IDENTIFICATION, PATHOGENICITY AND DISEASE PROGRESS OF FUNGI ASSOCIATED WITH WATER HYACINTH *Eichhornia crassipes* (Mart.) Solms IN KENYA.

BY

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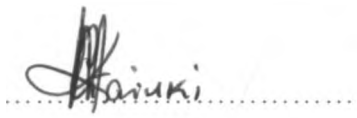
DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.



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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

Dedicated to my grandmother Mrs. Scolastica Nyambura Mbucho.

The determination with which she pursues her goals in life has been inspirational to much of my endeavour for success.

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LIST OF ABREVIATIONS

ADI	-Average disease index.
ANOVA	- Analysis of variance
CD	-Continuous darkness.
CABI	-Centre for Agriculture and Biosciences International
CSIRO	- Commonwealth Scientific and Industrial Research Organization
DI	- Disease incidence.
DL	- Diurnal light
DL – CD	- Diurnal light followed by continuous darkness.
DS	- Disease severity
FPDB	- Fresh potato dextrose broth.
IIBC	- International Institute of Biological Control
KARI	- Kenya Agricultural Research Institute
PPRI	-Plant Protection Research Institute
PDA	- Potato dextrose agar.
TWA	- Tap water agar.
USDA	- United States Department of Agriculture
WH3b1	- Water hyacinth fungal isolate No. 3b1.
WH3b2	- Water hyacinth fungal isolate No. 3b2.
WHDB	- Water hyacinth dextrose broth.
WHFPDB	- Water hyacinth fresh potato dextrose broth.

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ABSTRACT

A survey of plant pathogenic fungi associated with naturally infected water hyacinth (*Eichhornia crassipes*) was conducted at Lake Victoria, Lake Naivasha and Nairobi dam in Kenya. Twenty fungal isolates belonging to different genera were isolated. Two *Alternaria* species designated WH3b1 and WH3b2 were found to be pathogenic to the water hyacinth during the preliminary green house studies. The two exhibited different disease symptoms on the water hyacinth. On the basis of conidial measurements, growth characteristics, and pigmentation in culture, the two *Alternaria* species were identified as *A. Alternata* and *A. eichhorniae*.

A. eichhorniae was found to be ideal for biological control both under green house conditions and in the field. *A. eichhorniae* caused a severe disease characterised by leaf blight and discrete leaf lesions. Disease symptoms appeared between the 5th and the 7th day following inoculation. For *A. alternata*, symptoms appeared 3 days after inoculation as small yellowish, chlorotic lesions with necrotic brown centres. Later these lesions enlarged gradually and centres turned dark brown with pale yellow margins. When the two pathogens were compared for radial growth in three different solid media, *A. eichhorniae* did well in water hyacinth leaf decoction agar as compared to potato dextrose agar and V8 agar juice, while *A. alternata* grew fairly well in all the three. Water hyacinth leaf decoction agar (WHLDA) was the best media for growth and sporulation of *A. eichhorniae*.

Different broth media and cultural conditions were evaluated for the production highly pathogenic inoculum of *A. eichhorniae*. Inoculum of *A. eichhorniae* grown

on fresh potato dextrose broth (FPDB) for 1 week under diurnal light followed by another 1 week, under continuous darkness produced the highest disease incidence and severity.

Histopathological studies were only done for *A. eichhorniae*. Serial microtome sections of the inoculated leaves showed that the germinating conidia penetrated the leaf in three ways; 1) the germ tube formed an appressorium followed by an infection which punctured the epidermal cells, 2) the germ tube formed an infection peg without an appressorium and penetrated the leaf between the guard cell and epidermal cell and, 3) through the open stomata. Development of the fungus in the host tissue was the same irrespective of the mode of penetration. The fungus ramified through the host tissue both inter- and intra- cellularly destroying the host cells. The air chambers in water hyacinth, which are regular in shape, were seen to be irregular after 72 hours. This was followed by the formation of necrotic spots.

Complete death and defoliation of water hyacinth plants infected with *A. eichhorniae* occurred after the six week and disease development was noticed on uninoculated water hyacinth plants grown in neighbouring plot 0.5m away suggesting dispersal of spores by either wind or water. The result of this study suggests *A .eichhorniae* to be an aggressive pathogen on water hyacinth and hence a potential for development of a bioherbicide for water hyacinth in in Kenya.

CHAPTER 1

INTRODUCTION AND OBJECTIVES OF THE STUDY

1:1 Introduction

The deliberate use of plant pathogens to achieve economic control of weeds is a subject that brings with it a welcome attempt to integrate plant pathology, weed science and plant physiology in their broadest sense. There has been increasing world-wide interest in exploiting the potential of plant pathogens as biological control agents of aquatic weeds. However, only in the past three decades has serious attention been given to weed control by the use of plant pathogens such as fungi, bacteria, nematodes and viruses (Templeton, 1986; Templeton, 1988).

Pathogens used as microbial herbicides can be native or exotic, but the former has been used more commonly. A pathogen to be used as microbial herbicide is cultured *in vitro* on a large scale and applied in fairly high concentrations with the intent of killing the weed (Templeton, 1982). The need for culturing makes facultative saprophytes and facultative parasites the agents of choice. A glimpse of the potential of plant pathogens in weed control can be envisioned from the empirical successes of a few cases where they have been used successfully (Charudattan and Walker, 1982).

The well-known water hyacinth, *Eichhornia crassipes* (Mart.) Solms. (Pontederiaceae) is one of the most pernicious weeds in the world. Considerable economic losses have been attributed to it (Nag Raj and Ponnapa, 1970). The ravages caused by the weed are extensive. It impedes run-off in streams and promotes back

water and flood conditions in many areas. It also affects drainage of cultivated lands by choking off drainage channels by its dense growth. The wildlife resources of lakes and rivers are affected. The mat- like spread cuts off air and sunlight from the water below; decaying plant parts affect oxygen tension and therefore fish life. The weed's seriousness is as a result of its rapid rate of growth, vegetative reproduction, and ability to re-infest via the seedbank of flood – borne plants and lack of natural enemies. Excessive infestations deleteriously affect water traffic, water quality, and infrastructure for pumping and hydro-electricity generation, water use and thus affecting social economic structure of human communities. Other problems include property damage during floods, water loss due to evapo- transpiration and an increase in the population of vectors of human and animal diseases like bilharzia, cholera and malaria. Water hyacinth is now present in many countries of the tropics and in most of these it has become the most serious floating aquatic weed (Harley *et al* 1996).

The water hyacinth is increasingly becoming a subject of interest and concern for the scientists, environmentalists, water engineers, hydrologists, economists and politicians. It is also causing a great concern to the fishing communities on the shores of Lake Victoria and infested dams. This is due to the fact that it is seriously interfering with local fishing, village activities and even blocking some boat navigation routes and landing sites (Anon, 1995). With a surface area of 68,800 sq. km, Lake Victoria is the second largest fresh water Lake in the world after Lake Superior and supplies fresh water across half of Africa through the Sudan to the Mediterranean Sea. About thirty million people are economically dependent on the lake (Anon 1995). This water body is the largest connecting highway between the

great lake regions namely Kenya, Uganda, Tanzania, Burundi and the Democratic Republic of Congo (Mibey, pers. comm.1997).

Over the past decade the biological invasion of water hyacinth has posed a serious obstacle to the exploitation of Lake Victoria resources. The lake basin is a vital source of food, energy, drinking and irrigation water. Environmental protection is therefore essential to support the main activity of subsistence fishing. Surveillance satellites show an estimated one percent of the lake has already succumbed to the killer plant.

In Kenya, the weed is also to be found in Lake Naivasha, Nairobi Dam and various other water bodies such as those in ponds and water ways at the Bamburi cement factory and various restaurants in Nairobi and Mombasa (Anon, 1998).

Achieving control of the water hyacinth in Lake Victoria has become a major priority (Woomer, 1997). Integrated management of water hyacinth consists of chemical, biological, mechanical and manual clearance. Mechanical and manual clearance occupies an important role as an emergency measure especially in the decongestion of harbours and hydroelectric reservoirs (Woomer, 1997). Mechanical removal involving mechanical piling by means of elevators, grapples, crushers, rollers saw boats and other machinery especially constructed for the purpose, have been attempted in some countries. The weed is lifted out of the water conveyed to the bank of the river or channel, piled in heaps and allowed to rot (Anon, 1997). Mechanical removal and the use of herbicides have generally been inadequate and expensive control measures to apply on a large scale (Nag Raj and Ponnapa, 1970).

One of the problems in tackling the weed, is a divergence of views among the experts and disagreement between the Governments involved (Anon, 1997). The International Institute of Biological Control (IIBC) in Kenya says chemical, mechanical and biological control all have positive and negative aspects. Uganda, fearful for the impact on its hydroelectric production, favours chemical treatment, but the European Union has threatened to ban fish imports from the region if such a measure is implemented (Anon, 1997). The use of chemicals such as copper sulphate, lead nitrate and sodium arsenate has been suggested for the eradication of the water hyacinth. The minimum concentration of copper sulphate lethal to the plant is 0.018%; at this concentration fish and other fauna are affected. In regions where infested water is used for drinking purposes by humans and livestock, the use of poisonous chemicals in eradicating the weed is dangerous (Anon, 1962).

Some organisations, such as the Kenya Agricultural Research Institute (KARI), is applying biological control methods, which have successfully been implemented in other parts of the world, such as Australia. KARI has been breeding the weevil *Neochetina eichhorniae* which burrow down into the plant and kill it.

The one option available and which has not been explored but which may prove more effective in the long run is the use of plant pathogenic fungi on the water hyacinth. Furthermore, biological control is the only control method that offers economical and sustainable control. It is environmentally friendly and unlike chemical control it is perfectly safe where water is used for drinking and in fishing zones. Ones biological

control takes effect little further input is required, the agents are self regulating and they spread to suppress new growth as it appears (Harley *et al.* 1996).

An opportunity and incentive exists therefore to develop new, cost effective measures which can fill the gap between the short term and long term control and have minimal environmental impact. One promising possibility is the further development of a biological herbicide for water hyacinth based on local fungal pathogens of the weed.

In Kenya there is no recorded information on the identification, pathogencity, efficacy, histopathological relationships, host specificity, epidemiology and formulation of fungi associated with water hyacinth. All these are overriding concerns in the development of a mycoherbicide agent. This study was therefore designed with the following objectives: -

1. To isolate and identify fungal pathogens associated with water hyacinth in Kenya.
2. To carry out pathogenicity tests of the fungi isolated using water hyacinth plants.
3. To study the etiology and histopathological relationships of the key pathogens isolated from water hyacinth.
4. To study the rate of disease progress and spread under field conditions using the identified pathogens.

CHAPTER 2

LITERATURE REVIEW

2:1 The origin and spread of water hyacinth

Water hyacinth is an invasive, neotropical weed of fresh water. Its area of origin is the Amazon Basin, but it has been introduced around the world as an ornamental (Hill *et al.* 1997). Originally perceived as a practical problem for fishing and navigation, water hyacinth is now considered a threat to biological diversity, affecting fish faunas, plant diversity and other freshwater life and the food chains, which depend upon it. While water hyacinth has now spread around the tropical and subtropical world, no where has it posed greater risk to human activities or environmental conservation than in its most recent invasion, the tropical regions of Africa (Hill *et al* 1997). The first introduction of water hyacinth in Africa was in Egypt around 1890 originally to plant gardens in Cairo. It escaped into the Nile delta but has not spread beyond (Batanouny and El Fiky, 1984). Another early introduction was to Natal, South Africa in 1910. It has also been reported in Transvaal, Southern Mozambique and Zimbabwe (Gopal, 1987). The greatest spread of water hyacinth is thought to have resulted from its introduction to the river Congo in 1942. It was established along the entire length of the river by 1956 and it is believed to have crossed into the Nile basin through an interconnective swampy area in South Western Sudan (Bebawi, 1972). This route is most likely responsible for the current biological invasion in Lake Victoria.

Water hyacinth is reported in several other countries including Angola, Madagascar and Senegal, although it is not widespread in West Africa (Gopal, 1987). Currently, in East Africa the most serious problems are in the equatorial zone, especially in the

catchment of Lake Victoria, the upper Nile River, and the lower Shine River. There is considerable potential for water hyacinth to cause very serious damage. In lake Victoria, Kyoga and Albert and in the rivers Kagera, Nile and Shine, massive growths of water hyacinth are causing serious disruption to commercial fishing, boat transport and to infrastructure such as water supply intakes, port facilities and the hydro electricity generation at the Owen Falls Dam. In Southern Africa water hyacinth occurs in Zambia, Zimbabwe, Mozambique and South Africa. Of late the weed threatens to invade Botswana and has recently established in lake Kariba, occurring on both Zambia and Zimbabwe shores (Harley *et al.* 1996).

2:2 The biology of the water hyacinth

Water hyacinth belongs to the family Pontederiaceae, which contains eight other genera. Seven species are reported of which six are native to South America and one other, *E. natan* is native to Africa (Gopal, 1987). Only *E. crassipes* is regarded as a pan tropical weed of lakes, rivers and canals. The widespread distribution of water hyacinth is partly attributable to an attractive purple flower, promoting its establishment in many botanical gardens in the nineteenth century prior to its recognition as a noxious aquatic weed

Water hyacinth consists of a fibrous root system, a basal rhizome, elongated buoyant petioles and a small simple leaf. Flowers are lavender and borne on terminal inflorescence bearing up to 60 but usually 8-15 flowers. The bisexual flowers are blue with a central yellow area, borne on a single spike. The fruit is a thin walled capsule containing up to 450 seeds that sink on release. The plant reproduces sexually, producing small dark seeds and by vegetative propagules. Thousands of new plants

may develop during one season from one original plant (Klingman, 1982). Water hyacinth often grows with other aquatic weeds including *Salvinia molesta*, the grasses *Panicum repens* and *Paspalum distichum* (Woomer, 1997).

2:3 Productivity

Water hyacinth is one of the most productive plants on earth. Reports of this aquatic plant include 173, 123,160 tonnes per year in Florida and Indonesia respectively (Gopal, 1984). Wolverton and Mc Donald (1978) have reported a growth rate of 800 kg per ha per day. Productivity tend to be less with decreasing temperature and greater in nutrient rich wastewater (Haider, 1984; John, 1984) Batanounty and El – Fiky (1984) reported a 30-fold increase in biomass, which produced 43, offsets (vegetative propagules) over 50 days. The coverage of water hyacinth in the Crug Reservoir in Java changed from 3 to 48 ha in 50 days (Woomer, 1997).

2:4 Root Physiology

The roots are adventitious, unbranched, darkly pigmented ending in a conspicuous root cap and may extend up to 3m (Gopal, 1987). Water hyacinth has the capability to absorb chemical nutrients from solution, resulting in biological approaches to waste water treatment. Haider (1984) has reported selectivity in copper, zinc, and iron uptake by water hyacinth with most of these metal ions stored in the root and stem.

2:5 Biological control of weeds

Biological control is the use of host specific natural enemies to reduce the population density of a pest. The objective of biological weed control is not the eradication of

weeds but the reduction and long term stabilisation of weed density at a sub-economic level (Schroeder, 1983).

Though the biological control of plants by insects and plant diseases has been going on since the origin of land plants, the conscious use of the method by man to control weed pests is of fairly recent origin. Biological control of weeds was used initially only because other methods had failed. The reason for this limited use was the supposed dangers that following, introduction, a plant-feeding insect might change its feeding habit, and attack crop plants. However with the increasing interest in conserving our environment and the apprehension concerning pollution by pesticides, biological control of pests is getting increased support and research efforts are being expanded (Crafts, 1975).

The first attempt at biological control of a pest according to Crafts (1975) was carried out in the Hawaiian Islands. The pricky shrub *Lantana camara* had been introduced into the islands as an ornamental. Escaping from gardens, it spread to neighbouring hill and pasturelands becoming a potential threat. In 1902, Koebele an entomologist from the islands visited Mexico the native habitat of *L. camara* and sent back home some twenty-three species of insects, which he found, limited in their habits to this plant. Eight of these became established and large areas formerly infested with *Lantana camara* have been recovered for pasturage or cropping use (Crafts, 1975).

In Australia, the lace bug *Teleonemia scrupulosa* has been established as an effective biocontrol of *L. camara* (Schroeder, 1983).

Cactoblastis cactorum, a pyraid moth of South America origin was first released in Australia in 1926, where it was very effective in the biological control of *Opuntia stricta* (Schroeder, 1983).

2:5:1 Biological control of weeds with plant pathogens

The principles and practices of biological weed control with plant pathogens are generally similar to those of other biological control systems (Charudattan and Walker, 1982). Two distinct tactics have emerged for the biological control of weeds using plant pathogens; the classical or traditional tactic and the bioherbicide tactic.

The classical tactic is directed principally at plants that have been introduced into a new region or country and become weedy in the absence of their natural enemies. Pathogens are sought from the geographical origins of the plants for introduction into new regions with the expectation that they will become established, increase to epiphytic levels, and eventually become endemic when the weed is suppressed to subeconomic levels (Charudattan and Walker, 1982).

A variant of this rationale is the importation of pathogen strains from some or related host species for perpetuation on indigenous weed species that have evolved in the absence of the pathogen and thus have little or no genetic resistance to it. This rationale derives experience with such historically significant diseases as Chestnut blight, white pine blister rust, coffee leaf rust and potato late blight which were caused by accidentally introduced fungal pathogens that brought drastic changes in cultivated or native plant populations (Charudattan and Walker, 1982).

