SCREENING OF FUNGAL PATHOGENS FOR BIOCONTROL OF WATER HYACINTH (*EICHHORNIA CRASSIPES* (MART) SOLMS)

By

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THESIS

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DECLARATION

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

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CERTIFICATE

Certified that this thesis entitled "Screening of fungal pathogens for biocontrol of water hyacinth (Eichhornia crassipes (Mart.) Solms.)", is a record of research work done independently by Kum: Santhi Kammath, S. (91-11-44) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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INTRODUCTION

1. INTRODUCTION

Weeds are unwanted and undesirable plants which interfere with the utilization of land and water resources and thus adversely affect agriculture. Of the total annual loss of agriculture produce from various pests in India, weeds account for 45 per cent loss (Rao, 1983). Also weeds serve as alternative hosts to several crop insects, nematodes and pathogens and thus chances of their attack on crop plants are increased.

Aquatic weeds reduce markedly the flow of water in irrigation and drainage canals, channels and streams leading to flooding, seepage into adjoining areas, break in canal banks and inadequate delivery of irrigation water to fields. It necessitates more frequent mechanical cleaning. Aquatic weeds also form breeding grounds for obnoxious insects like mosquitoes. Some of the prominent aquatic weeds are Eichhornia crassipes (Mart) Solms, Salvinia molesta Mitchell, Hydrilla verticillata (L.F.) Royle, Lemna spp, Wolfia spp, Pistia stratiotes L. etc.

Increased interest has been generated in the use of microorganisms in weed control. An ideal material used to control weeds should be easy to produce and store, inexpensive to use, reliable at high and predictable level of control and safe for the user and environment. Many of these characteristics are exhibited by plant pathogenic fungi that infect plants we consider weeds in modern day agriculture.

Water hyacinth is the most gregariously growing aquatic weed in India. It belongs to the family Pontideriaceae. It was introduced to India from Brazil in 1896 as an ornamental pond plant. It later spread on the slow moving fresh water tanks and thus became a noxious weed. It reproduces mainly through offsets particularly during the monsoon season and forms thick blankets or tough mats. The weevil Neochetina eichhorniae Warner and grass carp (Ctenopharyan godon idella Val) have been used in biocontrol of the weed (Bhatia, 1970). No standard fungal pathogen for the biocontrol of the weed has been reported so far.

Based on the foregoing considerations, the aim of the present study was to explore the feasiblity of native fungal pathogens to control water hyacinth. The various steps undertaken in the study are:-

1. Survey of various fungal pathogens of water hyacinth, their periodical isolation and identification.

2. Testing the pathogenicity of the fungi obtained.

3. Host range studies with common cultivated crops and other weeds.

4. Fixing an optimum quantity of inoculum required for effective destruction of water hyacinth.

5. Mass multiplication and storage of the inoculum.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

A number of past and present successes in controlling weeds with plant pathogens had demonstrated the feasibility of this approach and point towards expanded activity. A perusal of literature revealed the following.

2.1 Reports of pathogens on weeds

As early as 1888, pathogens were reported from weeds. In 1888, Kellerman and Swingle reported Septoria cassiaecola Kell and Swingle on Cassia fistula L from India. In 1931 Stevens and Mendiola reported Endophyllum cassiae (Bresadola) Stevens and Mendiola on Cassia obtusifolia L from Ghana, Nigeria and Tanzania. It is a short cycled rust with aecioid teliospores. Arthur (1934) described Ravenalia cassiaecola Atk., R. cassiae covesii Long and Goodd from USA and Mexico on Cassia spp. These autoecious rusts occur predominantly on Leguminosae. Lingappa (1955) reported Synchytrium cassiae Lingappa isolated on Cassia pumila Lam from India. The pathogen caused marked hypertrophy of shoots. Alternaria cassiae Jurair and Khan was isolated from Cassia holoseria Fresn Jurair and Khan (1960).

During a search for natural enemies of water hyacinth Eichhornia crassipes (Mart) solms Nagraj and Ponnappa (1967) isolated Corticium solani (Prill and Delacr) Bourd and Galz in the Rhizoctonia phase and Myrothecium roridum Tode ex Fr. Nagraj and Ponnappa (1970) observed a blight of E. crassipes in and around Assam and Banglore and isolated Alternaria eichhorniae sp nov from the diseased plant parts.

During a survey conducted by Freeman and Zettler (1971) in the canal zone of Panama, to obtain fungal pathogens from Eichhornia azurea Kunth., observed a blight by Rhizoctonia solani Kuhn. It caused severe blighting of immersed portion of plants which frequently resulted in death of the entire plant. The sclerotia of the fungus remained viable for about nine months in the lake water without loss of virulence. Freeman and Charudattan (1974) reported the occurrence of Cercospora piaropi Tharp on water hyacinth in Florida.

Rakvidyasastra and Visarathanonth (1975) isolated thirteen fungi from water hyacinth and among them Alternaria eichhorniae, Myrothecium roridum and Rhizoctonia solani Kuhn

were found pathogenic. Charudattan and Conway (1976) reported Mycoleptodiscus terrestis (Gerdemann) causing leaf spot on water hyacinth for the first time. A fungi Cercospora rodmani Conway was isolated from declining water hyacinth in the Rodman reservoir in Florida by Conway (1976). A pathogenic fungus identified as Colletotrichum gloeosporioides (Penzig) and Penzig, Sacc. sp. Jussiaeae var glabrescens specific to Jussiaeae (Ludwigia) decurrens Walt was isolated from the weed plant by Boyette et al. (1979). The fungus was not pathogenic on Jussiaeae repens var glabrescens, rice, soybean and cotton.

Chattopadhyay and De (1979) reported a new leaf spot disease of Solanum torvum Sw caused by Alternaria solani (Soraeur). Andrews and Hecht (1981) isolated Fusarium sporotrichoides sherb from the aquatic weed Myriophyllum spicatum L and found it to be pathogenic. A new strain of Puccinia chondrillina Bubak and Syd was reported from skeleton weed in Australia by Hasan (1981). Rhizoctonia solani Kuhn was isolated from the weed Salvinia molesta Mitchell in the rice fields of Kerala and its pathogenicity was also demonstrated by Padmakumary et al. (1981). Satyaprasad and Usharani (1981) reported widespread

occurrence of Oidium parthenii sp nov causing powdery mildew on parthenium.

During a survey on the pathogen on weeds of the crop fields of Andhra Pradesh, Reddy and Rao (1982) obtained some new host records viz., Cochliobolus lunatus Nelson and Haasis on Dioscorea pentaphylla L, Dreschlera australiensis (Bugnicort) Subram and Jain. and Fusarium fusarioides (Frag and Cif) on Trianthenia portulacastrum L., Phoma exigua Desm on Melonchia corchorifolia Linn. Soharan et al. (1982) reported two new host records of Alternaria brassicae (Berk) Sacc., namely, Anagallis arvensis L. and convolvulus arvensis Linn. Balasooriya et al. (1984) worked on the fungi associated with water hyacinth in Northwest and Western provinces of Srilanka and found Penicillium oxalicum Currie and Thorn, Curvularia lunata (Wakker) Boedjin, Fusarium spp., Myrothecium roridum and a sterile fungus which colonises the leaf surfaces. A study was conducted to obtain biocontrol organism for the troublesome weed Eichhornia crassipes. Phoma sorghina (Sacc) Berenia Dorenbosch var kesteren was constantly isolated from the diseased plants 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 twenty five fungal and bacterial isolates only five were found pathogenic viz., Acremonium zonatum (Sawada) Gams., Dreschlera spicifera (Bain) Nicot, Fusarium equiseti (Corda) Sacc, Phoma sorghina and a Bacillus sp.

Serrone and Ialonga (1984) reported a new host of Alternaria tenuissema Nees. ex. Fr. on Abutilon theophrasti Medik. Siddaraimaiah et al. (1984) reported a new collor rot disease of parthenium caused by Sclerotium rolfsii Sacc. from India. It caused wilting and death of the weed plants.

Rao et al. (1985) reported a leaf blight of Euphorbia geniculata Orteg caused by Helminthosporium sp. Singh et al. (1985) worked on the mycoflora associated with water hyacinth in India during different 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, Curvularia sp., Pestalotia sp. Myrothecium roridum and Cercospora piaropi Tharp were found potentially pathogenic. A forma specialis of Colletotrichum gloeosporioides on Cuscuta spp was reported by Zhang (1985) from China.

Clay (1986) reported a new disease of purple nutsedge (Cyperus rotundus L.) caused by Balansia cyperi Edg in Lousiana. A survey on the mycoflora of water hyacinth in Andhra Pradesh was conducted by Jamil and Rajagopal (1986). They reported Fusarium oxysporum Schlect, Fusarium semitectum Berk and Rav, Alternaria sp. Curvularia sp., Helminthosporium sp. and a sterile fungus.

Rahim and Tawfig (1986) isolated Dreschlera spicifera (Bain) VArx causing leaf spot of E. crassipes.

Psuedocercospora nigricans (Cooke) Deighton causing foliar lesions on Cassia obtusifolia L. was isolated by Hofmeister and Charudattan (1987). Chang et al. (1989) reported Bipolaris halopense Chang, Leonard and Van Dyke a new species from Johnson's grass Sorghum halopense (L.) pers.

Aneja and Srinivas (1990) reported Cercospora rodmanii from diseased water hyacinth leaves. In another study Aneja et al. (1990) obtained three pathogenic fungi from water hyacinth namely, Fusarium chlamydosporium Wollenw

self perpetuating, they do not completely eliminate the host species and do not normally affect man or other animals. With this objective in view, Martyn and Freeman (1978) evaluated the potentiality of Acremonium zonatum (Sawada) Gams as a biocontrol agent of water hyacinth. They found that Eichhornia crassipes inoculated with A. zonatum reacted differently to infection depending on plant size. Infection altered the leaf production rates depending on the plant size, ie. with increase in the size of diseased plants, the rate of leaf production also increased.

Rakvidyasastra et al. (1978) studied the host range of fungi pathogenic to water hyacinth and found that Rhizoctonia solani was pathogenic to all the test plants viz. Hibiscus sabdariffa L., rice, maize, sorghum, cotton and tobacco. It also caused post emergence damping off of all except those plants belonging to family graminae at the seedling stage. All the tested plants except tobacco were susceptible to Myrothecium roridum. Alternaria eichhorniae infected only Hibiscus sabdariffa. Boyette et al. (1979) found that a spore mixture of Colletotrichum gloeosporioides f. sp. jussiaeae and C. gloeosporioides aeschynomene at concentrations of one to two million spores per ml of each

fungus in 94 l of water per hectare, was effective against Jussiaeae decurrens and Aeschynomene virginica L.

Conway and Freeman (1979) conducted field evaluation of Cercospora rodmani as a biocontrol agent for water hyacinth. They found that the pathogen spread from an area of infection and caused large areas of the weed to die and sink below the surface of water and the fungus could overwinter on older leaves to provide inoculum sources for the next season. Cheney et al. (1980) studied the influence of Puccinia chondrillina on flowering, seeding, plant height and biomass' of rush skeleton weed during different periods of the establishment of rust fungi. It was found that the leaf dry weight reduced significantly five weeks after infection and the root dry weight significantly reduced seven weeks after infection. The greatest reduction in seeds occurred where rust had been established for two years, while greatest reduction in flowering occurred after one year.

Host range studies of Albugo sp. from common rag weed, was conducted to evaluate its potential as biocontrol agent of the weed by Hartmann and Watson (1980). It was observed that among the fifty nine plant species tested only

sunflower was infected. Walker and Sciumbato (1981) conducted host range studies of Alternaria sp. pathogenic to Anoda cristata (L.) Schlect and found that the fungi caused slight to severe injury on cotton.

Phatak et al. (1983) worked on biocontrol of Cyperus esculentus L. (Yellow nutsedge) using Puccinia canaliculata Schw. It inhibits flowering, tuber formation and caused dehydration of plants finally killing them. Jamil et al. (1984) found that of the three fungi pathogenic to E. crassipes, Alternaria eichhorniae caused more damage than Cercospora sp. or Fusarium solani (Mart) Sacc. Fusarium solani showed remarkable selectivity in attacking older leaves and its use as a co-pathogen with cercospora appeared feasible.

Boyette and walker (1985a) studied the factors influencing the biocontrol of velvet leaf (Abutilon theophrasti Medik) and prickly sida (Sida spinosa L.) with Fusarium lateritium Nees. ex. Fr., higher level of control was obtained for both weeds with inoculum concentrations of 7.5×10^5 and 1.5×10^6 macroconidia per ml and post inoculative air temperature above 25^oC. A dew period of 12 hours at 25^oC was required for control of velvet leaf.

Crawley et al. (1985) conducted studies on interaction of Alternaria macrospora Zimm and Fusarium lateritium on spurred anoda. They observed highest levels of plant death when A. macrospora was applied five days before F. lateritium. This interaction is potentially useful to increase the effectiveness of the two pathogens as mycoherbicides.

Leth (1985) worked on bio-control of canada thistle with a species of Phomopsis. This fungus was found to cause die-back of shoots and showed greatest degree of specificity. Alber et al. (1986) tested nineteen species of senecio weeds and seven most common crops for susceptibility to Puccinia expansa Link. It was seen that the weeds Senecio alpinus L. and S. jacobaea L. were severely attacked. So Puccinia expansa Link could be used in the biocontrol of S. alpinus and S. jacobaea. Bronsten and Sands (1986) conducted field trials of Sclerotinia sclerotiorum Bary to control canada thistle in Montana. In addition to attacking weeds and causing wilting and death of shoots, it also infects root system. There was 20-80 per cent death of shoots, followed by reduction in plant density in the next year.

Charudattan (1986) observed that for integrated control of water hyacinth Cereospora rodmani and the arthropods (Neochetina bruchi Hustache and N. eichhorniae Warner) appeared to provide 98 per cent control of the weed.

Ridings (1986) suggested biological control of Strangler vine, Morrenia odorata L. in citrus orchards using Phytophthora palmivora (Butl.) Butl. Effective vine killing was obtained at eight chlamydospores per cm^2 of the soil. Smith (1986) reported the biocontrol of Northern joint vetch (Aeschynomene virginica) in rice fields using Colletotrichum gloeosporiodes f. sp. aeschynomene. Trijillo et al. (1986) worked on biocontrol of Clidemia hirta (L.) D. Don using Colletotrichum gloeosporioides in Hawaii. Host range studies indicated the appressoria formation on leaves of all the eleven ornamental species of family Melastomataceae. Galbraith (1987) developed biocontrol of Eichhornia crassipes using Acremonium zonatum along with Neochetina eichhorniae. Feeding by the weevil increased the infection by the fungus in relatively dry conditions. Spores of the fungus were transported by feet and digestive tract of the weevil. Mortenson (1988) reported that Round leaved mallow (Malva pusilla Sm) and velvet leaf (Abutilon theophrasti) were found

to be killed within seventeen to twenty days when inoculated with spore suspension of Colletotrichum gloeosporioides f. sp. malvae. Morin (1989) worked on the efficacy of Phomopsis convolvulus Ormeno for 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 the three to five leaf stage weeds were controlled with 10^9 conidia per ml.

Morris (1989) reported that a dried formulation of Colletotrichum gloeosporioides in wheat bran when sprinkled on young Hakea serica (schrad) seedlings, caused death of the seedlings from the stem tips. He also found that application of bran inoculum during early winter when seedlings were in cotyledonary to twenty leaf stage was more effective, causing ninety eight per cent mortality.

Joye (1990) reported that when Macrophomina phaseolina (Maubl) Ashby, was inoculated on Hydrilla verticillata (L.) Royle, 58-61 per cent reduction in dry weight of the weed was obtained. Tomley (1990) reported the

control of Parthenium hysterophorus L, using Puccinia abrupta var. partheniicola in Queensland.

Anwar (1991) showed that Promising control of Salvinia molesta Mitchell and E. crassipes was obtained using Myrothecium roridum integrated with Neochetina eichhorniae. Boyette et al. (1991) observed that when Jimson weed was inoculated with conidial suspension of Alternaria crassa (Sacc.) Rands, in Arkansas and Missisipi gave 96 and 87 per cent control respectively.

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

Jones (1990) worked on the use of Gliocladium virens Miller, Giddens and Foster in preemergence weed control. G. virens was cultured with sucrose and ammonium nitrate. This reduced a broad range of weeds by atleast ninety per cent and those seedlings which emerged were severely stunted.

In field trials conducted by Hildebrand and Jenson (1991) to evaluate the effectiveness of Colletotrichum gloeosporioides as biocontrol agent of St. John's weed Hypericum perforatum L, 72.2 and 83.3 per cent mortality was obtained at $2x10^6$ and $8x10^6$spores per ml respectively. Lakshmanan et al. (1991) reported 98 per cent control of Euphorbia geniculata Orteg by spraying with aqueous suspension of 5 x 10^6 spores per ml of Cochliobolus carbonum Nelson and Haasis.

2.3 Phyotoxins in weed control

A toxic metabolite was isolated from fourteen day old culture filtrates of Alternaria eichhorniae by Maity and Samaddar (1977). This was heat stable, dialysable and retarted in bio gel 200. In acid solution (pH 5) it was stable during storage at 4^oC. The partially purified toxin showed some degree of host specificity. At lower concentrations it reproduced typical blight symptoms on water hyacinth leaves. Robeson et al. (1984) obtained an unusual phytotoxin alteichin, from liquid culture of Alternaria eichhorniae, a fungal pathogen of water hyacinth. Alteichin is a doubly hydrated form of 4, 9, dihydroxy perylene - 3, 10

quinone. The herbicidal activity of Gliocladium virens, a soil borne fungus was reported by Jones and Hancock (1990). They could isolate a steroidal phytotoxin viridol which caused severe necrosis of roots.

A diketopiperazine compound, Maculosin was isolated and identified from a strain of Alternaria alternata (Fr.) Keissler infecting spotted Knapweed (Centaurea maculosa Lam) by strobel et al 1990. This toxin was phytotoxic and plant host specific at 10^{-3} and 10^{-5} M concentrations. Another phytotoxin, tenuzoic acid was also produced by Alternaria alternata, which has synergistic action with Maculosin.

A major phytotoxin Fumonisin B1, was isolated from Fusarium moniliformae by Abbas et al. (1991). Fumonisin B1 killed 95 per cent of the Jimson weed plants. On the weeds the toxin caused soft rot diffusing along leaf veins. Sharon and Gressel (1991) isolated a single flavanoid phytoalexin from Alternaria cassiae., pathogenic to Cassia obtusifolia. Stierele et al. (1991) isolated Cyperine, a phytotoxin from Aschochyta cypericola, a fungal pathogen of Cyperus rotundus.

2.4 Use of Mycoherbicides in Weed control

Mycoherbicides refers to the use of fungi for biological control of weeds. This is a relatively new concept. Only two mycoherbicides have been commercialized so far, viz., Devine (a formulation of Phytophthora palmivora) and Collego (formulation of Colletotrichum gloeosporioides f. sp. aeschynomene).

