Natural occurrence and potential of pathogenic fungi for integrated management of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach: Pontederiaceae, in Lake Victoria.

By

Kusewa Mwende Teresia

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Kusewa Mwende Teresia

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School of Environmental Studies
Moi University
P.O. Box 3900
Eldoret.

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TITLE

Natural occurrence of pathogenic fungi on diseased water hyacinth (*Eichhornia crassipes*), and their potential in integrated biological management of the weed in Lake Victoria.

Declaration

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Signature **Date**

Kusewa Mwende Teresia

SES/PBG/03/98

Declaration by Supervisors

This research thesis has been submitted with our approval.

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Signature **Date**

Dr. J.B. Okeyo Owuor

Division of Environmental Biological Sciences

School of Environmental Studies

Moi University.

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Signature **Date**

Dr. Gerald R.S. Ochiel

National Fibre Research Centre, Kibos

Kenya Agricultural Research Institute.

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DEDICATION

To my children, Peter Kitonga and Peris Wairimu

ABSTRACT

These studies were conducted to determine the natural occurrence of pathogenic fungi assciated with water hyacinth in Lakes Victoria and Naivasha in Kenya. The aim was to investigate the potential of integrating some of the most virulent fungal species identified, with *Neochetina* weevils for control of the water hyacinth in Lake Victoria.

Two surveys to collect, isolate and identify fungi associated with diseased water hyacinth plants were conducted in December 1999 (dry season) and May 2000 (rainy season) at different sites in Lakes Victoria and Naivasha. A total of 22 fungal species were isolated and some were found pathogenic to water hyacinth. In Lake Naivasha, 7 species were isolated and identified of which 80% were found to be pathogenic. In Lake Victoria, 22 species were isolated and identified and 69% were found to be pathogenic on water hyacinth. Two new pathogenic taxa of *Fusarium* and *Alternaria* were recorded for the first time in the study area. Fungal occurrence was higher during the rainy season recording a total of 17 species as compared to 12 fungal species recorded during the dry season.

Pathogencity tests were carried out in the laboratory and screen house at Muguga against water hyacinth to determine the fungi that were virulent on the weed. *Alternaria alternata* and the new taxa of *Fusarium* sp. showed the highest mean disease incidence of 50.5% and 40.6% respectively at 2.5% w/v in 0.2% Tween-20 solution, six weeks after infection. Although the disease severity (DS) did not show any significant difference at P=0.05, the new taxa of *Fusarium* performed better than other species (DS=27.8%). Both the disease incidence and severity were gave a strong negative effect on the water hyacinth plant's ability to generate new leaves. Correlation analysis between new leaf turnover with disease incidence and disease severity gave correlation co-efficient values of -0.92 and -0.99 respectively.

Screen house experiments showed that integration of both fungus *Alternaria alternata* and the new *Fusarium* sp with *Neochetina* weevils increased both the disease incidence and

disease severity significantly. *Alternaria alternata* integrated with *Neochetina* weevils gave an increase of 31.1% in disease incidence and 16.1% in disease severity. Integrating *Fusarium* sp. with *Neochetina* weevils showed an increase of 14.6% in disease incidence and 76.0% in disease severity. The action of the fungi and *Neochetina* weevils was synergistic and this study recommends integration of the two control methods as part of the management strategy for water hyacinth in Lake Victoria.

ACKNOWLEDGEMENTS

CHAPTER 1

1 Introduction

1.1 General Introduction

The aquatic weed water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laubach: Pontederiaceae) is an erect free floating, stoloniferous perennial herb (Harley *et al.,* 1996) and is indigenous to Brazil. In Kenya, water hyacinth was first recorded in 1957 growing as an ornamental plant by Gopal (1987). Today the weed is found in many water bodies especially Lake Victoria, Lake Naivasha and Nairobi Dam as well as ponds and waterways. It is also in other places with fresh water for example at Bamburi Cement factory and various restaurants in Nairobi and Mombasa. The entry route into Lake Victoria is thought to be River Kagera between 1989 and 1992. Recent estimates of the weed cover put it at 6000 hectares on the Kenyan side of Lake Victoria by April 1998 (Synoptics, 1998).

In East Africa, the water hyacinth has recently caused serious disruption to artisanal and commercial fishing and water transport, and to infrastructure such as water supply intakes, port facilities and the hydroelectricity generation plant at Owen Falls Dam (Harley *et al.,* 1996). Due to its negative economic importance, the Kenya law (Cap. 324 Plant Protection Act) prohibits importation, growth and cultivation of water hyacinth in the country.

Water hyacinth thrives in warm, shallow, nutrient-rich water. Under favourable conditions it can reproduce itself every two weeks often vegetatively and also by seed. The side shoots (stolons) break off and develop into new plants. Its rapid growth and multiplication makes mechanical and chemical methods of control ineffective and expensive requiring repeated application. Therefore biological control offers the most suitable ecologically sustainable control strategy for this weed (Harley, 1990). If biological control is used alone, expenditure ceases after the first few years, control continues indefinitely and it is environmentally safe.