Chandelier juncea, a weed originating from the Mediterranean region, the Near East and Southern Russia, was accidentally introduced in Australia and North America. In view of its economic importance in wheat\fallow cultivation in south-east Australia, a survey of biocontrol agents commenced in 1967 in Europe. Among the organisms found, the rust *Puccinia chondrillina* was most damaging. It was observed that this macrocyclic and monoecious rust occurs over a wide climatic and geographic range, that it remains active through - out the year and damages all parts and all stages of host plant. A strain of the fungus collected from Italy was finally found to be highly virulent against the most abundant Australian form of *C. juncea*. After the specificity of the strain had been assured by screening 56 test plant species belonging to 30 plant families (Hasan, 1972), *P. Chondrillina* was cleared for introduction to Australia (Shroeder, 1983). *Rubus constrictus* and *R. ulmifolius* introductions from Europe spread in Chile from 2 million ha in 1952 to 5 million ha in 1973. The European rust fungus *Phragmidium violaceum* which attack several wild species of *Rubus fruticosus* aggregate was released in Southern Chile in 1973. Within two years the rust became widespread and caused a considerable reduction of *R. constrictus*, which was gradually replaced by other plants (Schroeder, 1983).

Trujillo (1976) reports the successful biological control of the *Ageratine riparia* in Hawaii by a fungal pathogen, *Cercospora riparia* imported from Jamaica. The fungus considerably reduced density of *A. riparia* at elevations ranging 500 –2200m, where it used to be a serious weed in range land.

Fusarium roseum "*culmorum*" which was isolated from diseased *stratiotes aloides* collected in the Netherlands, was shown to be lethal to *Hydrilla vericillata*, an introduced water weed in Southern USA (Charudattan *et al*. 1978).

The bioherbicide tactic, on the other hand, is directed at indigenous or exotics plants that have become naturalised and have become weed for one reason or another. Some may have reached an equilibrium state with their natural enemies that are above economically acceptable levels. Others may have become weedy as a result of human activities such as cultivation, fertilisation, including enrichment of water ways with nutrient pollution; selective pesticide usage; or pasture management practice that disrupts or eliminates the pathogen life cycle (Charudattan and Walker, 1982).

Pathogens are sought among the indigenous weeds particularly in undisturbed areas, that may be grown and induced to sporulate in large-scale fermentation tanks. They are applied usually annually as herbicide so that each plant is inundated with inoculum at the most propitious time for infection Infection and pathogenesis thus create an epidemic with a pathogen that normally persists at an endemic level. Epidemics of these diseases may occur naturally in undisturbed areas because environment (including host density) favourable to development occur only periodically or conversely, because the environment may be usually favourable but the pathogen is irregular in its occurrences a result of poor overwintering capacity, weak saprophytic ability, lack of vectors, or other constrains to dissemination.

Theoretically all kinds of plant pathogens can be considered as potential weed control agents including viruses, bacteria, fungi and nematodes. So far, the number of fungi considerably exceeds that of other pathogens (Templeton, 1981). Out of a total of 83 pathogens studied or used 71 are fungi, 6 viruses, 3 bacteria and 3 nematodes. Among the fungi the mitosporic fungi are of great importance, followed by basidiomycetes. The fact that numerous conidial fungi are being studied for biological weed control is not surprising as they are more commonly encountered as pathogens of higher plants, and they can be cultured and induced to sporulate (Templeton, 1981).

Fungi are also easier to identify than bacteria and viruses, their taxonomic position is better defined and some information is already available on their host ranges. Further, the dispersal of most fungi does not depend on insects or other invertebrate, although spores are distributed by visiting host-feeding insects (Hasan, 1980). Fungal pathogens such as rusts are known to be highly damaging and host specific, the most important consideration for the selection of biocontrol agents.

A protocol for assessing the efficacy of mycoherbicide candidates as stipulated by Harris (1971); Charudattan (1989) and Schroeder, (1983) is most commonly used. The protocol entails;

a) Choice: = Determine the pathogen's destructiveness

This should be done from literature and pathogencity trials; in general, those causing anthracnoses, blights, rots, and wilts are more likely to kill the weed than leaf-spotters, obligate parasites (e g. rust fungi) and facultative saprophytes (smut fungi). If a choice = can be made, select those capable of killing the weed; if not, select the

most destructive. In principle, the search for pathogens should begin with a literature survey.

Although as wide a range of localities as possible should be surveyed to collect a variety of local strains, field surveys in areas which are ecologically homologous with the area where control is required are of particular interest. Due to the cyclical nature of diseases, field surveys should cover the entire growing season of the weed. When a suitable pathogen has been found, its taxonomic identity must be determined before its safety and efficacy can be investigated.

b) Determination of destructiveness:

Quantify under controlled conditions (growth chamber or greenhouse) disease damage and weed control efficacy on the basis of the amount and speed of control. In general, spores are preferred for inoculum since they are the infective structures in most fungi. A minimum of 10^5(ideally10^6) spores should be used to guarantee that inoculum was not a limiting factor to disease development. Pictorial keys (Freeman and Charudattan, 1974) are useful for quantifying disease levels.

c) Determination of moisture, temperature, and inoculum requirements for infection:

Under controlled conditions, determine the amount and duration of dew, temperature optimum, type and amount of inoculum (spores or mycelial preparations) needed to incite an epidemic in the field. This should give clues to the third aspect of efficacy, namely how easy is it to use the pathogen as a mycoherbicide.

d) Field evaluations

Applying principles from step 1 through 3, evaluate the pathogen's efficacy u field conditions that are relevant to weed and crop biology. In addition to the cr for weed control assessment listed under step 2, it is essential to derive a cons with weed scientists and agronomists on what constitute a satisfactory level of co for a given weed problem. At this point, disease assessment may be based on v analysis to facilitate direct assessment.

e) Further development

If the level of efficacy based on the amount, speed, and ease of control ap promising, industrial collaboration may be warranted for large- scale studies. I attempts may be made to determine if efficacy could be improved by removin obvious constraints to the destructiveness of the pathogen and the development epidemic.

2:6 Biological control of the water hyacinth.

The United States Department of Agriculture (USDA) in 1961 initiated biol control of water hyacinth. Subsequently CSIRO, IIBC, PPRI and USDA have c out surveys for natural enemies in the native range of the weed in South An (Harley *et.al.* 1996). Several species of insects and fungi have been identified as host specific, potential control agents.

Both arthropods and micro-organisms have been collected, tested and releas control water hyacinth. The most widely released organism is the smooth hy weevil (*Neochetina eichhorniae*) belonging to the beetle family . A closely r

species *N. bruchi* has also been studied but tend to be less effective when comp with *N. eichhorniae*. Both larvae and adults of these species feed on water hyaci but *N. eichhorniae* has also been documented to feed and oviposit upon other p species, including a few economic ones.

Two moths that produce larvae that feed upon the leaves and petioles of w hyacinth, *Sameodes albiguttalis* and *Acigana infusella* have also been release Queensland. Control levels resulting from these arthropods reported in Austr include 57% of the leaf area and 62% of the original water hyacinth coverage. O arthropods which are reported to attack water hyacinth include grasshoppers, o lepidoptera and mites (Harley, *et al.* 1996).

Water hyacinth is a good candidate for biological control because its main mean reproduction and spread is asexual through off shoots. Burdon and Marshall (19 examined the degree of biological control achieved in 81 different attempts in a t of 45 weed species. They demonstrated a significant correlation between the deg of control and the predominant mode of reproduction of the target species. Asexu reproducing species were effectively controlled more often than those reproducing sexual means. Successful biological control is favoured by limited amounts of gen variation in weed population. Many aquatic weeds that reproduce predominantly clonal propagation for instance *Alternanthera philoxeroides*, *Elodea canaden Eichhornia crassipes*, *Hydrilla verticillata*, *Pistia stratiotes* and *Salvinia mole* seem to be excellent target of biological control (Charudattan and Walker, 1982). low levels or absence of sexual reproduction in many populations of these spec

would reduce the likelihood of co-evolutionary responses following the introduction of a plant pathogen.

Insects have been the organisms used most in biological control of water hyacinth (Freeman *et al.* 1973). However in 1970, a biological control program for aquatic weeds using plant pathogens was initiated at the University of Florida USA. As part of this program, foreign and domestic surveys were made on water hyacinth and other target aquatic weeds (Charudattan, 1973; Conway *et al.* 1974).

Extensive general surveys were conducted to find potential pathogen biological control agents for water hyacinth. Rakvidhyasastra and Visarathanonth (1975) isolated 13 fungal species from diseased water hyacinth and found that *Alternaria eichhorniae* Nag Raj and Ponnapa, *Myrothecium roridum* Tode ex Fr. and *Rhizoctonia solani* were pathogenic. Freeman (1977) found that *Acremonium zonatum* (Sawada) Gams, *Bipolaris stenospila* Drechs., *Cercospora rodmanii* Conway, *Rhizoctonia spp.* and *Uredo eichhorniae* Gonz.-Frag. and Cif were pathogenic to the water hyacinth. Syed *et al.* (1978) isolated 51 fungi from naturally infected water hyacinth, of which three *Myrothecium* spp., two *Rhizoctonia* spp., and one *Pestalotia* sp. were highly pathogenic. Caunter (1982) isolated 10 fungi in different genera from diseased leaves and leaf stalks and found that the species of *Helminthosporium*, *Myrothecium* and *Chaetomella* were pathogenic to the water hyacinth.

Freeman and Charudattan (1974) reported *Cercospora piaropi* Tharp. on water hyacinth in Florida. In the Sudan, Abdel-Rahim (1984) found that the fungus *Phoma*

sagina (Sacc.) Boerema, Dornbosch & Van Kesteren caused leaf spotting on water hyacinth 7 to 10 days after inoculation. Abdel- Rahim and Tawfig (1984) studied the effect of several fungi and bacteria from water hyacinth, and found that the following were pathogenic: *A. zonatum*, *Drechslera specifera* (Bain) v. Arx, *Fusarium equiseti* (Corda) Sacc., *P. sorghina* and *Bacillus* sp. In Egypt, Mansour *et al.* (1980) tested the capability of dematiaceous hyphomycetes fungi for attacking water hyacinth. They found that *Alternaria grisea* Szilvinyi was highly pathogenic to water hyacinth, but *Alternaria alternata* (Fr.) Keissler, *A. humicola* Oudem., *Cladosporium cladosporoides* (Fresen) de Vries and *C. herbarum* (Pers.) Link ex Fr., were slight or very weak pathogens that induce small, zonate, yellowish-brown spots on the leaves.

Freeman *et al* (1981) stated that *C. rodmanii*, *A. zonatum* and *U. eichhorniae* exhibited high potential to control water hyacinth. Several studies have examined this possibility. Martyn and Freeman (1978) inoculated water hyacinth with *A. zonatum*, and found that the plants responded differently to infection depending on size, and that the fungus caused necrosis equal to 40% of the leaf size 2 weeks after inoculation.

Conway (1976a,b) reported *C. rodmanii* as a new pathogen on water hyacinth with potential to control the weed. Freeman *et al.* (1978) showed that *C. rodmanii* was host specific to water hyacinth and concluded that the fungus could be used either alone or in combination with other pathogens and insects. Conway and Freeman (1978) showed that *C. rodmanii* spread widely from the infected area, causing large areas of water hyacinth to die and sink. The fungus overwintered on older leaves, providing a source of inoculum for the next season. Field evaluation by *C. rodmanii*

(Conway *et al.*1979) showed that the fungus severely affected water hyacinth resulting in reduction of plant growth. Secondary infestations occured with disease spreading from the inoculated plants. Charudattan *et al.* (1985) reported efficacy of *C. rodmanii* on water hyacinth. Sanders and Theriot (1980) also evaluated *C. rodmanii* formulations for biological control of water hyacinth. They treated water hyacinth in spring with 5 to 20 g/m^2 of formulated pathogen containing 10^6 viable propagules /g. A significantly higher average disease index (ADI) was evident on the leaves of daughter plants, which indicated secondary infection. Using low-pressure equipment (25 lb/in^2) with *C. rodmanii* resulted in significantly higher ADI values than those obtained with high-pressure equipment (150 lb/in^2). They indicated that the high-pressure equipment destroys some of viable propagules in the formulation.

Charudattan *et al.* (1976) found that *Uredo eichhorniae* was a successful biological control agent for water hyacinth.

A series of surveys were conducted through out 1988-92 to identify naturally occurring fungal pathogens of water hyacinth in India. Infection of water hyacinth leaves by *Fusarium chlamydosporum* was characterised by small punctate leaf spots with ash coloured centres, which became elliptical to irregular shaped structures. Small and young leaves were less susceptible to infection compared to the older leaves, both in the field and in experimental pits (Aneja, 1993).

In Egypt Elwakil *et al.* (1989) carried out a survey of plant pathogenic fungi associated with naturally- infected water hyacinth. Two hundred fungal isolates belonging to different genera were isolated. *Alternaria alternata*, isolate number 5,

was found to be the best candidate for biological control of water hyacinth, both under greenhouse conditions and in the field. Shabana *et al.* (1995) reported that two of three Egyptian *Alternaria* spp. (isolates 3 and 5) isolated from water hyacinth were identified as *A. eichhorniae* and the third, isolate six, as *A. alternata*.

CHAPTER 3

MATERIALS AND METHODS

3:1 Collection of sample materials

Samples of diseased materials were collected from different sites on the shores of Lake Victoria, Lake Naivasha and Nairobi dam, between April and December 1997. These samples were taken to the Mycology laboratory Department of Botany, University of Nairobi for examination, isolation and maintenance.

3:2 Laboratory studies

3.2.1 Isolation and culturing of fungal isolates

a) Isolation

Isolation of the fungi was done from water hyacinth plants which had leaf spots, blights and chlorosis using a modification of the method used by Latch and Hanson (1961) and Elwakil *et al.* (1989). Diseased leaves and petioles were carefully washed several times in running water. Small pieces (0.5-1 cm) of the margin of the lesions were cut and surface sterilised with 0.5% sodium hypochlorite for 2 min then washed thoroughly with sterile distilled water. The surface sterilised pieces were dried between two sterile filter plates, then incubated in moist chamber for 24 to 48 hrs to allow for sporulation. Single conidia were then picked from lesions on incubated leaves and aseptically seeded on-to the surface of water agar using the tip of a sharp sterile inoculating needle. Inoculated plates were incubated on a laboratory bench under normal room temperature (20-24^0 C) and lightning conditions (12-hrs daylight).

b) Maintenance of cultures

Cultures of 20 isolated fungi were maintained using a modification of the technique described by Boesewinkel. (1976). PDA blocks (6-mm^2) bearing actively growing monosporic cultures were aseptically cut-out and transferred into sterile universal bottle slants containing PDA and maintained at 7^0 C.

3.3 Pathogenicity tests

3.3.1 Preparation of the inoculum

Sufficient quantities of conidia of all the isolated cultures were obtained by the method described by Jones (1944) and Latch and Hanson (1961) Conidial suspension was prepared from 7-14-day old monosporic cultures grown on PDA and the suspension spread on the surface of fresh PDA plates. Conidia were dislodged by gentle scrapping the surface using sterile glass slide rods and the suspension produced was strained through two layers of sterile cheesecloth to remove mycelia distilled water (10ml/plate).

Conidia were dislodged as described above and the spore suspension adjusted to 10^6 spores/ml with the help of Neuber improved haemocytometer.

3:3:2 Test plants

Water hyacinth plants were collected from field locations in Nairobi Dam and Lake Victoria and maintained in a greenhouse in tap water supplemented with phostrogen fertiliser at the rate of 2- teaspoonful dissolved in 5 litres of water. The plants were maintained for two weeks. Only the healthy plants were transferred a day before

inoculation to plastic pots (one plant per pot of 18cm diameter and 20 cm depth) containing 1.5 litres of tap water.

3.3.3 Inoculation and incubation of test plants

Individual water hyacinth plants were inoculated using a modification of the techniques described by Van der Vossen *et.al.* (1979) and Elwakil *et al.* (1989). The primary stage involved the mass screening of pathogenicity of all the 20 fungi isolated on the water hyacinth. Individual water hyacinth plants were placed in beakers containing 750 ml of tap water supplemented with phostrogen fertiliser at the rate of 2-teaspoonful dissolved in 5 litres of water every 7-14 days. Inoculum was sprayed to run-off on both sides of all leaves present on the plant using a 0.5 litre Baygon atomiser (Bayer East Africa Ltd). Three buckets were used for each isolate arranged in a completely randomised design. Each bucket had three water hyacinth plants. A second inoculation was applied 48-hours later. Control plants were sprayed with sterile distilled water. All inoculated plants were covered with moistened polythene papers and incubated in a greenhouse where temperatures ranged from 22 to 28^{0}c. The polythene bags were removed after 48- hrs.

3.3.4 Testing of Koch's postulates.

Inoculated plants were examined daily for symptom appearance and the colour, shape and size of lesions produced on leaves and the stems noted periodically for 40 days. Leaves showing any disease symptoms were detached and isolation of the causal fungus performed to fulfil Koch's postulates.

3:4 Identification of the pathogenic isolates

Identification of all the isolated fungi isolated from the water hyacinth was done up to the genera level. Only those found to be pathogenic to the water hyacinth during the preliminary green house tests were identified up to the species level. Identification of dematiaceous hyphomycetes was based on Ellis, (1971) key to genera. The identification was based on ways in which conidia were attached to the conidiogenous cells, shape and type of conidia and the mechanism by which the conidia are liberated. All the other fungal isolates were identified with the assistance of Prof. R.K. Mibey, Mycologist (University of Nairobi) and Dr Paul Kirk, Mycologist (CABI Bioscience UK).

3.4.1 Identification of the pathogenic *Alternaria* isolates

The identification of the pathogenic *Alternaria* isolates was based on colony growth and morphology in three different kinds of media, conidial measurements and ability to produce red colour on potato dextrose agar (PDA). The cultures were then compared with the published descriptions of *A. eichhorniae* and of *A. alternata* (Nag Raj and Ponnappa, 1970; Shabana *et al.* 1995; Ellis, 1971).