Various substrates were tried for large scale production of Fusarium oxysporum f. sp. cannabis Snyd, a pathogen of Cannabis sativa, L. by Hildebrand and Mccain (1978). Large scale inoculum production was achieved on a mixture of barley straw plus either glycine succinate, sodium nitrate solution, Alfa-alfa straw, cotton seed meal or soybean meal. Chlamydospores on Glycine Succinate Sodium nitrate, barley straw substrate retained their disease potential for over six months at room temperature. Walker and Riley (1982) described a method for producing inoculum of Alternaria cassiae for biocontrol of Cassia obtusifolia Eight gram of this conidial preparation contained 1 x 10^8 conidia per gram. Maximum weed control was obtained with a spray solution of more than or equal to 5 x 10^4 conidia per ml, applied at cotyledon to first leaf stage.

Walker and Connick (1983) used sodium alginate for pelletized formulation of mycoherbicides using Alternaria alternata, Fusarium lateritium, Colletotrichum malvarum, Alternaria macrospora and Phyllosticta. Boyette and Walker (1984) found that Fusarium lateritium was effective in suppressing the growth of velvet leaf and prickly sida when applied as granules. Here the fungus infested sodium alginate Kaolin clay granules controlled the weeds Velvet leaf and prickly sida giving 40 and 50 per cent mortality respectively in corn, soybean and cotton fields, without affecting the crops.

Boyette and Walker (1985b) developed a procedure for granulation of mycelial inoculum of Cerospora kikuchii Mats and Tommy, containing mixture of sodium alginate, Kaolin clay and mycelium in a 0.25 M calcium chloride solution, containing an average of 3.8×10^6 conidia per gm of air dried granules.

Devine, marketed by Abbott Laboratories, is the first registered mycoherbicide. This is a formulation of

Phytophthora palmivora. For producing stable form, chlamydospores of the fungus were produced in 50 ml of V-8 juice medium. This was allowed to incubate for 48-72 hrs and overlayed with water for 5-6 weeks (Kenney 1985).

Bowers (1986) gave the procedure for field application of Collego, a postemergence mycoherbicide for the control of Northern joint vetch in rice and soybean fields Collego is a two component product, component A is a water soluble spore rehydrating agent while component B is a water suspendible dried spore preparation of the fungus Colletotrichum gloeosporioides f. sp. aeschynomene.

Connick et al. (1991) developed an oil phase emulsion of Alternaria cassiae, a pathogen of Cassia obtusifolia. The oil phase contained paraffin wax, paraffin spray oil and an unsaturated monoglyceride emulsifier. The oil phase was mixed with 1:1 W/W with water. The Abbott Laboratories, USA developed an experimental formulation of Cercospora rodmani against Eichhornia crassipes. The

formulation is named ABG-5003, which consisted of mycelial fragments and spores and was applied as wettable powder (Tebeest 1991). Holder (1992) developed method of preserving Puccinia abrupta var. partheniicola. Here the dry harvested spores of the pathogen is cooled and stored at - 190°C. These spores remained viable for 32 days following thawing and were able to cause normal infection, thus enhancing its use as mycoherbicide.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

An experiment to screen the fungal pathogens for biocontrol of water hyacinth (Eichhornia crassipes (Mart) solms) was conducted at the College of Agriculture, Vellayani. The materials used and the methods adopted for the conduct of the experiment are summarised below.

3.1 Survey on the various fungal pathogens of water hyacinth

A survey was conducted for various fungal pathogen of water hyacinth in different parts of Trivandrum district (Veli, Ambalathara, Akulam) at monthly intervals from April 92 to July 93, covering the two seasons namely summer (April-May) and rainy season (June-November).

3.1.1 Effect of environmental factors on intensity of infection

The weather data viz temperature, relative humidity, number of rainy days and rainfall of each locality

were collected and the correlation of environmental factors on the intensity of infection was worked out using statistical methods.

3.1.2. Incidence of insect pests on water hyacinth

Presence of weevil (Neochetina eichhorniae Warner) or any other insect pests on the weed was observed.

3.2 Periodical isolation and identification of fungal pathogens of water hyacinth

Periodical isolation of fungal pathogens was done from diseased specimens once in a month from April 92 to July '93 covering the summer season (April-May) and rainy season (June-November).

The fungi isolated were maintained on potato dextrose agar slants. The species level identification of the fungi was done by International Mycological Institute, London.

3.3 Anastamosis grouping

Anastamosis grouping of the isolate of Rhizoctonia obtained from water hyacinth was done. The ability of the isolate to anastomose with the isolate from rice was tested by the method described by Parmeter et al. (1969). Sterilised discs of cellophane were placed over solidified two per cent water agar in nine cm petridishes. In each dish mycelial discs from actively growing culture of the two isolates of the fungus on potato dextrose agar were placed three cm apart over the cellophane. The dishes were then incubated at room ($28\pm3^{o}C$) temperature. Until the advancing hyphae came in contact and slightly overlapped. A two square centimeter portion of the area of contact of the growth was removed, stained with a dilute solution of cotton blue lactophenol, mounted on a glass slide and examined under microscope for anastomosis of the isolates

3.4 Testing of the pathogenicity

For this study, the following fungi were tested:-

1. Colletotrichum gloeosporioides (Penzig) Penzig and Sacc.
2. Curvularia lunata (Wakker) Boedjin.

3. Fusarium equiseti (Corda) Sacc.
4. Fusarium semitectum Berk and Rav.
5. Fusarium solani (Mart.) Sacc.
6. Rhizoctonia solani Kuhn.
7. Sterile fungus.

The water hyacinth plants were allowed to establish in pots for 1-2 weeks. The leaves and stem to be inoculated were given slight injury by gently puncturing with pins. Culture bits from seven day old culture of the pathogens were placed on the injured portion and the inoculated area was covered with small bit of cotton wool soaked in sterile water. Control plants were maintained by applying sterile water on the punctured leaves. The inoculated plants were covered with wet polythene bag to maintain humidity.

In the case of Fusarium spp., pathogenicity test was done using culture filtrates also. For this purpose the fungus was cultured in potato dextrose broth for 4-5 days. It was then filtered through filter paper. The filtrate was used for spraying on healthy water hyacinth plants. The plants were covered with wet polythene bags to maintain humidity.

3.5 Host range studies with common cultivated crops and other weeds

Host range studies were conducted to test the pathogenicity of the fungi on the common crop plants and weeds in and around the rice fields. Pot culture experiment was laid out with the seven isolates of the pathogenic fungi on six crop plants. Pathogenicity test was conducted using seven day old cultures of the respective pathogens by inoculating on the leaves, stem and collar region of the plants. The inoculated plants were covered with polythene bags to retain humidity. The experiment was repeated using six weed plants as hosts. For this experiment three replications and one control were maintained. The plants used for host range studies were:-

Crop plants

1. Amaranthus (Amaranthus spp.)
2. Chilli (Capsicum annuum L.)
3. Cowpea (Vigna sinensis L.)
4. Cucumber (Cucumis sativus L.)
5. Rice (Oryza sativa Linn)
6. Tomato (Lycopersicon esculentum Mill.)

Weed plants

1. Tropical spiderwort - Commelina benghalensis L.
2. Joria - Fimbristylsis miliaceae Vahl.
3. Indian pennywort - Hydrocotyl asiatica (Centella asiatica (L.)
4. Neerthamara - Monochoria vaginalis presl.
5. Ginger grass - Panicum repens L.
6. Water primrose - Ludwigia parviflora L.

3.6 Pot culture trials to select the most promising fungal pathogens

Water hyacinth plants were grown in pots for 1-2 weeks. The pathogenic fungi isolated and identified were used for the study. The fungi used for the study were :

1. Colletotrichum gloeosporioides.
2. Curvularia lunata.
3. Fusarium equiseti.
4. Fusarium semitectum.
5. Fusarium solani.
6. Rhizoctonia solani.

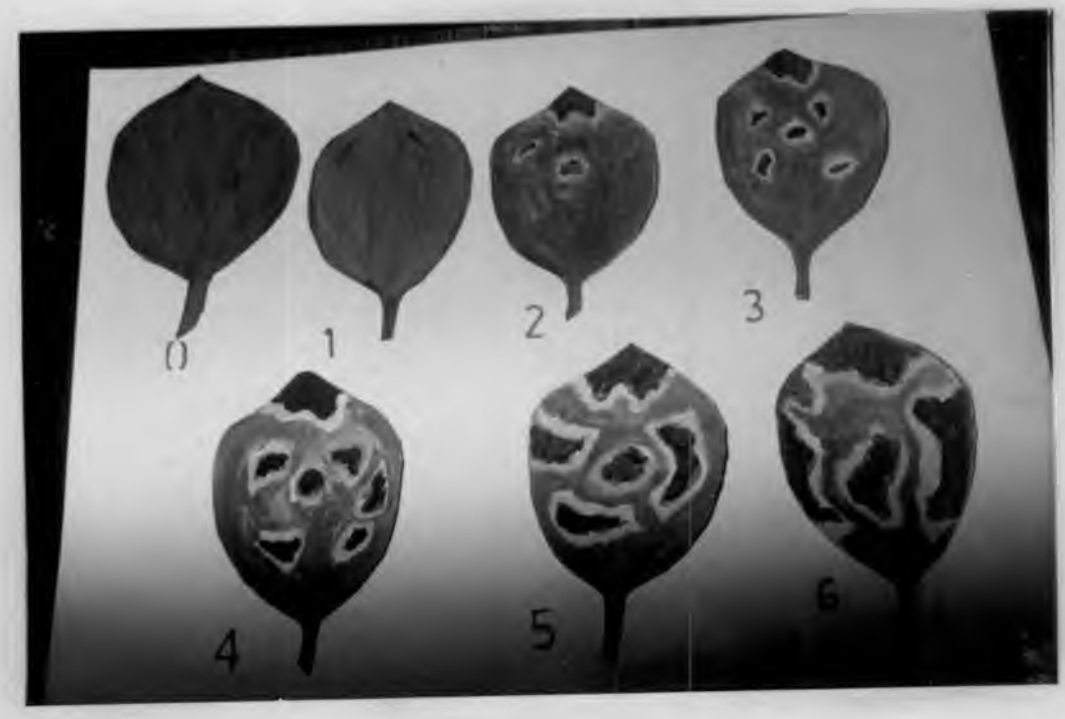
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Plate 1. Score chart for *Colletotrichum gloeosporioides*

The test pathogens were grown in potato dextrose agar media in sterile petri dishes. Water hyacinth plants were inoculated with culture bits from seven day old cultures of the respective pathogen. Inoculation was carried out on the leaves and stem of the test plant. Humidity was provided by covering with moistened polythene bags. The intensity of infection was calculated using score charts prepared for each pathogen.

Score chart for each pathogen

Colletotrichum gloeosporioides

0 - No symptom

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

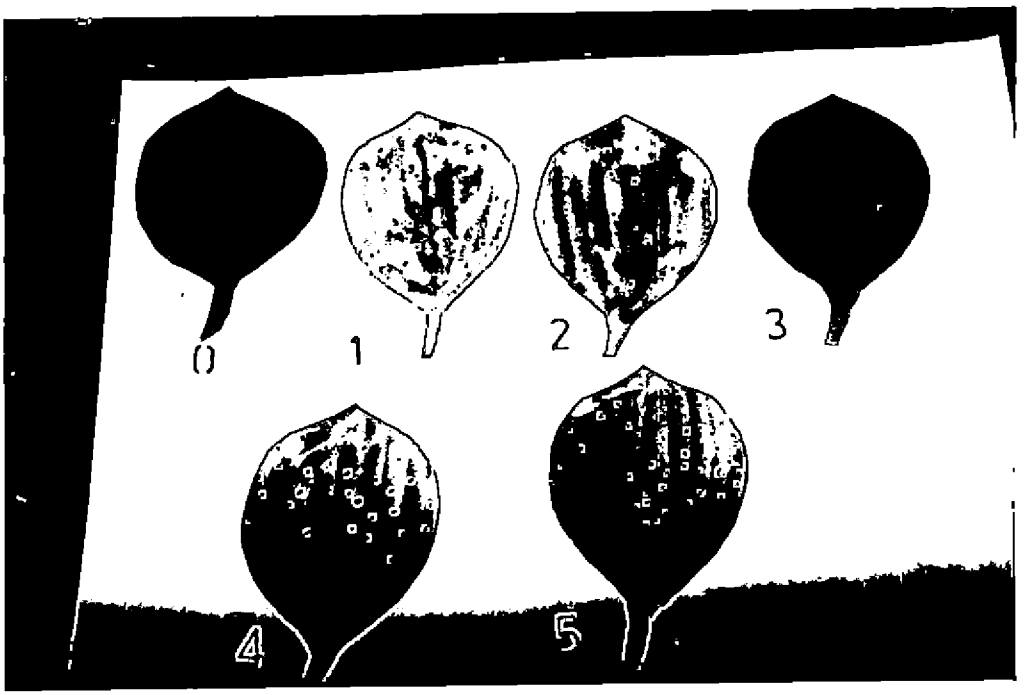
2 - Small spots covering 1-10 per cent leaf area

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

4 - Lesions coalesing covering 25-50 per cent leaf area

5 - Blighting covering 51-75 per cent leaf area

6 - Blighting covering more than 75 per cent leaf area (plate -1;)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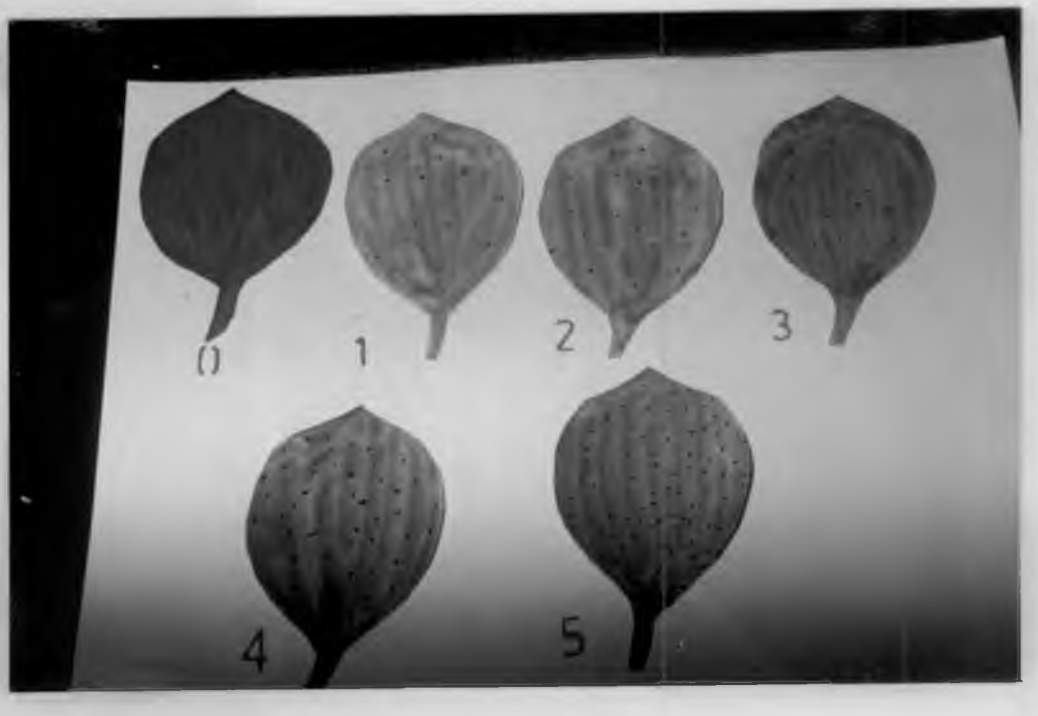
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Plate 3. Score chart for *Fusarium* spp.

Curvularia lunata

0 - No symptom.

1 - Small spots covering 1 per cent or less leaf area

2 - Spots covering 1-10 per cent of the leaf area

3 - Spots covering 11-25 per cent of the leaf area

4 - Spots covering 26-50 per cent of the leaf area

5 - Spots covering more than 51 per cent of the leaf area (plate -2)

Fusarium equiseti, F. semitectum and F. solani

0 - No symptom

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

2 - Blighting covering 25 per cent of leaf area

3 - Blighting covering 26-50 per cent leaf area

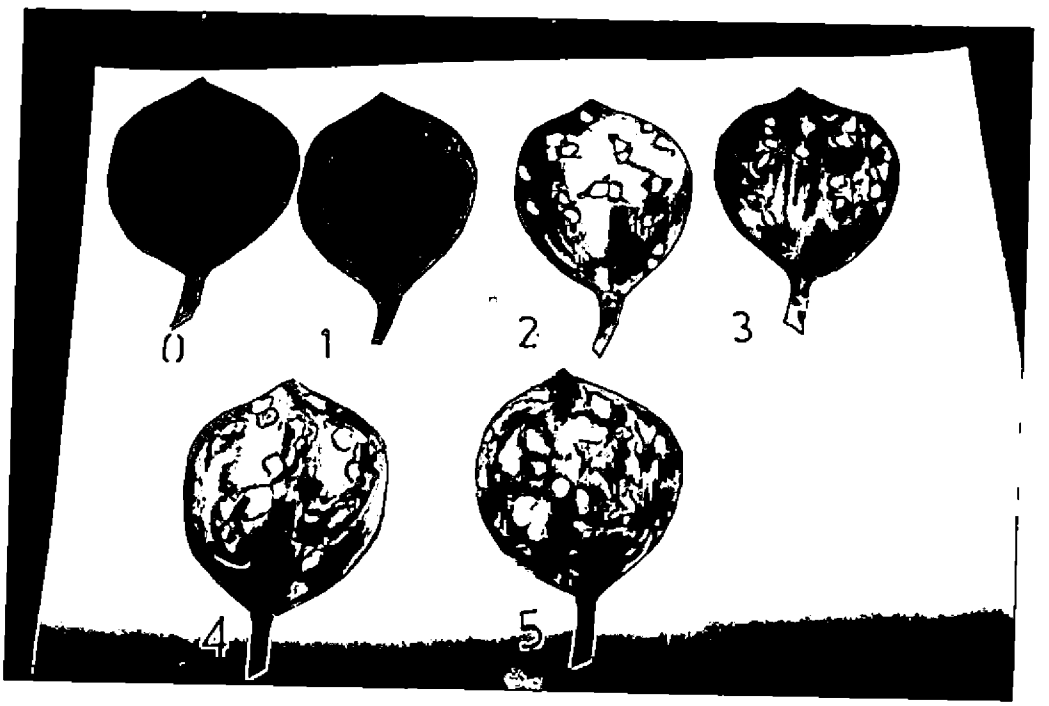
4 - Blighting covering 51-75 per cent leaf area

5 - Blighting covering more than 75 per cent leaf area (Plate-3)

Rhizoctonia solani

0 - No symptom

1 - Lesions on stem only


0
1
2
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2 - Lesions on stem and half of leaf area

3 - Lesions on stem and more than half of leaf area

4 - Lesions on stem and more than 75 per cent of leaf area

5 - Rotting of the leaves and stem
(Plate-4)

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

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

3.7 Quantity of inoculum required for effective destruction of water hyacinth

The promising fungal pathogens obtained from the above experiment viz., Colletotrichum gloeosporioides, Fusarium equiseti, Fusarium semitectum and Fusarium solani were used for this experiment and the quantity of inoculum of these fungi required for effective destruction of the weed was found out.