A number of biological control agents have been identified. The most successful of these include two phytophagous weevils, *Neochetina eichhorniae* (Warner) and *N. bruchi* (Hustache) and a moth *Niphograptha (=Sameodes) albiguttalis* (Warren) (Harley, 1990). The potential of phytopathogens as biological control agents for the weed has not yet been fully and fairly studied. However, a few fungal pathogens have been found to infect water hyacinth. These include *Acremonium zonatum* (Saw.) Gams (Hyphomycetes), *Cercospora rodmanii* Conway, *Alternaria eichhorniae* Nag Raj & Ponnappa, *A. alternata* (Fr.) Keissler and *Rhizoctonia solani* Kühn (all Hyphomycetes) and *Uredo eichhorniae* Gonz.-Frag. & Cif. (Teliomycetes). Since these fungi are known to occur naturally in several countries in the tropics, it should be possible to isolate promising native strains and thus eliminate fears about introducing exotic pathogens.

Studies by Charudattan *et al.* (1978) showed the possibility of integrated control systems for water hyacinth. Interaction between arthropods and several saprophytic and parasitic fungi and bacteria are common on arthropod-damaged water hyacinth. They observed that usually, there is an increase in the incidence of fungal infection following arthropod attack. They reported that the occurrence of water hyacinth leaf spot disease caused by the fungus *A. zonatum* was correlated to the incidence of arthropod damage in South Florida. These findings suggest the potential use of fungal pathogens either singly or integrated with other control methods that would sustainably manage water hyacinth on Lake Victoria Kenya.

1.2 Statement of the problem

The population in the Winam Gulf of Lake Victoria basin region is estimated to be over 7.5 million (CBS, 2001). This situation continues to exert environmental degradation of the lake's water resources resulting in water pollution, depletion of oxygen, algal blooms and proliferation of exotic water weeds such as water hyacinth all of which result into adverse socio-economic effects on the area's population.

Whereas indigenous plants are in equilibrium with their habitat, water hyacinth has grown excessively. Many factors favouring proliferation of the weed in Lake Victoria have lead to this. These include the absence of natural enemies and the new nutrient-rich habitat. In Kenya the

infestation of water hyacinth oscillates during the year amongst the bays of Kisumu, Kendu, Osodo, Nyakach, Homa and Asembo. The weed occurs also in other beaches such as Wichlum and Uhanya in Siaya; Rukala and Mabinju in the Nzoia River and Delta in Busia District, where it causes serious environmental problems and hinders socio-economic development. The plant is threatening the survival of other wetland plants in Lake Victoria that are of economic importance such as papyrus. The large mats pose major obstruction to fisheries operations.

Research on the development of sustainable and integrated methods for control of water hyacinth in Kenya, though recently initiated by the Kenya Agricultural Research Institute, still lacks sufficient data. Hitherto, no data has been collected on the potential of phytopathogens for control of the weed, and yet the problems caused by water hyacinth on the lake and its surroundings escalate.

1.3 Justification

The goal of an effective weed management system is to maintain an environment detrimental to the weed populations through the use of preventive, managerial, physical, biological and/or chemical methods. Each particular method of control has its advantages and disadvantages, which are often related to the characteristics of the weed or weed-complex. Thus, there is need for integrating two or more methods to obtain satisfactory control (Watson *et al.*, 1988). Usually, the aim would be to increase the level of biotic stress to curtail the plant capacity for compensatory growth and population resurgence. Integration can be viewed as vertical integration where a combination of various control tactics is used against a single pest (e.g., a weed species) or as horizontal integration used across different pest groups (insects, diseases and weeds) in one crop. This study was primarily concerned with the vertical integration of fungal pathogens and *Neochetina* weevils to control water hyacinth in Lake Victoria. Improved levels of biological control are possible if additional agents are deployed to complement the existing ones.

In the USA, Charudattan (1986) reported that the fungus *C. rodmanii* or the weevils *N. eichhorniae* and *N. bruchi* alone did not completely control water hyacinth. Individually, the arthropods significantly reduced shoot height (by about 50%), while the pathogen caused about

2% reduction in shoot length. In contrast, integrating both pathogen and arthropod achieved 99% control of water hyacinth in the experimental plots within 7 months clearly suggesting that their effects are synergistic. This position needs to be explored for controlling the water hyacinth menace in Lake Victoria. Although the biological control studies using weevils are at an advanced stage, the potential of integrating it with fungal pathogens offers opportunities for effective control of this weed.

1.4 Objectives

Overall objective:

To study the occurrence of isolates and identify indigenous pathogenic fungi and establish the effectiveness when integrated with *Neochetina* weevils for biological control of water hyacinth.

The specific objectives of the study are:

(i) To survey, isolate, identify and document the occurrence of local fungal pathogens on diseased water hyacinth plants around Lake Victoria and Lake Naivasha

(ii) To determine the pathogenicity of the isolated fungal species on water hyacinth

(iii) To evaluate the potential for integrating pathogenic fungi and *Neochetina* weevils in the control of water hyacinth.

Hypothesis:

(i) Ho: Different species and strains of pathogenic fungi naturally occur on water hyacinth in Lake Victoria and Lake Naivasha.

(ii) Ho: Some locally occurring pathogens are highly virulent on water hyacinth and can reduce the incidence of the weed significantly.

(iii) Ho: Integrating pathogenic fungi and weevils are synergistic for water hyacinth control.

1.5 Study areas

The study was carried out on the Kenyan side of the Lake Victoria, at the Winam Gulf and at Lake Naivasha in the Rift Valley.