3.4.1.1 Colony growth and morphology

Three types of media were used to compare the radial growth of the two pathogenic *Alternaria* isolates. The types of media tested were potato dextrose agar (PDA), V8 juice agar and water hyacinth leaf decoction agar (WHLDA).

a) Culture Media and their composition

All media were sterilised by autoclaving (15-lb/inch^2) pressure for 15 minutes at 121^{0}C. Culture media and their composition were as follows;

Water hyacinth leaf decoction agar (WHLDA)

The medium was prepared by dissolving 20 g of agar and boiling 200 g of water hyacinth leaves in a liter of tap water and filtering then amended with 20 g of D-glucose.

Potato dextrose agar (PDA)

The medium was prepared by dissolving 39 g of commercially prepared potato dextrose powder containing 4.0 g potato extract, 20 g Dextrose and 15 g LAB BM agar in 1 litre of distilled water.

V8 Agar (V8A)

The medium was prepared by dissolving 20 g of agar and adding 200 ml of V8 vegetable juice in 1 liter of distilled water.

The different media were then aseptically pipetted into sterile 9-cm diameter Petri dishes. Inoculum was prepared from 7 day monosporic cultures that had been grown on PDA as described in 3.2.1 The inoculum comprised of mycelial discs (5-mm diameter each) prepared by auguring the culture plates using a sterile hollow glass rod.

b) Media inoculation and incubation.

Mycelial discs were transferred on to the surface of cooled culture media using the tip of a sterile inoculating needle (1 disc/plate). Inoculated media were placed on the laboratory bench and incubated for 10 days under normal room temperature (20 to 24^0 C) and lighting conditions (12-hr day light).

C) Experimental design, data collection and analysis

The design of the experiment was a complete randomised design replicated six times. The treatments were the three types of media. A standard medium depth of 25-ml was used throughout the study. Fungal growth was expressed as radial growth. After the incubation period of 10 days, radial growth in each Petri-plate was measured by the average of two diameters taken at right angles for each colony. Analysis of variance (ANOVA) was carried out at the 5% probability level of the F test. Significant differences in the treatments effect were identified using Duncan multiple range tests at the 5% probability level.

3.4.1.2 Conidial measurements

This particular study was carried out to measure the conidial sizes of the two *Alternaria* species pathogenic on the water hyacinth. The procedure used was a modification of that used by Shabana *et al.* (1995).

a) Media preparation, inoculation and incubation

Two types of media were used namely, potato dextrose agar (PDA) and V8 juice agar. The media was prepared as in section 3.4.1.1 a. Mycelial discs were transferred to the surface of the culture media as described in section 3.4 1.1b. The media was placed on the laboratory bench and incubated for 14 days under normal room temperatures (20 to 24^0c) and lighting conditions (12-hr day light).

b) Sporulation, conidial harvesting and measurements

This was achieved using a modification of the slide culture technique as described by Ridell (1950). Two sheets of filter paper, a bent glassrod, a microscope slide and

C) Experimental design, data collection and analysis

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a) Media preparation, inoculation and incubation

Two types of media were used namely, potato dextrose agar (PDA) and V8 juice agar. The media was prepared as in section 3.4.1.1 a. Mycelial discs were transferred to the surface of the culture media as described in section 3.4.1.1b. The media was placed on the laboratory bench and incubated for 14 days under normal room temperatures (20 to 24^{0}c) and lighting conditions (12-hr day light).

b) Sporulation, conidial harvesting and measurements

This was achieved using a modification of the slide culture technique as described by Ridell (1950). Two sheets of filter paper, a bent glassrod, a microscope slide and

cover slip were placed in a Petri-dish in that order and sterilised by autoclaving. Sterilised PDA medium was poured into sterile Petri-plates to form a layer of about 2-mm depth.

Upon solidification, 1-cm 2 agar blocks were aseptically cut-out using a sharp sterile scalpel and placed on the microscope slide. The centre edges of each agar block were seeded with conidium of inoculum obtained as described in section 3.2.1 using a sterile inoculating needle and the cover slip placed centrally on the block Petri-plates covered with lids and incubated as described in section 3.2.1.

When suitable growth and sporulation had occurred, the cover slip was gently lifted using a clean pair of forceps and the agar block discarded. The cover slip was then mounted in a drop of clear lactophenol on a slide and viewed under the light microscope. The colour, size shape and septation of 40 different conidia were recorded. For the size of the conidia, the length of the conidium and the broadest width of the conidium body, were measured as described by Shabana *et al.* (1995).

3.4.1.3 Pigmentation in culture of PDA

This study was carried out to investigate which of the two *Alternaria* spp. pathogenic to the water hyacinth produced a red colour in PDA. The procedure used was a modification of Shabana *et al.* (1995). Fungal cultures of the two *Alternaria* spp. were grown as described in section 3.2.1.

Fresh potato dextrose broth (FPDB) was prepared by boiling 200 g of peeled fresh potato chips for 1-hr and filtering then amended with 20 g of D-glucose per litre. This

was then autoclaved for 15 minutes at 121^0 C. The media was then allowed to cool at room temperature and then dispensed into 50-ml per 250-ml Erlenmeyer flasks. Mycelial discs were then transferred to the surface of cool fresh potato dextrose broth using the tip of a sterile inoculating needle (5 discs/flask). The flasks were then left standing on the laboratory bench at normal room temperature (20 to 24^0 C) and lighting conditions for 7-14 days and colour change observed.

3:5 Effect of inoculum concentration of *Alternaria* spp. on disease incidence and severity.

This investigation was undertaken to establish the level of inoculum concentration of the pathogenic *Alternaria* isolates required to incite the highest amount of disease under green house conditions. A modification of the procedure used by Shabana *et al.* (1995) was used.

a) Inoculum preparation

Inoculum was prepared separately for each isolate as described in section 3.3.1 and conidial concentrations adjusted using haemocytometer counts and standardised at 5.6×10^6, 1.4×10^6, 1.4×10^5 and 1.4×10^4.

b) Inoculation

Inoculum was sprayed to run-off on both sides of all leaves present on plants using a 0.5 litre Baygon atomiser with a second application being made after 48 hours. Control plants were sprayed with sterile distilled water. All inoculated plants were covered with moistened polythene papers for 48 hours in the greenhouse.

c) Experimental design

The experiment was arranged in a completely randomised design replicated four times. The treatments being the four inoculum levels for the two *Alternaria* isolates.

c) Data collection and analysis

Ten days after inoculation the plants were rated for disease incidence (DI) and disease severity (DS). Disease incidence was determined as the presence or absence of a disease expressed as percentage, while disease severity as the severity of disease damage based on a pictorial disease scale developed for water hyacinth – *Cercospora rodmanii* pathosystem (Conway, 1976a) where;

0- No infection.

0.5- One to two spots on leaves.

1- Several scattered spots on leaves.

2- Greater than 25% of the leaf surface covered with lesions

3- Greater than 50% of the leaf surface covered with lesions.

4- Greater than 50% of the leaf surface covered with lesion and tip dieback.

5- Greater than 75% of the leaf surface covered with lesions; leaf and petiole death.

Values scored for individual leaves were summed up and averaged to derive disease severity for the whole plant. Analysis of variance (ANOVA) was carried out at the 5% probability level of the F test. Significant differences in the treatment means were examined using Duncan's multiple range tests at 5% probability level.

3:6 Effect of media and light regime on the Pathogencity of *Alternaria eichhorniae*.

Investigations were carried out to determine the influence of media and light regime on the pathogencity of *A. eichhorniae*. A modification of the procedure used by Shabana *et al*. (1995) was used. Three types of media namely, Fresh potato dextrose broth (FPDB), Water hyacinth dextrose broth (WHDB) and water hyacinth fresh potato dextrose broth (WHFPDB) were used. Light regimes tested were diurnal light - DL (12-hr daylight, 12-hr darkness), continuous darkness –CD and diurnal light for one week followed by continuous darkness for one week (DL –CD).

a) Culture media and their composition.

The term autoclave refers to sterilization by steaming for 15 minutes at 121^0c (i.e. 15 lb/m^2).

Fresh potato dextrose broth

The medium was prepared by boiling 200 g of fresh cut potato in 800 ml of water and filtering then amended with 20 g of D-glucose per liter then autoclaved.

Water hyacinth dextrose broth (WHDB)

The medium was prepared by boiling 200 g of fresh water hyacinth leaves (macerated) in 800 ml of water and filtering then amended with 20 g of D-glucose per liter then autoclaved

Water hyacinth fresh potato dextrose broth (WHFPDB)

The medium was prepared by boiling 200 g of water hyacinth leaves in a liter of water and filtering and mixed fresh potato extract prepared as above in the ratio of 3:1v/v.

b) Inoculation

Mycelia discs were transferred from culture plates prepared as in 3.4.1.1.b on to Erlenmeyer flasks containing 50-ml of broth media prepared as described above. Inoculated flasks were incubated on shelves. The interior of the incubators was illuminated continuously using 30 watts white fluorescent tubes (Philips FL &D) mounted 50-cm above the first shelf. Erlenmeyer flasks for assessing effects of continuous darkness and alternating light/dark cycles were wrapped using Aluminum foil through out the experiment and at 12-hr intervals respectively. After two weeks the conidial and mycelial suspension was prepared by blending about 5 g wet mycelium of mycelium from a flask in sterile water for 6 s at high speed in waring blender (commercial two –speed model No.91-262, waring Products Division, New Hartford, CT)

c) Experimental design, data collection and analysis

The experiment was arranged in a completely randomised design replicated three times. Two factors, type of media and light regime with three levels each were studied in a factorial combination. Test plants as described in section 3.3.2 were inoculated as described in section 3.3.3. Inoculated test plants were examined for lesion development after 10 days. Disease assessment was done in terms of disease severity and disease incidence as described in 3.5 (c).

3:6 Histopathological tests

Histopathological studies were carried out for the *Alternaria* isolate WH3b2. The inoculum was prepared as described in section 3:3.1. Six water hyacinth plants were used for histological studies as described by Okoth (1989). Inoculation of the test plants was done as described in section 3.3.3. Leaves from inoculated three water hyacinth plants were cut into small pieces (approx. 1 by 1cm discs) at 3hr intervals for up to 72 hours, each time the discs were placed in vials two thirds full of farmers fluid (mixture of absolute ethanol and glacial acetic acid in the ratio of 2:1). This fixative also decolourised the discs after 2 days.

Half of the total number of discs in each vial were carefully laid on wire gauze and then thoroughly washed in running water for 30 minutes. The leaf discs were then dehydrated in a series of alcohol namely 50%, 70%, 90% for one day for each of the concentration. The process was repeated once. The alcohol was then washed off with the following different concentrations of xylene each treatment lasting one day; 25%, 50%, 70%. The discs were then infiltrated with paraplast tissue embedding medium (wax) in the following series; mixture of xylene + wax (1:1) in an oven at 59^0c, just above the melting point of the wax pellets, for a day followed by two changes of pure wax each change lasting 12 hours.

To avoid artifacts, due to contamination during microscopic studies, and to ensure ribbon continuity with serial sectioning, fresh wax pellets (unused) were used.

The cleared leaf discs were then embedded in melted wax placed in special wooden blocks keeping the blocks under ice water. The blocks were then trimmed and sectioned serially 15µm thick, using a microtome.

The ribbons (cut leaf sections) were then broken into short pieces, using a camel brush, and floated on lukewarm water to straighten. Cover slips thinly smeared with diluted office glue (diluted with water) were used to pick the ribbons from the water bath, placed on the slides and left to dry on the slide dryer for two days. The slides were then dipped in pure xylene to remove the wax, and then dipped in cotton blue in lactophenol to stain for one minute. Excess stain was removed with 70% alcohol and the slides observed under the microscope.

3:7 FIELD EXPERIMENTS

Field studies were conducted outside the greenhouse in Chiromo campus of the University of Nairobi between the period March 1998 to August 1998, to study the rate of disease progress and spread under field conditions. These experiments were meant to supplement work being carried out on the biological control of the water hyacinth in Nairobi dam. A modification of the procedure used by Charudattan *et al.* (1985) was used.

3.7.1 Inoculum preparation

The two *Alternaria* isolates WH3b1 and WH3b2 were grown for 3 weeks in roux bottles on potato dextrose broth containing 5% yeast extract. The bottles were left to stand in the laboratory at room temperature and normal lighting conditions. Culture broth from several bottles were blended for about 10 seconds, then transferred in roux bottles containing sterile distilled water to encourage sporulation. Conidial concentration of 1.4×10^6 spores/ml was used. Triton X - 100 (a polyethylene ether;

Sigma, St. Louis. M.O) was used at the rate of 0.05 % v/v as a wetting agent. Triton X - 100 without the fungus or the broth was used as control.

Inoculation was done using a low-pressure knapsack sprayer. Inoculum was sprayed to run off on both sides of the leaves. Inoculation was done late in the evening, to avoid drying and desiccation of the inoculum. Both inoculated and control plants were maintained out doors.

3.7.2 Test plants

Water hyacinth plants were collected from field locations in Nairobi Dam and Lake Victoria and maintained in a greenhouse in tap water supplemented with phostrogen fertiliser at the rate of 2- teaspoonful dissolved in 5 litres of water for two weeks. Only the healthy plants were transferred a day before inoculation to plastic pots (one plant per pot of 18 cm diameter and 20 cm depth) containing 1.5 litres of tap water.

3.7.3 Design of the field experiments

The first experiment was designed to study the rate of disease progress in the field. The experiment was arranged in a completely randomised design replicated six times. Each replicate consisted of a bucket in which 3 plants were placed. Further scores for disease severity for each pathogen for a period of 6 weeks were averaged for each bucket, computed and used to calculate the area under disease progress curves (AUDPC).

The second experiment was meant to study the rate of disease spread in the field. The procedure used was a modification of the procedure used by Charudattan *et al.* (1985). The experiment was arranged in a completely randomised block design replicated four times. Each replicate consisted of a bucket in which 3 water hyacinth plants were placed. Further scores of disease incidence for each pathogen for a period of 6 weeks were averaged for each bucket, computed and used to compute the score for each plot. Analysis of variance (ANOVA) was carried out at the 5% probability level of the F test. Significant differences in the treatment means between the three plots were examined using Duncan's multiple range tests at 5% probability level. Inoculated plants were examined after every 7 days for 6 weeks. Four leaves per plant selected randomly from top to the bottom of the plant were tagged and used through out the experiment.

3.7.4 Data collection

a) Disease progress in the field

In each of the plant, disease scores for disease severity as described in section 3.5 (c) were taken and average values computed for subsequent data analysis.

b) Disease spread in the field

Inoculated plants were examined after the 6th week. Four leaves were tagged during inoculation and used for disease assessment at the end of the experiment. In each of the plant, disease scores for disease incidence were calculated as described in section 3.5.c. and the average values computed for subsequent data analysis.

CHAPTER 4

RESULTS

4.1 Identification of fungi isolated from diseased leaf lesion

Various fungal colonies, identified as *Alternaria* Nees, *Fusarium* Link, *Botrydiplodia* (Sacc.) Sacc , *Rhizopus* Ehrenb, *Aspergillus* Link, *Pestalotia* De Not, *Bipolaris* Shoemaker, *Curvularia* Boedjin, *Penicillium* Fr., *Helminthosporium* Link *Rhizoctonia* DC, *Stemphyllium* Wallr and *Myrothecium* Tode were observed growing on plates containing diseased leaf lesion discs within 24-48 hours of inoculation. Isolations was carried out through out the period collections were made.

a) *Alternaria*

Colonies effuse, usually grey, dark blackish brown or black. Hyphae colourless, and olivaceous brown. Conidiogenous cells integrated, terminal becoming intercalary, polytretic, sympodial, or sometimes monotretic, cicatrized. Conidia catenate or solitary, dry typically ovoid or obclavate, often rostrate, pale or mid olivaceous brown, smooth or verrucose with transverse and frequently also oblique or longitudinal septa (Ellis 1971).

b) *Fusarium*

Conidia produced hyaline, septate canoe-shaped produced in sporodochia. Conidia phialidic. Chlamydospores produced intecalary on the mycelium (Kirk, Pers Comm.1998).

c) *Aspergillus*

Conidiogenous cells enteroblastic and monophialidic with no sporodochium or stroma. Conidiogenous cells discrete variously arranged with phialides forming a more or less complex head at the apex of a stipe. Conidiophore each swelled at the apex with a spherical vesicle the surface of which is covered by numerous phialides or short branches bearing phialides (Kirk, Pers Comm. 1998).

d) *Pestalotia*

Conidiophore hyaline branched irregulary septate smooth, formed from the upper cells of pseudoparenchyma. Conidiogenous cells holoblastic, annellidic, indeterminate, integrated, cylindrical, hyaline smooth, with 1-3 percurrent poliferations. Conidia fusiform straight or slightly curved 6-celled. Basal cell hyaline, thin walled, truncate, with an endogenous cellular, simple or dichotomously branched appendage (Ellis 1971).

e) *Curvularia*

Conidiogenous cells enteroblastic and acrauxic and mostly polytretic and sympodial. Conidiophore mononematous and not nodose. Conidiophore not branched at the apex. Conidia short with 3 or more septa often curved with the end cells frequently paler than the intermediate cells (Ellis 1971).

f) *Penicillium*

Mycelia produced on 14 -day old culture of PDA, simple long with erect conidiophores that branches towards the tip in characteristic symmetrical or

asymmetrical broom like fashion. Conidiogenous cells phialidic forming a long conidial chain. The conidia are globose to ovoid, greenish sometimes yellow in colour. Conidia produced depicted the existence of several species of *Penicillium* (Kirk, Pers Comm.1998).

g) *Helminthosporium*

As genus conidia of *Helminthosporium* pseudoseptate, mostly obclavate, developing laterally, often in verticils through minute channels beneath septa borne on conidiophores, which are unbranched. The conidiophores are acroauxic bearing conidiogenous cells, which are enteroblastic, polytretic and determinate (Ellis 1971).

h) *Stemphylium*

Conidiogenous cell holoblastic integrated attached to conidiophores, which are acroauxic and macronematous. Conidiophores are mostly unbranched, thin walled and collapsing at the apex. The tip of the conidiophore swollen, rounded, thin walled, sometimes collapsing and becoming cupulate (Ellis, 1971).

i) *Myrothecium*

As genera, the conidiogenous cells enteroblastic and the conidiophore are acrauxic. Conidiogenous cells phialidic, sometimes monophialidic. Conidia semi-endogenous and acrogenous suggesting existence of more than one species. Sporodochia sessile or stalked, a viscid green to black; conidial mass found surrounded by a white zone of hyphae from which setae project. Phialides have necks (Ellis 1971).