The spore concentrations were fixed at three levels each for the selected fungi. For Fusarium spp. it was fixed

Plate 4. Score chart for Rhizoctonia solani

at $1x10^3$, $1x10^6$ and 1×10^9 spores per ml and for Colletotrichum gloeosporioides, it was 2×10^3, 2×10^6 and 2×10^9 spores per ml. Suspensions of each spore concentration was made in sterile water and made upto one litre each and taken in a hand sprayer and sprayed on the plants in pots. Three replications were maintained for each fungus for each concentration. The inoculated plants were covered with wet polythene bags, to maintain humidity. Suitable control was kept for each fungus. Intensity of infection was measured using score charts.

3.8 Mass multiplication and storage of inoculum with different carrier materials

Fifty grams each of the following carrier material, viz., Paddy straw, Coir pith, Peat moss, Wheat bran and Rice bran were taken in conical flasks and added enough water to moisten them. The materials were sterilised at 15 lbs pressure for 20 minutes for two successive days. Five mm discs from seven day old cultures of the test pathogens in potato dextrose agar medium were taken and inoculated on the carrier material. Three replications were maintained for each carrier material. This was incubated at room temperature of

(28±3°C). The spore count was taken at weekly intervals. For this a loopful of the inoculum was taken and a spore suspension made in five ml water. From this one drop was taken and placed on slide and stained using cotton blue lactophenol. Number of spores in one microscopic field was counted.

3.9 Viability of the spores in carrier materials

Ten ml of Czapeck's (Dox) broth was prepared in test tubes and sterilised. Into these tubes, loopful of the inoculum from each carrier material was taken and added to it. One drop of this spore suspension was placed on glass slide and kept in moist chamber to allow the spores to germinate. Germination count per microscopic field was taken after 24 hours. This was repeated at weekly intervals and the per cent germination of spores was worked out.

3.10 Field application

Pot culture experiment was conducted to evaluate the performance of the fungal pathogens using the inoculum in effective carrier materials (rice bran, wheat bran and coir pith). Three methods of application were tested.

1. Dusting the inoculum, uniformly @ 5 g/pot

2. Placing bits of inoculum on leaves and stem

3. Spraying the inoculum. For this two g of the inoculum was taken in 100 ml water. This suspension was filtered and the filtrate was used for spraying.

For this experiment about two to three week old inoculum was used.

3.11 Toxin production by Fusarium spp.

Toxin produced by Fusarium spp. was 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 this 7-10 day old cultures, five mm discs were cut and inoculated on autoclaved rice. After fourteen days of growth this inoculum of fungus infested rice was dried at room temperature ($28\pm3^{o}C$) for five days. The inoculum was ground into fine powder. Five gm of inoculum was added to 50 ml distilled water, stirred for 1-2 minutes and sieved through double cheese cloth to remove large particles. Then this filtrate was applied on healthy

water hyacinth plants and the symptom development was recorded. Suitable control was also maintained.

3.12 Statistical analysis

The data obtained during the study were analysed statistically by applying the techniques of analysis of variance. Correlation and regression were also worked out to determine the relation between disease incidence and weather parameters (Panse and Sukhatme 1967).

RESULTS

4. RESULTS

The results of the study conducted to screen the fungal Pathogens for biocontrol of water hyacinth are presented below.

4.1 Survey on the various Fungal pathogens of water hyacinth

A survey was conducted in two seasons viz., Summer and rainy seasons from three locations, viz., Veli, Ambalathara and Akulam from April 92 to July 93 to isolate the different fungi infecting water hyacinth.

During the survey, seven fungi were isolated namely,

1. Colletotrichum gloeosporiodes (Penzig) Penzig and Sacc.
2. Curvularia lunata (Wakker) Boedjin.
3. Fusarium equiseti (Corda) Sacc.
4. Fusarium semitectum Berk and Rav.
5. Fusarium solani (Mart.) Sacc.
6. Rhizoctonia solani Kuhn.
7. Sterile Fungus

Table 1. Seasonal occurrence of fungi on water hyacinth.

Sl. No.	Season	Location	Temperature (°C) Max.	Min.	Relative humidity (%) Mor.	Eve.	No.of rainy days	Rainfall in mm	Fungi isolated
1.	Summer Season (March-May)	Veli	32.34	25.47	92.39	69	5	22.8	Fusarim semitectum F. equiseti, Curvularia lunata, F. solani
		Ambalathara	31.33	24.99	90.57	83.5	10	175.75	Fusarium semitectum F. equiseti, F. solani
		Akulam	32.65	23.20	87	64	2	36.20	F. semitectum F. equiseti, F. solani and sterile fngus
2.	Rainy season (June-Aug)	Veli	30.19	24.16	90.65	78	21	500.4	C. gloeosporioides F. semitectum R. solani F. equiseti, F. solani
		Ambalathara	28.34	22.50	86.58	80.95	18	160.20	Fusarium semitectum F. equiseti, F. solani
		Akulam	30.32	23.80	89.8	78.14	25	171.60	F. semitectum F. solani F. equiseti, C. gloeosporioides
3.	Rainy season (Sep-Nov)	Veli	30.78	24.89	88.83	74.43	13	56.4	C. gloeosporioides Fusarium semitectum F. equiseti, F. solani
		Ambalathara	30.56	24.51	88.32	76.74	13	415.00	Fusarium semitectum F. solani F. equiseti, C. gloeosporioides
		Aakulam	28.70	23.03	91.90	75.03	10	270.70	Fusarium semitectum F. solani F. equiseti, sterile fungus

The seasonal occurrence of various fungi infecting water hyacinth was studied (Table - 1).

Observations on the occurrence of various fungi on water hyacinth and also the variations in the occurrence of these fungi from season to season were made.

Fusarium spp. were present throughout the year in all the three locations, viz., (Veli, Ambalathara and Akulam) Colletotrichum gloeosporioides was prevalent during the rainy season in all the three locations viz Veli, Ambalathara, and Akulam. Curvularia lunata was present during the summer only in Veli. Rhizoctonia solani was also isolated during the rainy season from veli whereas the sterile fungus was isolated during the summer season and rainy season, but the frequency of occurrence was low in both the locations ie Ambalathara and Akulam.

On statistical analysis of the data, it was observed that there was positive correlation between the occurrence of fungi and all the weather parameters viz., temperature, relative humidity, number of rainy days and rainfall. But none of the correlations were significant

Table 2 (a). Coefficient of correlation between weather parameters and occurrence of fungi on water hyacinth.

	Maximum temperature	Minimum temperature	Relative humidity (morning)	Relative humidity (evening)	Number of rainy days	Rainfall	Occurrence of fungi
Maximum temperature	1.0000						
Minimum temperature	0.6136	1.0000					
Relative humidity (morning)	0.1377	0.4981	1.0002				
Relative humidity (evening)	-0.5854	-0.0160	-0.0056	1.0000			
Number of rainy days	-0.5966	-0.1964	-0.1049	0.6700	1.0000		
Rainfall	-0.4405	-0.0950	0.1049	0.4700	0.5009	1.0000	
Occurrence of fungi	0.1311	0.1515	0.3304	0.3734	0.1691	0.4551	1.0000

Table 2 (b). Regression equation relating weather parameters and occurrence of fungi on water hyacinth.

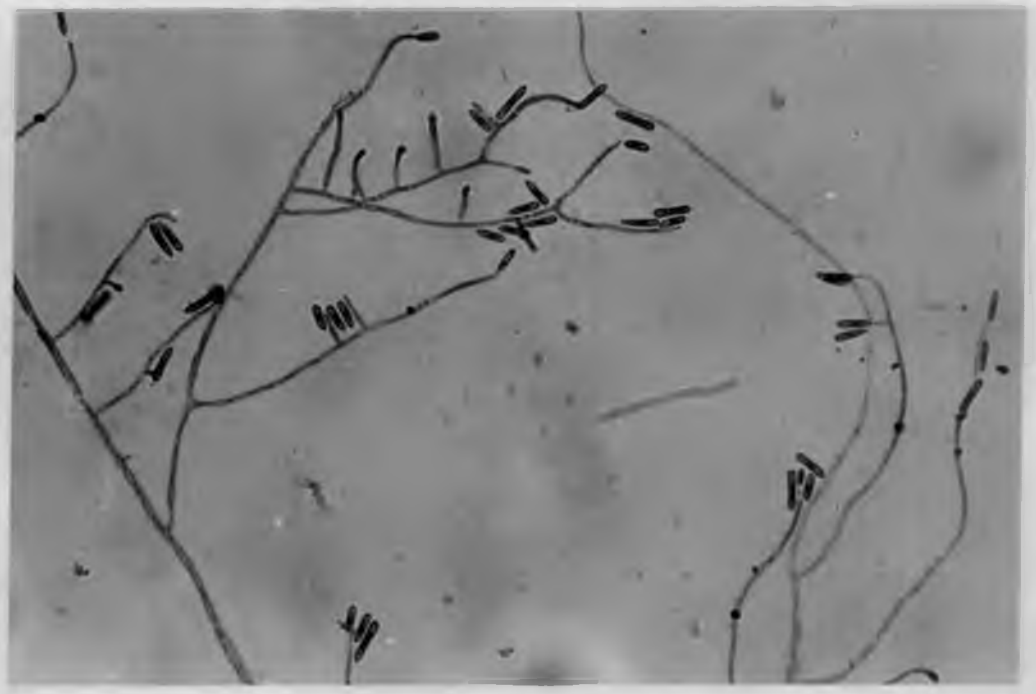
Y = Occurrence of fungi

Y = 0.5400801 - 0.10107 Maximum temperature + 0.1395 Minimum temperature + 0.0644 Relative humidity (morning) - 0.1138 Relative humidity (evening) + 0.0530 Number of rainy days + 0.0023 Rainfall.

R^2 = 0.9250651.

Plate 5. Conidia and conidiophores of *Colletotrichum gloeosporioides*

Plate 6. Conidia and conidiophores of *Curvularia lunata*



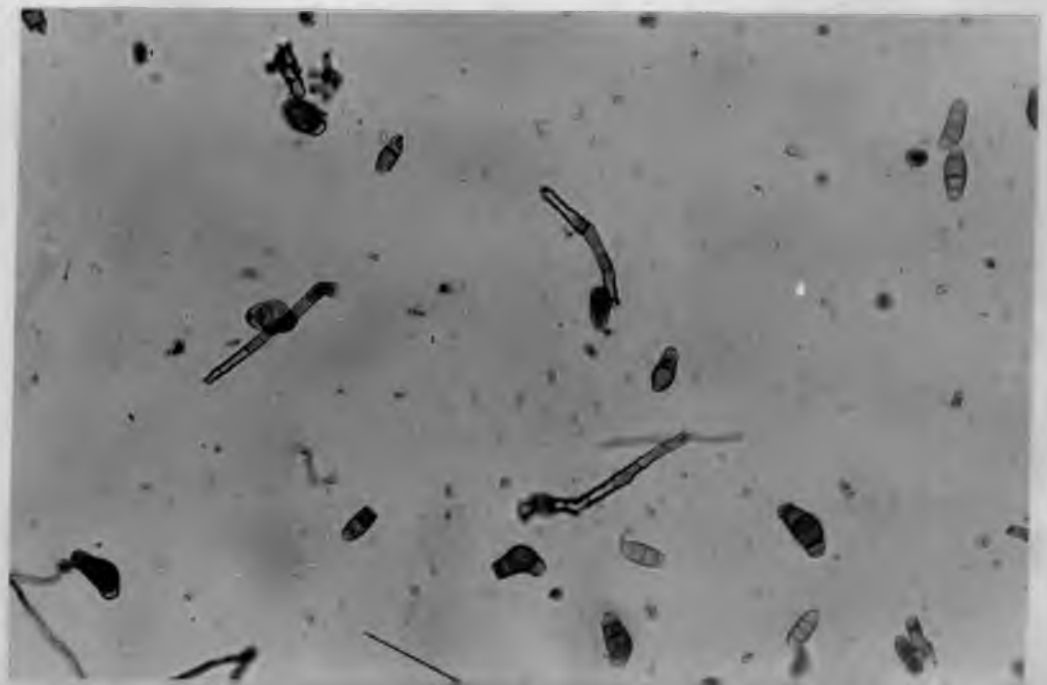
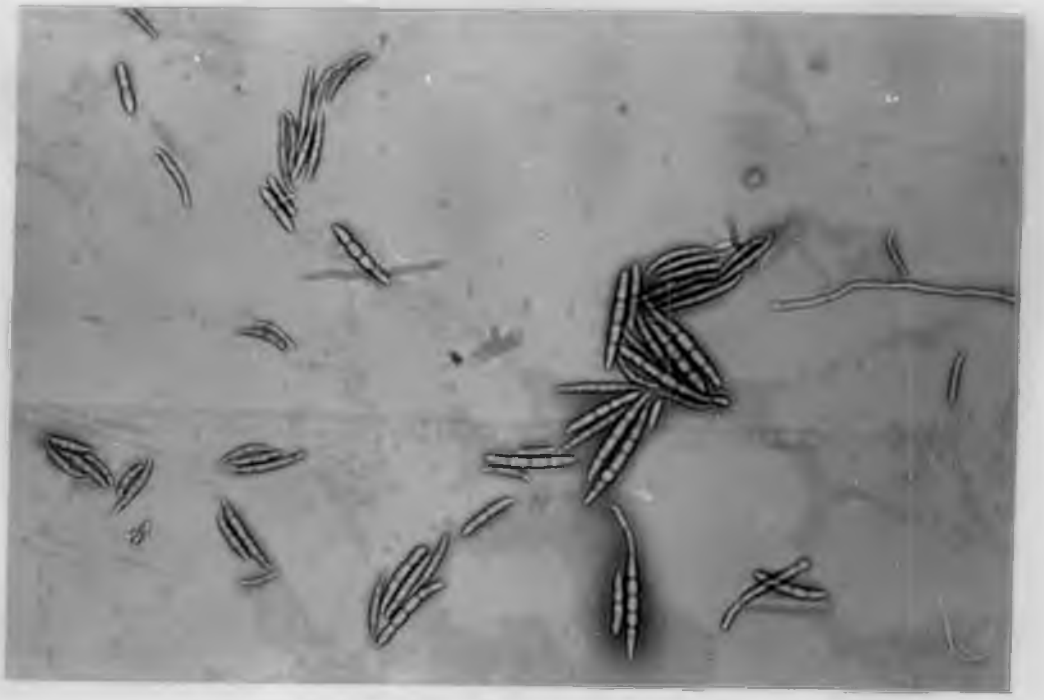


Table 3. Morphological characters of the Fungi isolated from water hyacinth.

Sl. No.	Fungus	IMI Acession Number	Morphological Characters
1.	Colletotrichum gloeosporioides (penzig) penzig and Sacc.	357143	Hyphae branched, Septate, hyaline - Fungus produces large number of acervuli in the culture. Acervuli globose, dark brown to black coloured. Conidiophores non-septate, hyaline. Conidia are single celled, hyaline, straight with blunt ends and oil globule in the centre Conidia measures 12.2 to 17.5 µm x 3.8 µm in size (Plate 5)
2.	Curvularia lunata (wakker) Boedjin	357146	Hyphae branched, septate and dark brown in colour, conidiophores septate and dark brown coloured. Conidia are three celled the middle cell slightly curved. The conidia measures 20-32 µm x 9-15 µm (Plate 6).



Sl. No.	Fungus	IMI Acession Number	Morphological Characters
3.	Fusarium equiseti (Corda) Sacc	357141	Aerial mycelium abundant, woolly and white, it gradually becomes cream colour. Macro and micro conidia abundant. Macro - conidia are larger in size with 5-7 Septa, and measuring to 35-60 x3-5 µm. They are long, hyaline, rounded at the tips. Size of Micro conidia ranges from 5-14 µm x 3.5 - 5 um (Plate 7).
4.	Fusarium semitectum Berk and Rav.	357140	Cultures at first white with peach tinge and peach coloured from below. Micro Conidia are fewer in number Macro conidia are abundant. They are curved, with slightly pointed ends and 3-4 septate the size of macro conidia ranges from 27-46 x 3-5 µm and that of the micro conidia is 5.1 to 8.3 µm x 1.8 to 3.4 µm (Plate 8).

Plate 9. Conidia and conidiophores of Fusarium solani

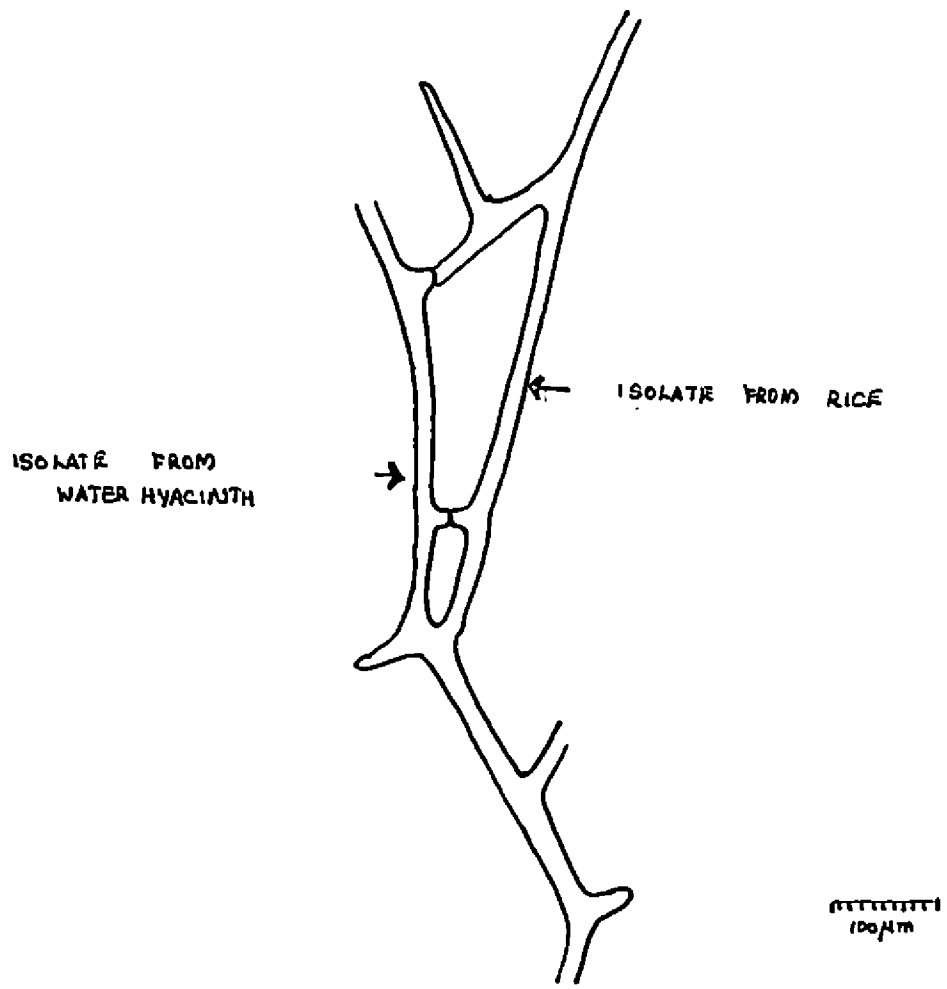


FIG - 1 ANASTAMOSIS GROUPING OF RHIZOCTONIA ISOLATE FROM WATER HYACINTH.