1.5.1 Lake Naivasha

1.5.1.1 Introduction

Lake Naivasha lies on the floor of the Rift Valley, between 0°45'S and 36°20'E, some 80 km north-west of Nairobi. It consists of a shallow fresh water lake and is fringed by *Acacia* dominated woodland. The lake is of recent geological origin and is ringed by extinct or dormant volcanoes. Lake Naivasha includes three chemically distinct water bodies. The main lake (about 15,000 ha, max. depth c. 8 m) incorporates a partially submerged crater, the Crescent Island lagoon (max. depth c. 18 m), at its eastern end. The lagoon is largely isolated at low water levels. To the southeast, separated by Papyrus, *Cyperus papyrus*, swamp and an isthmus of Acacia woodland, is a small (c. 550 ha), somewhat alkaline Lake Oloidein. Lake Naivasha is a significant natural resource both domestically and internationally. In addition to serving as a freshwater source for the growing community of Naivasha and satisfying the irrigation needs of the horticultural sector, it also provides cover and habitat for a number of different wildlife species.

1.5.1.2 Geophysical features

The lake level measured in 1993 was 1886.54 metres above sea level. Soils on the lacustrine plains around the lake have developed on sediments from volcanic ashes and do vary from well to poorly drained, fine to sandy silts and clay loams of varying colour, but often pale (LNROA, 1993). Fertility is variable and in some areas the soil can be sodic or saline. In the catchment soils are generally well developed but of moderate to low fertility, deep clayish loams, greyish brown to black, often with drainage problems. Soils often degenerate into black cotton soils with impended drainage in low-lying areas.

JICA (1990) gave the total catchment area of the Lake Naivasha basin as 3401 km^2. The discharge from the catchment surface is wholly internal and gravitates from higher altitudes.

There are a number of rivers around the lake but only two of these Malewa or Morenda and Gilgil are important and give substantial flow into the lake. They together account for 90% of the waters flowing to the lake. There is a definite sequence of vegetation species from the submerged hydrophytes in the lake, working towards the Acacia-grassland on dryland. The most common vegetative cover around the lakes natural environment is dense *Tarchonanthus camphoratus/Acacia drepanolobium* shrubland.

Another important feature around the lake region is the generation of geothermal energy, which is comparatively cheap, clean and environmentally acceptable. The geothermal fields at Olkaria generates 45 MW.

1.5.1.3 Climate and rainfall

Mean maximum ambient temperatures for Naivasha range from 24.6 to 27.3°C with the highest temperatures in January and February (Jaetzold and Schmidt, 1983). The mean monthly minimum temperature ranges from 6.8^{o}C to 8.0°C with the coldest months in July and August. Rainfall is bimodal with main pulses in April/May and again in November. Average rainfall for the lake is 608 mm per annum with a variation round the mean from 443 to 939 mm.

1.5.1.4 Demography and socio-ecomonics.

The population living within the Lake Naivasha catchment area according to the 1999 National Census report, is approximately 1.2 million people (CBS, 2001). The land within the catchment area is classified as suitable for ranching, but with increasing land pressure it is being subdivided and forced to host an intensive smallholder system of production more suitable to the high potential areas. Major irrigation development and pumping from the lake has been established over the last 20 years. The growing of cut flowers and vegetables for export is now big business around the lake, and of national importance due to the foreign exchange the industry generates and employment that it offers to an estimated 30,000 work force. Fishing is also a major activity in the lake. But the lake has received major environmental problems especially from agricultural pollutants, water abstraction and invasion of two main aquatic weeds, *Salvinia molesta* and *Eichhornia crassipes.*

1.5.1.5 Map Of Lake Naivasha

Fig. 1 Map of Lake Naivasha

1.5.2 Lake Victoria (Winam Gulf)

Lake Victoria is the largest freshwater lake in Africa and the second largest fresh-water lake in the world. It covers an area of 68,800 km^2, of which 6% of the lake falls on the Kenyan side whereas 43% and 51% fall in Uganda and Tanzania respectively. Lake Victoria Basin is endowed with rich and unique terrestrial and aquatic bio-diversity, ranging from forests, wildlife and fisheries. However, Lake Victoria is an extremely fragile ecosystem. It is shallow- only 80 metres at its deepest and depends on rainfall for 85 percent of its water.

1.5.2.1 Geo-physical features

The Lake Victoria region ranges from 1120-1500m above sea level. The terrain is generally flat but somewhat gently undulating in some areas. Large areas of this region are composed of granitic rocks, and these have been only small areas affected by volcanic activity. Most parts of the Winam Gulf are extremely shallow and have irregular shoreslines, being a typical feature of Lake Victoria. There are many bays on the western shores of central and south Nyanza.

The major rivers, which drain into the lake such as Nzoia, Nyando, Sondu, Yala and Kuja, originate from Kenyan Highlands such as Mt. Elgon, the Cherangani Hills, Mau and Elgeyo Escarpments. During their course many tributaries join the rivers. The Rivers Yala and Nyando rivers flow into broad swamps near the lake.

With the wide variety of rocks there is considerable diversity in the soil types of the lake region. In the volcanic areas, on the higher hills, dark-red clays have developed into rich and fairly well drained soils. Around the gulf and the shores of the lake, the sedimentary rocks have broken down into sandy loam, peaty swamp soils rich in humus and large areas of 'black cotton' soils.

Little remains of the original vegetation in this region since most of it has been cleared for agricultural development. Along the main watercourses there are some areas overgrown with dense tropical forests and especially the upper slopes highland grass and forest. But most of the region there is savanna vegetation, with scattered low trees and fairly high grass.

