cent on the ninetieth day.

When coir pith was used as the carrier material, the average spore germination was 50.99 which was the highest among the carrier materials tested. The average germination percentage of spores in guinea grass straw was 29.85. This was the lowest among the carrier materials tested. Here initial per cent germination was 82.33 and reached zero after eighty days of storage.

In the case of rice bran the spore viability was comparatively high. The percentage germination on the tenth day of observation was 95.00 and decreased to 8.00 on the ninetieth day with an average germination percentage of 49.66. In rice straw the initial germination percentage of spore was 99.00 and it decreased to zero after ninety days of storage.

The average germination percentage of spores of C. gloeosporioides on water hyacinth leaf was 30.70. The per cent germination of spores was 81.0 on the tenth day and decreased to

zero on ninetieth day of storage. When wheat bran was used as carrier material the per cent germination was 97.00 on the tenth day of storage this was decreased to 0.66 on the ninetieth day of storage. The average germination per cent of the spore being 47.40.

On statistical analysis of the data, it was observed that the per cent spore viability was maximum on rice straw and coir pith on the tenth day (99.00 and 98.00 respectively) and on twentieth day (97.66) on rice straw on twentieth day (97.33) on wheat bran on tenth day (97.0) and on rice bran on tenth day (95.0) of observation and were statistically on par. Germination percentage of spores was zero for rice straw, guinea grass straw and water hyacinth leaf on the ninetieth day of observation.

4.6.2.2 *Fusarium equiseti*

Statistical analysis of the per cent germination of spores in different carrier materials showed significant differnce between the treatments (Table 12, Fig. 8). The spore germination percentages were higher in rice bran (59.11) and coir pith (54.27) and were statistically on par. The per cent germination was the least in water hyacinth leaf (22.48). It was also found that with increasing storage time, the viability of spores decreased. The germination percentage was 88.44 on the tenth day of storage and it declined to 9.33 on ninetieth day of storage.

Table 12 Effect of different carrier materials on the spore viability of *Fusarium equiseti*

Carrier material	* Mean percent viable spores at 10 days interval									
	1	2	3	4	5	6	7	8	9	Mean
Coir pith	98.33 (85.68)	84.33 (66.76)	76.66 (61.12)	71.00 (59.58)	61.66 (51.73)	42.00 (40.37)	25.83 (30.19)	19.33 (26.03)	9.33 (17.62)	54.27 (48.79)
Guinea grass straw	74.33 (59.54)	71.00 (57.42)	70.33 (56.99)	64.33 (53.31)	58.38 (49.98)	37.00 (38.42)	10.60 (19.04)	0 (0.00)	0 (0.00)	42.92 (37.19)
Rice bran	91.00 (72.76)	86.00 (68.03)	84.00 (66.40)	76.00 (60.65)	63.00 (52.53)	51.66 (45.94)	47.33 (39.99)	23.66 (29.06)	9.33 (17.76)	59.11 (50.35)
Rice straw	99.33 (87.28)	97.66 (82.90)	81.00 (64.20)	50.00 (44.98)	29.33 (32.74)	11.00 (19.33)	0 (0.00)	0 (0.00)	0 (0.00)	40.92 (36.83)
Water hyacinth leaf	76.66 (61.12)	55.66 (48.25)	28.00 (31.83)	25.00 (29.99)	20.66 (27.01)	5.33 (13.10)	0 (0.00)	0 (0.00)	0 (0.00)	22.48 (23.48)
Wheat bran	91.00 (72.76)	65.33 (53.95)	55.33 (48.05)	52.66 (46.51)	51.33 (45.75)	25.33 (30.15)	9.00 (17.20)	0 (0.00)	0 (0.00)	38.89 (34.93)
Mean	88.44 (73.19)	76.66 (62.90)	65.89 (54.77)	56.50 (49.17)	47.44 (43.29)	28.72 (31.22)	15.46 (17.74)	7.17 (9.18)	9.33 (5.90)	

Figures in parantheses indicate transformed values.

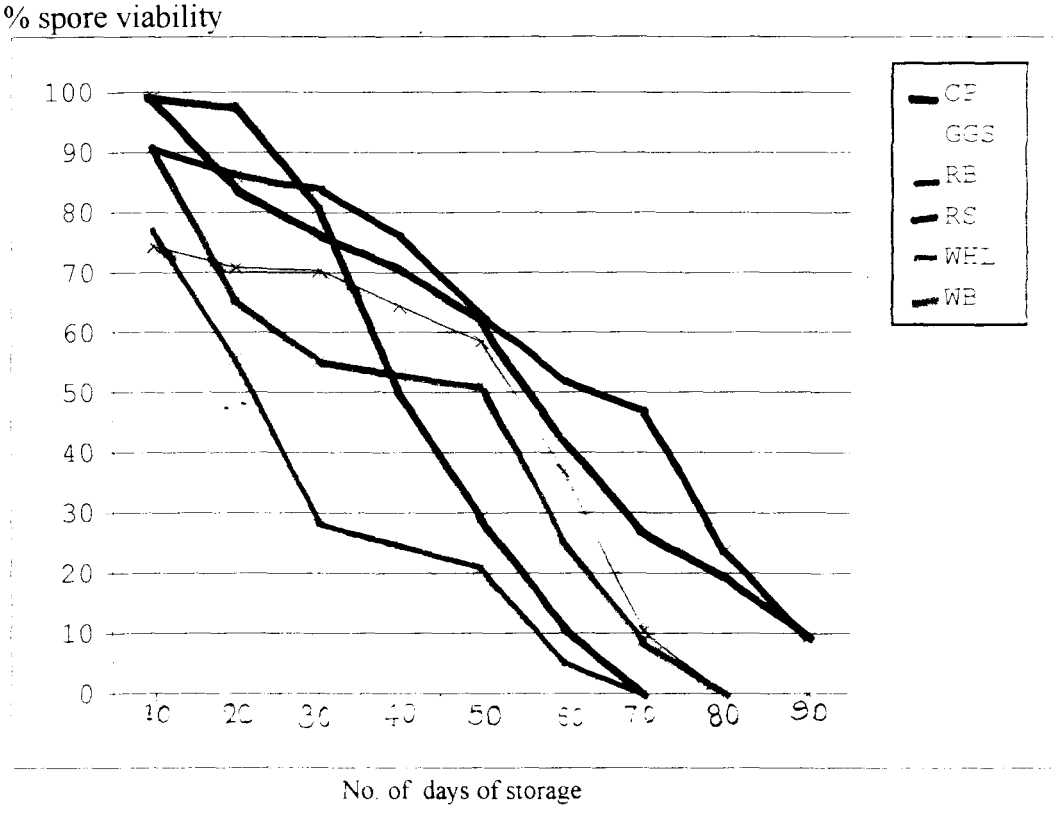
* Average of three replications

CD (0.05) for carrier material - 2.54

CD (0.05) for period of storage - 1.63

CD (0.05) for interaction - 3.99

Fig-8 Effect of different carrier materials on the spore viability of *F. equiseti*



CP- Coir Pith
GGS- Guinea Grass Straw
RB- Rice Bran
RS- Rice Straw
WHL- Water Hyacinth Leaf
WB- Wheat Bran

In coir pith, the initial germination percentage on the tenth day of storage was 98.33 and it decreased to 9.33 on ninetieth day. When guinea grass straw was used as the carrier material, the initial per cent germination was 74.33 and it decreased to zero on eightieth day leaf.