Sl. No.	Fungus	IMI Acession Number	Morphological Characters
5.	Fusarium solani (Mart) Sacc.	357142	Aerial mycelium abundant white in color later turns to slight brown color. Macro conida is 3-5 septate, curved and tips pointed. The size ranges from 35-40 x 3.7-4 µm. The macro conidia are fewer in number, their size ranges from 5.2 to 8 µm x 1.8 to 3 µm (Plate 9)
6.	Rhizoctonia solani Kuhn		The hyphae branched and hyaline with a thickness of 5.26 to 7.95 µm. Sclerotia produced were white in color at first and later turns brown in color. The size of sclerotia ranges from 152.3 - 271.5 x 145 - 220 µm. The ability of this isolate to anastamose with the isolate from rice was studied. It was seen that the isolate from E.crassipes anastomoses with the isolate from rice (Fig 1).

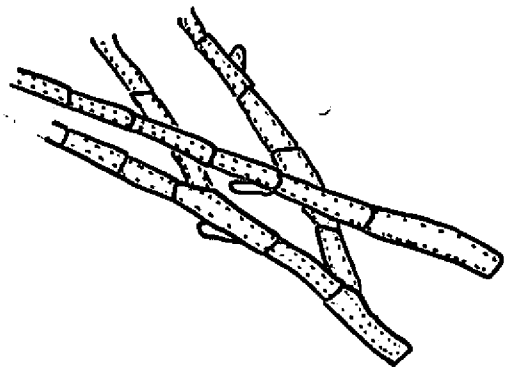


FIG-2 MYCELIA OF STERILE FUNGUS

Sl. No.	Fungus	IMI Acession Number	Morphological Characters
7.	Sterile fungus	357145	The fungus is sterile, without any sporulation The hyphae are branched, septate and dark brown coloured. The thickness of the hyphae range from 6.1 to 7.5 µm (Fig. 2).

(Table 2a). Ninety three per cent of the variation in the occurrence of fungi was attributed to the weather parameters (Table 2b).

During the survey, no incidence of the weevil, Neochetina eichhorniae was observed on the weed.

4.2 Morphological characters of the fungal pathogens

The morphological characters of the fungal pathogens isolated are presented in Table – 3.

4.3 Pathogenicity tests

The pathogenicity of various fungi isolated from water hyacinth was tested by artificially inoculating with culture bits on healthy water hyacinth plants. It was observed that all the fungi isolated were pathogenic. The symptoms produced by each pathogen are described below.

Colletotrichum gloeosporioides

The symptoms first appeared as small dark brown spots with yellow halo around each spot on the leaf lamina,

Plate 10. Symptoms produced by C. gloeosporioides on water hyacinth.

Plate 11. Symptoms produced by Curvularia lunata on water hyacinth.

Plate 12 A. Symptoms produced by *Fusarium equiseti* on water hyacinth.

Plate 12 B. Symptom produced by *Fusarium semitectum* on water hyacinth.

about 7-10 days after inoculation. These spots gradually enlarged and adjacent spots coalesced to form large patches. Symptoms were limited to leaf lamina only (plate 10).

Curvularia lunata

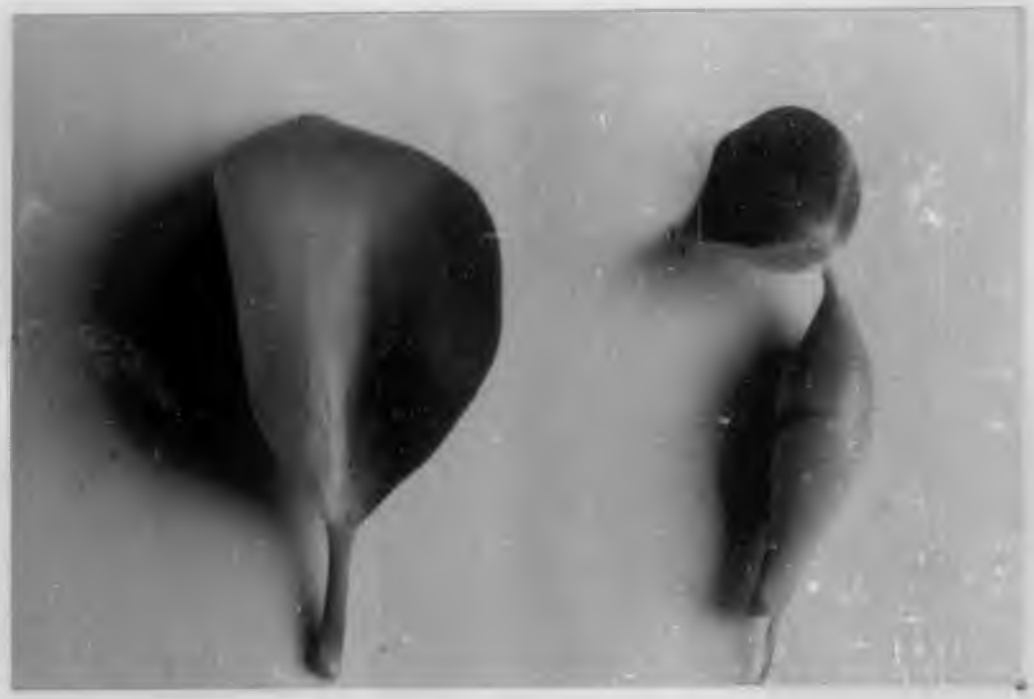
The symptoms appeared as isolated small pin head sized black spots scattered on the leaf lamina (plate 11) about ten days after inoculation.

Fusarium equiseti, F. semitectum and F. solani.

In the case of all the three fungi symptoms appeared about 7-10 days after inoculation. The initial symptoms of the disease appeared as small brownish spots with a characteristic yellow halo especially towards the tips and margins of the leaves. As the disease advances, these spots enlarge to form brown lesions spreading from the tip downwards covering major area of the leaves resulting in the blighting and drying of the entire leaves. No symptoms were seen on the stalk and stem (Plate 12a, b, c).

Plate 12 C. Symptom produced by Fusarium solani on water hyacinth .

Plate 13. Symptom produced by Rhizoctonia solani on water hyacinth .



In the case of Fusarium spp. culture filtrates were also used for pathogenicity tests, the symptoms observed were more or less similar to those in the case of inoculation with the culture bits. The symptoms appeared 10-12 days after inoculation.

Rhizoctonia solani

The symptoms appeared on the plant parts about a week after inoculation. The symptoms were observed on the leaf and leaf stalk. The symptoms appeared as irregular straw colored spots with a dark brown margin. These spots enlarged to form large lesions. Similar type of spots and lesions developed on the leaf stalk also (plate 13).

Sterile fungus

Symptoms were seen on the leaves of water hyacinth about a week after inoculation. Initially small light brown colored spots develop on the leaf lamina. These spots later enlarged with a prominent yellow halo around each spot.

Table 4. Susceptibility of the host plants to the fungi tested.

Sl. No.	Host plant	*Fusarium semitectum*	*Fusarium equiseti*	*Fusarium solani*	*Colletotrichum gloeosporioides*	*Curvularia lunata*	*Rhizoctonia solani*	Sterile fungus
1.	Amaranthus	-	-	-	-	-	+	-
2.	Chilli	-	-	-	+	-	-	-
3.	Cowpea	-	-	-	-	-	+	-
4.	Cucumber	-	-	-	-	-	-	-
5.	Rice	-	-	-	-	-	+	-
6.	Tomato	-	-	-	-	-	-	-
7.	*Commelina benghalensis*	-	-	-	+	-	-	-
8.	*Fimbristylis miliaceae*	-	-	-	-	-	+	-
9.	*Hydrocotyl asiatica*	-	-	-	+	-	-	-
10.	*Ludwigia parviflora*	-	-	-	+	-	-	-
11.	*Monochoria Vaginalis*	+	+	+	-	-	+	-
12.	*Panicum repens*	-	-	-	-	-	+	-

* + Suspectible - Not suspectible

Plate 14. Symptom produced by Rhizoctonia solani on amaranthus

Plate 15. Symptom produced by Colletotrichum gloeosporioides on chilli

4.4 Host range studies

Host range studies with six cultivated plants and six weeds were carried out. The susceptibility of the plants to the pathogens tested are given in Table 4.

The symptoms observed on the various crop plants are as follows.

Amaranthus

Of all the pathogens tested on amaranthus, R. solani alone caused infection 5-7 days after inoculation. It produced creamy colored irregular spots on the leaf margin (Plate 14).

Chilli

Among the various pathogens tested on chilli, C. gloeosporicides alone developed symptoms 7-10 days after inoculation. It produced small brown specks which enlarge into larger spots. As the spots become old shot hole symptoms developed (Plate 15).

Plate 16. Symptom produced by *Rhizoctonia solani* on rice

Plate 17. Symptoms produced by

A. *Colletotrichum gloeosporioides* on *Commelina benghalensis*

B. *Colletotrichum gloeosporioides* on *Ludwigia parviflora*

C. *Rhizoctonia solani* on *Fimbristylis miliaceae*

A B C

Plate 18. Symptom produced by *Colletotrichum gloeosporioides* on *Hydrocotyl asiatica*

Plate 19.A Symptom produced by *Fusarium* spp. on *Monochoria vaginalis*

B *R. solani* on *Monochoria vaginalis*

Cowpea

Of all the pathogens tested on cowpea, R. solani was found to be pathogenic to cowpea seedlings causing damping off. Symptoms appeared about 7-10 days after inoculation as characteristic dark brown coloured lesions at the collar region resulting in the wilting of seedlings.

Cucumber

Among the pathogens tested, none was pathogenic to cucumber seedlings.

Rice

Of the various pathogens tested, R. solani alone produced symptoms on rice. The initial symptoms appeared as small brown spots about 7-10 days after inoculation which later enlarged. Typical sheath blight lesions with greyish white centre and pale brown margins were produced on the leaf sheath (Plate 16).

52

Tomato

Of the various pathogens tested, none was pathogenic to tomato seedlings.

Symptoms observed on various weed plants tested are as follows:-

Commelina benghalensis

C. gloeosporioides when inoculated on C. benghalensis caused leaf spots. It appeared as round spots with brown to reddish brown margin and light brown centre. The symptoms appeared one week after inoculation. The other pathogens tested did not produce symptoms on the weed (Plate 17).

Fimbristylis miliaceae

Among the various fungi tested, R. solani alone caused symptoms on the weed. symptoms were seen on the stem of the weed plant about 7-10 days after inoculation. Symptoms appeared as dark brown irregular patches on the stem of the weed (Plate 17).

A B

Hydrocotyl asiatica

Among the various fungi tested, C. gloeosporioides alone developed symptoms on the weed. Symptoms appeared about one week after inoculation. The symptoms were seen initially as small brown spots on the leaf lamina. These spots do not enlarge (Plate 18).

Ludwigia parviflora

Of the various fungi tested, C. gloeosporioides alone produced symptoms on leaves of the weed. One week after inoculation leaves showed small spots with dark reddish brown margins. Later the centre of the spots fall off leaving shot hole symptoms (Plate 17).

Monochoria vaginalis

Of the fungi tested, all the species of Fusarium semitectum, F. equiseti, F. solani and R. solani developed symptoms on the leaves of the weed. Blighting of the leaves was seen from the tip proceeding downwards on the leaf lamina. R. solani on inoculation caused blighting of the

Plate 20. Symptom produced by Rhizoctonia solani on Panicum repens

whole plant from tip of the leaves to the stem resulting in drying up of the whole plant (Plate 19).

Panicum repens

Of the various fungi inoculated in P. repens, R. solani, alone was found to be pathogenic to the weed. The symptoms appeared as oval shaped spots with cream to light brown colour, without any definite margin (Plate 20).

4.5 Selection of the promising fungal pathogens of water hyacinth

For this experiment Colletotrichum gloeosporioides, Curvularia lunata, Fusarium equiseti, F. semitectum, F. solani and Rhizoctonia solani were included. On statistical analysis of the intensity of infection it was found that among the pathogens of water hyacinth all the three species of Fusarium were found to have a higher intensity infection rates (Table 5) The per cent intensity of infection was highest in the case of F. semitectum being the 51.10. In the case of F. equiseti and F. solani there was 48.88 per

Table 5. Intensity of infection produced by inoculation of different fungal pathogens on water hyacinth.

Sl. No.	Fungal pathogens	Intensity of infection (in %)
1.	*Colletotrichum gloeosporioides*	44.44
2.	*Curvularia lunata*	20.00
3.	*Fusarium equiseti*	48.88
4.	*Fusarium semitectum*	51.10
5.	*Fusarium solani*	48.88
6.	*Rhizoctonia solani*	45.76

CD for treatments - 10.481

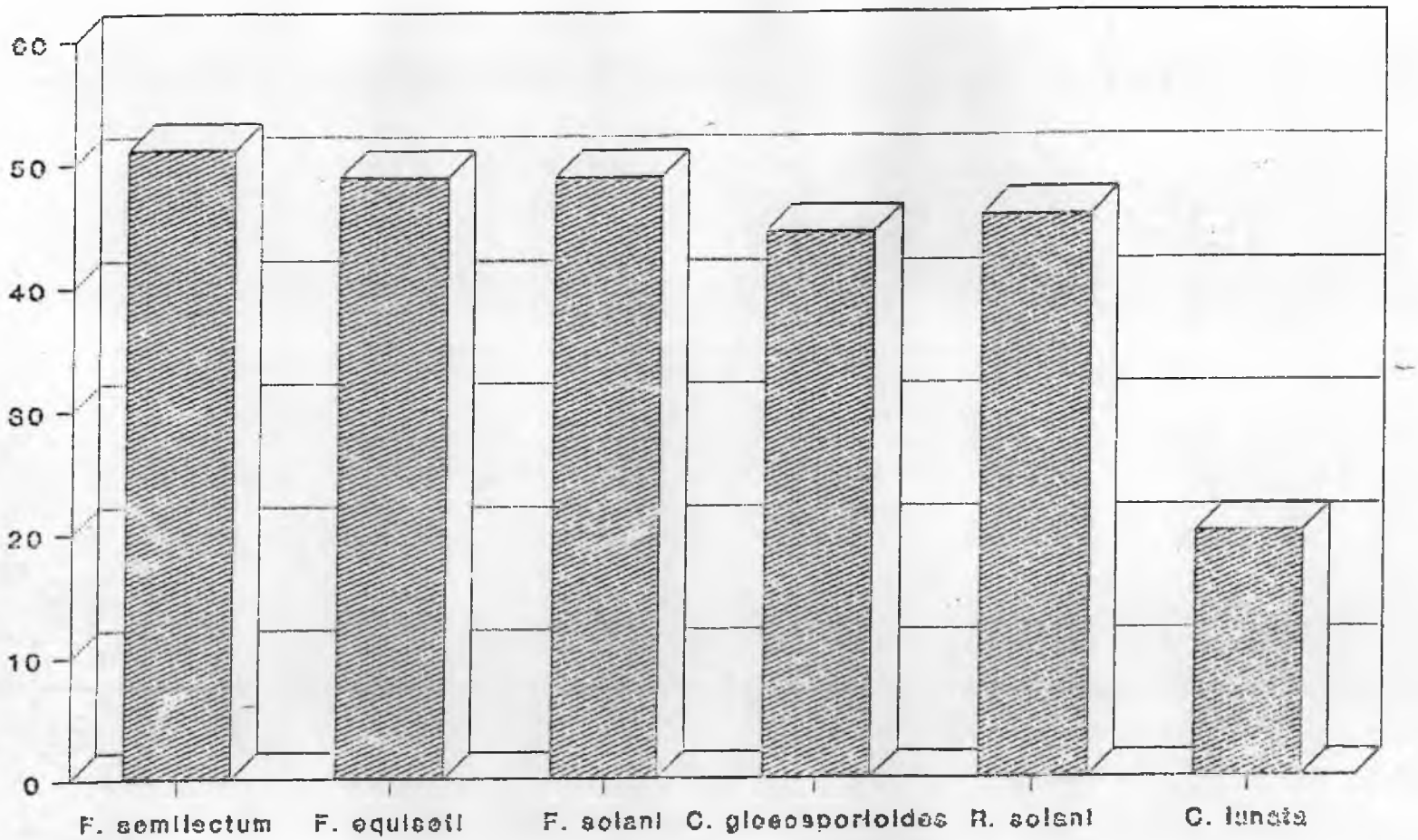


Fig. 3. Intensity of infection produced by different pathogens on water hyacinth

cent infection whereas R. solani showed 45.76 per cent infection. In the case of C. gloeosporioides the percentage intensity of infection was 44.44. C. lunata gave the lowest intensity of infection (20 per cent) (Table - 5, Fig - 3).

4.6 Quantity of inoculum of promising fungal pathogens required for effective destruction of water hyacinth

All the three isolates of Fusarium spp and Colletotrichum gloeosporioides were selected for further studies. Since Rhizoctonia solani was found to be pathogenic to many crop plants it was not included for further studies. Similarly since the symptoms produced by C. lunata was in the form of small isolated specks, it was not considered for further studies.

On statistical analysis of the intensity of infection caused by different spore concentrations of promising fungal pathogens, there was significant difference between the treatments (Table 6).

In the case of C. gloeosporioides. on statistical analysis of per cent intensity of infection it was observed

Table 6. Effect of different spore concentrations of promising fungal pathogens on water hyacinth .

Sl. No.	Promising pathogens	concentration (spores per ml)	Mean percent intensity of infection	CD for comparison
1.	Colletotrichum gloeosporioides	$2x10^3$	20.36 (4.59)	
		$2x10^6$	46.29 (6.89)	0.145
		$2x10^9$	59.26 (7.76)	
2.	Fusarium equiseti	$1x10^3$	22.22 (4.81)	
		$1x10^6$	48.89 (7.04)	0.164
		$1x10^9$	64.44 (8.09)	
3.	Fusarium semitectum	$1x10^3$	15.55 (3.98)	
		$1x10^6$	51.11 (7.19)	0.249
		$1x10^9$	64.44 (8.09)	
4.	Fusarium solani	$1x10^3$	15.55 (3.98)	
		$1x10^6$	53.33 (7.37)	0.207
		$1x10^9$	64.44 (8.09)	

* Figures in paranthesis indicate transformed values

Plate 21. Symptom produced by Colletotrichum gloeosporioides at concentration of 2×10^9 spores/ml.

Plate 22. Symptom produced by Fusarium semitectum at concentration of 1×10^9 spores/ml.

that there was significant difference between the spore concentrations tested. Among the three concentrations tested [(1) 2 x 10^3 spores/ml. (2) 2 x 10^6 spores/ml. (3) 2 x 10^9 spores/ml], the third concentration viz., 2 x 10^9 spores/ml was most effective followed by the second concentration ie 2 x 10^6 spores/ml. The first concentration of 2 x 10^3 spores/ml was the least effective (Table 6 & Plate 21).