1.5.2.2 Climate and rainfall

The temperatures around the lake region are considerably high. Kisumu's mean annual temperature for instance, is 29.4°C., with maximum temperatures recorded between 27°C - 35°C (KMD, 1984). The relative humidity is also high due to the closeness of Lake Victoria, and afternoon thunderstorms often occur. Kisumu receives an average annual rainfall of 1323mm and has a very short dry season. The lowest rainfall occurs in January (~ 60mm) and October (~75mm). The wettest period is from March to June, with peaks in April (195.5mm) and May (254mm). In the north, east and south where the land rises annual rainfalls of 1524mm are recorded (KMD, 1984).

1.5.2.3 Demography and socio-economics

The population living within the Lake Victoria region, according to the 1999 National Census report, is approximately 7.5 million people (CBS, 2001). The growth rate in the region is reported to be among the highest in the world and currently stands at 6% per annum. The lake is very important to the riparian community by providing food, freshwater for livestock, domestic, agricultural and industrial use, hydroelectric energy generation, recreation, tourism, transport and is a storehouse of vast genetic resources. Increasing population pressure and socio-economic activities in the lake basin have resulted in changes in land use, water quality, wetland biodiversity and fisheries in and around the lake.

The main economic activities within the lake region are based on agriculture and fisheries. Current estimates show that annual fish catch from Lake Victoria is about half million metric tons with Tanzania landing 40%, Kenya 35% and Uganda 25%. This generates some US$ 300-400 million. Over 70% of the population in the lake region are small-scale farmers, and grow mainly sugar, tea, coffee, maize and horticultural crops as well as keep livestock.

Fig.2　Map of the Winum Gulf.

CHAPTER 2

2 Literature review

2.1 Water Hyacinth

2.1.1 Biology and distribution.

Water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach is a flowering plant in the family Ponterideriaceae (Harley, 1990). It is a free-floating aquatic weed with rosettes of leaves supported by the petiole. It spreads both vegetatively by stolons that develop from the base of the rosette and by seed. The rate of reproduction results in doubling in the area infested, if growth conditions are favourable, every 6-15 days.

According to Gopal (1987) the water hyacinth shoot comprises of a sympodially branched, stoloniferous rhizome with several short internodes. The rhizomes grow up to 6 cm in diameter and up to 30 cm in length. Each node bears a leaf and roots. The leaf consists of a petiole, an isthmus (the thin part between the petiole and the blade) and a blade. The petioles may be elongated, swollen in the middle and tapering slowly towards the blade or may form a bulbous float. They are spongy, measure up to 5 cm in diameter and generally 30-50 cm long. The leaf lamina is orbicular to ovoid in shape with a nearly cordate base. The laminae usually measure upto 15 x 30 cm. Roots are adventitious, fibrous, unbranched and have a conspicuous root cap. The roots produce a large number of laterals of limited growth giving them a fine feathery appearance. Each lateral has a prominent root cap. The roots exhibit little variation in their thickness but length varies from 10 to 300 cm. The roots are normally dark violet to bluish or pinkish violet in colour.

The flowers are borne on a terminal inflorescence, which is an ‘attractive lavender coluoured spike subtended by two bracts and surmounted on an elongated peduncle’. Each spike has 4 to 25 flowers. The flowers are sessile, beautifully coloured but short-lived. Each flower develops into a fruiting capsules with up to 400 minute seeds (Harley, 1994).

Water hyacinth grows in a variety of freshwater habitats from shallow temporary ponds, marshes and sluggish flowing waters to large lakes, reservoirs and rivers. These habitats present a broad spectrum of physio-chemical environments. Water hyacinth growth is greatly influenced by the levels of nutrients in the water particularly nitrogen, phosphorous and potassium (Reddy *et al.*, 1989; 1990; 1991). These nutrients often emanate from fertilizers applied to agricultural land reaching water bodies through run-off or via agricultural drains and urban and industrial effluents (Harley, 1994). The plant has been reported in areas with temperatures ranging from as low as 1° C during winter in northern latitudes to over 40°C summer temperatures in dry tropics.

Water hyacinth is one of the most economically important aquatic plants (Holm *et al.*, 1977). The weed is widely distributed in the tropics and sub-tropics and may extend to latitude 40°N and 45°S. The weed, which is native to South America, was first introduced into Africa in Egypt around 1880. It has now spread to most water bodies on the continent (Gopal, 1987). In Kenya, water hyacinth is reported to have been introduced as an ornamental in 1957. It first appeared in Lake Naivasha between 1982-1983. The first reports of the weed in Lake Victoria were made during the early 1990's (C.W. Kariuki, pers. comm.).

2.1.2 Ecological and economic importance.

The current rate of spread of water hyacinth in Africa appears to be much greater than elsewhere and is seriously affecting water resource management, ecology, conservation of biodiversity, and the economic well-being of humans in riverine communities. Research carried out by Hamdoun and Tigani (1977) showed that evapo-transpiration through a cover of water hyacinth is always greater than evaporation loss from an open water surface. They estimated that 7 billion m^3, or one-tenth of the average flow of the Nile, was lost every year through evapotranspiration by water hyacinth. This reduction in water can eventually convert open water into shallow mashes.

Water hyacinth plant cover causes obnoxious smell, and adds colouring matter and suspended particulate matter in the water. In general, it's cover lowers temperature, pH, bicarbonate alkalinity and dissolved oxygen content, and increases the free carbondioxide content, Biological Oxygen Demand (B.O.D), and nutrient levels (Gopal, 1987). The diffusion of oxygen into water

through the water-atmosphere interface is nearly completely blocked and the oxygen content of water under a mat of water hyacinth may reduce to zero affecting the fauna and flora in the water bodies (Harley, 1990; 1994). Fish production is heavily reduced. Shading reduces or completely eliminates photosynthetic activity. However, water hyacinth, if it does not develop a complete cover, may prove useful to fish which find shelter in the root masses and possibly also feed upon the organisms and detritus there (Gopal, 1987).