In rice bran decrease in the spore viability was gradual. The average spore germination percentage was 59.11 which was the highest among the carrier materials tested. It was also observed that the germination percentages of F. equiseti spores in rice bran were 91.00 and 9.33 respectively during the tenth and ninetieth day of storage. The initial percentage of germination was the highest for rice straw ie., 99.33, but spore viability reduced abruptly and became zero on seventieth day of storage. In water hyacinth leaf, the average germination percentage of spores of F. equiseti was the least (22.48) among the carrier materials tested. During tenth day the per cent germination of spores of F. equiseti on water hyacinth leaf was 76.66 and reached zero on seventieth day of storage.

Statistical analysis of the per cent germination of spores on different carrier materials at different periods of storage showed that rice straw and coir pith yielded maximum on tenth day of storage . Germination percentage became zero on seventieth day in rice straw and eightieth day in guinea grass straw and wheat bran.

4.6.2.3 *Fusarium pallidoroseum*

On statistical analysis of the germination percentage of spores of *F. pallidoroseum*, it was observed that there was significant difference between the treatments (Table 13, Fig. 9). Rice bran and water hyacinth leaf yielded the least germination percentage of spores (32.25 and 30.07 respectively) which were statistically on par.

Of the various carrier materials tried, coir pith yielded maximum average per cent germination of 54.02. The initial per cent spore germination was 98.66 and it decreased to 14.30 on ninetieth day of storage.

In guinea grass straw the percentage of germination was 70.66 on tenth day of storage and then it decreased gradually and reached 3.33 per cent on ninetieth day with an average per cent germination of 33.99.

Rice bran yielded an average per cent germination of 32.25. On tenth day, the per cent germination was 85.66 and on ninetieth day it was 4.60. In the case of rice straw, the average percentage of germination was 51.77. Initial germination percentage was 99.33, which was the maximum among the carrier materials tested.

The average percentage of germination of spores of *F. pallidoroseum* on water hyacinth leaf was 30.07. The initial

Table 13 Effect of different carrier material on the spore viability of *Fusarium pallidoroseum*

Carrier material	* Mean percent viable spores at 10 days interval									
	1	2	3	4	5	6	7	8	9	Mean
Coir pith	98.66 (86.14)	97.60 (82.96)	89.00 (70.75)	50.66 (45.17)	42.66 (40.70)	40.33 (39.40)	29.33 (32.74)	23.66 (29.06)	14.30 (22.10)	54.02 (49.90)
Guinea grass straw	70.66 (57.19)	54.66 (47.67)	46.33 (42.88)	40.33 (39.41)	34.66 (36.04)	25.66 (30.41)	19.66 (26.28)	10.66 (19.04)	3.33 (10.34)	33.99 (34.36)
Rice bran	85.66 (67.80)	53.66 (47.09)	43.66 (41.34)	37.66 (37.84)	23.33 (28.82)	17.33 (24.54)	14.66 (22.49)	9.66 (18.07)	4.60 (12.35)	32.25 (33.37)
Rice straw	99.33 (87.28)	98.00 (83.42)	66.00 (54.38)	58.66 (49.98)	43.66 (41.33)	36.66 (37.25)	32.66 (34.83)	20.66 (26.99)	10.33 (18.60)	51.77 (48.24)
Water hyacinth leaf	80.00 (63.52)	60.33 (50.95)	44.00 (41.52)	24.33 (29.49)	20.00 (26.55)	16.33 (23.81)	12.66 (20.78)	9.33 (17.76)	3.66 (10.76)	30.07 (31.68)
Wheat bran	83.66 (66.30)	60.66 (51.14)	51.66 (45.94)	39.00 (38.63)	31.33 (34.00)	27.33 (31.49)	23.33 (28.86)	21.33 (27.49)	18.00 (25.07)	39.59 (38.77)
Mean	86.33 (71.37)	70.82 (60.54)	56.78 (49.47)	41.77 (40.00)	32.61 (34.59)	27.27 (31.15)	14.70 (27.67)	15.88 (23.07)	9.04 (16.55)	

Figures in parantheses indicate transformed values

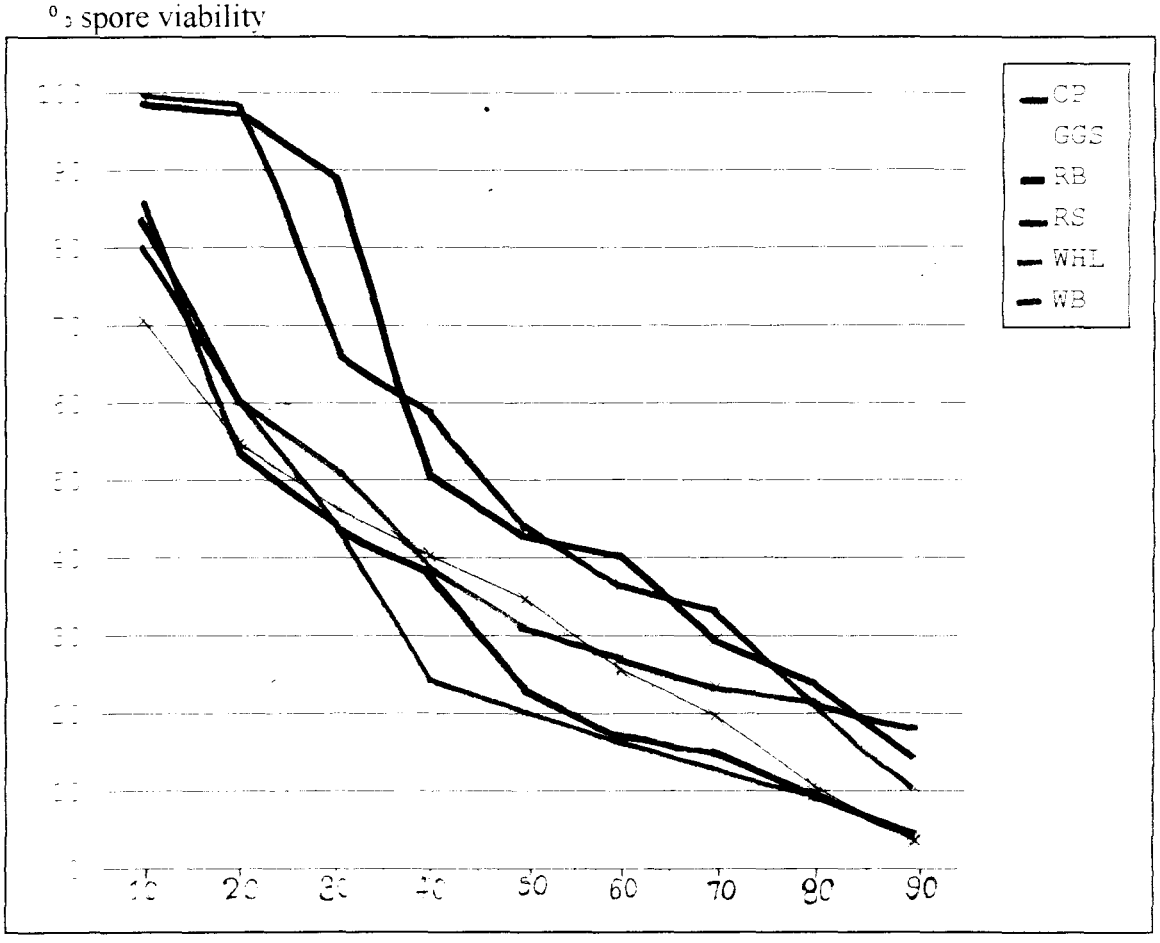
* Average of three replications

CD (0.05) for carrier material - 1.25

CD (0.05) for period of storage - 1.82

CD (0.05) for interaction - 4.45

Fig-9 Effect of different carrier materials on the spore viability of *F. pallidoroseum*



CP- Coir Pith
GGS- Guinea Grass Straw
RB- Rice Bran
RS- Rice Straw
WHL- Water Hyacinth Leaf
WB- Wheat Bran

germination percentage was 80.00 and reduced to 3.66 in ninety days of storage. When wheat bran was used as carrier material, the per cent germination was 83.66 on tenth day and it decreased to 18.00 on ninetieth day of observation.