On statistical analysis of the percentage intensity of infection produced by different spore concentrations of Fusarium equiseti, significant difference was found between the three concentrations tested, namely, (1) 1 x 10^3 spores/ml 2) 1 x 10^6 spores/ ml 3) 1x 10^9 spores /ml. Among the three concentrations tested, the third concentration ie 1 x 10^9 spores/ml was the most effective followed by the second concentration viz., 1 x 10^6 spores/ml. The first concentration ie 1 x 10^3 spores/ml was found to be the least effective (Table 6).

In the case of Fusarium semitectum, among the three spore concentrations tested viz., (1) 1 x 10^3 spores/ml, (2) 1 x 10^6 spores/ ml. (3) 1x 10^9 spores/ml, the third concentration (1 x 10^9 spores/ml) was found to be the most

effective one, followed by the second concentration ie 1 x 10^6 spores / ml. The first concentration ie 1 x 10^3 spores/ml was found to be the least effective (Plate 22).

In the case of Fusarium solani statistical analysis of the data revealed that there was significant difference between the concentrations tested. Among the three concentrations tested (1x10^3 spores/ml, 1 x 10^6 spores/ml and 1 x 10^9 spores/ml), the third concentration viz., 1 x 10^9 spores/ml was the most effective, followed by a concentration of 1 x 10^6 spores/ml. The first concentration of 1 x 10^3 spores/ml was the least effective (Table 6).

4.7 Mass multiplication and storage of inoculum in different carrier materials

An experiment was carried out to study the use of different carrier materials for storage of potential pathogens of water hyacinth. The spore count was taken at weekly intervals starting from one week after inoculation upto the seventh week, in the various carrier materials tried.

In rice bran, wheat bran and paddy straw visible mycelial growth of the fungi could be observed about three days after inoculation whereas in coir pith, the mycelial growth started only one week after inoculation. In rice bran and wheat bran inoculated with Colletotrichum gloeosporioides acervuli formation was noted about 2-3 weeks after inoculation. In peat moss neither mycelial growth nor sporulation of the fungi could be observed throughout the period of observation (7 weeks).

Colletotrichum gloeosporioides

On statistical analysis of the spore count of C. gloeosporioides in different carrier materials it was found that there was significant difference between the treatments. Coir pith yielded an average spore count of 23.31. In the first week the spore count was 22.27, in the second and third week respectively the spore count was 42.63 and 41.63. The spore count showed a decreasing trend being 20.17, 15.20, 10.73 and 10.50 in the fourth, fifth, sixth and seventh week respectively.

In paddy straw, an initial spore count of 17.07 was obtained in the first week and it increased to 32.67 in the

Table 7. Effect of different carrier materials on the sporulation of Colletotrichum gloeosporioides .

Sl. No.	Carrier material	* Mean spore count per microscopic field at weekly intervals							Mean
		1	2	3	4	5	6	7	
1.	Coir pith	22.27	42.63	41.63	20.17	15.20	10.73	10.50	23.31
2.	Paddy straw	17.07	32.67	24.67	18.57	15.83	14.60	12.43	19.40
3.	Peat moss	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4.	Rice bran	38.87	97.50	101.97	106.73	49.70	40.60	26.90	65.61
5.	Wheat bran	37.17	106.77	109.93	97.97	40.07	26.83	22.90	63.09

CD for treatments - 4.01

* Average of three replications

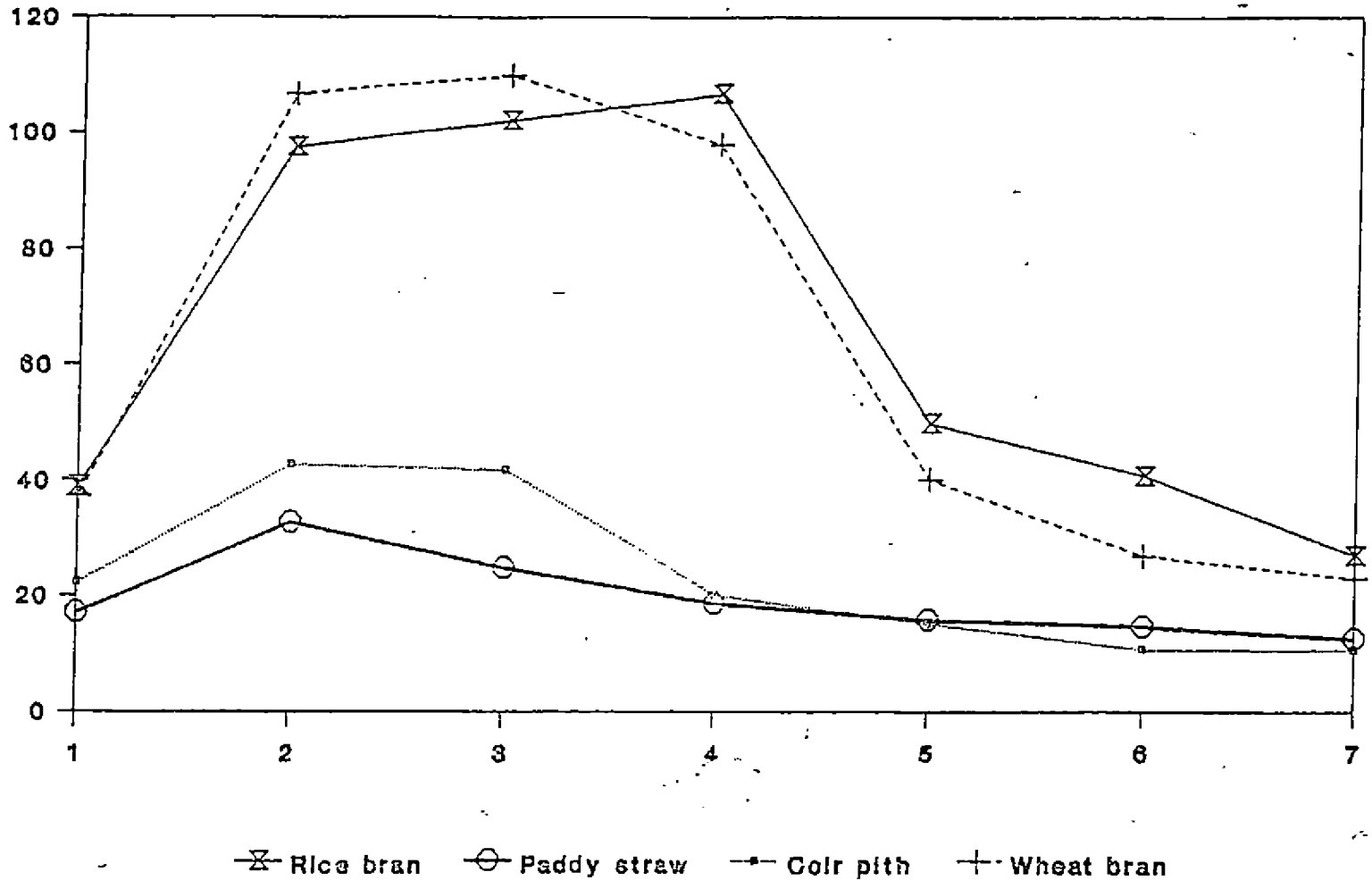


Fig. 4. Effect of different carrier materials on the sporulation of C. gloeosporioides

second week. From the third week onwards a decreasing trend in spore count was observed being 18.57, and reached 15.83 and 14.60 respectively in the fifth and sixth week. The final spore count was 12.43 in the seventh week of observation. And average spore count of 19.40 was obtained in paddy straw.

The average spore count in rice bran was 65.61 which was the highest among the carrier materials tested. In the first week, the spore count was 38.87 it increased to 97.50 in the second week and reached 101.97 in the third week A maximum spore count of 106.73 was obtained in the fourth week. In the fifth week, a sharp decline in spore count was observed being 49.70. It further decreased to 40.60 in the sixth week and reached 26.90 in the seventh week.

Wheat bran yielded an average spore count of 63.09. Here the initial spore count being 37.17. Then a steep increase in spore count to 106.77 and 109.93 in the second and third week respectively. From the fourth week onwards the spore count decreased being 97.97. It further decreased to 40.07, 26.83 and 22.90 in the fifth, sixth and seventh week respectively. (Table 7 & Fig. 4)

Table 8. Effect of different carrier materials on the sporulation of Fusarium equiseti,

Sl. No.	Carrier material	* Mean spore count per microscopic field at weekly intervals							Mean
		1	2	3	4	5	6	7	
1.	Coir pith	17.87	30.87	56.23	66.40	67.87	42.63	27.57	44.21
2.	Paddy straw	13.17	30.73	30.93	40.33	30.20	17.77	17.13	25.75
3.	Peat moss	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4.	Rice bran	42.90	84.03	100.93	106.60	47.70	35.17	26.47	63.40
5.	Wheat bran	33.70	82.77	93.17	104.17	65.13	51.93	43.37	67.73

CD for treatments - 4.002

* Average of three replications

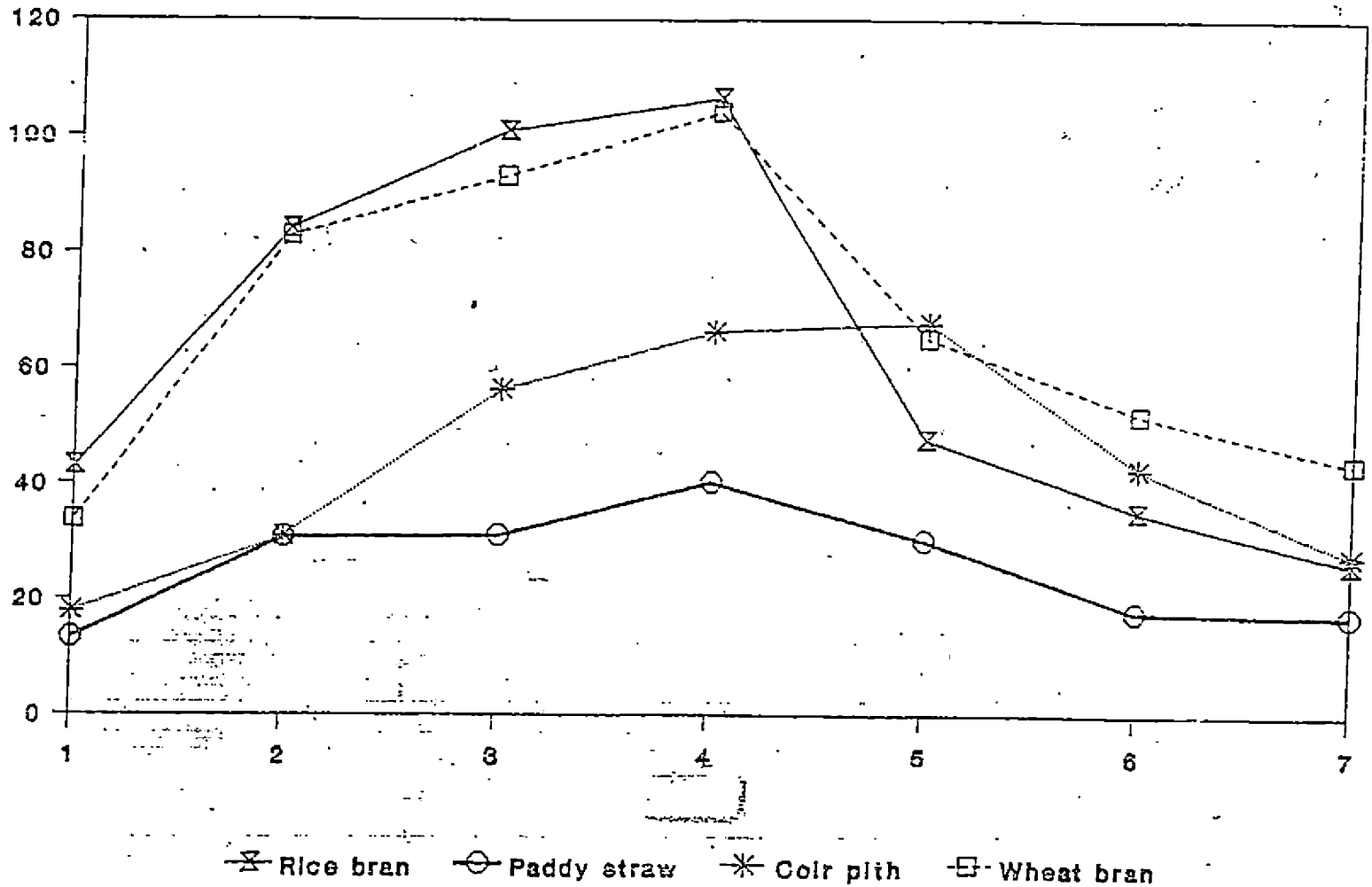


Fig. 5. Effect of different carrier materials on the sporulation of Fusarium equiseti

Fusarium equiseti

On statistical analysis of the spore count it was found that there was significant difference between the carrier materials tested for storage of Fusarium equiseti (Table 8 and Fig. 5).

In coir pith, during the first week of observation, the spore count being 17.87, it increased to 30.87 in the second week, and again increased to 56.23 in the third week. Maximum spore count of 66.4 and 67.87 were obtained in the fourth and fifth week respectively. the spore count decreased to 42.63 in the sixth week and reached 27.57 in the seventh week. The average spore count of F. equiseti in coir pith being 44.21.

In paddy straw, an initial spore count of 13.17, was observed in the first week. In the second week it reached 30.73 and remained stable in the third week. In the next week, it increased to 40.33 and showed a decreased spore count of 30.20 in the fifth week. Then it further decreased to 17.77 in the sixth week and remained so during the seventh week also. The average spore count of F. equiseti, was lowest in paddy straw being 25.75.

In the case of rice bran, the initial spore count was 42.90 and it increased to 84.03 in the second week of observation. In the next week, the spore count was 100.93 and it reached a maximum of 106.60 in the fourth week. Then it showed a steep decrease and reached 47.70 in the fifth week. It further decreased to 35.17 in the sixth week and finally reached 26.47 in the seventh week. The average spore count of F. equiseti in rice bran, being 63.40.

In wheat bran, an average spore count of 67.73 was obtained, which was the highest among the five carrier materials tested. The initial spore count of 33.70 was obtained in the first week whereas in the second and third week, the spore count increased to 82.77 and 93.17 respectively. In the fourth week a high spore count of 104.17 was obtained. In the fifth week it showed a decrease to 65.13 and reached 51.93 and 43.37 in the sixth and seventh week respectively.

Fusarium semitectum

Stastical analysis of the spore count of Fusarium semitectum in different carrier materials revealed that there

Table 9. Effect of different carrier materials on the sporulation of Fusarium semitectum.

Sl. No.	Carrier material	*Mean spore count per microscopic field at weekly intervals							Mean
		1	2	3	4	5	6	7	
1.	Coir pith	16.13	25.70	38.33	57.97	59.10	39.06	20.40	36.68
2.	Paddy straw	15.47	29.27	30.67	33.77	22.20	19.00	15.07	23.62
3.	Peat moss	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4.	Rice bran	33.90	86.30	91.93	93.40	31.90	31.30	23.17	56.71
5.	Wheat bran	33.57	83.20	93.77	105.40	68.47	50.36	37.57	67.48

CD for treatments - 4.139

* Average of three replications

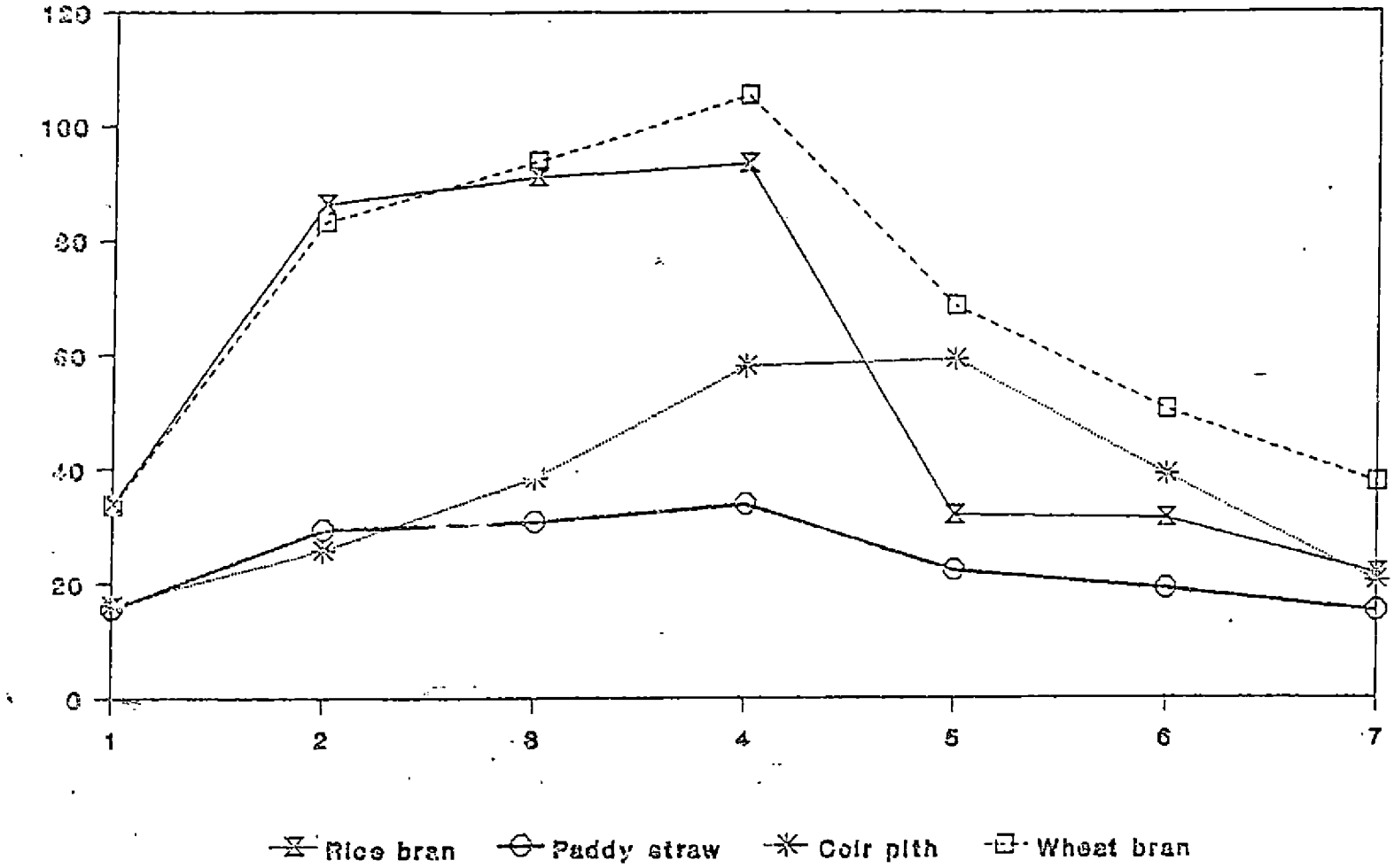


Fig. 6. Effect of different carrier materials on the sporulation of Fusarium semitectum

was significant difference between the treatments. (Table 9 and Fig. 6).