These changes to the aquatic environment affect the health of riverine communities directly through reduced fish catches and by increasing the incidence of diseases such as malaria, encephalitis, schistosomiasis, and river blindness (Holm, 1969). Thick mats of water hyacinth also impede access to fishing areas, destroy fish traps and increase breeding of vectors of human and animal diseases (Harley, 1996). Dawood *et al.* (1965) showed that in Egypt, the bilhazia snails prefer inhabiting areas of *Potamogeton crispus* followed by *Eichhornia crassipes*, and then *Panicum repens.* In addition they claimed that the cholera organisms concentrate around the roots of water hyacinth.

Water hyacinth infestation of canals and drains causes a reduction in the flow of water below designed levels thereby preventing delivery of irrigation water and drainage of fields. Dams become choked and silted resulting in reduced capacity and useful life. In addition, plants swept into the intakes of hydroelectricity generating plants and irrigation headworks can cause costly damage. When rivers are infested, navigation is impeded and landings are blocked and damaged (Charudattan, 1986).

Nutrient rich water in particular by nitrogen, phosphorous and potassium favour growth of water hyacinth. In addition to these, calcium, magnesium, sulphur, iron, manganese, aluminum, boron, copper, molybdenum and zinc and have also been found to enhance water hyacinth growth. On a positive note therefore the ability of water hyacinth to extract nutrients and heavy metals is exploited in sewage or industrial wastes treatment plants by passing them through channels containing the weed (Harley, 1994). In order for successful treatment to be achieved, plants must be kept growing actively by regular removal of excess vegetative material. However, water

hyacinth should never be introduced into a region where it does not occur, as there is risk of creating a serious weed problem.

2.2 Management of water hyacinth

Management strategies for water hyacinth include chemical control, mechanical /physical removal, utilization and biological control. Chemical control and mechanical/ physical removal requires a costly and continuous input. In contrast, biological control is more flexible in its application. Chemical control may be used for controlling small infestations accessible by land or boat and eradicating some small infestations in regions that are climatically unfavorable to growth of water hyacinth (Smith *et al.,* 1984).

2.2.1 Chemical control

Chemical control is expensive and requires a high input of manpower, chemicals and mechanical equipment. The advantage of chemical control is that it is quick and effective. However, the direct and indirect impacts of the herbicides on the environment are enough to invite caution in their use. The rapid kill of large mats of weed adds a huge quantity of organic matter to the water body (Anon 1985). The detritus formed after chemical control cannot be removed and its decay releases large amounts of nutrients. This results in rapid degradation of water quality, development of algal blooms and other changes associated with eutrophication. More often, the organic matter creates anoxic conditions in shallow water bodies resulting in large-scale death of fish and other aquatic organisms. Most chemicals are broad-spectrum in action and often not confined in their action to the target organisms alone but affect other organisms also in and around the water body. If residues are excessive the water will be unsuitable for human consumption or irrigation.

In spite of this, a few chemicals have been recommended for use in water hyacinth control. Among a number of chemicals so far used against water hyacinth, 2,4-dichlorophenoxy acetic acid (2,4-D) which is widespread in use and efficacious. Plants sprayed with 2,4-D exhibit twisting, curling and elongation of leaves within 24 hrs. The symptoms are later intensified, the

plants start sinking within a few weeks and later decompose under water. The herbicide biodegrades rapidly and lasts for about a week. The U.S. Federal Food, Drug and Cosmetic Act established a tolerance level of 1 mg/kg 2,4-D in fish and shellfish and 0.1 mg/l in potable water (Gopal, 1987). The drift of the herbicide by wind during its aerial spray can cause great damage to crops and other plants and animals in the surrounding area.

Another chemical, N-(phosphonomethyl) glycine (glyphosate) was reported to achieve complete kill in 14 days at a concentration of 2 kg/ha. Glyphosate acts slowly but may prove better than 2,4-D and other chemicals. It has the advantage of low toxicity to fish and rapid biodegradation in soil and water (Gopal, 1987). However, the cost of application may be prohibitive in some countries (Labrada, 1996).

Harley (1994) reviewed the negative effect on biocontrol agents by the chemical herbicides. If not applied carefully and selectively, they can nullify the effect of biocontrol agents by disrupting or eliminating local populations of parasitoids and predators. When a large population of water hyacinth is killed within a short period, a large population of natural enemies especially arthropods may die of starvation. He stated that any surviving adults and immature stages might not be able to migrate to chemically untreated populations of the weed and face adverse physical conditions in the drying of the weed. The normal cyclic increase in arthropod population will be upset and the biocontrol pressure on subsequently emerging host populations will be disrupted.

2.2.2 Mechanical/physical control

Physical control involves pulling out the plants by hand and by employing boats, rakes and booms to help in the collection and transport of the plants to the land in cases of large and deeper water-bodies. This is practiced in many developing countries. Another method is to construct floating barriers, which help in accumulating the weed in a certain part of the water body to also prevent it from reaching other waterbodies. Physical control has obvious limitations of scale and re-infestation by remnant plant fragments and seeds (Harley, 1994).

Mechanical control involves the use of more specialized equipment and machinery specifically designed for the mechanical removal of water hyacinth. This involves use of grapplers, conveyor belts, cutter and destroyer boats, saw boats and forks (Gopal, 1987).