Statistical analysis of the data revealed that the germination percentage of spores of F. pallidoroseum in rice straw on tenth day (99.33) and twentieth day (98.00), coir pith on tenth (98.66) and twentieth day (97.60) were statistically on par and percentage germination was the least in ninety days of storage in rice bran (4.60) water hyacinth leaf (3.66) and guinea grass straw (3.33).

4.6.3 Storage life of metabolites produced by the pathogenic fungi

For testing the storage life of metabolites, healthy water hyacinth plants were applied with filtrates of the pathogenic fungi viz., F. equiseti, F. pallidoroseum, and C. gloeosporioides stored for different periods of time. Statistical analysis of the per cent damage intensity showed that there was significant difference between the treatments in each of the fungi tested (Table 14).

4.6.3.1 Colletotrichum gloeosporioides

Analysis of the percentage of damage caused by the metabolite of C. gloeosporioides stored for different periods of

Table 14 Extent of damage caused by metabolites of pathogenic fungi stored for different periods of time

Treatments		* Mean per cent intensity of damage					
		Metabolites of					
		C. gloeosporioides		F. equiseti		F. pallidoroseum	
Metabolite on the day of preparation		41.73	(39.20)	55.00	(47.94)	72.33	(60.16)
metabolite stored	One day	40.73	(39.80)	74.86	(64.48)	48.46	(44.23)
	Three days	16.99	(23.46)	83.80	(71.72)	44.00	(41.49)
	Five days	10.93	(18.62)	88.66	(75.60)	42.33	(40.51)
	Seven days	9.20	(17.20)	92.20	(79.47)	38.06	(37.99)
	under refrigerated condition for one weak	10.33	(17.71)	56.37	(48.82)	34.93	(36.12)
CD (0.05)		1.58		2.00		1.48	

Figures in parantheses indicate transformed values
*Average of three replications

time revealed that metabolite on the day of preparation and one day after preparation gave maximum per cent damage of 41.73 and 40.73 respectively and were statistically on par. The least per cent damage was produced by the metabolite stored for five days, seven days and stored at 5°C,(10.93, 9.20 and 10.33)

4.6.3.2 Fusarium equiseti

Statistical analysis of the percentage of damage caused by metabolite of F. equiseti at different intervals of storage revealed that the treatments differ significantly. The per cent damage was maximum for the metabolite stored for seven days i.e. 92.20. This was followed by the metabolite stored for five days (88.66) The symptom production was the least for metabolite stored at 5°C ie., in refrigerator (56.37) and for metabolite applied on the day of preparation (55.00) and both were statistically on par.

4.6.3.2 Fusarium pallidoroseum

Significant difference was observed among the treatments and the maximum damage was observed in plants which were applied with metabolite on the day of preparation itself (72.33 per cent). On storage of the metabolite, the intensity of damage was reduced to 48.46, 44.00, 42.33, 38.06 and 34.93 per cent on one day, three days, five days, seven days after

preparation and stored under refrigerated condition respectively.

4.6.4 Extent of damage caused by fungi grown on different carrier materials

Healthy plants were inoculated with fungi, viz., F. equiseti, F. pallidoroseum and C. gloeosporioides grown in different carrier materials like coir pith, guinea grass straw, rice bran, rice straw, water hyacinth leaf and wheat bran (Table 15).

On statistical analysis of the percentage intensity of infection produced by C. gloeosporioides in different carrier materials, it was found that there was significant difference among the carrier materials tested. Guinea grass straw and rice straw gave maximum damage of 29.30 per cent and 28.95 per cent respectively. This was followed by rice bran (23.61) water hyacinth leaf (18.60) wheat bran (16.34) and coir pith (15.98) which were statistically on par.

In the case of F. equiseti grown in six carrier material tested, maximum infection was produced by the fungus on guinea grass straw (94.63), coir pith (90.69) and water hyacinth leaf (89.22) which were statistically on par. The least damage was caused by fungus on rice bran (32.65 per cent) and rice straw (27.97 per cent).

Table 15 Extent of damage caused by the pathogenic fungi grown on different carrier materials

Carrier materials	* Mean per cent infection		
	C.gloeosporioides	F. equiseti	F.pallidoroseum
Coir pith	15.98 (23.55)	90.69 (72.20)	46.31 (42.87)
Guinea grass straw	29.30 (32.76)	94.63 (76.57)	72.03 (58.05)
Rice bran	23.61 (29.06)	32.65 (34.83)	81.20 (64.28)
Rice straw	28.95 (32.54)	27.97 (31.91)	48.33 (44.03)
Water hyacinth leaf	18.60 (25.54)	89.22 (70.81)	43.95 (41.51)
Wheat bran	16.33 (23.83)	75.75 (60.47)	59.10 (50.22)
CD (0.05)	6.19	9.50	6.92

Figures in parantheses indicate transformed values
* Average of three replications

Statistical analysis of the intensity of infection produced by F. pallidoroseum, revealed significant difference among treatments. Out of the six carrier materials used, the fungus produced maximum infection when grown on rice bran (89.20) and guinea grass straw (72.03). The percentage intensity of infection produced by the inoculum on wheat bran, rice straw, coir pith and water hyacinth leaf were 59.10, 48.33, 46.31 and 43.95 respectively.

4.7 Standardisation of method of application of pathogens for biocontrol of water hyacinth

An experiment was conducted to standardize the methods of application of pathogens. The different methods tested were (1) placement of bits of inoculum (2) dusting the inoculum along with carrier material @ 5 g/plant (3) spraying spore suspension in water (4) dusting the spores in diatomaceous earth and (5) spraying the spore suspension in diatomaceous earth. The results are presented in Table 16.

All the three fungi tested showed same trend towards the treatments. Maximum intensity was observed in the treatments, placement of bits of inoculum and dusting inoculum along with carrier material. The percentage intensity of infection were 42.99, 94.28 and 92.89 for C. gloeosporioides, F. equiseti and F. pallidoroseum when bits of inoculum were placed on leaves and 42.00, 87.74 and 85.24 per cent infection for three fungi

Table 16 Extent of damage caused by pathogenic fungi by different methods of application

Treatments	* Mean per cent infection					
	C. gloeosporioides		F. equiseti		F. pallidoroseum	
Placement of bits of inoculum	42.99	(40.95)	94.28	(76.13)	92.89	(74.50)
Dusting the inoculum along with carrier material	42.00	(40.38)	87.74	(69.48)	85.24	(67.38)
Spraying of spore suspension	27.76	(31.78)	75.11	(60.05)	77.44	(61.22)
Dusting the spores in diatomaceous earth	20.93	(27.21)	45.99	(42.68)	43.33	(41.15)
Spraying the spore suspension along diatomaceous earth	30.31	(33.39)	70.80	(57.27)	66.91	(54.86)
CD (0.05)	5.10		9.26		7.32	

Figures in parantheses indicate transformed values
* Average of three replications

respectively when inoculum was dusted along with carrier materials. The least infection of 20.93, 45.99 and 43.33 per cent were observed when spores were dusted along with diatomaceous earth.

4.8 Characterization of the toxin

For characterizing the toxin from Fusarium spp. the toxins from the filtrates were concentrated and paper chromatogram was developed in butanol : formic acid : water solvent system. The paper was dried for 14 - 16 h under a hood and sprayed with bromophenol blue. This produced an yellow colour to the area and was the indication of the presence of fusaric acid.