4.2 Green house tests

4.2:1 Pathogencity tests

Symptom development for all the fungal isolates was monitored periodically for 40 days. Of all the isolates only the *Alternaria* isolates were found to be pathogenic to the water hyacinth after a series of repeated experiments. The disease caused by the *Alternaria* isolate was characterised by chlorotic lesions with varying degree of necrosis.

Microscopic observations of the *Alternaria* isolates depicted a variable population of conidia, some of which were small and others were elongated with a long pedicel. A pigment was produced in some of the PDA plates. From the single spore isolations some of the plates grew faster than others on PDA. The two isolates were separated and designated as WH3b1 for the fast growing one and WH3b2 for the slow growing one.

The two isolates were shown to produce different symptoms on the water hyacinth plants. Water hyacinth plants inoculated with *Alternaria* isolate WH3b1, symptoms appeared 3 days after inoculation. The symptoms first appeared as chlorotic flecks with some necrosis on the upper surface of the leaves. Lesions then coalesced and formed blights in many areas of the leaves after the 12^{th} day. After 20^{th} day symptoms extended to petioles, while partial defoliation became prominent after 30^{th} day. This was followed by death at the 40^{th} day.

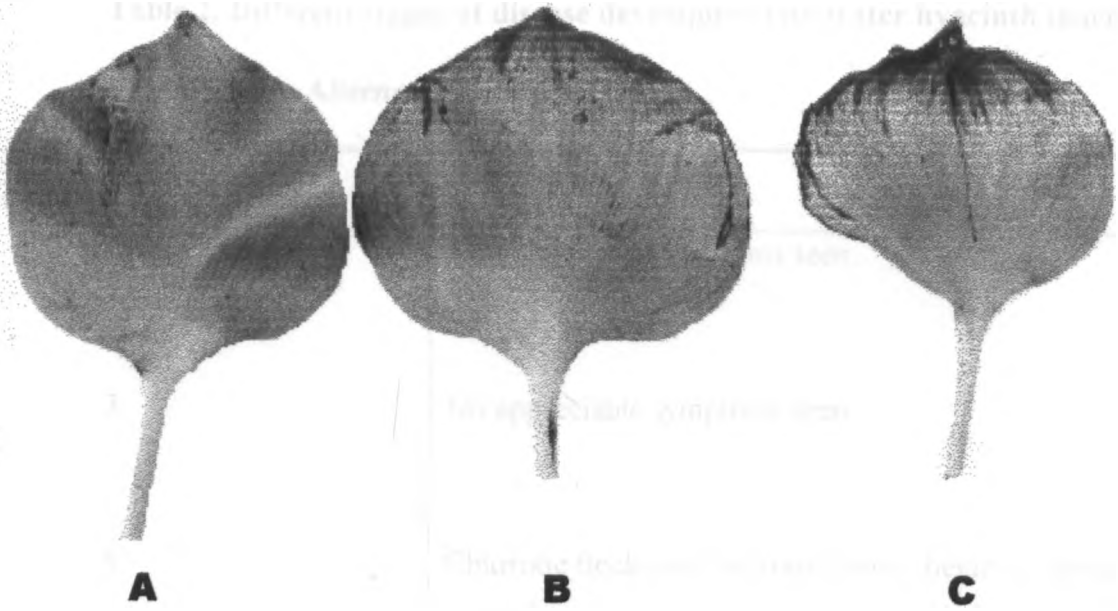
Water hyacinth plants inoculated with *Alternaria* isolate WH3b2, developed symptoms 5 to 7 days after inoculation. The symptoms first appeared as yellowish

chlorotic flecks and developed into small lesions with necrotic brown centres. After the 9th day enlarged lesions of deep yellow colour with brown coloured centres were observed in some of the leaves. Older leaves were more susceptible than the younger and emergent leaves. After the 12th day some of the lesions coalesced to form brown spots while others turned necrotic. Hypophyllous lesions appeared sunken and light brown with distinct margins at the 20th day. Later, some leafspots extended to the petioles (plate 3). Severe infections in the form of several spots culminated in the premature death of the leaf followed by desiccation after the 40th day.

Re – isolations from the artificially infected tissues yielded monosporic cultures of the two *Alternaria* isolates, which were identical with the original isolates, confirming Koch's postulates while repeated inoculation tests confirmed the pathogencity of the fungus to the water hyacinth.

Table 1. Different stages of disease development on water hyacinth inoculated with *Alternaria* isolateWH3b1

Time in days after inoculation	Observations
1	No appreciable symptoms seen.
3	Chlorotic flecks observed on some leaves.
5	Lesions with some necrosis seen on upper surface of the leaves.
7	Chlorotic lesions with varying degrees of necrosis observed.
9	Enlarged lesions of deep yellow colour with brown coloured centres observed in some leaves.
12	Some of the lesions coalesced to form brown spots while others turned necrotic.
20	The lesions now extended to the petioles.
30	Lesions turned dry and there was partial defoliation
40	Death. Plants integrated and the contents of the beakers turned into a dirty dark brown coloured fluid.



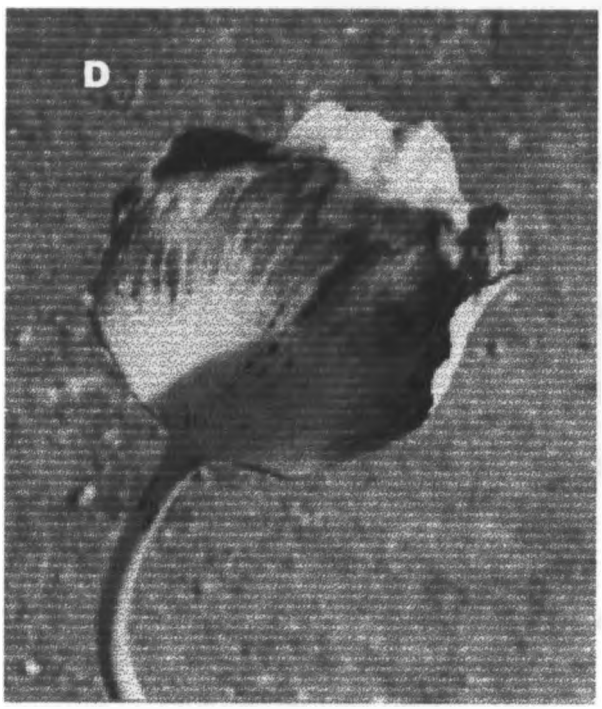
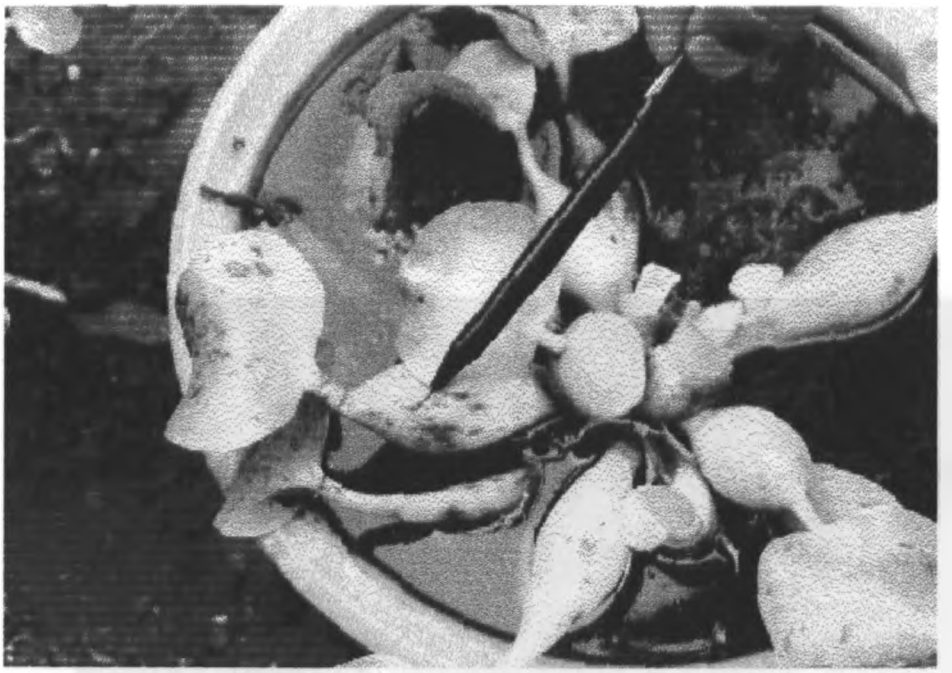


Plate 1: Water hyacinth leaves showing symptoms induced by *Alternaria* isolate WH3b1 (A), 1 week after inoculation; (B), 2 weeks after inoculation; (C), 3 weeks after inoculation; and (D), 4 weeks after inoculation;

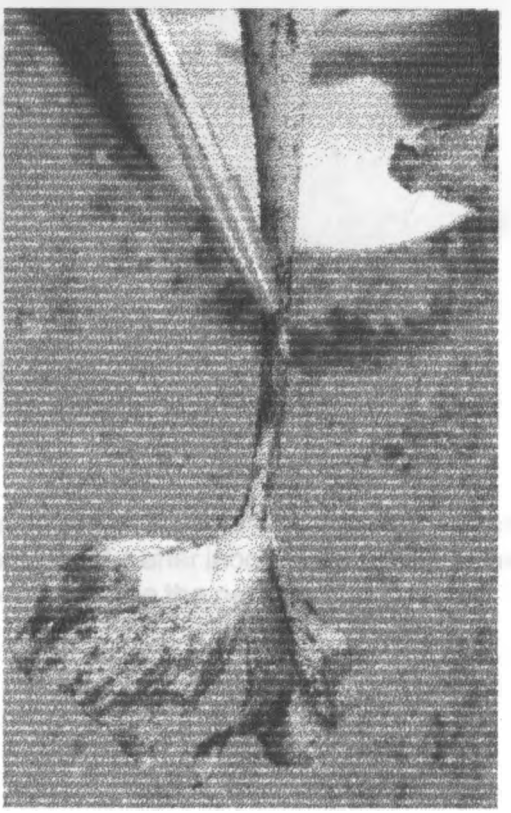
Table 2. Different stages of disease development on water hyacinth inoculated with *Alternaria* isolate WH3b2

Time in days after inoculation	Observations
1	No appreciable symptoms seen.
3	No appreciable symptoms seen.
5	Chlorotic flecks and necrotic lesions begin to appear.
7	Chlorotic lesions with varying degrees of necrosis observed.
9	Lesions enlarged gradually and coalesced into blotches with brownish to dark brown centres and faint yellow margins.
12	Symptoms extended to the bulbous petioles on which hypophyllus lesions were sunken and dark brown.
20	Lesions coalescing formed extensive blights on the leaves and petioles.
30	Some of the lesions that coalesced turned dry and there was partial defoliation.
40	Severe infection caused premature death of the leaves.

Plate 2: Water hyacinth leaves showing symptoms induced by *Alternaria* isolate WH3b2.Larger lesions are oval to elliptical running parallel to the longitudinal axis on the leaf.



A



B

Plate 3: Water hyacinth leaves showing symptoms induced by *Alternaria* isolate WH3b2 on the petioles.

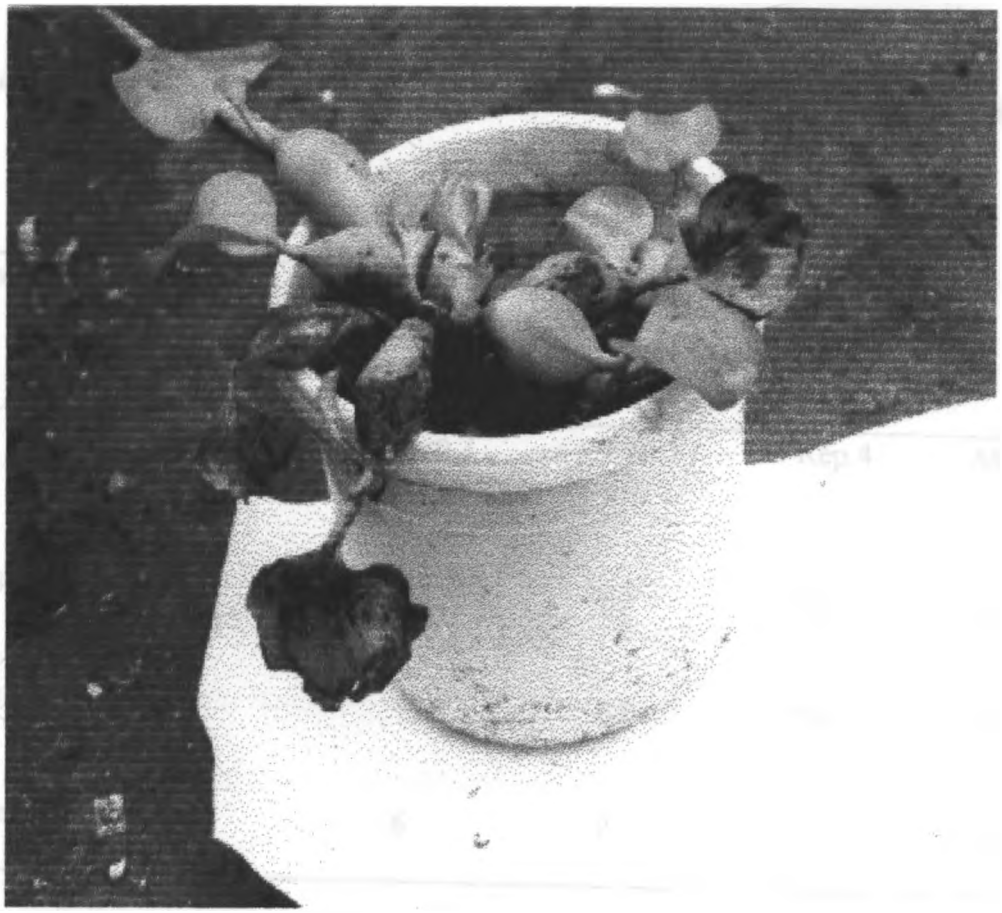


Plate 4: Water hyacinth plant inoculated with 1.4 x 10^6 conidia/ml of *Alternaria* isolate WH3b2, three weeks after inoculation. Note the die-back on the older leaves and the severe infection on the petioles.

Table 3. Percent infected area of water hyacinth 20 days after inoculation with the *Alternaria* isolates

Isolate[a]	Percent infected area (estimated in terms of disease severity)[b]				
	Rep.1	Rep.2	Rep.3	Rep.4	Mean [c]
WH3b1	69	75	69	69	70.5A
WH3b2	85	75	65	75	75.0A
Control	0	6	8	6	5.0B

[a] 10^6 conidia/ml of each of the isolate was used
[b] Disease severity measured using a disease rating scale of 0-5 (Conway, 1976a)
[c] Means within a column followed by the same letter(s) are not significantly different according to the Duncan multiple range test(P=0.05)

4.3 Further greenhouse tests

4.3:1 Effect of inoculum concentration on disease incidence and severity

Both disease incidence and disease severity increased remarkably with increase in the inoculum concentration for both the two *Alternaria* isolates (table 4). At the higher levels of inoculum concentration of 1.4 $x10^6$ and 5.6 x 10^6 conidia/ml, *Alternaria* isolate WH3b1 depicted a high level of disease incidence of 70% and 72% respectively while the disease severity was such that greater than 50% of the leaf surfaces were covered with lesions and tip die back experienced. The disease severity levels were not significantly different at the 5% probability level according to the Duncan multiple range tests.

For the lower levels of inoculum concentration of 1.4 x 10^5 and 1.4 x 10^4 conidia/ml there was a significant variation in the levels of disease incidence with the lower inoculum level having 61% of the total leaf population covered with necrotic lesions and the lower having 52.5% of the total leaf population covered with lesions. The difference between the two-inoculum levels was significantly different at 5% probability level based on the Duncan multiple range test. Disease severity levels were not significantly different (P=0.05) for the lower levels of inoculum concentration of 1.4 $x10^5$ and 1.4 x 10^4 conidia/ml for *Alternaria* isolate Wh3b1 with greater than 50% of the leaf surface covered with lesions. Inoculum level of 1.4 x 10^4 conidial/ml induced a higher level of disease severity than that of 1.4 x 10^5 conidia/ml, but this was not significantly different at P=0.05

For *Alternaria* isolate WH3b2, disease incidence and severity increased remarkably with increase in inoculum concentration with the higher levels of inoculum

concentration of 5.6 x 10^6 and 1.4 x10^6 conidia/ml having no significant difference (P=0.05). Disease incidence scores of 72.5% and 71.75% were achieved for the two levels respectively. For disease severity, greater than 50% of the leaf surfaces were covered with lesion and tip die back. At the lower levels of inoculum concentration, both disease incidence and disease severity were not significantly different at P=0.05 according to the Duncan multiple range test.