In coir pith, in the first week of observation yielded a low spore count of 16.13. It further increased to 38.33 in the third week. In the fourth week it was 57.87. The highest spore count was in the fifth week of observation being 59.10. From the sixth week onwards, the spore count showed a decreasing trend being 39.06 and 20.40 in the seventh week.

In paddy straw, the initial spore count of F. semitectum was very low, being 15.47, there was a gradual increase in spore count from the first to the fourth week, reaching 33.77. Then there was a gradual decrease in the spore count to 22.20 in the fifth week and reached 15.07 in the seventh week. Among the carrier materials tested, the spore count was lowest for paddy straw, the average being 23.62.

In the rice bran, the average spore count was 56.71. In the first week of observation, the spore count was 33.90, then there was a steep increase in the spore count to 86.30 in the second week. In the third week, it further

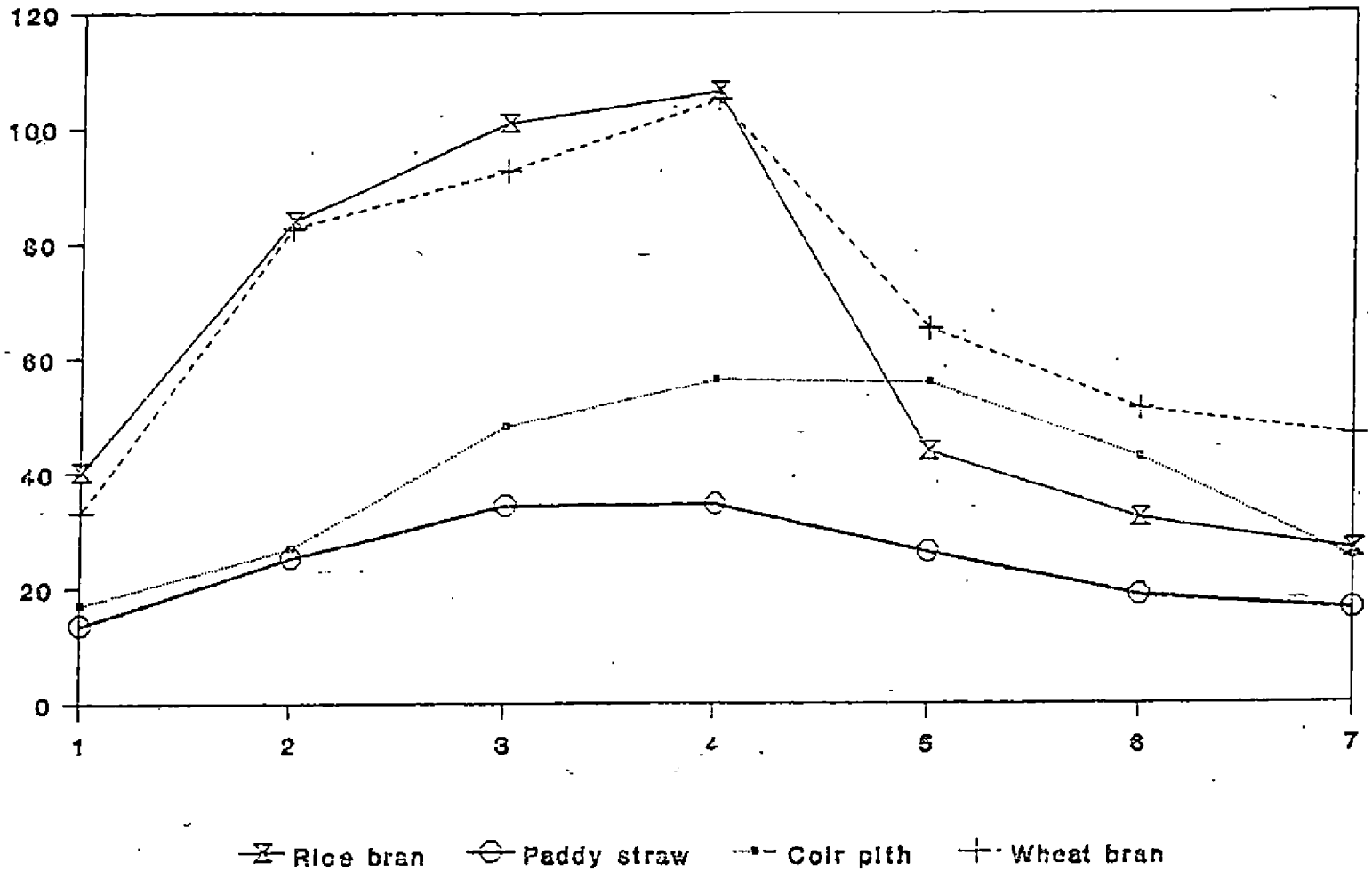


Fig. 7. Effect of different carrier materials on the sporulation of Fusarium solani

increased to 91.93 and remained stable in the fourth week (93.40). From fifth week onwards, there was a decline in the spore count from 31.90 to 23.17 in the seventh week.

Wheat bran yielded maximum spore count of F. semitectum, the average being 67.48. An initial spore count of 33.57 was obtained in the first week. It increased at a fast rate to 83.20 in the next week. The peak time of spore harvest was in the fourth week being 105.40. Then it decreased to 68.47 in the fifth week and reached 37.57 in the seventh week. The graph showed a steep rise and decline in the spore count in the case of wheat bran. The optimum time for spore harvest is the fourth week of observation.

Fusarium solani

Statistical analysis of the spore count revealed that there was significant diference between the carrier materials used for storage of Fusarium solani (Table 10 and Fig. 7).

In coir pith, an average spore count of 38.80 was obtained. In the first week the spore count was 17.07, it increased to 26.87 in the second week and reached 48.03 in

the third week. The highest spore count were obtained in the fourth and fifth week, being 56.27 and 55.67 respectively. In the sixth week it decreased to 42.67 and further decreased to 25.07 in the seventh week.

Paddy straw yielded lowest spore count of Fusarium solani the average spore count being 24.08. In the first week of observation, the spore count was 13.47, it increased to 25.23 in the second week and further increased to 34.27 in the third week and remained stable in the fourth week at 34.50. From the fifth week onwards a gradual decline in spore count was observed. In the fifth and sixth week, the spore count were 26.23 and 18.67 respectively, and reached 16.17 in the seventh week.

In the case of rice bran, the average spore count was 61.98. Here the initial spore count was 40.17 in the first week, which increased sharply to 83.97 in the second week, followed by 100.93 in the third week. A maximum spore count of 106.47 was obtained in the fourth week. In the fifth week of observation, there was a steep decline in the spore count of 43.70. Which again decreased to 32.17 in the sixth week and reached 26.47 in the seventh week.

On using wheat bran as carrier material the average spore count of Fusarium solani was 68.11, which was the highest among the carrier materials tested. Here the initial spore count was 33.67, followed by a sudden increase in the second week being 82.70 and further increased in the third week being 92.63. The spore count further increased to 105.13 in the fourth week of observation. In the fifth week the spore count decreased to 65.13 and a further decrease to 51.07 and 46.43 in the sixth and seventh week respectively.

4.8 Effect of different carrier materials on the viability of spores of the pathogens of water hyacinth

Effect of different carrier materials on the viability of the spores of potent pathogens of water hyacinth was tested. Observations on the number of spores germinated per microscopic field was taken from the fourteenth day of inoculation and the percentage spore viability was calculated.

Colletotrichum gloeosporioides

On statistical analysis of the per cent germination of C. gloeosporioides it was observed that among the carrier

Table 11. Effect of different carrier materials on the viability of spores of C. gloeosporioides .

Sl. No.	Carrier material	Percentage germination of spores at weekly intervals 1	2	3	4	Mean
1.	Coir pith	19.10 (4.37)	10.55 (3.25)	2.85 (1.69)	2.05 (1.43)	8.63 (2.68)
2.	Paddy straw	13.90 (3.73)	5.83 (2.413)	2.00 (1.41)	1.90 (1.38)	5.90 (2.23)
3	Rice bran	39.80 (6.31)	19.75 (4.444)	12.69 (3.569)	7.35 (2.713)	19.89 (4.256)
4.	Wheat bran	43.10 (6.57)	17.70 (4.21)	13.89 (3.73)	10.90 (3.30)	21.39 (4.45)

CD for Media week - 0.197

* Figures in paranthesis indicate transformed values

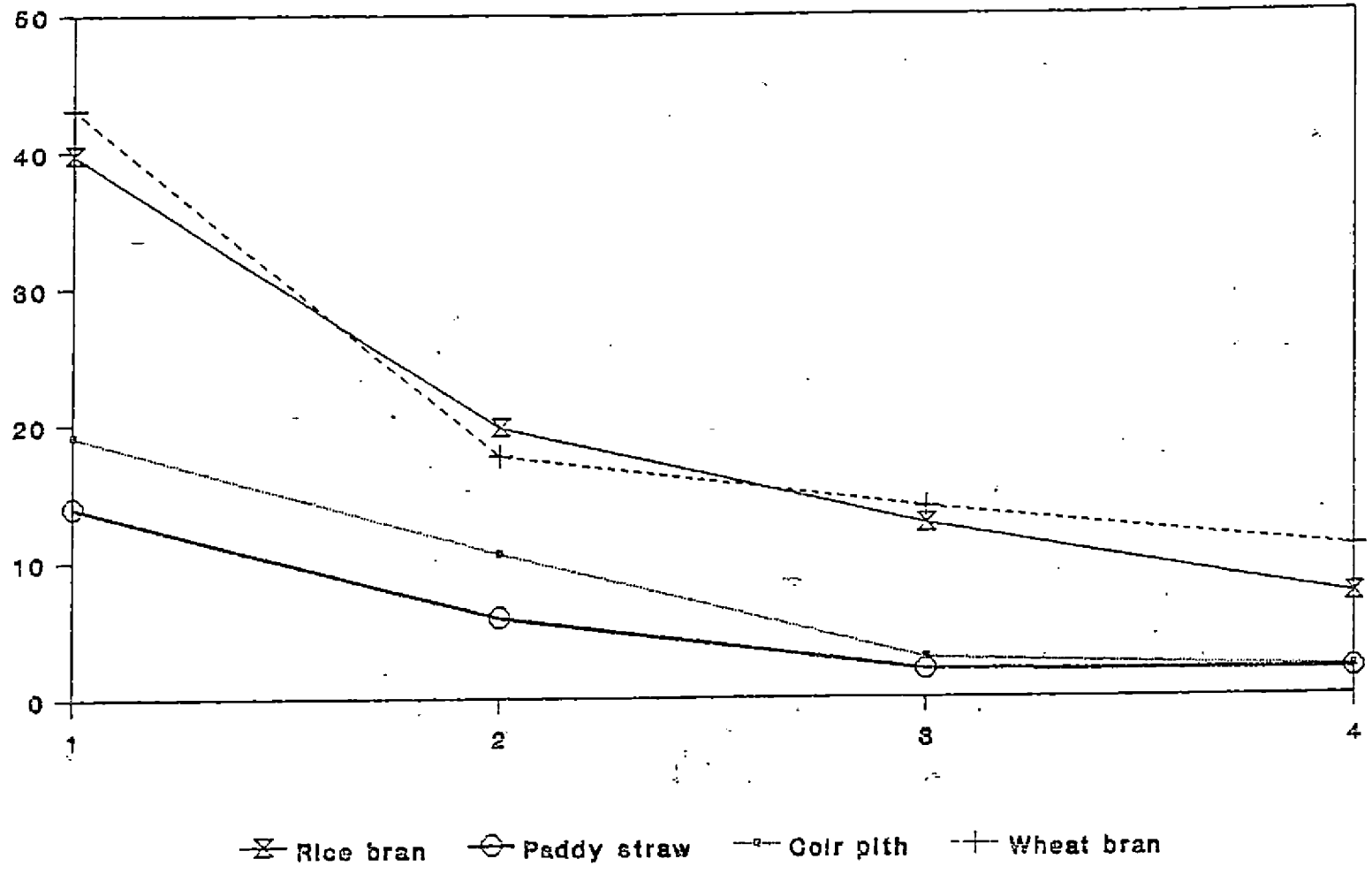


Fig. 8. Effect of different carrier materials on the viability of the spores of C. gloeosporioides

materials tested, wheat bran and rice bran were on par and gave maximum average per cent germination of spores, being 21.39 and 19.89 respectively (Table 11 and Fig. 8).

In coir pith the average per cent germination of spores of C. gloeosporioides being 8.63. During the first, second, third and fourth week of observation, the per cent germination of spores of C. gloeosporioides in coir pith were 19.10, 10.55, 2.85 and 2.05 respectively.

When paddy straw was used as carrier material, C. gloeosporioides had a germination percentage of 5.90. The initial germination percentage was 13.90 in the first week of observation, it decreased to 5.83 per cent in the second week. It further decreased to 2.00 per cent and 1.90 per cent in the third and fourth week of observation respectively.

In rice bran, the average germination percentage of C. gloeosporioides being 19.89. It was found that the per cent germination of spores of C. gloeosporioides stored in rice bran being 39.80, 19.75, 12.69 and 7.35 respectively during the first, second, third and fourth week of observation.

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Table 12. Effect of different carrier materials on the viability of spores of Fusarium equiseti.

Sl. No.	Carrier material	Percentage germination of spores at weekly intervals 1	2	3	4	Mean
1.	Coir pith	31.10 (5.60)	17.50 (4.19)	9.95 (3.15)	4.29 (2.07)	15.78 (3.75)
2.	Paddy straw	19.39 (4.40)	19.30 (4.28)	4.25 (2.06)	0 (0.00)	10.49 (2.68)
3.	Rice bran	48.85 (6.89)	48.59 (6.97)	46.00 (6.78)	8.77 (2.96)	38.05 (5.92)
4.	Wheat bran	50.20 (7.09)	29.60 (5.44)	19.75 (4.44)	4.15 (2.04)	25.92 (4.75)

CD value for Media week - 0.125

* Figures in paranthesis indicate transformed values

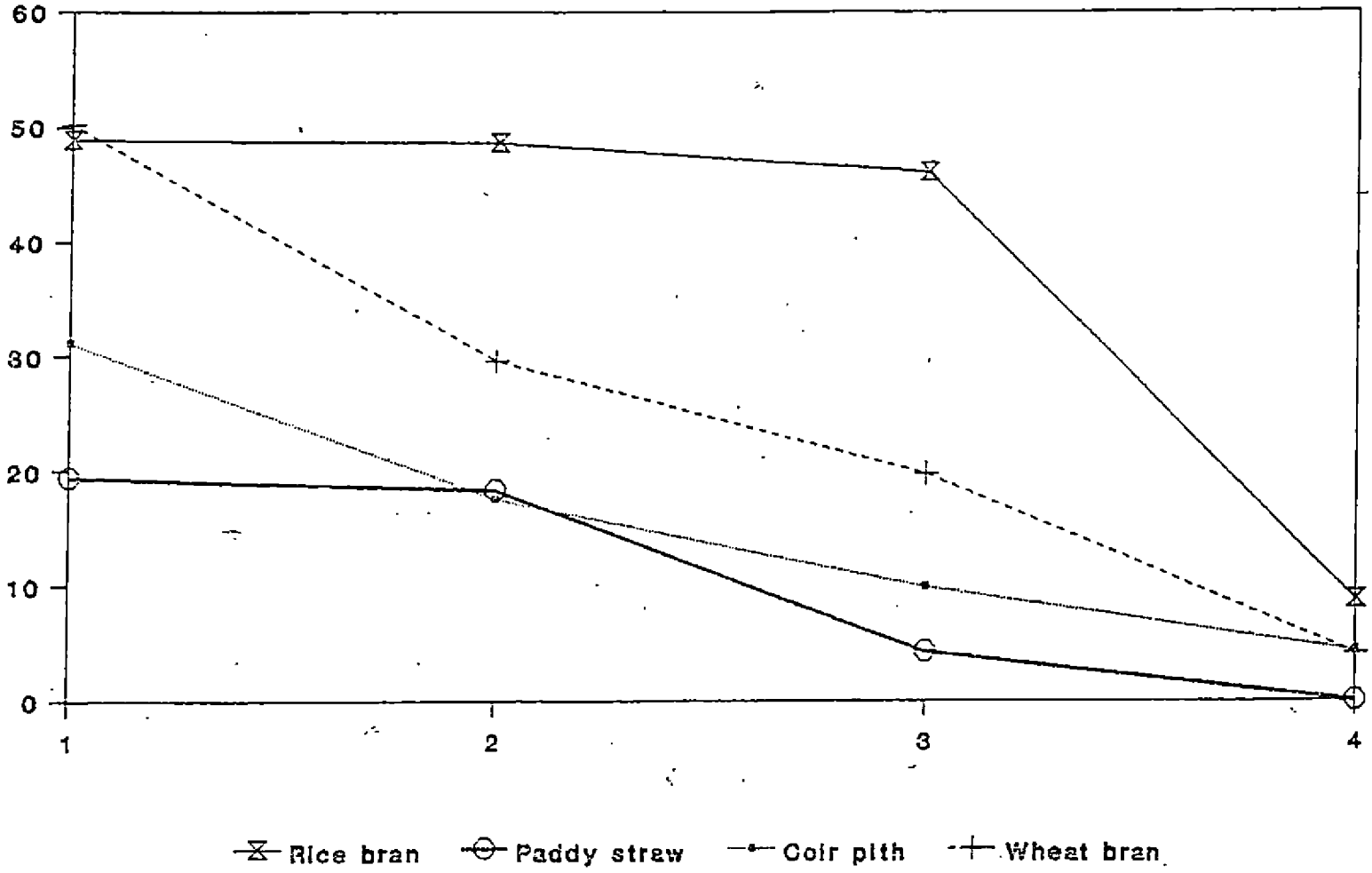


Fig. 9. Effect of different carrier materials on the viability of the spores of F. equiseti

In wheat bran, the average per cent germination of spores of C. gloeosporioides was 21.39. In the first week the per cent germination was 43.10. The per cent germination decreased to 17.70, 13.89 and 10.89 respectively in the second, third and fourth week respectively (Fig. 9).

Fusarium equiseti

On statistical analysis of the data, it was observed that there was significant difference between the per cent germination of spores in different carrier materials tested (Table 12, Fig. 9).

In coir pith an average per cent germination of 15.78 was obtained. The germination percentage of the spores of F. equiseti in coir pith was maximum in the first week of observation being 31.10 it decreased to 17.50 in the second week. The per cent germination was 9.95 and 4.29 respectively in the third and fourth week of observation.

When paddy straw was used, an average percentage germination of 10.49 was obtained. The per cent germination of spores of F. equiseti in paddy straw during the first,

Table 13. Effect of different carrier materials on the viability of spores of Fusarium semitectum.