DISCUSSION

5. DISCUSSION

Detailed host range studies were conducted with thirty cultivated plants and forty one common weed plants to investigate the host range of fungi pathogenic to water hyacinth, viz., C. gleoesporioides, F. equiseti and F. pallidoroseum. It was observed that among the thirty cultivated plants and forty one weed plants tested C. gloeosporioides was found to be pathogenic to amaranthus, bhindi, chilli, Euphorbia hirta, Hydrocotyl asiatica and Phyllanthus niruri. The pathogenicity of C. gloeosporioides to chilli, Commelina benghalensis, Hydrocotyl asiatica and Ludwigia parviflora have been observed earlier by Santhi Kamath(1994).

The present host range study revealed that F. equiseti produced symptoms on Amaranthus tricolor , A. viridis, Commelina benghalensis, C. jacobi and Monochoria vaginalis. Rahim and Tawfig(1984) reported that the host range of F. equiseti pathogenic to water hyacinth included the following crops, viz., Allium cepa, Beta vulgaris, Chenopodium amaranticolour, Hordeum vulgare, Cyperus rotundus, Hibiscus esculentus and Zea mays. In 1994 Santhi Kamath also conducted host range studies of F. equiseti in twelve plants and found that the fungus can infect Monochoria vaginalis.

In the present study, F. pallidoroseum was found to produce symptoms on napier grass, Axonopsis sp. Boerhaavia diffusa, Calatropis gigantea, Cassia occidentalis, Commelina benghalensis, C. jacobi, Echinochloa colonum, Euphorbia hirta, Justicia diffusa, J. prostrata, Monochoria vaginalis, Oldenlandia umbellata, Phyllanthus niruri and Scoparia dulcis. Nagalingam (1983) found that F. semitectum was safe to plants like chillies, cabbage,brinjal and tobacco which is in agreement with the present study. Santhi Kamath (1994) also observed that F. pallidoroseum could infect Monochoria vaginalis.

In the experiment conducted to standardise the dosage of inoculum required for effective destruction of the weed revealed that C .gloeosporioides, Fusarium equiseti and F. pallidoroseum caused maximum infection of 89.7, 98.01 and 74.3 percentage respectively, when $1x10^{11}$ spores per ml was used. This concentration was more effective than $1x10^{9}$ and $1x10^{10}$ spores per ml. The field trials conducted by Hildebrand and Jenson (1991) revealed that 72.2 and 83.3 per cent mortality of Hypericum perforatum was obtained at $2x10^{6}$ and $8x10^{6}$ spores per ml of C. gloeosporioides. According to Santhi Kamath (1994) $1x10^{9}$ spores per ml of Fusarium sp. (64.44) and $2x10^{9}$ spores per ml of C. gloeosporioides (59.26) were found to be effective in the destruction of the weed. When a higher concentration of $1x10^{10}$ and $1x10^{11}$ spores per ml was used in the present study, the intensity of infection also increased (Table 2).

In the present study, culture filtrates of fungi were collected and applied to the healthy water hyacinth plants. It was observed that the culture filtrates produced symptom same as that does the pathogens. A major phytotoxin fumonisin B1 was isolated by Abbas et al. (1991) from Fusarium moniliformae that killed 95 per cent of the Jimson weed plants. Santhi Kamath (1994) observed that the culture filtrate of the three species of Fusarium, viz., F. equiseti, F. pallidoroseum and F. solani produced symptoms on water hyacinth as that by the pathogen because the culture filtrates contain toxin.

Study 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 synergistic effect (96.45). F. pallidoroseum in combination with C. gloeosporioides produced less infection (59.34) than F. pallidoroseum alone (78.36). When C. gloeosporioides was used alone, it gave only 19.63 per cent infection. But when used with F. equiseti it produced 69.68 per cent infection. When C. gloeosporioides used along with F. pallidoroseum the intensity of infection was 59.34. Combination of the three fungi produced 64.34 per cent infection. Eventhough C. gloeosporioides gave the least infection, it can be used as a co-pathogen with F. equiseti. The same trend was observed in field condition also but, in general intensity of infection was less. Jamil et al. (1984) found that Alternaria eichhorniae, Cercospora sp

and F. solani were pathogenic to water hyacinth. It was also found that F. solani was selective in attacking older leaves and it can be used as a co-pathogen with Cercospora sp. Charudattan (1986) demonstrated the combined use of two mycoherbicides to control weed. He found that showy crotalaria could be controlled by the combined application of Colletotrichum dematium f. sp crotalariae and Fusarium udum f.sp crotalariae.

Experiments were conducted to study the effect of metabolites of the pathogens individually and in combination for the destruction of water hyacinth plants under in vitro and field condition. The metabolite of F. pallidoroseum showed maximum damage under laboratory and field condition. It's combination with F. equiseti and the combination of all the three fungi showed synergistic action, whereas, C. gloeosporioides alone produced the least damage and its combination with F. equiseti and F. pallidoroseum also produced lesser damage. Strobel et al. (1990) found that maculosin and tenuzoic acid produced by Alternaria alternata infecting spotted knapweed when used in combination have a synergistic action against the weed.

When different carrier materials were tried for storage of fungi, sand-maize medium and vermiculite supported neither mycelial growth nor sporulation throughout the period of observation for all the three fungi.

In the case of C. gloeosporioides maximum spore count was observed on water hyacinth leaf followed by guinea grass straw. The spore count was found to increase upto twentieth day and decreased gradually upto ninetieth day of storage. The spore count of F. equiseti increased for the first forty days and then decreased in an increasing rate. Maximum spore count was observed on thirtieth and fortieth day of storage. The spore count was the least from seventy days onwards. Among the different carrier materials, maximum spore count was observed in guinea grass straw. The spore count was least for wheat bran. The spore count was zero in wheat bran, , , rice straw and water hyacinth leaf in eightieth and ninetieth days of storage. The spore count was maximum on guinea grass straw on thirtieth day followed by fourtieth day. F. pallidoroseum produced maximum spore in rice bran followed by guinea grass straw. At different periods of storage, spore count increased upto twentieth day and then decreased gradually. Maximum spore count was observed in rice bran on twentieth day followed by tenth day. From the present study, it was concluded that all the above fungi can grow and sporulate well in guinea grass straw and can be stored upto twenty days with maximum number of spores except F. equiseti which can be stored upto forty days.

Effect of different carrier materials on the viability of spores of the pathogens of water hyacinth was tested and

it was revealed that spore viability reduced on storage. C. gloeosporioides had maximum per cent spore germination in coir pith and rice bran. The viability was minimum for water hyacinth leaf and guinea grass straw. For F. equiseti the percentage germination of spore was maximum in rice bran and coir pith and the least in water hyacinth leaf. On increasing the time of storage spore viability was decreased. Eventhough, the percentage of germination of spores on guinea grass straw was 74.33 on the tenth day, it decreased to zero on eightieth day. In the case of F. pallidoroseum maximum spore viability was observed in coir pith and the least in water hyacinth leaf. Hareendranath (1989) reported broken maize grain as a suitable medium for mass multiplication of F. pallidoroseum followed by tapioca chips and jack seeds as they produced maximum number of spores. The present observation particularly with respect to sporulation of the fungi was not in agreement with that of Faizal (1992) who reported wheat bran and rice bran as good substrates for growth, sporulation and virulence of F. pallidoroseum. Work done by Santhi Kamath(1994) also concluded that wheat bran and rice bran were good carrier materials for Fusarium spp. and C. gloeosporioides.

In the study for testing the storage life of metabolites, it was observed that the efficiency to produce symptoms was reduced on storage in the case of C. gloeosporioides and F. pallidoroseum. Maximum damage was

produced by the culture filtrate on the day of preparation. Metabolite stored for seven days and under refrigerated condition had the least effect for both. But for F. equiseti, on storage the effectiveness of metabolite increased. Maximum destruction was observed in metabolite stored for seven days. The performance of the metabolite stored under refrigerated condition was poor for all the three fungi.