Comparatively, at the higher inoculum levels of inoculum concentration *Alternaria* WH3b2 had higher levels of disease incidence and severity than *Alternaria* isolate WH3b1 (table 4 & 5). In the case of the *Alternaria* isolate WH3b2 levels of disease incidence and severity increased with increase in the level of inoculum concentration whereas in the case of *Alternaria* isolate WH3b1 disease incidence increased with increase in the levels of inoculum concentration but disease severity scores did not show a similar tread and remained constant (P=0.05)

Table 4 : Effect of the level of inoculum on disease incidence and severity ; *Alternaria* isolate WH3b1

Level of inoculum	Disease incidence (DI) (%)[a]					Disease severity (DS)[a]				
(Conidia/ml)	Replicates				mean[b]	Replicates				mean[b]
5.6×10^6	75	70	73	70	**72.00A**	4	4	4	4	**4.00A**
1.4×10^6	70	65	70	75	**70.00A**	4	4	3	4	**3.75A**
1.4×10^5	60	64	60	60	**61.00B**	3	3	3	4	**3.25A**
1.4×10^4	50	54	56	50	**52.50C**	3	4	3	4	**3.50A**

[a] Disease severity (DS) obtained using a disease severity score of 0 –5 developed for water hyacinth, *Cercospora rodmanii* pathosystem, Disease incidence (DI) proportion of the number of leaves with necrotic spots in a plant expressed as a percentage.

[b] Means within a column followed by the same letter (s) are not significantly different according to Duncan multiple range test (P=0.05).

Table 5: Effect of the level of inoculum on disease incidence and severity;. *Alternaria* isolate WH3b2

Level of inoculum	Disease incidence (DI) %[a]					Disease severity (DS)[a]				
(Conidia/ml)	Replicates				mean[b]	Replicates				mean
5.6×10^6	75	70	75	70	**72.5A**	4	4	4	5	**4.25A**
1.4×10^6	75	70	74	68	**71.75A**	4	4	4	4	**4.00A**
1.4×10^5	50	54	55	50	**52.25B**	3	3	3	4	**3.25B**
1.4×10^4	50	45	50	55	**50.00B**	3	3	4	3	**3.25B**

[a] Disease severity (DS) obtained using a disease severity score of 0 –5 developed for water hyacinth, *Cercospora rodmanii* pathosystem, Disease incidence (DI) proportion of the number of leaves with necrotic spots in a plant expressed as a percentage.

[b] Means within a column followed by the same letter (s) are not significantly different according to Duncan multiple range test (P=0.05).

4.4 Identification of the *Alternaria* sp.

Identification of the two *Alternaria* isolates was based on colony growth and morphology, conidial measurements and pigment production on potato dextrose agar.

a) *Alternaria* isolate WH3b1

The fungus produced pale to light brown, smooth, sometimes verruculose conidia with up to 8 transverse and several longitudinal septa (plate 9). The conidial measurements ranged from 22-34 μm x 7- 12 μm. The conidia were formed in long, often, branched chains, obclavate, obypyriform ovoid or ellipsoidal often with a short conical cylindrical beak.

Radial growth (diameter) on PDA after 10 days was 62mm, V8 juice agar, 62 mm and WHLDA, 47 mm (Table 6). On PDA the colonies appeared black or olivaceous black and sometimes grey.

b) ***Alternaria* isolate WH3b2**

The fungus produced conidia which were ovate, obclavate, obpyriform or simply ellipsoidal usually with an indistinct basal pore, beakless when ellipsoidal or with a short conical narrowly tapered or almost cylindrical beak (plate 7). Conidial measurements ranged from 31 – 103 μm x 9-16 μm with four to ten longitudinal or oblique septa and media cells bulging considerably, yellow brown to medium golden brown. The conidium wall was minutely verruculose.

Growth on PDA was velvety to cottony, rising to a height of 6mm pale Vinaceous lilac in colour. Sectoring was evident. The medium coloured "Tourmaline pink" to

"Indian Lake". Colonies 24 mm diameter at room temperature (24^0 C to 27^0 C). Sporulation was very poor to scant or absent. In fresh potato dextrose broth the fungus grew well imparting a "Vinaceous Red" colour to the medium (plate 6A). The intensity of this pigment deepened with age.

On V8 juice agar colonies 31 mm in diameter growth after 10 days. Sporulation was abundant with virtually no mycelial growth at the 10^{th} day.

On water hyacinth leaf decoction agar (WHLDA) colonies 62 mm in diameter growth in 10 days at room temperature. The fungus grew and sporulated well. In old cultures (more than 10 days) conidia were subject to secondary growth resulting in a change in form, size and colour. Such conidia were twice as thick, the number of septa increased accompanied by deepening of the constrictions at the septa and darkening of spore walls (plate 7B) Spore germination in older cultures was also evident (Plate 7 A).

On the basis of these characteristics *Alternaria* isolate WH3b1 was identified as *A. alternata* and *Alternaria* isolate WH3b2 as *A. eichhorniae* as described by Nag Raj and Ponnapa, 1970; Ellis, 1971; Shabana *et al.* 1995.

Table 6. Colony radial growth in mm after 10 days of the two *Alternaria* isolates WH3b1 and WH3b2 when grown on three different media.

Media type	*Alternaria* isolates*	
	WH3b1	WH3b2
V8	61.7[a]	31.3[a]
PDA	61.9[a]	24.3[b]
WHLDA	47.2[b]	61.5[c]

*Means within a column followed by the same letter(s) are not significantly different according to the Duncan multiple range test (P=0.05)

*Each value in the above table represents a mean of 6 replicates

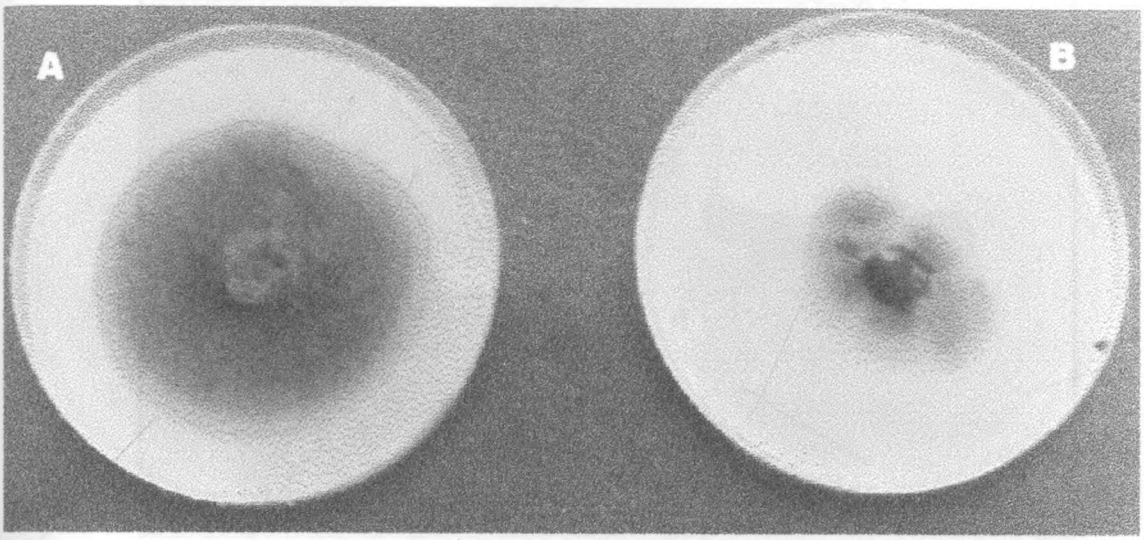


Plate 5: Ten day old cultures of *Alternaria* spp. (A), *A. alternata,* and (B), *A. eichhorniae* grown on V8 juice agar.

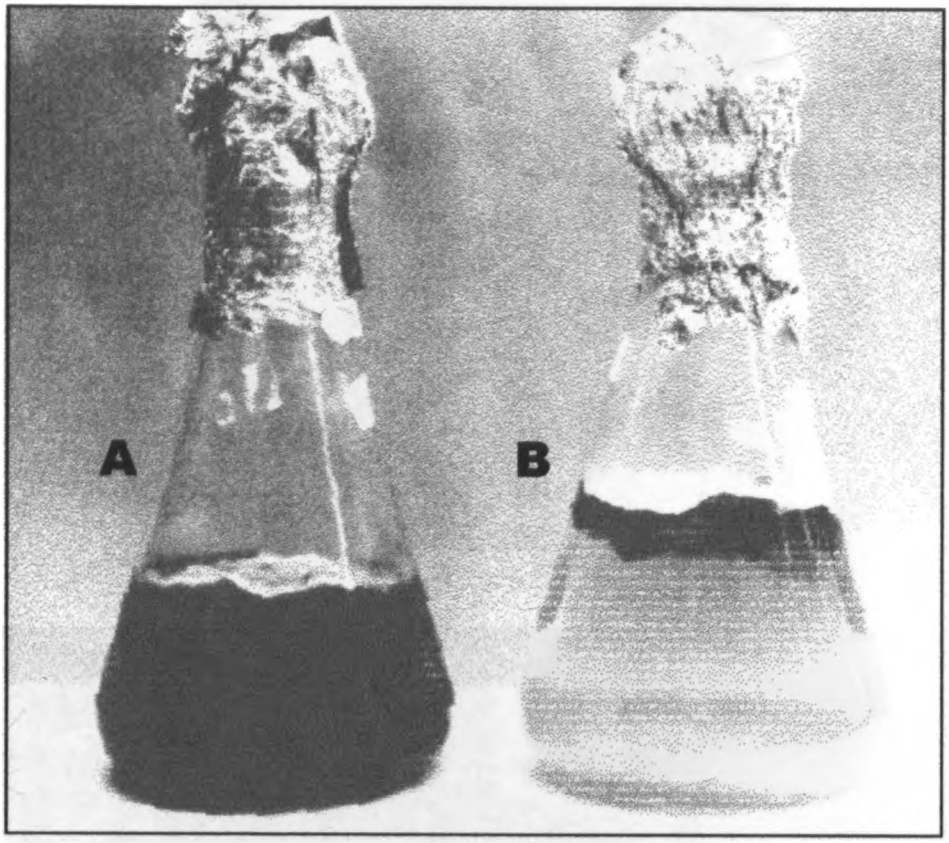
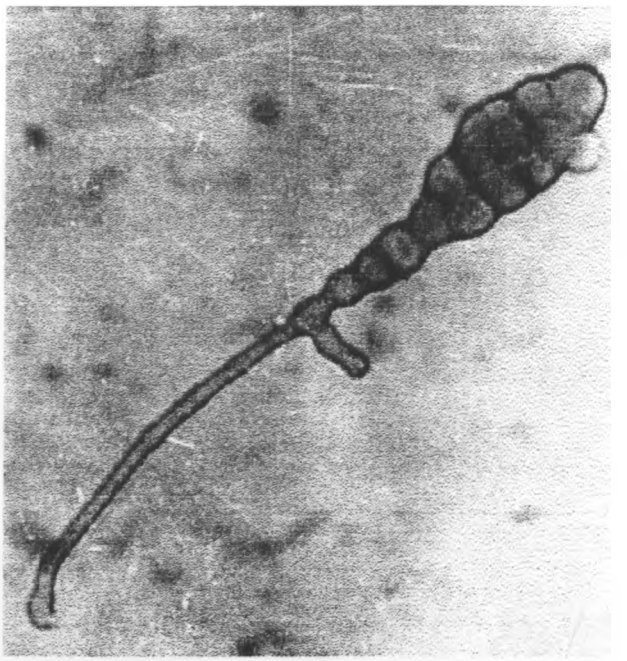


Plate 6: Cultures of *Alternaria* spp. (A), *A. eichhorniae* and (B), *A. alternata*, grown on FPDB (Fresh potato dextrose broth) for four weeks.



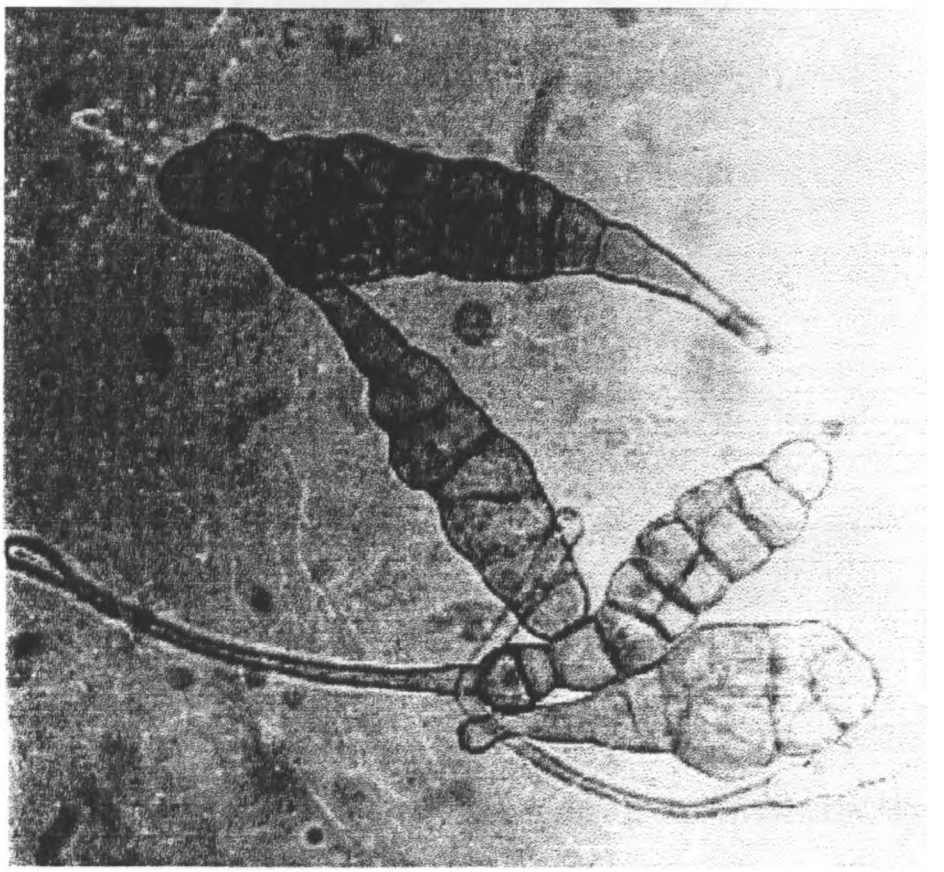


Plate 7: (A), Conidium of *A. eichhorniae* grown on V8 juice agar. (B), mass conidia of *A. eichhorniae* grown on V8 juice agar. Mg x 400.

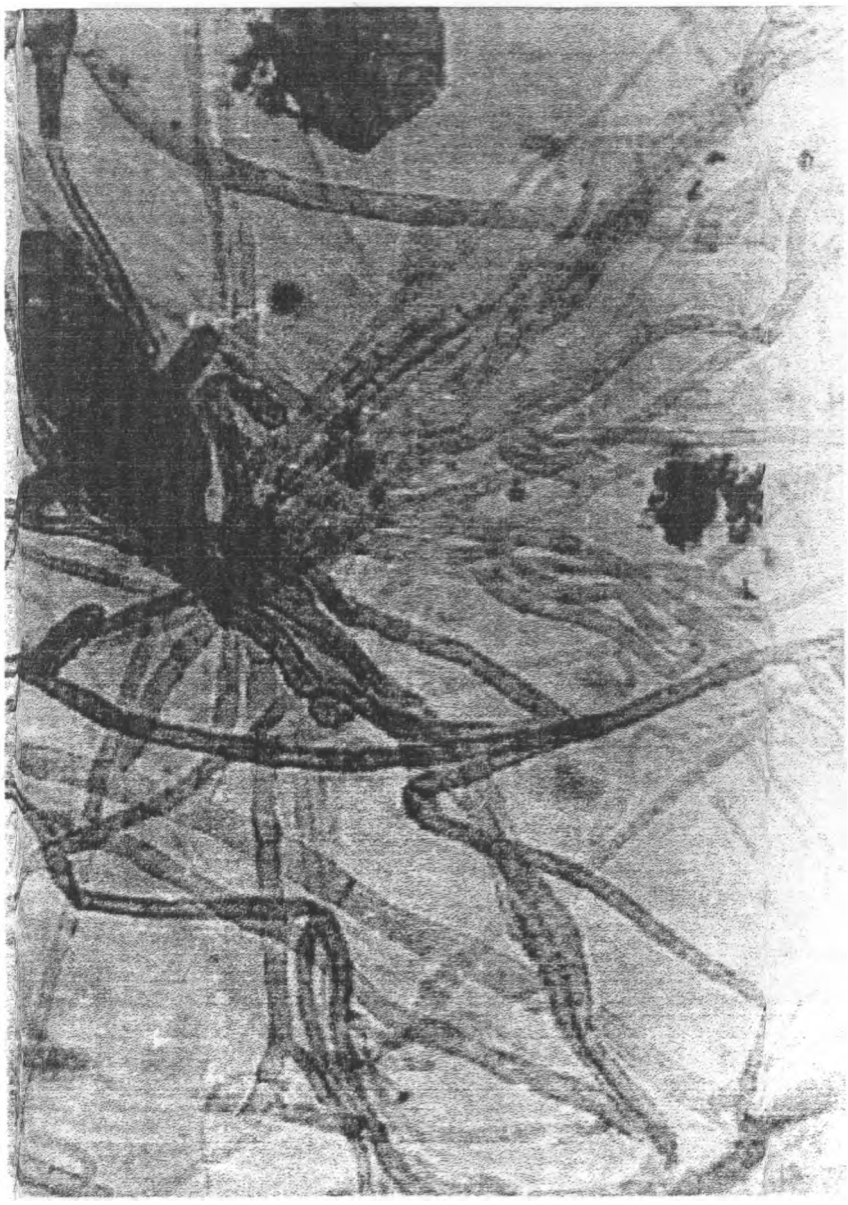


Plate 8: Colla and Conidiophore of *A. eichhomiae* grown on WHDA. Mg x 400.