Mechanical and manual/physical removal of the water hyacinth has been practiced in Egypt, Ghana, Congo and Uganda (Fish, 1988; Khattab, 1988; de Graft-Johnson, 1988). The methods present very little environmental hazards and provide room for plant utilization. They are useful methods for reducing small infestations and for maintaining canals. The techniques, manual and mechanical removal, are costly, as wages per working day and machine running and maintenance costs are prohibitively high.

2.2.3 Biological control

Pieterse (1990) defined biological control of aquatic weeds as 'activities aimed at decreasing the population of an aquatic weed to acceptable levels by means of a living organism or virus'. In general there are three approaches for biological control of aquatic weeds:

i) the use of selective organisms, i.e. organisms that attack one or only a few species;

ii) the use of non-selective organisms i.e. organisms which attack all (or nearly all) weed species present and;

iii) the use of competitive plants species i.e. plants which compete with a weed species for one or more critical growth factors.

Biological weed control technologies are being developed world wide to augment current cultural and chemical control methods and to replace herbicides which have lost registration or where existing weed control technologies are ineffective or environmentally unacceptable (Johnson *et al.,* 1996). In the Amazon Basin (South America), the area of origin of water hyacinth, the weed is not much of a problem because the natural enemies present attack the weed and keep its population below the nuisance level (Oso, 1988).

2.2.3.1 Control by means of arthropods

Research into biological control of water hyacinth began in 1961. The control agents were first released about 10 years later and one or more species have been introduced in many countries worldwide (Harley and Forno, 1990). Arthropod control agents such as *Neochetina eichhorniae* and *N. bruchi* (Coleoptera: Curculionidae), the moths *Niphograptha (=Sameodes) albiguttalis, Acigona infusella* and *Arzema densa* (Lepidoptera: Pyralidae) and the mite *Orthogallum terebrantis* (Acarina: Galumnidae) have been found to control water hyacinth in its native habitats (Center *et al.,* 1988).

According to Gopal (1987), the *Neochetina* weevils have attained the highest levels of control among all the agents so far released. The two species *Neochetina bruchi* Hustache and *N. eichhorniae* Warner feed on the petioles and leaves respectively of water hyacinth. The two species can be distinguished by the colour and pattern of scales covering the elytra. *N. eichhorniae* has gray mottled with brown scales and hence the name mottled weevil. In *N. bruchi* (chevroned weevil), the tubercles (which appear as dark shiny lines on the elytra) are situated very near mid-length while in *N. eichhorniae* they are located further forward. *N. bruchi* has much finer striae on the elytra than those of *N. eichhorniae.*

The egg, larvae and pupae of the two species are almost indistinguishable from one another. The two species have different ovipositional sites and hence co-exist. *N. bruchi* oviposits (several eggs per hole) in the 2nd or 3rd layer of aerenchymatous cells in the middle third of older bulbous petioles and *N. eichhorniae* places eggs singly just beneath the epidermal layer of the tender young leaf or sheathing bases of older leaves and in ligules. Maximum oviposition is 8.5 eggs/female/day. The insects have up to three generations per year.

The larvae are the most damaging stage of growth. Upon hatching, they mine towards the petiole bases and feed in the stems particularly in the vicinity of the roots where they leave characteristic blackish tunnels. The adults are almost entirely nocturnal. As they move on the leaf surface, they scrap off the epidermis in parallel lines until a small scar is formed. One adult produces an average of 20 feeding spots per day and damage by five adults can kill a medium sized plant in the laboratory in about 10 days. While the damage by the adults is only mechanical, the damage

by the larvae is very often followed by secondary invasion of pathogens further weakening the plants (Gopal, 1987).

2.3 Biological control of aquatic weeds with plant pathogens.

Attempts have been made in several countries to use pathogens for water hyacinth control. For instance the use of *Cercospora* sp in Rodmanii Reservoir (USA) and in South Africa (Waterhouse, 1994). However, it is worth noting that plant pathogens exert a limiting pressure on target water hyacinth populations but seldom eliminate it (Freeman *et al.*, 1974). A susceptible host, a virulent pathogen and favourable environmental conditions are essential components for plant disease to occur (Charudattan, 1986). Several important factors apparently limit the development of natural epidermics of plant pathogens of weeds. These factors may include host heterogeneity, a narrow range of temperature or moisture conditions conducive to infection, poor survival of inoculum from season to season, and limiting dispersal mechanisms (Charudattan *et al.*,, 1990). Altogether these factors moderate the amount of disease and decrease the likelihood of eruptive epidermics.

According to Charudattan (1986), the dew or moisture requirements needed to establish infection are a major concern. In most cases 12 hrs of moisture appear sufficient to establish infection at an incidence high enough to establish control. Post inoculation/incubation temperatures are critical to establishment of the disease by affecting the latent period (Charudattan, 1988). Successful biological control agents require relatively short moisture periods and have relatively wide temperature latitude for rapid disease development. The relative survival of the biological control agents appears to depend on the amount of inoculum and specialized structures possessed by each pathogen. Pathogens that have specialized fruiting structures such as teliospores, oospores, ascospores, or sclerotia may be expected to survive with relative success (Charudattan 1988).

Charudattan *et al.* (1990) stated that biological control of weeds using plant pathogens could be accomplished by one of two main strategies: the classical and the microbial herbicide strategies. The classical or inoculative biocontrol approach involves the importation and release of one or

more natural enemies that attack the target weed in its native range into areas where the weed is introduced and is troublesome and where its natural enemies are absent. Pathogens utilized for classical weed control have been mostly rust fungi but certain other fungi also capable of self-dissemination through air-borne spores or capable of a sustained residual occupation of soil are under consideration for the classical approach (Watson, 1991).