Sl. No.	Carrier material	Percentage germination of spores at weekly intervals 1	2	3	4	Mean
1.	Coir pith	24.37 (4.94)	17.05 (4.13)	6.80 (2.68)	2.30 (1.51)	12.63 (3.29)
2.	Paddy straw	19.30 (4.39)	19.60 (4.43)	9.40 (3.07)	6.95 (2.63)	13.81 (3.63)
3.	Rice bran	53.15 (7.29)	52.35 (7.24)	15.90 (3.99)	4.51 (2.13)	31.72 (5.16)
4.	Wheat bran	47.27 (6.88)	31.33 (5.60)	22.58 (4.75)	15.54 (3.94)	29.16 (5.29)

CD for Media - week - 0.274

* Figures in paranthesis indicate transformed values

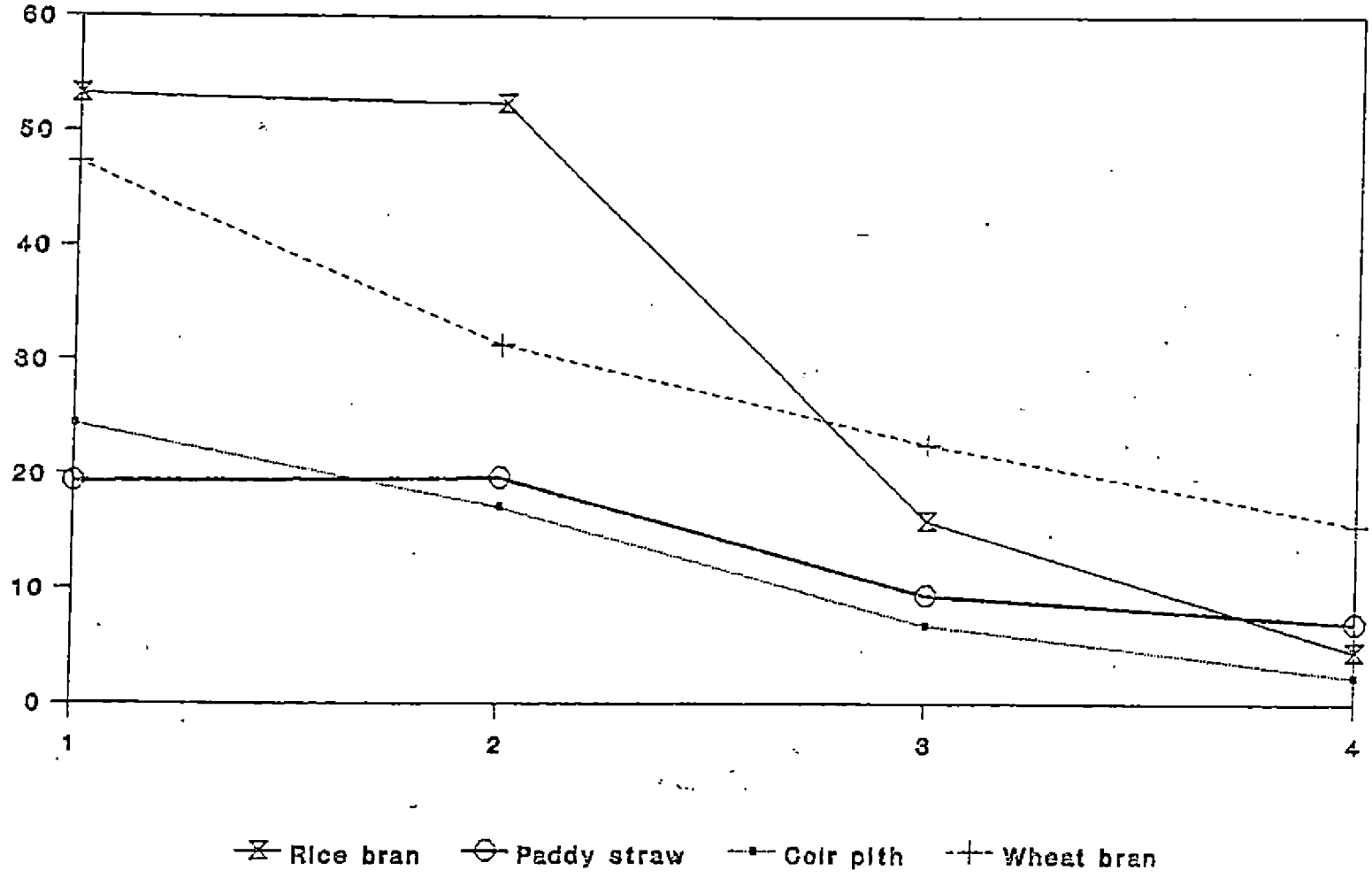


Fig. 10. Effect of different carrier materials on the viability of the spores of F. semitectum

second, third and fourth week of observation being 19.39, 19.30, 4.25 and zero respectively.

In rice bran, the average per cent germination of spores of F. equiseti being 38.05. During the first two weeks of observation, the per cent germination of spores remained constant being 48.85 and 48.59 respectively. In the third week a slight decrease in germination percentage to 46.00 was observed. In the fourth week, there was a steep decrease in the germination percentage to 8.77.

Wheat bran yielded an average germination per cent of 25.92. The per cent germination of F. equiseti in wheat bran being 50.20, 29.60, 19.75 and 4.15 in the first, second third and fourth week of observation respectively.

Fusarium semitectum

On statistical analysis of the per cent viability of spores of Fusarium semitectum in different carrier materials, it was observed that, rice bran and wheat bran were on par and were the best among the carrier materials tested. (Table 13 fig. 10).

When coir pith was used, it yielded an average germination percentge of 12.63. In the first week, the germination per cent was 24.37, it decreased to 17.05 in the second week and further decreased to 6.80 and 2.30 per cent in the third and fourth week respecticely.

In the case of paddy straw the germination percentage remained stable during first two weeks of observation being 19.30 and 19.60 respectively. In the third and fourth week of observations it reached to 9.40 and 6.95 per cent respectively. The average per cent viability of spores in paddy straw, being 13.81.

In the case of rice bran, the per cent germination was 53.15 and 52.35 in the first and second week respectively. In the third week it showed a sharp decline to 15.90 per cent and reached 4.51 in the fourth week. The average per cent germination of spores of F. semitectum in rice bran being 31.72.

Wheat bran yielded average per cent germination of spores of Fusarium semitectum of 29.16. In the first week the percentage germination was 47.27 and it decreased to 31.33 per cent in the second week. It reached 22.58 and 15.54 per cent in the third and fourth week respectively.

Fusarium solani

On statistical analysis of the data, it was observed that there was significant difference between the per cent germination of spores in different carrier materials. But rice bran and wheat bran were found equally effective ie. they were on par.

In coir pith, F. solani had an average per cent germination of 14.57. In the first two weeks of observation the percentage of germination were 19.85 and 21.49 respectively. Then it decreased to 12.25 in the third week and further decreased to 4.70 during the fourth week of observation.

In paddy straw an average germination percentage of 10.21 was obtained. During the first, second, third and forth week of observation, the germination percentage of F. solani in paddy straw being 17.75, 18.40, 4.70 and zero respectively.

In the case of rice bran, the average per cent germination was 30.44. In the first week of observation, the per cent germination was 39.75 and increased to 54.35 in the second week. It further decreased to 19.75 per cent in the

Table 14. Effect of different carrier materials on the viability of spores of Fusarium solani .

Sl. No.	Carrier material	Percentage germination of spores at weekly intervals 1	2	3	4	Mean
1.	Coir pith	19.85 (4.46)	21.49 (4.64)	12.25 (3.50)	4.70 (2.17)	14.57 (3.70)
2.	Paddy straw	17.75 (4.21)	18.40 (4.29)	4.70 (2.17)	0 (0.00)	10.21 (2.67)
3.	Rice bran	39.75 (6.30)	54.35 (7.37)	19.75 (4.44)	7.89 (2.81)	30.44 (5.23)
4.	Wheat bran	48.80 (6.99)	33.40 (5.78)	21.00 (4.58)	17.50 (4.18)	29.94 (5.38)

CD for Media week - 0.274

* Figures in paranthesis indicate transformed values

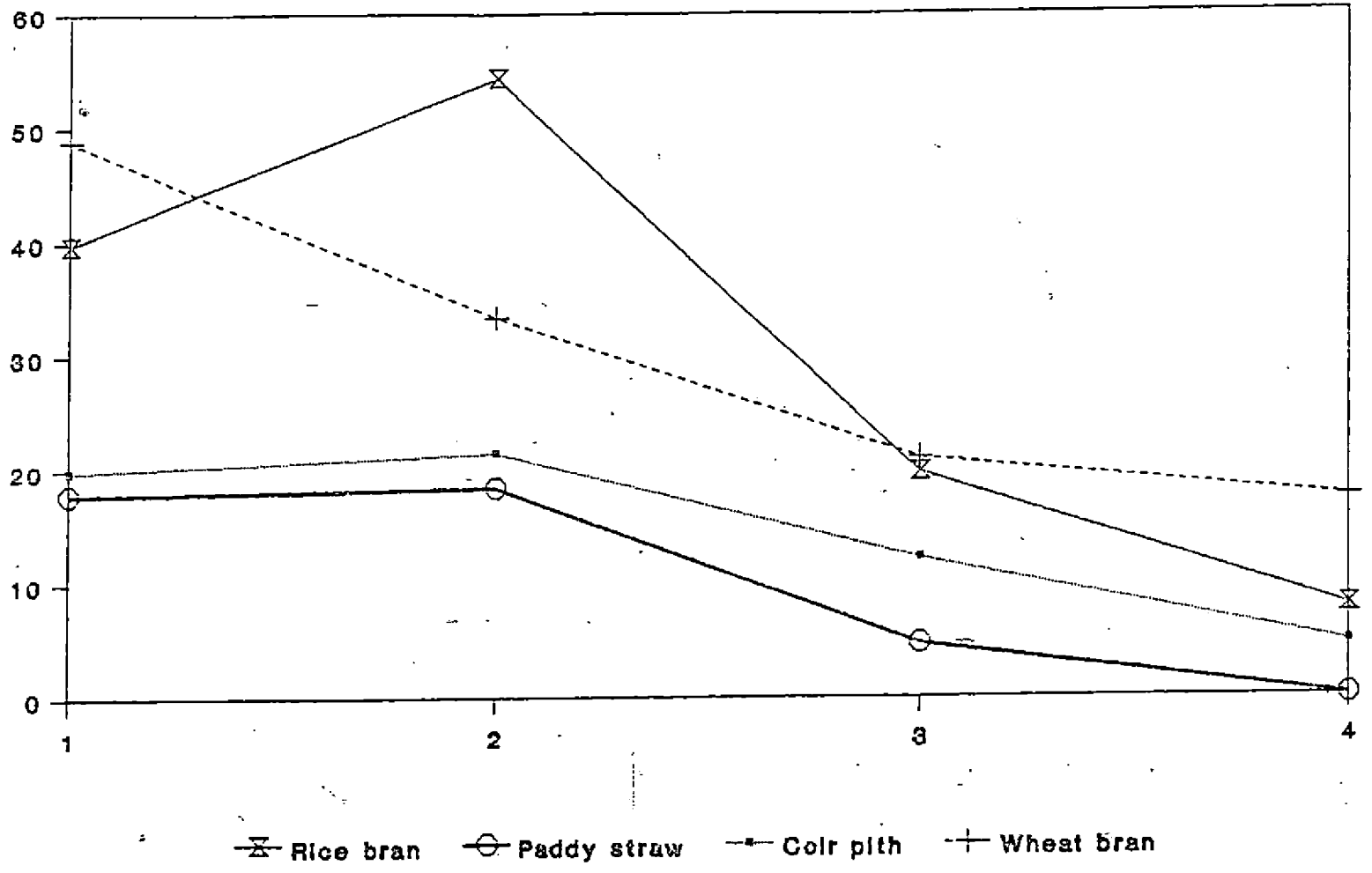


Fig. 11. Effect of different carrier materials on the viability of the spores of F. solani

third week and reached 7.89 per cent in the fourth week. (Table 14 and Fig 11).

The average per cent germination of F. solani in wheat bran being 29.94. In the first week of observation, the per cent germination was 48.80. In second week it decreased to 33.40 per cent, then to 21.00 per cent on the third week. During the fourth week of observation the germination percentage was 17.50.

4.9. Field application

A pot culture experiment was laid out to evaluate the field performance of promising fungal pathogens of water hyacinth in different carrier materials viz. coir pith, rice bran and wheat bran. Three methods of application were tested viz. 1). Dusting the inoculum uniformly @ 5 g/pot. 2). By placing bits of inoculum on leaves and stem. 3). By spraying the inoculum. Of the three methods of application of inoculum tried, in the case of methods 2 and 3 viz., placing bits of inoculum on leaves and stem and by spraying the inoculum on the plants, symptoms were observed 12-14 days after inoculation in the case of C. gloeosporioides, Fusarium

Table 15. Field performance of promising fungal pathogens of water hyacinth in different carrier materials.

Sl. No.	Carrier material	Fungi	Method of application: Dusting	placing bits of inoculum	spraying
1.	Coir pith	C. gloeosporioides	-	+	+
		F. equiseti	-	+	+
		F. semitectum	-	+	+
		F. solani	-	+	+
2.	Rice bran	C. gloeosporioides	+	++	++
		F. equiseti	+	++	++
		F. semitectum	+	++	++
		F. solani	+	++	++
3.	Wheat bran	C. gloeosporioides	+	++	++
		F. equiseti	+	++	++
		F. semitectum	+	++	++
		F. solani	+	++	++

\+ Poor symptom development

++ Good symptom development

\- No symptom

Table 16. Symptom produced by culture filtrates of *Fusarium* spp. on water hyacinth.

Sl. No.	Pathogen	Time taken for symptom development	Symptom developed
1.	*F. equiseti*	7-10 days	Small brown spots with yellow halo towards the margin of the leaves, later these spots enlarge and spread downwards
2.	*F. semitectum*	7-10 days	Small brown spots with yellow halo towards the margin of the leaves, later these spots enlarge and spread downwards
3.	*F. solani*	7-10 days	Small brown spots with yellow halo towards the margin of the leaves, later these spots enlarge and spread downwards

Plate 23. Symptom produced on water hyacinth by toxin from *F. equiseti*.

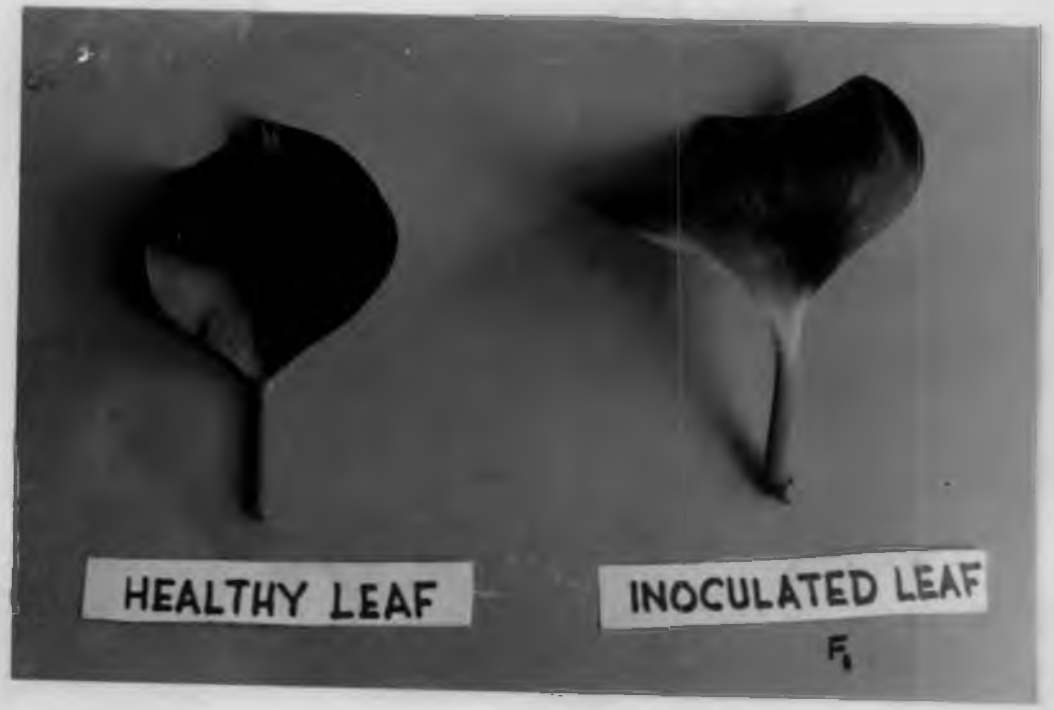
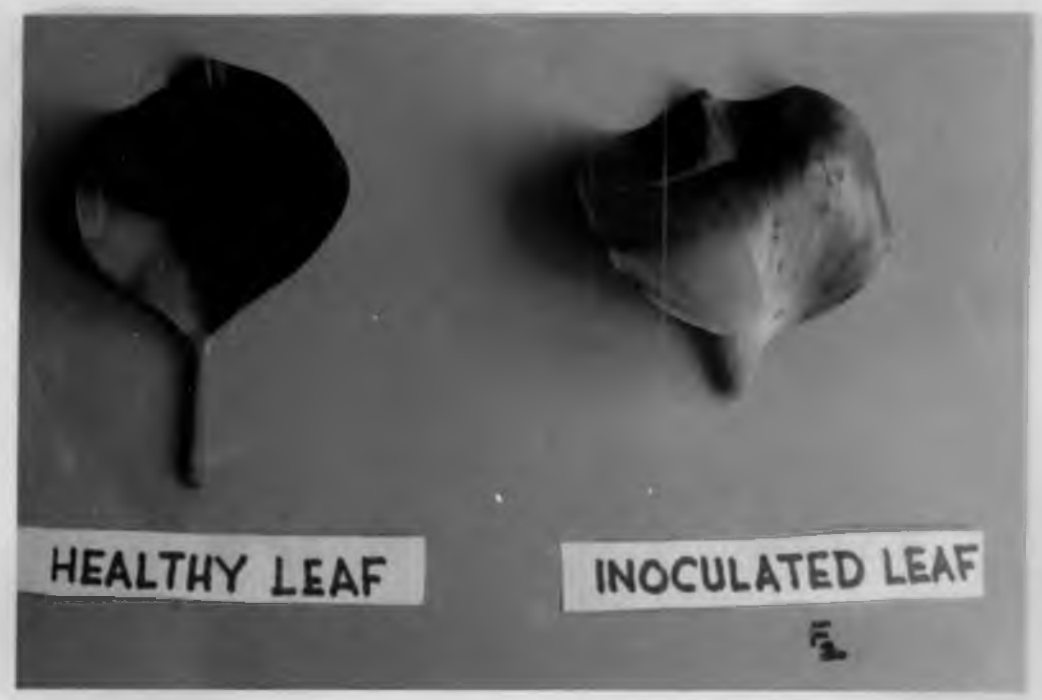

HEALTHY LEAF
INOCULATED LEAF
F1

Plate 24. Symptom produced on water hyacinth by toxin from *F. semitectum*

Plate 25. Symptom produced on water hyacinth by toxin from *F. solani*


HEALTHY LEAF
INOCULATED LEAF
F1


HEALTHY LEAF
INOCULATED LEAF
F2

equiseti, F. semitectum and F. solani. Whereas in the first method ie. dusting the inoculum, the symptom development was poor, only small chlorotic specks were observed. In the case of coir pith, the symptom development was poor or negligible compared to the other two carrier materials viz., rice bran and wheat bran (Table 15).