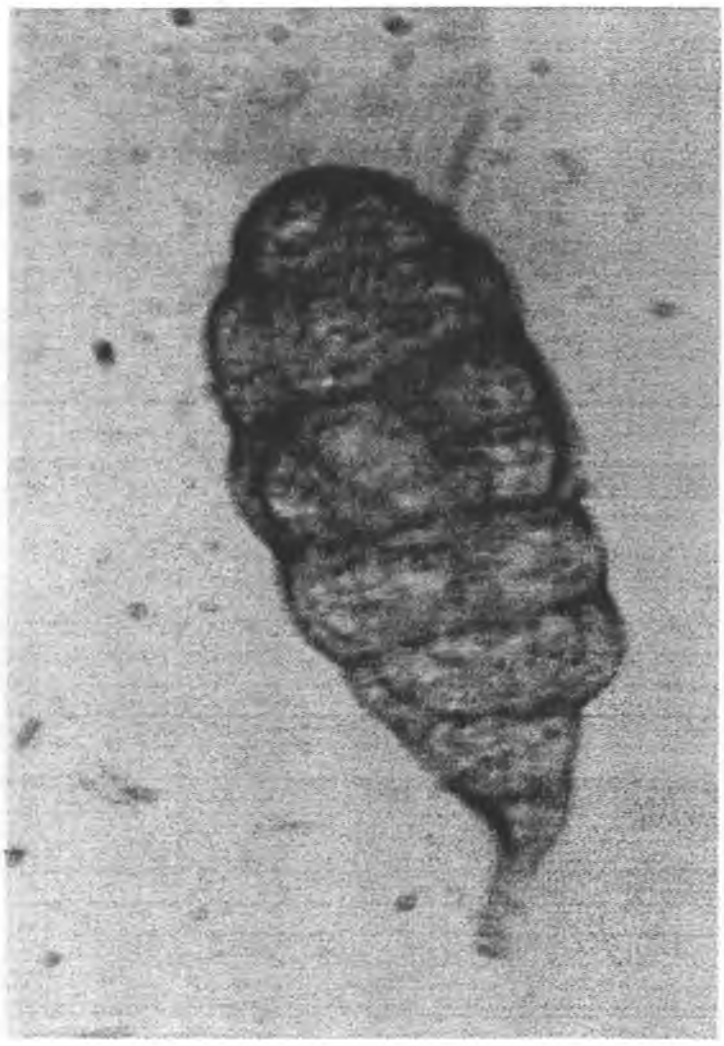


Plate 9: Conidium of *A. alternata* grown on PDA. Mg x 1000.

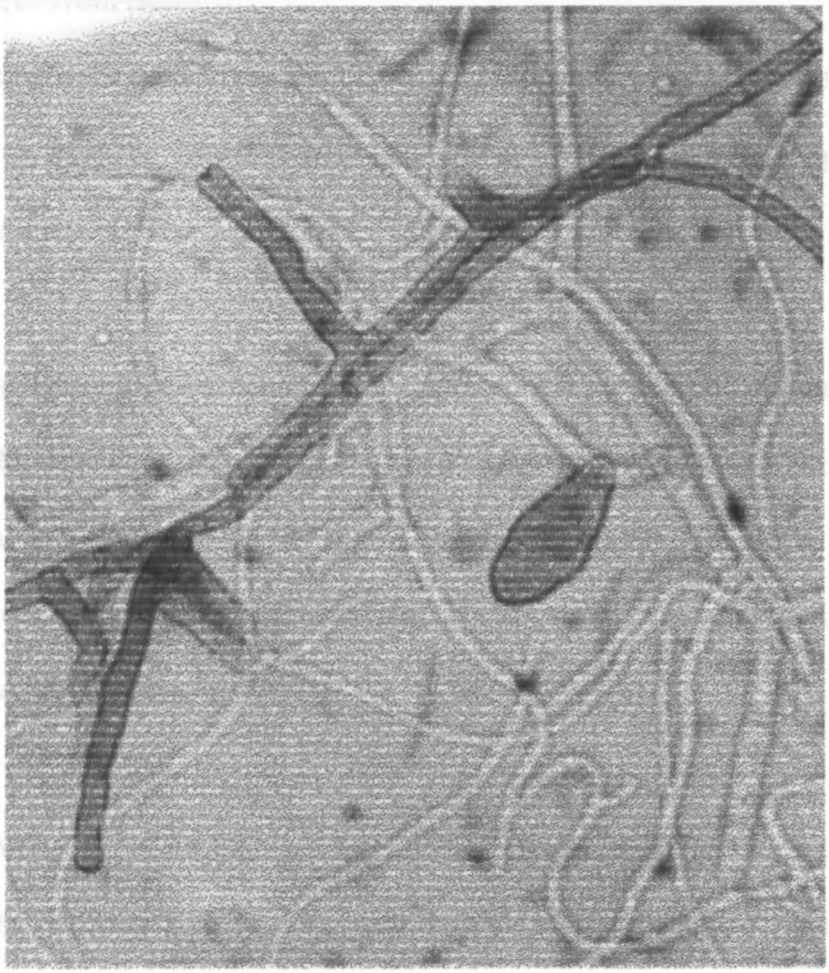


Plate 10: Conidiophore and conidium of *A. eichhorniae* grown on V8 juice agar. Mg x 400.

4.5 Effect of media , light regime on the pathogencity of *A. eichhorniae*

The Duncan multiple range test results were used to rank the effectiveness of the three broth media in promoting DI and DS. Based on the effectiveness of DI and DS the three broth media were ranked as fresh potato dextrose broth (FPDB), followed by water hyacinth dextrose broth (WHDB) and water hyacinth fresh potato dextrose broth (WHFPDB). The latter two (WHDB and WHFDB) were not significantly different at P=0.05.

The light regime had a significant effect on DI. On the basis of Duncan multiple range test, the three light regimes could be ranked for their effectiveness in promoting DI as diurnal light (DL) followed by continuous darkness (CD) followed by diurnal light for one week followed by continuous darkness for another one week (DL – CD), all significantly different at P=0.05.

The interaction of medium and light regime had a significant effect on DS but not on DI. The treatment FPDB – DL + CD gave the highest DI and DS values of 90 % and 3.67 respectively (table 7). The implication here being that the *A. eichhorniae* inoculum grown on FPDB for 1 week, under diurnal light followed by another 1 week, under continuous darkness was the most effective in producing the highest DS and DI values.

Table 7: Influence of type of medium and light regime on the Pathogencity of *A. eichhorniae*

Medium[a]	Light regime[b]	DI (%) [c]	DS [c]
FPDM	DL	82.00A[d]	0.83A
FPDM	CD	76.33A	1.33A
FPDM	DL – CD	90.00A	3.67B
WHDB	DL	50.00B	0.83A
WHDB	CD	50.00B	0.83A
WHDB	DL – CD	51.67B	0.83A
WHFPDB	DL	54.67B	1.00A
WHFPDB	CD	57.33B	0.83A
WHFPDB	DL – CD	60.67B	0.83A

[a] A suspension of conidia of 1.4 x10^6 conidia/ml was used

[b] DL, diurnal light; CD continuous darkness

[c] DI, disease incidence (expressed as a percentage); DS, disease severity (from a disease severity scale, 0-5), were quantified after 10 days

[d] Means within a column followed by the same letter (s) are not significantly different according to Duncan multiple range test P =0.05

4.6 Histopathological studies

Pre-penetration, penetration and colonisation of host tissues in the infection process of water hyacinth by *A. eichhorniae*

On the study of the mode of penetration of epidermal cells of water hyacinth by *A. eichhorniae*, five phases were distinguished in the infection process. These were conidial germination, appressorium formation, germtube entry, inter-, and intra-cellular penetration followed by necrosis of the host cells.

After inoculation, germination did not occur until after the six hours. By the ninth hour 95% of the germinating conidia had a germ tube. The germ tube appeared to emerge from the tip of the pedicel. Okoth, (1989) while working on *A. crassa* on *Datura stramonium* reported that the germ tube emerged from one or more cells as the tip of the pedicel, this was not observed with *A. eichhorniae*. After 12 hours most of the conidia had germinated and some were penetrating the host tissue through the epidermis and forming a globose appressorium.

There was an indention of the cuticle and cell wall prior to penetration. The germ tube stained deeply as compared to the rest of the conidia in cotton blue lactophenol. In a few instances the conidia were observed penetrating the host through the stomata, in which case, no appressorium was formed. Similar observations were made by Okoth (1989) while working on the mode of penetration of *A. crassa* on the weed *Datura stramonium*. There were cases when conidial germ tubes produced an infection peg similar to that of *Botrytis cinerea* as described by McKeen (1973) where infection

frequently occurred when the germ tube was short 10-20 µm. Some germ tubes passed over the stomata without penetrating. Similar findings have been reported for *Colletotrichum kahawae* on *Coffea arabica* (Mwang'ombe and Shanker, 1994).

Following penetration, germ tubes continued growing in the host in a similar manner following either stomatal or direct penetration. The infection hyphae branched repeatedly and ramified through the host tissue and became intercellular. The hyphae elongated in a radial manner proceeding inter- and intra-cellurarly.

Seventy-two hours after inoculation, the hyphae eventually destroyed cell contents and many cell walls leaving small cavities and an irregular network of cell wall fragments. Mostly affected were the air chambers whose intercellular spaces normally regular in shape and which serve as an adaptive feature to floating plants (Fahn, 1982). Invasion of the spongy parenchyma resulted in destruction and collapse of the tissue. Chloroplasts were observed irregularly scattered in the spongy mesophyll.

From the water hyacinth plants inoculated with *A. eichhorniae* and from which leaf sections were not cut, necrotic spots appeared within 6-7 days after inoculation. The lesions enlarged due to intercellular growth of hyphae and subsequent destruction of all leaf tissues. By the 10^{th} day the fungus had sporulated on the surface of the lesion which often coalesced to form large necrotic areas followed by premature defoliation. The lesions were similar to those of leaves of water hyacinth inoculated with *A. eichhorniae* in the pathogenicity tests.

These lesions were cut, surface sterilised as described in section 3.2.1 a. and plated on PDA plates and incubated at room temperature (20-24^{0}c). *A. eichhorniae* was re-isolated from the diseased lesions thus confirming Koch's postulates.

4.7 FIELD STUDIES

4.7.1 The rate of disease progress in the field

Table 8 : Numeric area under disease progress curves (NAUDPC) from field test showing the rate of disease progress in the field for six weeks

Pathogen[a]	Disease severity scores Time in weeks[b]						Numeric AUDPC[c]
	1[d]	2	3	4	5	6	
A. alternata	0.75	0.83	1.17	2.00	2.50	2.83	58.030
A. eichhorniae	0.67	2.50	3.50	4.30	4.67	4.83	124.040
Control	0.17	0.33	0.50	0.58	0.67	0.67	17.500

[a]1.4 x 10^6 conidia/ml was used for each isolate. Sterile distilled water was used to inoculate the control

[b]Disease severity scores taken after every one week for six weeks

[c]AUDPC –Numeric area under disease progress curve computed directly from disease severity scores

[d] Each value for disease severity score is a mean of 6 replicates

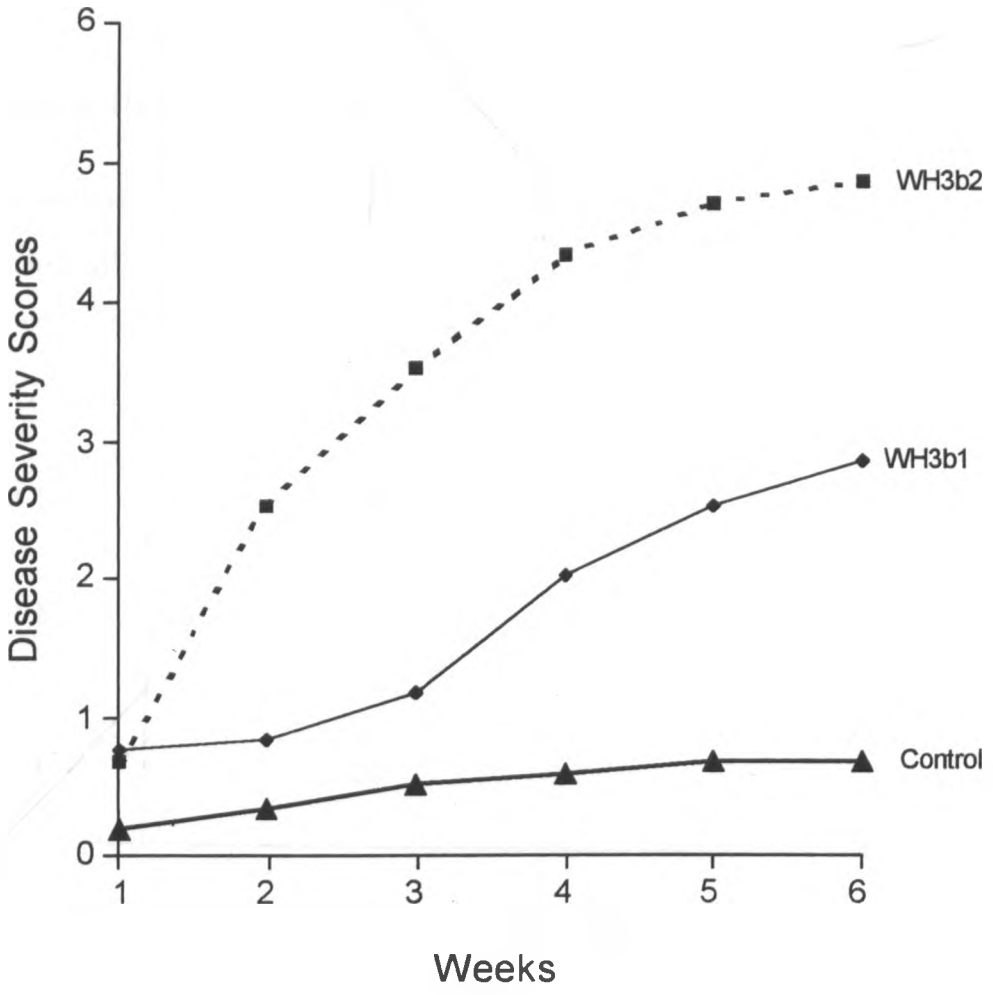


Fig.1: Rate of disease progress with time for the two *Alternaria* spp.

4.7.2 The rate of disease spread in the field

Table: 9 Rate of disease spread in the field for *Alternaria alternata*

Treatment[a]	Disease incidence expressed as a percentage[b]				
	Rep. 1	Rep.2	Rep.3	Rep. 4	Mean [c]
Plot 1	50	52	48	54	51.00A
Plot 2	25	20	16	20	20.25B
Plot 3	20	20	0	0	10.00B

[a] 3 treatments: plot 1- inoculated with 1.4×10^6 conidial/ml. plot 2- uninoculated and located 0.5-m from plot 1. plot 3- uninoculated and located 3-m away from plot 2

[b] Disease incidence- calculated on the basis of individual plants in a plot. then total added then averaged as per the plot and expressed as a percentage.

[c] Means within a column followed by the same letter(s) are not significantly different according to Duncan multiple range test P =0.05

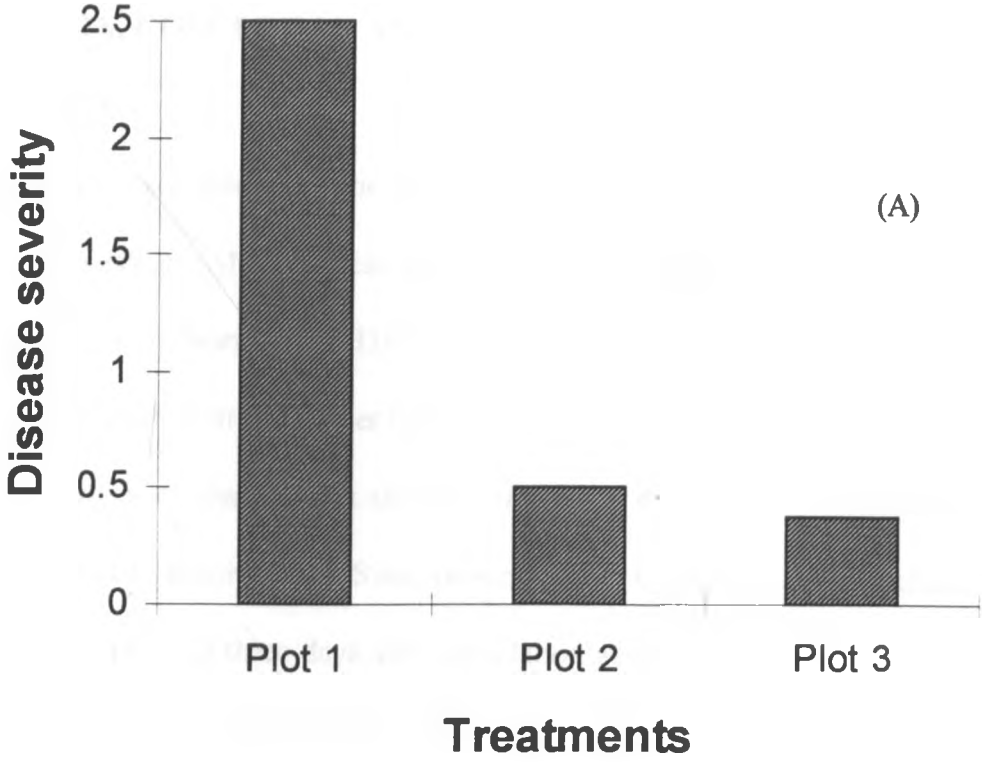
Table: 10 Rate of disease spread in the field for *Alternaria eichhorniae*

Treatment[a]	Disease incidence expressed as a percentage[b]				
	Rep. 1	Rep.2	Rep.3	Rep. 4	Mean[c]
Plot 1	74	60	76	64	68.50A
Plot 2	50	54	54	48	51.50B
Plot 3	20	0	24	16	14.00C

[a] 3 treatments: plot 1 – inoculated with 1.4×10^6 conidial/ml. plot 2- uninoculated and located 0.5-m from plot 1, plot 3- uninoculated and located 3-m away from plot 2

[b] Disease incidence- calculated on the basis of individual plants in a plot. then total added then averaged as per the plot and expressed as a percentage.