Studies on the extent of damage caused by the pathogens grown on different carrier materials revealed that the performance of the pathogens varied with the media. C. gloeosporioides caused maximum damage when grown on guinea grass straw and rice straw. In the case of F. equiseti also guinea grass straw was found to be the best substrate. F. pallidoroseum grown on rice bran and guinea grass straw were found to infect more virulently. It was concluded that the virulency of pathogens grown on guinea grass straw was comparatively higher.

Different methods of application of C. gloeosporioides, F. equiseti and F. pallidoroseum were tried under laboratory conditions. All the three fungi showed the same trend in the different methods of application. Of the five treatments, placement of bits of inoculum and dusting the inoculum along with the carrier material produced maximum infection. Infection was less when spores were dusted along with diatomaceous earth.

Faizal (1992) conducted pot culture studies on the effect of spore formulation of F. pallidoroseum and showed that the fungal spore suspension containing diatomaceous earth as inert material and spore suspension in water were equally effective. Apart from these methods various other methods viz., dusting of inoculum in carrier materials, placing of bits of inoculum etc. were tested and found as better methods. This was not in agreement with the study by Santhi Kamath (1994) who tried different methods for the application of the fungi, viz, C. gloeosporioides, F. equiseti and F. pallidoroseum and found that placing bits of inoculum on the plants and spraying of the inoculum of the fungi were effective. However Morris (1989) sprinkled a dried formulation of C. gloeosporioides in wheat bran inoculum @ 10 kg/ha on Hakea serica seedlings and obtained good results.

In the present study, the toxin in the cell free metabolite of Fusarium spp. which are pathogenic to water hyacinth was identified as Fusaric acid by paperchromatography method. In 1969 Davis also isolated the phytotoxin Fusaric acid from the infected plants which caused wilt of plants. Other compounds such as Fumonisins (Abbas et al., 1995) and a polypeptide (Jin et al., 1996) were isolated from Fusarium spp.

SUMMARY

SUMMARY

A study was conducted for the biological control of water hyacinth using fungal pathogens. The study aims at exploring the possibility of using these pathogens as effective mycoherbicides and making suitable formulations of these mycoherbicides for field application. The different aspects included were detailed host range study of the already identified fungal pathogens of water hyacinth viz. Colletotrichum gloeosporioides, Fusarium equiseti and F. pallidoroseum, standardization of dosage of inoculum for the effective destruction of the weed, field evaluation of pathogens and metabolities singly and in combination on the target weed, standardization of carrier materials for storage and field application of the pathogens and its metabolites, standardization of methods of field application and characterisation of the toxin present.

Detailed host range studies of the fungal pathogens viz. C. gloeosporioides, F. equiseti and F. pallidoroseum were conducted on thirty cultivated plants and forty one common weed plants seen in and around water ways infested with water hyacinth. Of the thirty cultivated plants tested C. gloeosporioides was seen to be pathogenic to crop plants viz. amaranthus, bhindi, chilli and mango. Among the forty one weed

plants, it produced symptoms on Euphorbia hirta, Hydrocotyl asiatica and Phyllanthus niruri. F. equiseti produced symptoms only on amaranthus among crop plants and Amaranthus viridis, Commelina benghalensis, C. jacobi and Monochoria vaginalis among the weed plants tested. In the case of F. pallidoroseum, symptoms were produced on 14 weed plants out of 41 plants tested. The plants that were susceptible are Axonopus sp., Boerhaavia diffusa, Calotropis gigantea, Cassia occidentalis, Commelina benghalensis, C. jacobi, Echinochloa colonum, Euphorbia hirta, Justicia diffusa, J. prostrata, Monochoria vaginalis, Oldenlandia umbellata, Phyllanthus niruri and Scoparia dulcis.

Studies carried out to standardize the dosage of inoculum required for the effective destruction of the weed revealed that for all the three fungi tested the spore concentration of $1x10^{11}$ spores per ml was the most effective one causing maximum intensity of infection on water hyacinth leaves. Cell free metabolites were isolated from the fungi tested. The metabolities from F. pallidoroseum and F. equiseti were found to be effective in destroying the weed, whereas, the performance of the metabolite of C. gloeosporioides was very poor.

Study on the effect of pathogens singly and in combination on water hyacinth plants revealed that the combined application of F. pallidoroseum and F. equiseti had synergistic effect. The infection was the least when C. gloeosporioides was

used alone. However, the percentage of infection was higher when it was used along with F. equiseti and F. pallidoroseum. In the case of metabolites, F. pallidoroseum showed maximum damage . It's combination with F. equiseti and the combination of all the three fungi showed synergistic action, whereas, C. gloeosporioides and it's combination with F. equiseti and F. pallidoroseum produced the least damage . .

Of the various carrier materials viz. coir pith, guinea grass straw, rice bran, rice straw, sand-maize medium, vermiculite, water hyacinth leaf and wheat bran tested for the storage and mass multiplication of pathogens, guinea grass straw was found to be effective for all the three fungi tested. For C. gloeosporioides and F. pallidoroseum, spore count increased upto twentieth day and decreased gradually upto ninetieth day of storage. For F. equiseti, spore count increased for the first forty days and then decreased in an increasing rate. In the case of C. gloeosporioides, maximum spore count was observed on water hyacinth leaf followed by guinea grass straw. For F. pallidoroseum the spore count was maximum in rice bran followed by guinea gras straw. On increasing the time of storage spore viability was found decreased. C. gloeosporioides and F. equiseti had maximum per cent spore germination in coir pith and rice bran and F. pallidoroseum had maximum spore viability in coir pith.

When the metabolities of pathogenic fungi were stored, the efficiency to produce symptoms was reduced in the case of C. gloeosporioides and F. pallidoroseum. Maximum damage was caused by the culture filtrate of these fungi on the day of preparation itself. But for F. equiseti, the effectiveness of metabolite increased on storage and maximum destruction was observed in the metabolite stored for seven days. Metabolite stored under refrigerated condition performed poorly for all the three fungi. The extent of damage caused by pathogen grown on different carrier materials differed. Guinea grass straw was found to be the best substrate for all the three fungi tested. However, for C. gloeosporioides and F. pallidoroseum, rice straw and rice bran also were equally effective.

Among the different methods of application of the pathogenic fungi tested, placement of bits of inoculum and dusting the inoculum along with the carrier materials produced maximum infection.

In the present study, the pathogenic fungi tested were found to produce toxin as these caused damage to healthy water hyacinth plants. The toxin present in the culture filtrate of Fusarium spp. was identified as Fusaric acid.

In conclusion, of the three pathogenic fungi tested, F. equiseti and F. pallidoroseum had narrow host range and their

intensity of infection was also high. A spore concentration of $1x10^{11}$ spores per ml was found to be effective in the destruction of water hyacinth for all the three fungi tested. The combined application of F. pallidoroseum and F. equiseti and also their metabolites had synergistic effect on the destruction of the weed. Guinea grass straw, coir pith and rice bran can be used for the storage and mass multiplication of these fungi [illegible]. The optimum time for the storage was upto twenty days for C. gloeosporioides and F. pallidoroseum and upto forty days for F. equiseti. It was observed that the cell free metabolites on the day of preparation for C. gloeosporioides and F. pallidoroseum and metabolite of F. equiseti on storage increased effectiveness in the destruction of the weed. Dusting of the inoculum along with carrier material and placing bits of inoculum were found to be good methods of application of the pathogen.