4.10. Toxin production by Fusarium spp.

The culture filtrates of the three isolates of Fusarium spp. pathogenic to water hyacinth viz. F. equiseti, F. semitectum, F. solani were sprayed on healthy water hyacinth plants. Culture filtrates of all the three isolates of Fusarium spp. produced symptoms within 7-10 days of spraying as small brown spots with a characteristic yellow halo towards the margin of the leaves (Table -16 & Plates 23, 24 and 25).

DISCUSSION

5. DISCUSSION

The survey of fungal pathogens of water hyacinth in Trivandrum district in two seasons revealed the presence of seven fungi viz. Colletotrichum gloeosporioides (Penzig) Penzig and Sacc, Curvularia lunata (Wakker) Boedjin, F. equiseti Berk and Rav, F. semitectum (Corda) Sacc, F. solani (Mart) Sacc, Rhizoctonia solani Kuhn and sterile fungus.

A perusal of literature revealed that the following fungi were reported on the weed viz., Curvularia lunata (wakker) Boedjin, (Rahim and Tawfig, 1984), F. equiseti (corda) sacc, (Agharkar and Banerjee 1932), F. solani (Mart) sacc, (Jamil et al., 1984), F. moniliformae Shelden and a Fusarium sp Berk and Rav (Rahim and Tawfig, 1984), F. oxysporum and F. semitectum (Jamil and Rajagopal, 1986), F. chlamydosporium Wollenw and Reinking (Aneja et al., 1990), Rhizoctonia solani Kuhn (Rakvidyasastra and Visarathanonth, 1975) and a sterile fungus was reported by Jamil and Rajagopal (1986). Of the various fungi isolated from water hyacinth Colletotrichum gloeosporioides is a new record.

In regard to the occurrence of different fungi on water hyacinth it was noticed that all the three Fusarium spp. viz., F. equiseti, F. semitectum and F. solani were present throughout the year, whereas, Curvularia lunata was prevalent during the summer season only. Colletotrichum gloeosporioides and Rhizoctonia solani were isolated during the rainy season, whereas, the sterile fungus was present during the summer and rainy season, but the frequency of occurrence was less. Work conducted by Jamil and Rajagopal (1986) revealed that species of Fusarium, Alternaria and Helminthosporium appeared in the winter season, Aspergillus, Penicilluim and sterile fungus were associated on the leaves of water hyacinth in the early days of summer only. In the present study, the presence of Fusarium spp. throughout the period of observation lead to the conclusion that this pathogen can survive in the off season and make its presence during the rainy season, when the host plants have a thick vegetative growth. Also this fungus has the capacity to thrive in moist condition for a long time. Observations indicate that rainfall is the most important factor affecting the natural occurrence of Colletotrichum gloeosporioides and R. solani. Butler (1951) also observed that anthracnose of

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Bathurst burr (Xanthium spinosum L.) caused by Colletotrichum xanthii Halst was present during the rainy season.

Eventhough Galbraith (1987) reported that feeding by the weevil, Neochetina eichhorniae Warner increased infection by Acremonium zonatum in water hyacinth, in the present study no incidence of the weevil was recorded on the weed.

The pathogenicity tests revealed that all the fungi isolated were pathogenic to water hyacinth. Colletotrichum gloeosporioides produced small dark brown spots with yellow halo which later enlarged to form irregular patches. Curvularia lunata produced black pin head sized spots on the leaf lamina which do not enlarge as it becomes old. Jamil and Rajagopal (1986) observed that Curvularia spp. produced yellow spots which later caused drying up of the leaves.

All the three species of Fusarium were found to cause similar symptoms of blighting of leaves within 7-10 days of inoculation. Rahim and Tawfig (1984) found that Fusarium equiseti could cause severe damage to water hyacinth leaves. According to Aneja et al. (1990) the symptom produced by Fusarium chlamydosporium was leaf spots with ash

coloured centre later becoming irregular shaped. In the present study, when water hyacinth plants were inoculated with culture filtrates of Fusarium spp. they produced the similar symptoms as those obtained by placing the bits of inoculum.

In the case of Rhizoctonia solani irregular straw coloured spots with brown margin on the leaves and leaf stalk was produced. The sterile fungus caused light brown coloured spots with yellow halo on the leaves of the weed. Jamil and Rajagopal (1986) found that the sterile fungus caused similar symptom on the leaf and petioles of water hyacinth.

Host range studies were conducted with twelve plants (Six crop plants and six weed plants) to investigate the host range of fungi pathogenic to water hyacinth. It was observed that Fusarium spp. could not infect any of the cultivated plants tested, whereas, it was pathogenic to Monochoria vaginalis only. The host range of Fusarium 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. (Rahim and Tawfig, 1984).

In the present study, of the various plants tested Colletotrichum gloeosporioides was found to be pathogenic on chillies, Commelina benghalensis, Hydrocotyl asiatica and Ludwigia parviflora. Boyette et al. (1979) had reported the fungus Colletotrichum gloeosporioides f. sp. jussiaeae from Jussiaeae (Ludwigia) decurrens (Walt) Dc. Rhizoctonia solani was found to infect the maximum number of crop plants included in the study viz., amaranthus, cowpea and rice. Among the weed plants, Fimbristylis miliaceae, Monochoria vaginalis and Panicum repens were found suspectable to R. solani.

The present host range study revealed that C. lunata and the sterile fungus were non pathogenic to the test plants.

The lack of cross infectivity of Fusarium spp. to cultivated plants and other weeds except Monochoria vaginalis demonstrate the specificity of the fungus. Therefore the use of Fusarium spp. as a biological control agent for water hyacinth would not expect to create problems for the plants grown in our ecosystem.

The experiment conducted to measure the percentage intensity of infection produced by the pathogens of water hyacinth viz., Colletotrichum gloeosporioides, Curvularia lunata, Fusarium semitectum, Fusarium equiseti, Fusarium solani and Rhizoctonia solani revealed that all the three species of Fusarium gave higher rate of diseases intensity. Further the narrow host range of Fusarium spp. seems to qualify better as a biocontrol agent. The per cent intensity of infection by C. lunata was the least. Eventhough R. solani gave a high per cent intensity of infection, it was found to be pathogenic to the common crop plants especially rice, cowpea and amaranthus. So the wide host specificity of R. solani limits its practical use as a biocontrol agent. Eventhough C. lunata was not pathogenic to all the test plants it was not considered as an efficient biocontrol agent because the intensity of infection produced by this fungus was very low. It caused only isolated small pin head spots, on the leaf lamina which remain as such without causing any further damage.

In the experiment carried out to fix the quantity of inoculum of promising fungal pathogens of water hyacinth for effective destruction of the weed, it was found that for

F. equiseti, F. semitectum and F. solani, the spore concentration of 1 x 10^9 spores per ml was most effective causing 64.44 per cent intensity of infection. In the case of Colletotrichum gloeosporioides 2 x 10^9 spores per ml gave the highest per cent intensity of infection ie 59 per cent. In similar work conducted by Boyette et al. (1979), it was reported that spore concentrations of 5 x 10^5, 1 x 10^6, 2 x 10^6 spores per ml of Colletotrichum gloeosporioides f. sp. jussiaeae killed 67, 87 and 100 per cent of the weed water primrose respectively. Lakshmanan et al. (1991) also reported that 5 x 10^6 spores per ml of Cochliobolus carbonum gave 98 per cent control of Euphorbia geniculata.

Among the different carrier materials tried to store the promising pathogens of water hyacinth, wheat bran was found to be the best substrate for Fusarium equiseti, F. semitectum and F. solani. Rice bran was the second best for all the three fungi. In the case of all the three fungi, the spore count first showed an increasing trend till the fourth week in both the carrier marterials. After the fourth week, the spore count showed a decreasing trend till the seventh week. In paddy straw also the increase in spore count was observed till the fourth week and then the spore count

started decreasing. In coir pith, the increasing trend was observed till the fifth week for Fusarium equiseti and F. semitectum, whereas, for F. solani the increase in spore count was observed till the fourth week only. From the present study it can be concluded that wheat bran is the best substrate for effective sporulation of all the three Fusarium spp.

Effect of different carrier materials on the viability of the spores of F. equiseti, F. semitectum and F. solani revealed that, in case of F. semitectum and F. solani wheat bran had highest average germination percentage followed by rice bran, paddy straw and coir pith respectively. Spores of F. equiseti had highest average germination percentage in rice bran followed by wheat bran, paddy straw and coir pith respectively. In all the four carrier materials, the viability of spores of the three Fusarium spp. showed a decreasing trend with increase in storage time. The viability of spores decreased from the first to fourth week of observation.

For Colletotrichum gloeosporioides rice bran gave highest average spore count, the second highest being in

wheat bran. The lowest average spore count was in paddy straw. In rice bran and wheat bran increasing trend in spore count was observed till the fourth week, after this the decreasing trend started and continued till the seventh week. In paddy straw and coir pith, the increasing trend in spore count was observed till the third week. The spore count showed a decrease from the fourth week onwards. In the case of viability of C. gloeosporioides spores, it was the highest in wheat bran. Wheat bran and rice bran were on par. A decreasing trend was seen in the per cent viability from the first to the fourth week of observation in all the carrier materials tested. So from the present study it was found that wheat bran and rice bran were best carrier materials for Fusarium spp. and Colletotrichum gloeosporioides. Moreover bran inoculum are lighter in weight and can be easily distributed in the field. The best time for the harvest of spores is about two weeks after inoculation.. Morris (1989) reported that Colletotrichum gloeosporioides (Penz) Sacc when cultured on wheat bran inoculum the spores remained viable for sixteen days.

Different methods of application of the fungi (C. gloeosporioides, F. equiseti, F. semitectum and F. solani)

were tried in the field. Placing bits of inoculum of the fungi on the plants and spraying of the inoculum of the fungi were found to be equally effective. Good symptom development was observed in both the methods after 7-10 days of inoculation. When the inoculum was dusted on the plant parts @ 5 g/pot, the symptom development was very poor, as chlorotic specks only. The field performance of the inoculum of the fungi in different carrier materials showed that, symptom development was good in the case of rice bran and wheat bran. But the symptom produced by applying coir pith inoculum was poor. Morris (1989) when tested spraying of wheat bran inoculum of C. gloeosporioides @ 10 kg/ha on Hakea serica seedlings obtained good mortality rates of the weed.

Plant pathogens produce a variety of substances toxic to plants, several of which have been identified (Scheffer and Yoder, 1972). The production of an apparently narrow host spectrum phytotoxin by Alternaria eichhorniae was demonstrated by Nagraj and Ponnappa (1970). In the present investigation all the three species of Fusarium viz. F. equiseti, F. semitectum and F. solani were tested for toxin production by spraying the culture filtrates of the

fungi on healthy water hyacinth plants. It was observed that all the three species of Fusarium produced similar symptoms as those developed by inoculating the culture bits of the fungi. Rahim and Tawfig (1984) found that culture filtrates of Acremonium zonatum, Fusarium equiseti, Phoma sorghi and and Bacillus sp were toxic to water hyacinth.

SUMMARY

6. SUMMARY

A survey of fungal pathogens of water hyacinth in three localities of Trivandrum district viz., Veli, Ambalathara and Akulam yielded the following fungi Colletotrichum gloeosporioides (penzig) Penzig and sacc., Curvularia lunata (Wakker) Boedjin, Fusarium equiseti (corda) sacc., Fusarium semitectum Berk and Rav, Fusarium solani (Mart) sacc., Rhizoctonia solani Kuhn and a Sterile fungus. Of the fungi reported on water hyacinth C. gloeosporioides is a new record. F. equiseti, F. semitectum and F. solani were present throughout the period of survey whereas C. gloeosporioides and R. solani were present only in the rainy season. C. lunata was isolated during the summer season only.

The pathogenicity of all the fungi was proved by artificial inoculation. The host range of these fungi was tested with six cultivated plants and six weed plants. R. solani was found to infect maximum number of crop plants viz., amaranthus, cowpea and rice. Among the weeds tested R.

solani was pathogenic to Monochoria vaginalis and Panicum repens. C. gloeosporioides was pathogenic to chilli only. Among the weed plants it infected Commelina benghalensis, Hydrocotyl asiatica and Ludwigia parviflora. F. equiseti, F. semitectum and F. solani were found to infect Monochoria vaginalis only.

The experiment conducted to select the promising fungal pathogens of water hyacinth revealed that Fusarium semitectum caused highest intensity of infection of 51.10 per cent followed by F. equiseti and F. solani at 48.88 per cent. In the case of C. gloeosporioides the per cent intensity of infection was 44.44 per cent and for R. solani it was 45.76. Curvularia lunata gave the lowest intensity of infection of 20 per cent.

All the three isolates of Fusarium and C. gloeosporioides were selected for further studies, to fix the quantity of inoculum of these pathogens required for effective destruction of water hyacinth. As R. solani was found to infect many crop plants it was not included for this experiment. Curvularia lunata caused only very low intensity of infection so it was also avoided for further

studies. For F. equiseti, F. semitectum and F. solani the spore concentration of 1 x 10^9 spores/ml was the most effective one causing maximum intensity of infection. A spore concentration of 2 x 10^9 spores/ml of C. gloeosporioides caused maximum intensity of infection on water hyacinth leaves. Of the various carrier materials (coir pith, paddy straw, peat moss, rice bran, wheat bran) used for mass multiplication and storage of the fungal pathogens wheat bran was found to be the best substrate for F. equiseti, F. semitectum and F. solani. In the case of all the three fungi, the spore count first showed an increasing trend till the fourth week after inoculation. After the fourth week, the spore count decreased. The viability of spores of F. equiseti, F. semitectum and F. solani was highest in wheat bran followed by rice bran, paddy straw and coir pith. For C. gloeosporioides rice bran gave the highest average spore count, followed by wheat bran, coir pith and paddy straw respectively. The spore count showed an increasing trend till the fourth week. In the case of viability of spores of C. gloeosporioides it was the highest in wheat bran. Wheat bran and rice bran were on par. In peat moss none of the fungi was found to grow.

Different methods of application of the fungi viz., C. gloeosporioides, F. equiseti, F. semitectum and F. solani were tested in the field. Placing bits of inoculum of the fungi on the plants and spraying of inoculum of the fungi were found to be the most effective methods. The field performance of the inoculum of the fungi in different carrier materials (coir pith, rice bran and wheat bran) showed that symptom development was good in the case of rice bran and wheat bran inoculum, but the symptom produced by coir pith inoculum was poor.

In the present study, F. equiseti, F. semitectum and F. solani were found to produce toxins which produced symptoms similar to those caused by inoculation of the culture bits.

The following conclusions can be made from the above study. Fusarium equiseti, F. semitectum and F. solani were the major pathogens of water hyacinth in and around Trivandrum district. These three fungi were found to be present throughout the period of study. This lead to the conclusion that these pathogens can survive in the off season and make their presence during the rainy season, when the host

plant has thick vegetative growth. The narrow host range of Fusarium spp. and their high rates of intensity of infection qualify it as a good biocontrol agent of water hyacinth. For mass multiplication and storage of the Fusarium spp. and Colletotrichum gloeosporioides wheat bran and rice bran were the best suited carrier materials. The optimum time for harvest of spores is about two weeks after inoculation.

APPENDIX

APPENDIX – I

Composition of Media

1. Potato Dextrose Agar

Peeled potatoes	–	200 g
Agar	–	12 g
Dextrose	–	10 g
Water	–	1 litre

2. Czapek's (Dox) Agar

Sucrose	–	30 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	–	15 g or 20 g
Distilled water	–	1 litre

3. Oatmeal Agar

Oatmeal	–	30 g
Agar	–	20 g
Water	–	1 litre

4. CMC medium used for sporulation of Fusarium graminearum

Carboxy methyl cellulose	–	15 g
Ammonium nitrate	–	1 g
Potassium Dihydrogen phosphate	–	1 g
Magnesium sulphate	–	0.5 g
Yeast extract	–	1 g
Distilled water	–	1 litre

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* Orginals not seen

SCREENING OF FUNGAL PATHOGENS FOR BIOCONTROL OF WATER HYACINTH (*EICHHORNIA CRASSIPES* (MART) SOLMS)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

A survey was conducted in and around Trivandrum district viz., in Veli, Ambalathara and Akulam to obtain the fungal pathogens of water hyacinth for its biocontrol. Colletotrichum gloeosporioides (Penzig) Penzig and Sacc Curvularia lunata (Wakker) Boedjin, Fusarium equiseti (Corda) sacc., Fusarium semitectum Berk and Rav, Fusarium solani (Mart) sacc., R. solani Kuhn and sterile fungus were found infecting the plants. The seasonal occurrence of the fungi isolated was studied and it was found that Fusarium spp. were present throughout the period of study. C. gloeosporioides and R. solani were present in the rainy season only.

The pathogenicity of all the above fungi to the water hyacinth plants was established by artificial inoculation. Host range studies revealed that R. solani had a wide host range, which included amaranthus, cowpea, rice, Monochoria vaginalis and Panicum repens. The host range of C. gloeosporioides included chilli, Commelina benghalensis, Hydrocotyl asiatica and Ludwigia parviflora Fusarium spp. were found to infect Monochoria vaginalis only.

Among the fungal pathogens isolated from water hyacinth, F. semitectum caused highest intensity of infection of 51.10 per cent followed by F. equiseti and F. solani (48.88 per cent) C. gloeosporioides and R. solani caused 44.44 and 45.76 per cent intensity of infection respectively. Curvularia lunata caused the lowest intensity of infection of 20 per cent.

An experiment was conducted to fix the concentration of inoculum required for effective destruction of water hyacinth. The spore concentration of 1×10^9 spores/ml was the most effective one in the case of F. equiseti, F. semitectum and F. solani For C. gloeosporioides spore concentration of 2×10^9 spores/ml was the most effective one.

Different carrier materials were tried for mass multiplication and storage of the promising fungal pathogens of water hyacinth. The different carrier materials tested were coir pith, paddy straw, peat moss, rice bran and wheat bran. Wheat bran was found to be the most suitable media for F. equiseti, F. semitectum and F. solani. In wheat bran, the spore count and viability of the spores of these fungi were

maximum. For C. gloeosporioides, in rice bran maximum spore count was obtained whereas, in the case of viability of the spores, rice bran and wheat bran were on par. In peat moss none of the fungi grew.

In the field tests conducted to try different methods of application of the fungi viz., C. gloeosporioides, F. equiseti, F. semitectum and F. solani, applying bits of inoculum of the fungi and spraying of the inoculum of the fungi were found to be the best methods. Whereas, dusting of the inoculum produced very poor symptoms. The field performance of the fungi in different carrier materials showed that rice bran and wheat bran inoculum caused good symptom development on water hyacinth plants whereas, coir pith inoculum caused poor symptom development.

All the three Fusarium spp. viz., F. equiseti, F. semitectum and F. solani were found to produce toxin. Which could cause similar symptoms on the water hyacinth leaves as those produced by inoculating the culture bits.