[c] Means within a column followed by the same letter(s) are not significantly different according to Duncan multiple range test P =0.05



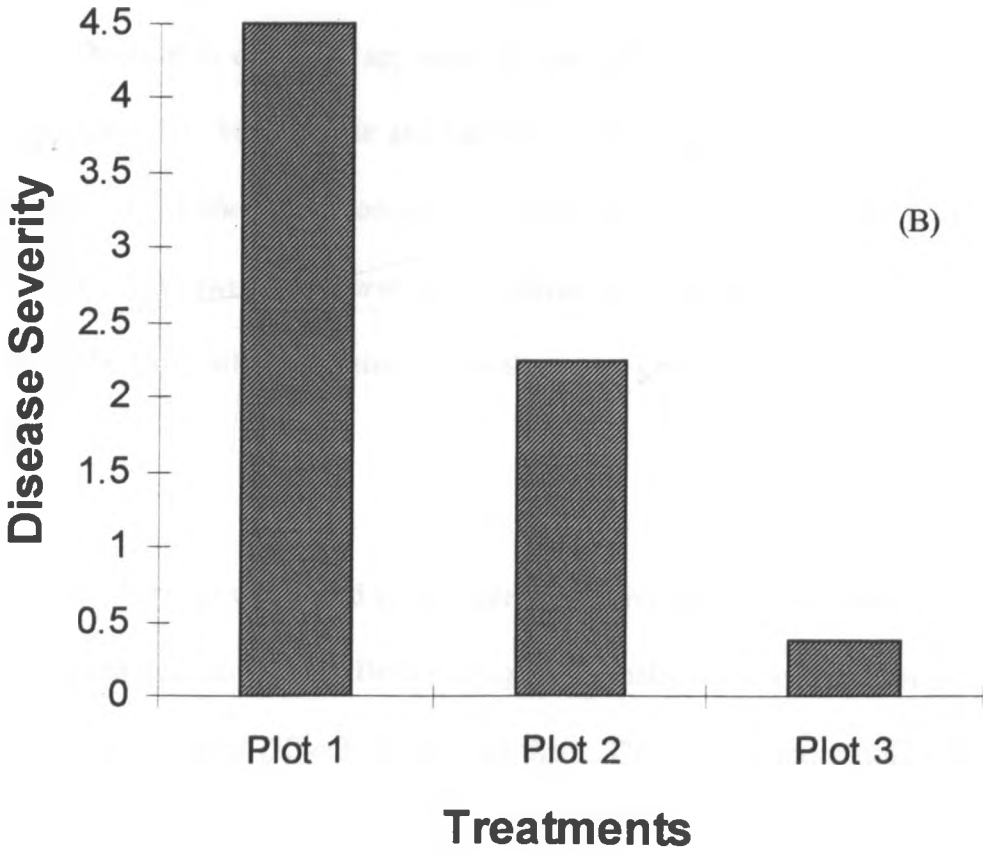


Fig 2: Disease spread in (A), *A. alternata,* and (B) *A. eichhorniae*, where: Plot 1 = Treated ; Plot 2 = Control No. 1; Plot 3 = Control No. 2.

5:0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

Surveys for fungi associated with the water hyacinth in Kenya yielded 20 fungal isolates in various genera. Most of these fungal isolates were not pathogenic on water hyacinth. *Alternaria eichhorniae* (WH3b2) was identified as the best potential fungal pathogen for biological control of water hyacinth followed by *A. alternata* (WH3b1). Initial green house tests showed that the two *Alternaria* species differed in disease symptoms on water hyacinth plants. Symptoms on water hyacinth plants inoculated with *A .alternata* appeared three days after inoculation as chlorotic flecks while that of *A .eichhorniae* appeared after the fifth day as tiny necrotic flecks surrounded by a yellow necrotic halo. After a week, the symptoms became very distinct with those of *A. eichhorniae* manifested as blotches with brown to dark brown centres and faint yellow margins. Those of *A. alternata* appeared as deep yellow with brown coloured centres after the ninth day. Microscopic and cultural studies helped in delimitation of the two pathogens. *A. eichhorniae* produced a pigment in culture of PDA while *A. alternata* did not. The two *Alternaria* spp differed in conidia sizes. Conidial measurements of the two pathogens fitted within the range given for their respective type specimens.

In this study, *A. eichhorniae* was found to be more aggressive than *A. alternata* both under greenhouse and field conditions. Both pathogens normally occurred together on the same lesions thus making it difficult to separate them. Thus, single spore isolation aided in the separation of the two isolates. Elwakil *et al.* (1989) while carrying out a similar study in Egypt made several isolations of *Alternaria,* which he described as *A. alternata*. Four years later Shabana *et al.* (1995) while working on the same isolates

separated *A. eichhorniae* from *A. alternata*. Although this was the first report of *A. eichhorniae* in Egypt and Africa in particular *A. eichhorniae* is not a new pathogen of water hyacinth as such. Nag Raj and Ponnappa (1970) reported *A. eichhorniae* as the causal agent of blight of water hyacinth in India. Nevertheless, this is the first time the pathogen has been reported in Kenya

The apparent narrow host range and production of a phytotoxin with a narrow host spectrum are the two factors that make *A. eichhorniae* to qualify as a biocontrol agent (Nag Raj and Ponnapa, 1970). Host range trials done in Egypt (Shabana *et al.* 1995) affirmed this. The occurrence of *A. alternata* as a pathogen of water hyacinth has been reported in Bangladesh, Australia, and India. *A. alternata* is a plurivorous species with several pathotypes and a saprophyte (Ellis, 1971) . This raises doubt on the suitability of the pathogen as a biocontrol agent.

According to Shabana *et al.* (1989) the presence of more than one species of *Alternaria* as causal agent of different diseases on the same host is not unusual. For instance, *A. alternata A. brassicae* and *A. brassicola* often occur in the same geographical region as pathogens of Brasicaceae such as broccoli, cabbage and cauliflower. Due to the possibility of co -occurrence of *A. alternata* and *A. eichhorniae* isolates, the two species could easily be confused unless they are grown under cultural conditions that promote chromogenesis. The occurrence of *A. eichhorniae* in the newly colonised (adventive) regions but not in the native region of this weed suggests that this is a recently evolved pathogen representing a new host pathogen association as theorised by Hokkanen and Pimentel (1984) and elaborated by Hokkanen (1985). Nag Raj and Ponnapa (1970) described *A. eichhorniae* on the

basis of three key characteristics; chromogenesis in culture, surmaturation of conidia and absence of gemmae.

Colony and conidial morphology and pathogencity to water hyacinth were used in describing *A. eichhorniae* (Shabana *et al.* 1995). Chromogenesis was observed in all the cultures of *A. eichhorniae*. Chromogenesis is the most reliable taxonomic feature of this species; the red colour being due to the production of a group of phytotoxic pigments (Shabana *et al.* 1995).

The levels of disease assessed in terms of disease severity and disease incidence increased with increase in inoculum levels of the two pathogens. However, at high levels of inoculum concentration, there was no significant difference between the higher inoculum levels on both disease incidence and severity. This may be explained by the fact that at higher levels of inoculum concentration, germtube formation in conidia was inhibited. Okoth (1989) reported similar observations on the studies of *A. crassa* as a pathogen of the weed *Datura stramonium*. The effect could be observed when the inoculated leaves were mounted under the microscope. In areas where spores were crowded, either very little or no germination occurred. This renders credence to the use of standardised inoculum if effective results are to be achieved.

Radial growth diameter was used to assess the *in vitro* growth of the two isolates. Although colony diameter is not recommended as a reliable parameter (Lilly and Barnett, 1951), it can nevertheless be used as a suitable indicator of a preference of a pathogen to a certain medium. In this particular experiment *A. eichhorniae* and *A. alternata* grew well on all solid media initially tested. It is therefore imperative that

colony diameter would be a reliable parameter for comparing *in-vitro* growth of the two species. *A. alternata* grew well in V8 agar and PDA but poorly in WHLDA whereas *A. eichhorniae* did poorly in V8 and PDA but extremely well in WHLDA. The conclusion here being that the strong affinity of *A. eichhorniae* on WHLDA further points to its specificity on the water hyacinth and that it's possible to culture the pathogen in the laboratory cheaply.

The fungus *A. eichhorniae* sporulates easily in both solid and liquid media and therefore multiplication of this pathogen would be fairly easy. Studies by Shabana *et al.* 1995 have affirmed *A. eichhorniae* as an aggressive and safe pathogen. Conidial and mycelial fragments of this pathogen are infective. This could be formulated as a wettable or a suspension inoculum. Development of *A. eichhorniae* as a bioherbicide is already under way in Egypt.

Studies on *A. eichhorniae* by Naj Raj and Ponnapa (1970) revealed that the fungus grew and sporulated well on steam sterilised petioles of water hyacinth. Sporulation on PDA for both the two pathogens was poor or entirely absent.

Nyanapah (1982) discourages the use of colony diameter more so when precise measurements are required due to the fact that some fungal colonies have a tendency to grow upwards rather than laterally. In the study of colony characteristics of *Cercospora sesami* it was observed that colonies of *C. sesami* (which grew laterally) were larger than those of *C. sesamicola* (which had an upward tendency). Mycelial dry weights of the two fungi were similar when cultured on liquid media for equal duration.

Among the various liquid media and cultural conditions tested for the production of highly pathogenic inoculum of *A. eichhorniae*, best results were obtained on FPDB under an alternating photo stimulus of diurnal light and continuous darkness. This study related very well with that of Shabana *et al.* (1995) who in addition found out that both the mycelial and conidial inocula of *A. eichhorniae* were equally virulent and that shake cultures were faster growing than stationary cultures. The effects of light regime and shaking could be due to their effects on pigmentation and phytotoxin production. Soderhall *et al.* (1978) found that white light stimulated the production of a red brown pigment of *A. alternata*. The two main phytotoxins produced by *A. eichhorniae* are red pigments which, unlike alternariol and alternariol mono methyl ether studied by Soderhall *et al.*(1978) are not inhibited by light. Maity and Samaddar, (1977) found that the shake culturing during the first three days of incubation reduced the time needed for maximum toxin production by *A. eichhorniae*. Phytotoxin producing mycelium may be more necrogenic and capable of inducing rapid pathogenesis than non-toxigenic mycelium and should be more desirable for bioherbicide use and *A. eichhorniae* presents such an opportunity.

Histological studies showed that the pathogen *A. eichhorniae* invaded water hyacinth between the sixth and the ninth hour indirectly through the stomata and directly through the epidermis. Since water hyacinth is an aquatic weed, numerous stomata are to be found on the adaxial side and remain open through out thus giving the fungus ample time for penetration. Open stomata have been reported to act as an attractive stimulus to germ tubes (Dickson, 1949; Rathiah, 1977). There were indications of conidia forming an infection peg without necessarily developing an appressorium,

causing an indentation of the cuticle and epidermal cells and thus suggesting mechanical penetration. Mwang'ombe and Shanker, (1992) while working on *Colletotrichum kahawae* noted that there were no appressorial attachments observed over the stomata but appressorium was formed above the epidermal cells. The anatomy of *Eichhornia* sp. is such that the leaves and stems have air chambers filled with gases. These chambers are inter-cellular, regularly shaped and pass through the entire leaf. The air chambers are usually separated from one another by thin partitions of one or two layers of chloroplasts containing cells called the diaphragms which apparently allows the passage of gases but not water (Fahn, 1982). This is a survival mechanism, which aids in floatation. Seventy-two hours after inoculation these cells were completely destroyed by the intra- cellular mycelium of *A. eichhorniae*.

Inter- and intra- mycelial development of the pathogen in the host tissues was the same regardless of the mode of penetration. After 72 hours there was substantial break down of the spongy mesophy ll and the adjacent air cells with chloloplasts being scattered and the host tissue extensively damaged by *A. eichhorniae*.

Water hyacinth plants were found to be susceptible to *A. eichhorniae* with the older leaves being more susceptible than the emergent younger leaves. The rate of disease spread and progress is fast such that after one month the whole plant is covered with lesions. This greatly impairs the photosynthetic ability of the plant rendering it more susceptible to attack even by other opportunistic fungi such as *Curvularia* and *Fusarium* spp.

From this study it is evident that *A. eichhorniae* is both wind and water disseminated Abundant spores were found in the necrotic lesions and readily splashed and wind spread to the adjacent plants. Controls placed 0.5m away from treated plants were found to be diseased after the 6th week. One of the most practical considerations for mycoherbicide candidate is its ability to easily spread in the field. Charudattan (1989) notes that a mycoherbicide that does not yield fairly rapid control cannot be fitted into the growers cultural and pest schedules and therefore may not find acceptance even if its other aspects are satisfactory. Rates of disease progress for both *A. eichhorniae* and *A. alternata* were monitored periodically for six weeks. After the 6th week the amount of disease quantified based on disease severity for the six weeks were computed and used to calculate the area under disease progress curves (AUDPC).

AUDPC for the two pathogens were significantly different from the control with that of *A. eichhorniae* being higher (124.040) than that of *A. alternata* (58.030) and the control (17.500).

Disease severity for both *A. eichhorniae* and *A alternata* progressed with time reaching a maximum at the sixth week (Figure 1). However in all cases *A. eichhorniae* had higher DS values than *A. alternata*. AUDPC as measure of the aggressiveness of a pathogen was used in this study to show the aggressiveness of the two pathogens. From the results obtained *A. eichhorniae* is an aggressive pathogen of the water hyacinth having greater than 50 % of the leaf surface covered with lesion and tip die back after the sixth week. *A. alternata*, though pathogenic was not as aggressive as *A. eichhorniae*..

Charudattan (1989) indicated that in considering potential mycoherbicide candidates, speed should be measured in terms of 2 to 6 weeks following application during which disease development and early signs of successful control should be evident, although complete or total control may be ensured several weeks later. This was depicted by the *A.eichhorniae* isolate in this investigation.

According to Figure 1, time aspect has an important role to play in the epidemiology of the two pathogens of water hyacinth. The fact that the initial inoculum applied was standard and that there was no multiple application thereafter shows there was progress of disease with time. There is an indication that after initial inoculation and subsequent infection, there is sporulation of the pathogen and production of secondary inoculum. *A. eichhorniae* is therefore a virulent and an aggressive pathogen. A disease severity score of 5 according to Conway (1976a) is equivalent to more than 75 % of the leaf surface covered with lesions and petiole death. Such was the case of *A. eichhorniae* after the 6th week.

5.2 Conclusion and recommendations

This study reveals that *A. eichhorniae* and *A. alternata* have the potential as biocontrol agents of water hyacinth in Kenya. *A. eichhorniae* meets the criterion of a mycoherbicide as far as virulence and aggressiveness are concerned.

The occurrence of *A. alternata* as a pathogen of water hyacinth has been reported in several countries. *A. alternata* is a plurivorous species with several pathotypes and a saprophyte . This raises doubt on the suitability of the pathogen as a biocontrol agent.

Histological studies showed that the pathogen *A. eichhorniae* invaded water hyacinth between the sixth and the ninth hour indirectly through the stomata and directly through the epidermis. This is an important factor in determining the most ideal conditions for field inoculations. The most likely period would be in the evening to take advantage of low temperatures and thus inoculum desiccation is minimal.

For *A. eichhorniae*, there was dispersal of the pathogen from the treated plot to the adjacent plot 0.5 m away, but not to the second control plot which was 3 m away. This could be attributed to the dissemination of the pathogen propagules by wind or water. This is an important attribute, which shows that the pathogen can be used as a mycoherbicide under field conditions.

A. eichhorniae therefore meets the criteria of a mycoherbicide candidate and it is recommended that;

1. Further detailed studies on cultural aspects of *A. eichhorniae* be carried out.
2. There is a need to carry out a study that describes the relationship of water hyacinth growth rate to *A. eichhorniae* efficacy as a predictive tool in the biocontrol system.
3. More studies on the host range of *A. eichhorniae* be done to certify that the pathogen does not pose any danger to the crops and the environment
4. The development of this pathogen as a bioherbicide in Kenya should commence once the above three concerns have been positively addressed.

LITERATURE CITED

Aneja, K.R. and B. Srinvas, 1993. Fungal pathogens of aquatic weed of Haryana. J. of Mycopathological Research. **30**:2.139- 152.

Abdel - Rahim, A.M. 1984. *Phoma sorghina* causing a leaf spot of water hyacinth in the Sudan. Plant path. **33**: 429.

Abdel - Rahim, A. M. and S. Tawfig, 1984. Pathogencity of fungi and bacteria from the Sudan to water hyacinth. *Weed Research*, UK **24**: 233-8.

Batanounty, K. H. and A.M. EL-Fiky, 1984. Water hyacinth in Egypt .*In*; Proceedings of the international conference on water hyacinth. Thyagarajan, G. (ed.) pp.127-144.UNEP.Nairobi. Kenya.

Bebawi, F. F. 1972. Studies on the ecology of *Eichhornia crassipes* (Mart.) Solms in the Sudan. MSc. thesis, University of Khartoum.

Boesewinkel, H. J. 1976. Storage of fungal cultures. Trans. Br. Mycol. Soc. 66(1): 183.

Burdon, J. J. and D. R. Marshal.1981. Biological control and the reproductive mode of weeds. J. APP. Ecol. 18,649 –658.

Caunter, I .G. 1982. Potential for biocontrol of water hyacinth with indigenous fungal Pathogens. Kuala Lumpur, Malaysia. *Malaysian plant prot. Soc.*, 489-92.