REFERENCES

REFERENCES

Abbas, H.K., Boyette, C.D., Hoagland, R.E. and Ronald, F.V. 1991. Bioherbicidal potential of Fusarium moniliformae and it's phytotoxin-Fumonisin. Weed Sci., **39**: 673-677

Abbas, H.K. and Ocamb, C.M. 1995. First report of production of Fumonisin B_1 by Fusarium polyphialidium collected from seeds of Pinus strobus. Plant Dis., **79**: 642

Abbas, H.K., Ocamb, C.M., Xie, W., Mirocha, C.T. and Shier, W.T. 1995. First report of Fumonisin B_1, B_2 and B_3 production by Fusarium oxysporum var. redolens. Plant Dis., **79**: 968

Anderson, R.N. and Walker, H.L. 1985. Colletotrichum coccodes, a pathogen of eastern black night shade. (Solanum ptycanthum). Weed Sci., **33**: 902-905

Andrews, J.H. and Hecht, E.P. 1981. Evidence for pathogenicity of Fusarium sporotrichoides to Eurasian watermilfoil (Myriophyllum spicatum). Can. J. Bot., **59**: 1069-1077

Aneja, K.R. and Srinivas, B. 1990. Leaf spot disease of water hyacinth, Eichhornia crassipes - a new disease record from India. Trop. Pest Management, **36**: 405-406

Aneja, K.R., Srinivas, B. and Singh, K. 1990. Three new pathogenic fungi of water hyacinth from India. Trop. Pest Management, **36**: 76

Backman, P.A and Rodrigus, K.R. A system for the growth and delivery of biological control agent to the soil. Phytopathology, **65**: 819-821

*Balasooriya, I., Gunasekhara, S.A., Hettiarachchi, S. and Gunasekhara, I.J. 1984. Biology of water hyacinth fungi associated with water hyacinth in Srilanka. Proc. Int. Conf. Water hyacinth. (Thyagarajan, G. Ed.). Nairobi, Kenya. VIY envt. Pragn. pp - 304-307

Beevi, N.S. 1979. Studies on entomogenic fungi associated with lady bird beetle (Henosepilachna vigintioctopunctata (Fabr.) Coccinellidae - Coleoptera). Master of Science - thesis. Kerala Agricultural University.

Bilgrami, K.S., Ahmad, M.S.S., Sahay, S.S. and Rahman, M.F. 1995. Production of fusarial toxin under varying conditions. Indian Phytopath., **48**: 347-351

Boyette, C.D., Templeton, G.E. and Oliver, L.R. 1984. Texas gourd (Cucurbita texana) control with Fusarium solani f. sp cucurbitae. Weed Sci., **32**: 649-655

Boyette, C.D., Templeton, G.E. and Smith, R.J. Jr. 1979. Control of winged water primrose (Jussiaeae decurrens) and Northern joint vetch (Aeschynomene virginica) with fungal pathogen. Weed Sci., **27**: 497-501

Boyette, C.D. and Walker, H.L. 1984. Evaluation of Fusarium lateritium as a biological herbicide for controlling velvet leaf - (Abutilon theophrasti) and prickly sida (Sida spinosa). Weed Sci., 32: 106-109

Boyette, C.D. and Walker, H.L. 1985a. Factors influencing biocontrol of velvet leaf (Abutilon theophrasti) and prickly sida (Sida spirosa) with Fusarium lateritium. Weed Sci., 33: 209-211

Boyette, C.D. and Walker, H.L. 1985b. Production and storage of inoculum of Cercospora kikuchi for field studies. Phytopathology, 75: 183-185

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

*Butler, F.C. 1951. Anthracnose and seedling blight of Bathrust Burr caused by Colletotrichum xanthii (Halst). Aust. J. Agric. Res. 2: 401-411

Cardina, J., Littrell, R.H. and Hanlin, R.T. 1988. Anthracnose of Florida begganweed (Desmodium tortuosum) caused by Colletotrichum truncantum. Weed Sci., 36: 329-334

*Counter, T.G. 1984. Potential for biological control of water hyacinth with indigenous fungal pathogens. In Proc. of the International conference on plant protection in the tropics (Heong, K.L. ed). Sch. of Biol. Sci. University Sains. Malaysia. pp. 489-492.

Chang, M.Y., Leonard, K.J. and Vnadyke, C.G. 1989a. Bipolaris halopense: a new species from Sorghum halopense. (Johnson's grass). Mycologia, 81: 532-538

Chang, M.Y., Vandyke, C.G. and Leonard, K.J. 1989b. Evaluation of endemic foliar fungi for potential biological control of Johnson's grass (Sorghum halopense). Screening and host range test. Plant Dis., 73: 459-464

*Charudattan, R. 1978. Biological control projects in Plant Pathology. A Directory. Misc. Publ. Plant Pathol. Devt. Univ. Florida, Graines villa, F.L.

*Charudattan, R. 1986. Biological control of showy crotalaria with two fungal pathogens. Weed Sci. Soc. Am. Abstr., 26: 51

Charudattan, R. and Conway, K.E. 1975. Comparison of Uredo eichhorniae, the waterhyacinth rust and Uromyces pontederiae. Mycologia, 67: 653-657

Charudattan, R. and Conway, K.E. 1976. Mycoleptodiscus terrestris leaf spot on water hyacinth. Pl. Dis. Reptr., 60:1

*Connick, W.J., Dougle, D.J. and Quimby, P.G. Jr. 1991. An improved emulsion with high water retension for mycoherbicides delivery. Weed Technol., 5: 442-444

Conway, K.E. 1976. Evaluation of Cercospora rodmanii as a biological control agent of water hyacinth. Phytopathology, 66: 914-917

Crawly, D.K., Walker, H.L. and Riley, J. A. 1985. Interaction of Alternaria macrospora and Fusarium lateritium on spurred anoda. Plant Dis., 69: 977-979

Daniel, J.T., Templeton, G.E., Smith, R.J. and Fox, W.T. 1973. Biological control of northern joint vetch in rice with an endemic fungal disease. Weed Sci., 21: 303-307

Davis, D. 1969. Fusaric acid in selective pathogenicity of Fusarium oxysporum. Phytopathology., 59: 1391-1395

De Frank, J. and Putnam, A.R. 1985. Screening procedure to identify soil borne actinomycetes that can produce herbicidal compounds. Weed Sci., 33: 271-274

Dinoor, A. and Eshed, N. 1984. Pathogens and natural plant communities. Annu. Rev. Phytopathol., 22: 443-466

Faizal, M.H. 1992. Studies on the entomogenous fungus Fusarium pallidoroseum (Cooke) Sacc. associated with cowpea aphid. Aphis craccivora Koch. Master of Science thesis. Kerala Agricultural University

Favel, D.R., Marois, J.J., Lumsden, R.D. and Connick, W.J. Jr. 1985. Encapsulation of potential biocontrol agent in an alginate clay matrix. Phytopathology., **75**: 774-777

Fravel, D.R., Lewis, J.A. and Chittans, J.L. 1995. Alginate prill formulation of Talaromyces flavus with organic carrier for biocontrol of Verticillium dahliae. Phytopathology., 85: 774-777

Freeman, T.E. and Charudattan, R. 1974. Occurrence of Cercospora piaropi on water hyacinth in Florida. Pl. Dis. Reptr., **58**: 277-278

Freeman, T.E. and Zettler, F.W. 1971. Rhizoctonia blight of water hyacinth. Phytopathology., **61**: 892

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

Hildebrand, D.C. and Mc Cain, A.H. 1978. The use of various substrates for large scale production of Fusarium oxysporum f.sp. cannabis inoculum. Phytopathology, **68**: 1099-1101

Hildebrand, D.C. and Jenson, K.I.N. 1991. Potential for the biological control of St. John's wort (Hypericum perforatum) with an endemic strain of Colletotrichum gloeosporioides. Can. J. Plant Pathol., **13**: 66-70