The mycoherbicide approach involves the use of native plant pathogenic fungi developed and used in the inundative strategy to control weeds in the way chemical herbicides are used or using living products that control specific weeds in agriculture as effectively as chemicals (Watson, 1991). Pathogens used as microbial herbicides can be native or exotic, but the former has been used more commonly. The pathogen is cultured *in vitro* on a large scale and applied in fairly high concentration to the weed (Charudattan, 1990). The need for culturing makes facultative saprophytes and facultative parasites the agents of choice for this strategy. If necessary, microbial herbicides can be applied repeatedly during the growing season or annually using conventional pesticide application techniques.

To be successful in this approach, it must be possible to produce abundant and durable inoculum in artificial culture, the pathogen must be genetically stable and specific to the target weed, and it must be possible to infect and kill the weed in environments of reasonably wide latitude (Charudattan, 1990). Efficacy and host specificity are two overriding concerns in the development of a mycoherbicide agent. For practical reasons Charudattan (1988) defined efficacy as 'the ability to yield a satisfactory amount, speed, and ease of weed control'.

In the bioherbicide approach opportunities exist for man to overcome restraints of disease development by increasing the quantity of inoculum, timing inoculation to coincide with increased host plant susceptibility, formulating infective propagules, or applying irrigation to overcome environmental limitations (Watson, 1991). In classical approach however, these opportunities are fewer.

Research has indicated that the evolution of new strains, varieties or even species is rather rapid among the microorganisms. The importance of phytopathogens as biocontrol agents is therefore limited by the possibility of a change in pathogenicity or development of new strains or varieties in relatively shorter time in the area of introduction and thereby infecting other important plants. The patterns of geographical distribution known for most of the important pathogens indicate that phytopathogens for biological control must be found from within the local or regional range of the weed's distribution, as attempts to import and introduce pathogens may cause more severe problems (Charudattan, 1990).

2.3.1 Biological control of water hyacinth using fungal pathogens

A literature survey reported by Charudattan (1990) revealed that 114 fungal taxa including many virulent pathogens have been reported worldwide on water hyacinth. However, most of these fungi pathogenic have a wide host range and diverse geographic distribution. The indeterminate, rapid growth and vegetative proliferation of water hyacinth also enables the weed to escape disease pressure by compensating for disease losses.

The fungus *Cercospora rodmanii* has been found to effectively control water hyacinth at Rodman Reservoir in Florida (Conway, 1976). *C. rodmanii* is host specific to water hyacinth and can spread from infected areas causing large areas of water hyacinth to die and sink (Conway and Zettler, 1971; Conway 1976a). Freeman and Charudattan (1984) described the fungus to cause small, punctate necrotic spots on the leaves and petioles of the plant. The spots are more numerous at the distal portion of the leaf but can occur over the entire leaf surface and the upper portions of the petiole. Because of these spots, the leaves die from the tip back, with death of the tissue gradually spreading towards the base of the leaf until the entire plant is killed. Plants with severely infected leaves become chlorotic and develop spindly petioles. In the later stages of disease, root deterioration is frequently evident.

Freeman and Charudattan (1984) also noted that in nutrient medium, cultures of *C. rodmanii* are light to dark grey on top and deep red on the bottom. A diffusible red pigment, identified as the

phytotoxin cercosporin, is present in the agar surrounding the culture. The toxin causes necrosis of water hyacinth leaves and may be involved in pathogenesis.

Field trials show that the fungus severely affects the weed in conditions that favour reduction of growth (Charudattan *et al., 1978;* Charudattan 1986). *C. rodmanii* has been formulated as a mycoherbicide to control the spread of the water hyacinth. Abbott Laboratories, Chicago, Illinois, explored the potential for commercialization of a mycoherbicide product of the pathogen. A wettable powder formulation was developed and obtained a U.S. Environmental Protection Agency Experimental Use Permit to evaluate the mycoherbicide. It was tested in Florida and Mississippi and the results indicated that the formulation was acceptable. However, it needed to be standardized with respect to the infectivity and virulence of the propagules.

Another fungi, *Alternaria eichhorniae* Nagraj and Ponappa is known to cause severe blight to water hyacinth. It has also been found to have a very narrow host range. It causes leaf lesions that are oval to elliptic, and are arranged along the longitudinal axis of the leaf. However, several large lesions may coalesce to form irregular blotches all over the leaf surface. It produces a distinct bright red coloration in the nutrient media caused by at least four toxic substances of which two, bostrycin and deoxybostrycin have been identified (Charudattan and Rao, 1982).

Other fungal pathogens such as *Acremonium zonatum, Rhizoctonia solani* and *Helminthosporiun bicolor* (all Hypomycetes) have also been evaluated and found to have a potential in biocontrol of water hyacinth (Table 1.) (El Wakil, 1990).

Table 1. A list of virulent fungal pathogens of waterhyacinth and their value for Biocontrol.