Charudattan, R. 1973. Pathogenicity of fungi and bacteria from India to hydrilla and water hyacinth. Hyacinth control. J .**11**:44-48.

Charudattan, R. 1989. Assessment of efficacy of mycoherbicide candidates. Proc, VII. Int. Symp. Biol. Contr. Weeds 6 -11 March 1988, Rome, Italy. Delfosse, E.S.(ed.). 1st. Sper. Patol. Veg. (MAF), pp. 455 -64.

Charudattan, R. and H. L. Walker, 1982. Biocontrol of weeds with plant pathogens. John Wiley and Sons. New York. 293pp.

Charudattan, R., D.E Mc Kinney, H.A.Cordo, and A. Silveira – Guido, 1976. *Uredo eichhorniae*. A potential biocontrol agent for water hyacinth. In Proc. Iv Int. Symp. Biol. Control Weeds (ed. T.E. Freeman), University of Florida, Gainesville, FL. pp. 210 -3.

Charudattan, R., S. B. Linda, S. B. Marjan kluepfel and Y.A. Osman,1985. Biocontrol efficacy of *Cercospora rodmanii* on water hyacinth. Phytopathology. **75**: 1263-9.

Conway, K.E, 1976a. *Cercospora rodmanii* a new pathogen of water hyacinth with biological control potential. Can. J. Bot. **54**: 1079-1083.

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

Conway, K. E and T.E. Freeman, 1978. The potential of *Cercospora rodmanii* as a biological control agent of water hyacinth (*Eichhornia crassipes*). Proceedings of the 4th international symposium on biological control of weeds, Gainesville, Florida,1976. 207-209.

Conway, K. E; T.E. Freeman and R. Charudattan, 1974. The fungal flora of water hyacinth in Florida. Part 1. Water resources research centre publ No.30.University of Florida. Gainesville, 11p.

Conway, K. E., R.E Cullen, T.E. Freeman, and J. A. Cornell, 1979. Field evaluation of *Cercospora rodmanii* as biological control of water hyacinth

(*Eichhornia crassipes*); Inoculum rate studies . Miscellaneous publication, Army Corps of Engineers, Water ways experiment station . WES- MP - A - 79 -6: 56p.

Crafts, S. A. 1975. Modern weed control. University of California press Berkeley. Los angels. London. 140 –159.

Dickinson, S. 1949. Studies in the physiology of obligate parasitism. The stimuli determining the direction of growth of the germtubes of rust and mildew Spores. *Anni- Bot.* (London) N. S. **13**: 89 –104.

Ellis, M. B. 1971 "Dematеacious Hyphomycetes". Commonwealth Mycol Inst., Kew England.465

Elwakil, M. A., E. A. Sadik, E.A. Fayzalla, and Y. M. Shabana, 1989 Biological control of water hyacinth with fungal pathogens in Egypt *In*. Proceedings of the 8th international symposium on biological control of weeds 483-4.

Fahn, A. 1982. Plant anatomy. 3rd edition. Oxford; Pergman. .221.

Freeman, T. E. 1977. Biological control of aquatic weeds with plant pathogens. *Aquat. Bot*. **3**: 175-8.

Freeman, T. E. and R. Charudattan, 1974. Occurrence of *Cercospora piaropi* on water hyacinth in Florida. Plant. Dis. Reptr. **58**: 277-8.

Freeman, T.E., R. Charudattan and F.W. Zettler, 1973. Biological control of water weeds with plant pathogen. Water resources Research Centre, publ. No 23.University of Florida. Gainesville 52 p.

Freeman, T.E., R. Charudattan and K. E. Conway, 1978. Biological control of water weeds with plant pathogens. Publication Water Resource Research Centre, **WRRC – PUB –45:** 76p.

Freeman, T. E., R. Charudattan, and K.E. Conway, 1981. Biological control of aquatic plants with pathogenic fungi. Technical Report, U.S. Army Corps of Engineers. Waterways experiment station. Vicksburg, MS, pp. 2760-1

Gopal, B. 1984. Utilization of water hyacinth as a new resource for its control. Some environmental considerations. In. *Proceedings of the international conference on water hyacinth*. Thyagarajan, G. (Ed.), pp.193-206. UNEP, Nairobi.

Gopal, B. 1987. *Water hyacinth*. Elsevier science publishers, Amsterdam.477pp

Haider, S. Z. 1984. Mechanisms of absorption of chemical species from aqueous medium by water hyacinth and prospects for utilization .*In*. Proceeding of the International conference on water hyacinth. Thyagarajan G.(ed.), pp. 41-57.UNEP.Nairobi.

Harley, K. L. S., M. H. Julien and A. D. Wright. 1996. Water hyacinth. A tropical Worldwide problem and methods for its control. *Paper prepared for the Second International weed control congress Copenhagen 1996*.

Harris, P. 1971. Biological control of weeds. Environ. Lett.2: 75- 88.

Hasan, S. 1972. Specificity and host specialization of *Puccinia chondrillina*. Annals of Applied Biology 72, 257 – 263.

Hasan, S. 1980. Plant pathogens and biological control of weeds. Review of plant Pathology 59: 349-356.

Hill, G., J. Waage and G. phiri, 1997. The water hyacinth problem in tropical Africa. A paper prepared for the first meeting of an International Water Hyacinth Consortium held at World Bank, Washington, 18-19 March 1997.

Hokkanen, H. and D. Pimentel, 1984. New approaches for selecting biological

control agents. Can. Entomol. **116**: 1109 –1121.

Hokkanen, H. 1985. Success in classical biological control. CRC Crit. Rev. Plant Sci. 3, 35 – 72.

John, C. K. 1984. Use of water hyacinth in the treatment of effluent from rubber industry. *In*. Proceedings of the international conference on water hyacinth. Thyagarajan G.(ed.) pp.699-612.UNEP. Nairobi.

Jenkins, W. A. 1938. Two fungi causing leafspot of peanuts. J. Agric. Research **50**:317-332.

Jones, F. R. 1944. Life history of *Cercospora* on sweet clover. Mycologia **36**: 518-525.

Jones, J. P. 1958. Isolation of a sporulating strain of *Cercospora kikuchii* by selective Subculturing. Phytopathology, 48: 287 –288.

Klingman, G. C. 1982. Weed science: Principles and practices. pp. 401

Latch, C. G. and E. W. Hanson, 1961. Comparison of three stem diseases of *Melilotus* and their causal agents. Phytopath. 52: 300-350.

Lilly, V.G. and L.H. Bernett, 1951. Physiology of fungi. McGraw Hill Book Company Inc. New Yolk. 464 pp.

Mansour, F. A., M. A. Zahran and M.A. Shady, 1980. Microbiological control of Water hyacinth (*Eichhornia crassipes*) in Egypt. *Proc of the seventh ann. Conf. On the restoration and creation of wetlands*. 16-17 May 1980, Tampa, Florida. Cole, D.P. (ed.), pp. 195 –208.

Maity, B. R., and Samaddar, K. R. 1977. A toxic metabolite from *Alternaria eichhorniae*: Production and properties. *Phytopathol .Z.* **88**, 78-84.

Martyn, R. D. and T. E. Freeman, 1978. Evaluation of *Acremonium zonatum* as potential biocontrol agent of water hyacinth. Plant. Dis. Rep. **62**, 604-8.

McKeen, W. E. 1973. Mode of penetration of epidermal cells of *Vicia faba* by *Botrytis Cinerea*. Phytopathology. **64** : 461 -467.

Mwang'ombe A. W. and M. Shanker, 1994. Pre - penetration events and sporulation of *Colletotrichum kahawe* Waller and Bridge (Syn. *Colletotrichum coffeanum* Noack) on different cultivars of *Coffea arabica*. E. Afric. For. J. 59 (4), 327 -336.

Nag. Raj, T. R. and K.M. Ponnapa. 1970. Blight of water hyacinth by *Alternaria eichhorniae* sp. nov. Trans. Brit. Mycol. Soc. 55, 123-30.

Nyanapah, J. O., P.O. Ayiecho, and J.O. Nyabundi, 1995. Evaluation of Sesame cultivars for resistance to *Cercospora* leaf spot. E. Afric. For. J.60 (3), 115 -121.

Okoth, S. A. 1989. Studies on *Alternaria crassa* (Sacc.) Rands as a possible Mycoherbicide on *Datura stramonium* L. Msc. Thesis University of Nairobi. 33 -39.

Pierterse, A. H. and K. J. Murphy, 1990. Fungi in biological control systems. (M. N. Burge, Ed), pp. 88-110.Manchester Univ. Press, Manchester, England.

Rakvidhyasatra, V. and N. Visarathanonth, 1975. Isolation and identification of fungi Pathogenic to water hyacinth (*Eichhornia crassipes* [Malt] Solms. Kaset sart. J. 9: 170 – 7.

Rathiah, Y. 1977. Stomatal tropism of *Cercospora beticola* in sugar beet. Phytopathology. **67**: 358 - 362.

Ridell, R. W. 1950. Permanent stained mycological preparations obtained by slide culture. Mycologia 42: 265-270.

Sanders, D.R. and E.A. Theriot, 1980. Preliminary evaluation of *Cercospora rodmanii* Formulation for the biological control of water hyacinth. Proc. Of the 33rd Annu. Meet. of the S. Weed. Scie. Soc., 15-17 January 1980, Hot springs, Arkansas, Truelove, B. (ed.) 19 3p.

Schroeder, D. 1983. Biological control of weeds. In. Recent advances in weed research. Fletcher W.W (ed.) Commonwealth Agricultural Bureau. 42-68pp.

Shabana, Y.M., R. Charudattan and M. A. Elwakil . 1995. Identification, pathogenicity and safety of *Alternaria eichhorniae* from Egypt as a bioherbicide agent for water hyacinth. Biological Control. **5**:123-135.

Shabana Y. M., R. Charudattan, J. T. Devalerio and M.A. Elwakil, 1997. An evaluation of hydrophilic polymers for formulating the Bioherbi-herbicide agents *Alternaria cassiae* and *A. eichhorniae*. *Weed Tech.* Vol.**11**: 212-220.

Soderhall, K., E. Svensson, and T. Unestam, 1978. Light inhibits the production of alternariol and alternariol monomethyl ether in *Alternaria alternata*. *App. Environ. Microbiol.* **36**: 655 -657.

Syed, R. A., O. Setyawati and Kasno. 1978. Biological control of some important Aquatic weeds (for BioTROP). Triniland, Commonwealth Institute of Biological Control, Report work carried out during 1976, pp. 187-9.

Templeton, G. E. 1981. Status of weed control with plant pathogens. In: Charudattan, R.; Walker, H. L. (Eds) Biological control of weeds with plant pathogens. New York; John Wiley & sons.

Templeton, G. E. 1982. Status of weed control with plant pathogens *In*. Biological control of weeds with plant pathogens. Charudattan, R and H. L. Walker (Eds.) 29- 39.

Templeton, G. E. 1982. Status of weed control with plant pathogens. In Biological Control of weeds with plant pathogens (ed. R. Charudattan and H. L. Walker 1982), pp. 29-44. John Wiley, New Yolk.

Templeton, G. E. 1985. Specific weed control using mycoherbicides. *In*. British crop protection conference weeds. 601-608.

Templeton, G. E. 1986. Mycoherbicide research at the University of Arkansas – past Present, and future. Weed sci. 34 (suppl. 1): 35- 37

Templeton, G. E. 1988. Biological control of weeds. AM. J. Alt. Agric. 3: 69 –72.

Trujillo, E. E. 1976. Biological control of *Hamakua pamaki* with plant pathogens. Proceedings of the American Phytopathological Society 3, 298.

Van der Vossen, R.T.A., A. Cook and G. N.N. Murakuru,1979. Breeding for resistance to Coffee Berry Disease caused by *Colletotrichum coffeanum* Noack (Sensu Hindrof) in *Coffea arabica* L.T. Methods of preselection for resistance. Euphytica 25: 733-745.

Wolverton, B. C and R. C. Mc Donald, 1978. Nutritional composition of water hyacinth grown on domestic sewage. Economic Botany. **32**.363- 370.

Woomer, L. P. 1997. Managing water hyacinth invasion through integrated control and utilization. Perspectives for lake Victoria. African Crop Science Journal. Vol. 5 No.3 309-324.

APPENDICES

Appendix 1: Analysis of variance (ANOVA) table for experiment **4.2:1** of percent infected area of each of the *Alternaria* isolates

Source	Degrees of freedom (df)	Mean square for % infected area	F
Main effects			
Isolates	2	5076.98	155.22***
Error	8	32.71	
Total	10		

*** Highly significant at probability level P = 0.05

Appendix 2 Analysis of variance (ANOVA) table for experiment **4.3:1** of effect of inoculum concentration Disease Incidence (DI) - *Alternaria* isolate WH3b1

Source	Degrees of freedom (df)	Mean square for DI	F
Main effects			
Conidial conc.	3	321.58	36.07***
Error	12	8.92	
Total	15		

*** Highly significant at probability level P = 0.05

Appendix 3: Analysis of variance (ANOVA) table for experiment **4.3:1** of conidia concentration on disease severity (DS) - *Alternaria* isolate WH3b1

Source	Degrees of freedom (df)	Mean square for DS	F
Main effects			
Conidial conc.	3	0.4167	0.678^{ns}
Error	12	0.2083	
Total	15		

^{ns} Not significant

Appendix 4 Analysis of variance (ANOVA) table for experiment **4.3:1** of effect of conidial concentration on disease incidence (DI) - *Alternaria* isolate WH3b2

Source	Degrees of freedom (df)	Mean square for DI	F
Main effects			
Con.Conc.	3	591.75	55.26***
Error	12	10.71	
Total	15		

*** Highly significant at probability level P = 0.05

Appendix 5: Analysis of variance (ANOVA) table for experiment **4.3:1** of effect of conidial concentration on disease severity (DS) - *Alternaria* isolate WH3b2

Source	Degrees of freedom (df)	Mean square for DS	F
Main effects			
Con. Conc.	3	1.062	5.68*
Error	12	0.1885	
Total	15		

* Significant at probability level P = 0.05

Appendix 6: Analysis of variance (ANOVA) table for experiment **4.4** of the effect of media on radial growth of the pathogen. *Alternaria* isolate WH3b1

Source	Degrees of freedom (df)	Mean square for DI	F
Main effects			
Media	2	425.39	17.873***
Error	15	23.8	
Total	17		

*** Highly significant at probability level P = 0.05

Appendix 7: Analysis of variance (ANOVA) table for experiment **4.4** the of effect of media on radial growth of the pathogen. *Alternaria* isolate WH3b2

Source	Degrees of freedom (df)	Mean square for DI	F
Main effects			
Media	2	2109.50	205.85***
Error	14	10.25	
Total	16		

*** Highly significant at probability level P = 0.05

Appendix 8: Analysis of variance (ANOVA) table for experiment **4.5** of effect of media and light regime on the pathogencity of *A. eichhorniae* inoculum. Disease incidence (DI).

Source	Degrees of freedom (df)	Mean square for DI	F
Main effects			
Light	2	2550.037	5.67*
Media	2	87.37	165.51***
Interaction			
Ligh * Medi	4	35.43	0.565^{ns}
Error	18	14.96	
Total	26		

*** Highly significant at probability level P = 0.05
* Significant at probability level P = 0.05

Appendix 9: Analysis of variance (ANOVA) table for experiment **4.5** of effect of media and light regime on the pathogencity of *A. eichhorniae* inoculum. Disease severity (DS).

Source	Degrees of freedom (df)	Mean square for DS	F
Main effects			
Light	2	3.53	16.28^{***}
Media	2	2.11	27.21^{***}
Interaction			
Ligh * Medi	4	2.25	18.42^{***}
Error	18	14.96	
Total	26		

*** Highly significant at probability level P = 0.05

Appendix 10: Analysis of variance (ANOVA) table for experiment **4.7.2** of the rate of disease spread in the field due to the effect of wind and water. *Alternaria* isolate WH3b1 (DS).

Source	Degrees of freedom (df)	Mean square for DS	F
Blocks	3	0.1875	0.5894^{ns}
Main effects			
Plot	2	5.6875	21.00^{**}
Error	6	0.2708	
Total	11		

*** Highly significant at probability level P = 0.05
ns Not significant at probability level P = 0.05

Appendix: 12 GLOSSARY

GLOSSARY

Acroauxic Growing and elongating at the peak.

Appressorium A flattened hyphal pressing organ from which a minute infection peg usually grows and enters the epidermal cells of the host.

Biodiversity The variety and value of life on earth from the genetic through the organismal to the ecological levels.

Blastic Blown out with marked enlargement of the conidium initial taking before it is delimited by a septum.

Conidium Any a sexual spore which when mature is liberated from a a conidiophpre or conidiogenous cell.

Conidiophore A simple or branched hyphal arising from a somatic hypha and bearing at its tip or side one or more conidiogenous cells. Sometimes used interchangeably with conidiogenous cell.

Ellipsoidal Elliptical in optical section.

Gemmae A thick walled cell similar to a chlamydospore.

Haustorium A special hyphal branch esp. one within a living cell of the host for absorption of food.

Inoculate To put a micro - organism or a substance containing one into an organism or a substratum.

Inoculum The substance generally a pathogen used for inoculating

Mononematous Conidiophore composed of a single thread or filament.

Mycelium Mass of hyphae constituting the body of the fungus

Nodose	Straight
Obclavate	The shape of a club upside down, thickened towards the base.
Ovoid	Egg shaped with the broad end at the base
Obypyriform	The shape of a pear upside down with broad end at the base.
Septum	A cross wall in hypha.
Sympodial	Proliferating the axis elongating by growth of a succession of each of which develops behind and one side of the previous apex where growth had ceased with the production of conidium or conidia
Tretic	Apparently enteroblastic, protrusion of the inner wall taking place through one channel or several channels in the inner wall
Verruculose	Finely warted