*Holder, A.N.G and Smith, D. 1992. Effects of cryo-preservation methods in liquid nitrogen on viability of Puccinia abrupta var. Parthenicola urediniospores. Mycol. Res., 90: 473-476

Howell, C.R. and Striponovic, R.D. 1984. Phytotoxicity to crop plants and herbicidal effect on weeds of viridiol produced by Gliocladium virens. Phytopathology., 74: 1346-1349

*Jamil, K., Narasaiah, J. and Thyagarajan, G. 1984. Studies on the evaluation of naturally occurring fungal pathogens of water hyacinth. Proc. Int. Conf. Water hyacinth. (Thyagarajan, Ed.) Nairoby, Kenya

Jamil, K. and Rajagopal, D. 1986. Studies on the mycoflora of water hyacinth. Their individual and combined effect on the phyllosphere. Indian J. Microbiol. 26: 70-77

Jeyarajan, R. and Ramkrishnan. 1995. Mass production of Trichoderma viride for biological control. Indian J. Mycol. Pl. Path., 25: 125

Jin, H., Hartman, G.L., Nickel, C.D. and Widholm, J.M. 1996. Characterisation and purification of a phytotoxin produced by Fusarium solani, the causal agent of soybean sudden death syndrom. Phytopathology, 86: 277-282

*Jones, R.W. and Hancock, J.G. 1990. Soil borne fungi for biological control of weeds. Acs Synopsium Series, **439**: 276-286

Joyner, R.G. and Freeman, T.E. 1973. Pathogenicity of Rhizoctonia solani to aquatic plants. Phytopathology., **63**: 681-685

*Kalidas, B. 1981. Phytopathogen as weed control agent. Proc. of the eighth Asian - Pacific Weed Sci. Soc. Conf.

Karunakaran, P., Nair, M.C. and Gokulapalan, C. 1980. Survival of the clove pathogen Colletotrichum gloeosporioides on the weed Clerodendron in India. Plant Dis., **64**: 415-416.

Kenney, D.S. 1986. DeVine - the way it was developed - an industrialist's view. Weed Sci., **34**: 15-16

Kirkpatrick, T.L., Templeton, G.F., Te Beest, D.O. and Smith, R.J. 1982. Potential of Colletotrichum malvarum for biological control of prickly sida. Plant Dis., 66: 323-325

Koning, G., Hanman, A., Eicker and Vandeventer, H.A. 1995. First report of Fusarium equiseti on South African soybean cultivars. Plant Dis., **79**: 754

Kuruvila, S. and Jacob, A. 1981. Mass culturing of Fusarium oxysporum Schtt on entomogenous fungus of brown plant hopper. (Nilaparvatha lugens Stal.) Agric. Res. J. Kerala., **19**: 66-68

*Lakshmanan, P., Jayarajan, R. and Vidhyasekharan, P. 1991. Cochliobolus carbonum, a potential biocontrol agent for Euphorbia geniculata. Zeitschrift fur pflanzenkranheiten und pflanzenschutz., **98**: 185-187

Mahadevan, A. and Sridhar, R. 1986. Isolation of fusaric acid from culture filtrates of Fusarium oxysporum. In Methods in Physiological Plant Pathology. Sivakami Publications, Madras pp. 93-95

*Maity, B.R. and Samaddar, K.R. 1977. A toxic metabolite from Alternaria eichhorniae production and properties. Phytopathologische zeitschrift, **88**: 78-84

Martin, R.D. and Freeman, T.E. 1978. Evaluation of Acremonium zonatum as potential biocontrol agent of waterhyacinth. Pl Dis. Reptr., **62**: 604

Mayee, C.D. and Datar, V.V. 1986. Phytopathometry. Technical bulletin-1 (Special bulletin-3). Marthwada Agricultural University. pp. 146

Mc Rea, C.F. and Auld, B.A. 1988. The influence of environmental factors on anthracnose of Xanthium spinosum. Phytopathology, **78**: 1182-1186

Mehta, R.D., Patel, K.A., Rao, K.K. and Mehta, M.H. 1995. Trichoderma harzianum as biocontrol agent in formulated product. Indian J. Mycol and Pl. Pathol., **25**: 129

Morin, L., Watson, A.K. and Reflender, R.D. 1989. Efficacy of Phomopsis convolvulus for control of field bind weed (Convolvulus aryensis). Weed Sci., **37**: 830-835

Morris, M.J. 1989. A method for controlling Hakea serica Schrad seedlings using the fungus Colletotrichum gloeosporioides (Penz.) Sacc. Weed Res., **29**: 449-454

Mortenson, K. 1988. The potential of an endemic fungus Colletotrichum gloeosporioides for biological control of round leaved mallow. (Malva pussila) and velvet leaf (Abutilon theophrasti) Weed Sci. **36**: 473-478

*Nagalingam, B. 1983. Studies on the ecology and bioagents of Myzuz persicae (Sulzer) Ph.D. thesis. Tamil Nadu Agric. Univ. Coimbatore

*Nagraj, T.R. and Ponnappa. 1970. Blight of water hyacinth caused by Alternaria eichhorniae. Trans. Br. Mycol. Soc., **55**: 123-130

Panse, V.G. and Sukhatme, P.V. 1967. Statistical methods for Agricultural workers. Indian Council of Agricultural Research, New Delhi. pp. 381

Papavizas, G.C., Fravel, D.R. and Lewis, J.A. 1987. Proliferation of Talaromyus flavus in soil and survival in alginate pellets. Phytopathology, 77: 131-136

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

Rahim, A.M. 1984. Phoma sorghina causing leaf spot of water hyacinth in Sudan. Plant Pathol., 33: 429

Rahim, A.M. and Tawfig, S. 1984. Pathogenicity of fungi and bacteria from Sudan to water hyacinth. Weed Res., 24: 233-238

Rahim, A.M. and Tawfig, S. 1985. Evaluation of Acremonium zonatum and Phoma sorghina for the biological control of water hyacinth. Trop. Pest Management, 31: 157-158

Rahim, A.M. and Tawfig, S. 1986. A leaf spot of water hyacinth caused by Drechslera spicifera. J. Phytopathol., 8: 233-240

*Rai, I.S. and Bridgmon, G.H. 1971. Studies on rot of canada thistle (Cirsium arvense) caused by Fusarium roseum. J. colo. Wyo. Acad. Sci. 17: 2

*Rakvidyasastra, V. and Visaranthanond,N. 1975. Isolation and identification of fungi pathogenic to water hyacinth. (Eichhornia crassipes (Mart) Solms.) Kasetsart J. 9: 170-177

Ridings, W.H. 1986. Biological control of strangler vine in citrus - a researcher's view. Weed Sci., **34** (Suppl-1): 31-32

*Robenson, D., Strobel, G., Matusumoto, G.K., Fisher, E.L., Chen, M.H. and Clandy, J. 1984. Altechin an unusual phytotoxin from Alternaria eichhorniae fungal pathogen of waterhyacinth. Experentia, **40**: 1248-1250

Santhi Kamath, S. 1994. Screening of fungal pathogens for biocontrol of water hyacinth. (Eichhornia crassipes (Mart.) Solms.) Master of Agriculture thesis, Kerala Agricultural University.

Sharma, B.K. and Basondrai, A.K. 1996. Biocontrol of plant pathogen. Potential and limitations. Farmer and Parliament, **1**: 17-18

*Singh, K.P., Singh, L. and Singh, R.P. 1985. Mycoflora associated with water hyacinth. Acta Botanica India., **13**: 272-275

Smith, R.J.Jr. 1986. Biocontrol of Northern joint vetch (Aeschynomene virginica) in rice (Oryza sativa) and soybeans (Glycine max). A research view. Weed Sci., **34**: 17-23

Stevens, K.L., Badar-nol-Dis, Ahmad, A. and Ahmad, M. 1979. The antibiotic bostrycin from Alternaria eichhorniae. Phytochemistry, **10**:

Stierle, A., Upadhyay, R. and Strobel, G. 1991. Cyperine, a phytotoxin produced by Aschochyta cypericola a fungal pathogen of Cyperus rotundus. Phytochemistry, **30**: 2191-2192.

Strobel, G., Stierk, A., Park, S.H. and Cardellina, J. 1990. Maculosin a host specific phytotoxin from Alternaria alternata on spotted knap weed. pp. 53-62 In Microbes and Microbial product as herbicides. (R.E. Hoagland, ed.) American Chemical Society, Washington.

Te Beest, D.O. 1988. Addition to host range of Colletotrichum gloeosporioides f. sp. aeschynomene. Plant Dis., 72: 16-18

Te Beest, D.O. 1991. Microbial control of weeds. Chapman and Hall, Newyork, London. pp. 282

Te Beest, D.O. and Templeton, G.E. 1985. Mycoherbicide - Progress in the biological control of weeds. Plant Dis., **69**: 6-10

Trijillo, E.E., Latterell, F.M. and Ross, A. 1986. Colletotrichum gloeosporioides, a possible biological control agent for Clidemia hirta in Hawaii forests. Plant Dis., **70**: 974-976

*Villarcorta, A. 1976. Technique for the mass culturing of the entomogenic fungus Metarrhizium anisopleae Metch. in granular form. Anais da Sociedade Entomologia do Brazil., **5**: 102-104

Walker, H.L. 1981 a. Granular formulation of Alternaria macrospora for control of spurred anoda (Anoda cristata). Weed Sci., **29**: 342-345

Walker, H.L. 1981 b. Fusarium lateritium, a pathogen of spurred anoda (Anoda cristata), prickly sida (Sida spinosa) and velvet leaf (Abutilon theophrasti). Weed Sci., **29**: 629-631

Walker, H.L. and Connck, W.J. Jr. 1983. Sodium alginate for production and formulation of mycoherbicides. Weed Sci., **31**: 333-338

Walker, H.L. and Riley, J.A. 1982 Evaluation of Alternaria cassiae for the biocontrol of sickle pod. (Cassia obtusifolia). Weed Sci., **30**: 651-654

Weidmann, G.J. 1991. Host range testing - Safety and Science. pp. 83-97. In Microbial control of weeds. (Te Beest, D.O. ed.) Chapman and Hall, Newyork, London.

Weidmann, G.J. and Templeton, G.E. 1988. Efficacy and soil persistence of Fusarium solani f. sp. Cucurbitae for control of Texas gourd. (Cucurbita texana). Plant Dis., **72**: 36-38

Wymore, L.A. and Watson, A.K. 1989. Interaction between velvet leaf isolate of Colletotrichum coccodes and the diazuron for velvet leaf (Abutilon theophrasti) control in the field. Weed Sci., **37**: 478-488

* Originals not seen

APPENDIX

APPENDIX - I

Composition of media

Czapek's (Dox) Agar

Sucrose	-	3 g
Sodium nitrate	-	2 g
Dipotassium phosphate	-	1 g
Magnesium sulphate	-	0.5 g
Potassium chloride	-	0.5 g
Ferrous sulphate	-	0.01 g
Agar	-	20 g
Water	-	1 l

Nutrient solution (Knop's solution)

Calcium nitrate	-	0.8 g
Potassium nitrate	-	0.2 g
Potassium dihydrogen phosphate	-	0.2 g
Magnesium sulphate	-	0.2 g
Ferrous sulphate	-	Trace
Water	-	1 l

Potato Dextrose Agar

Potato	-	200 g
Dextrose	-	20 g
Agar	-	20 g
Water	-	1 l

ABSTRACT

BIOCONTROL OF WATER HYACINTH USING FUNGAL PATHOGENS

By

SUSHA, S. THARA

ABSTRACT OF THE THESIS

SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENT FOR THE DEGREE

MASTER OF SCIENCE IN AGRICULTURE

FACULTY OF AGRICULTURE

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI - THIRUVANANTHAPURAM

1997

ABSTRACT

Detailed study was conducted on the host range of the already identified fungal pathogens of water hyacinth viz. *Colletotrichum gloeosporioides*, *Fusarium equiseti* and *F. pallidoroseum* on thirty cultivated plants including vegetables, pulses and oil seeds, field crops, fruits and forest crops and ornamental plants and forty one common weed plants which are seen in and around water ways infested with water hyacinth. It was observed that *C. gloeosporioides* could infect amaranthus, bhindi, chilli, *Euphorbia hirta*, *Hydrocotyl asiatica* and *Phyllanthus niruri*. Of the thirty cultivated plants and forty one weed plants tested *F. equiseti* was seen to be pathogenic to amaranthus, *Amaranthus viridis*, *Commelina bengalensis*, *C. jacobi* and *Monochoria vaginalis*.

F. pallidoroseum could produce symptoms on napier grass, *Axonopus* sp., *Boerhaavia diffusa*, *C. benghalensis*, *C. jacobi*, *Echinochloa colonum*, *Euphorbia hirta*, *Justicia diffusa*, *J. prostrata*, *M. vaginalis* and *Oldenlandia umbellata* and *Scoparia dulcis*.

For the effective destruction of the weed, $1x10^{11}$ spores per ml concentration of *C. gloeosporioides*, *F. equiseti* and *F. pallidoroseum* were found to be more effective than $1x10^{9}$ and $1x10^{10}$ spores per ml concentration.

Cell free metabolites of the pathogenic fungi were found to produce symptoms on water hyacinth plant. Metabolite produced by F. pallidoroseum caused considerable damage than by F. equiseti and C. gloeosporioides.

When pathogens were applied singly and in combination on water hyacinth it was observed that the combined application of F. pallidoroseum and F. equiseti followed by F. pallidoroseum alone gave maximum intensity of infection. Eventhough C. gloeospoirioides gave least intensity of infection it can be used as a co-pathogen with F. equiseti.

Metabolite of the pathogens individually and in combination when applied on healthy water hyacinth plants, maximum damage was caused by F. pallidoroseum alone and the combination of metabolite of three fungi viz. C. gloeosporioides, F. equiseti and F. pallidoroseum. Least damage was caused by metabolite of C. gloeosporioides.

An experiment was conducted to find out suitable carrier materials for the mass multiplication and storage of pathogen. It was observed that for C. gloeosporioides maximum sporulation was in water hyacinth leaf followed by guinea grass straw and rice bran. But the spore viability was maximum for rice straw, and on coir pith. Maximum infection was caused by fungus grown on guinea grass straw and rice straw.

In the case of F. equiseti spore counts was higher in guinea grass straw followed by coir pith. The spore germination was maximum in rice bran and coir pith. Maximum infection was produced by the fungi on guinea grass straw, coir pith and waterhyacinth leaf.

F. pallidoroseum produce maximum number of spores on rice bran followed by guinea grass straw. It was observed that the germination percentage of spores were maximum on coir pith. Out of the six carrier materials used the fungus produced maximum infection when grown on rice bran and guinea grass straw.

In the study for testing the storage life of metabolities, it was observed that the efficiency to produce symptom was reduced on storage in the case of C. gloeosporioides and F. pallidoroseum whereas for F. equiseti on storage the effeciency of the metabolite to cause damage increased. Metabolite stored on refrigerated condition performed poorly for all the three fungi.

Different methods of application of the three fungi were tried. Of the five treatments, placement of bits and dusting the inoculum along with the carrier materials produced maximum infection.

In the experiment conducted to characterize the toxin presented in the cell free metabolite of pathogenic fungi, observed the presence of Fusaric acid in the metabolite of Fusarium spp.