Name	Type of damage	Limitation(s) as a Biocontrol agent	Important References
Acremonium zonatum (=*Cephalosporium zonatum)* = *C. eichhorniae*	Zonate leaf spot, often damaging to the entire lamina	Slow rate disease progress, secondary spread is limited pathogen lacks acceptable	Rintz 1973, Martyn and Freman 1978, Charudattan 1984
Alternaria eichhorniae	Leaf spot and severe leaf blight	None confirmed, a rigorous assessment of the host range of the pathogen is needed to determine the biocontrol potential.	NagRaj and Ponnappa 1970, Charudattan and Rao 1982, Charudattan 1984
Bipolaris oryzae	Severe foliar blight	Pathogen lacks host specificity	Charudattan *et al.*1975 Charudattan 1984
Cercospora piaropi	Leaf spot, leaf necrosis, and general debilitation	None apparent, rate of disease progress may be too slow to afford sufficient pressure on the host.	Tharp 1917, Freeman and Charudattan 1974
Cercospora rodmanii	Leaf spot, leaf necrosis, and general debilitation	None, rate of disease progress can be too slow in relation to the rate of host growth, resulting in lack of control.	Conway 1976a,b, Conway and Freeman 1977, Conway *et al.* 1978, Freeman and Charudattan 1984.
Helminthosporium bicolor	Leaf spot surrounded by yellow halo	Information lacking; may have potential in biological control.	Rao 1970 Charudattan 1984
Marasmiellus inoderma	Foliar blight	Pathogen lacks host specificity and its growth rate and disease progress rate are too slow.	NagRaj 1965
Myrotherium roridum	Necrotic leaf spot	Lacks host specificity	NagRaj and Ponnappa 1967, Ponnappa 1970, 1971.
Rhizoctonia solani Incl. *Rhizoctonia* state of *Corticum solani,* and *Aquathanatephorus penulus*	Foliar blight	Lacks host specificity	NagRaj and Ponnappa Joyner and Freeman 1973
Uredo eichhorniae	Chlorotic and necrotic spot and pustule	Information on host specificity and efficacy are lacking.	Charudattan and Conway 1975, Charudattan *et al.* 1976, 1981.

Adapted from Gopal 1987

CHAPTER 3

3 Materials and methods

3.1 Source of Material

3.1.1 Survey for diseased water hyacinth plants

Two surveys to collect diseased water hyacinth plant parts were conducted at several sites around Lake Victoria and Lake Naivasha. One survey was carried out during the dry season (December 1999) and the other during the rainy season (May 2000). During the first survey, the sampling sites in the Southern part of Lake Victoria included Karungu Bay, Sango Rota Beach and Mbale Beach. Three sites around Kisumu town; at the Police Pier, Tourist Resort and the Yatch Club were also sampled. No sampling was done in the northern part of the lake. At Lake Naivasha, the samples were collected at Loldia, Fisherman's camp and Sher Agencies.

During the second survey, samples were collected from a pond at Budalangi on the northern part of Lake Victoria, River Kasat and Police pier around Kisumu and Kusa and Kendu Bay in the southern part of the Lake. At Lake Naivasha samples were collected at Sher Agencies, Elsa Mere and at Fisherman's Camp.

Plant parts with leaf spots, blights, leaf chlorosis and root rots were collected and carried separately in paper bags to eliminate saprophytic growth. A maximum of five plant parts exhibiting each symptom was collected per sampling bag. Where plants with particular symptom were few the maximum numbers available were collected. Where plants exhibiting particular symptom were greater than five, five plants were sampled randomly. The bags were well labeled with date, collection site and sample number. All disease symptoms exhibited at the sampling site were noted. The samples were then carried to the laboratory for isolation.

Parameters recorded in the field included the stage of growth, flowering, extent of infestation and associated plants. Other biological control agents present were also identified and noted.

3.1.2 Isolation and Purification of fungi.

Fungi were isolated from field collected water hyacinth plants which had leaf spots, blights, chlorosis and root rots. Diseased leaves and petioles were washed in running tap water several times. Small pieces (0.5-1 cm) off the margins of the lesions were cut and surface sterilized with 0.5% sodium hypochlorite for 2 min., then washed thoroughly with sterilized water. The sterilized pieces were then dried between two sterile filter papers, then plated on potato dextrose agar (PDA) media and incubated in a Hereaus incubator, model B6120 (German) at 25°C for four days to allow enough mycelial growth for re-isolation.

The P.D.A medium was prepared by mixing 39 gms of dehydrated PDA (Oxoid) powder per litre of distilled water in conical flask twice the volume of medium. For the suppression of any bacterial growth, 1 gm of chlorophenical antibiotic powder was added to the mixture. The mixture was then autoclaved in a Hirayama Steam Pressure Sterilizer model HL-42Ae manufactured in Japan, at an overpressure of 1 atm. at 120°C for 20-30 minutes. The molten agar was then poured out into petri dishes (15 mls per petri dish) under a Holten (model HVR2448 – Denmark) lamina airflow and allowed to cool.

After 4 days of incubation, the cultures were re-isolated using hyphal tip and single spore isolation techniques so as to obtain pure cultures of fungal pathogens. Conidia/spores or mycelia where streaked across the agar surface on three different points. During inoculation and incubation the cultures were kept upside down to prevent spread of conidia all over the plate. To ensure a sterile working environment, the benches were wiped with 70% ethanol solution before isolation and in the evenings.

The cultures were then observed for sporulation and spore identification was carried out using the Hughes-Tubaki-Barron system of classification as described in Barnett and Hunter (1990). Identification of species in this classification was based primarily on the development of the conidia and to a lesser extent on the development of conidiophores. Shape, pigmentation and septation of conidia were considered secondary characteristics for identification of the isolates to species level. For the cultures that did not sporulate making identification difficult, the cultures were coded using alphabetic letters. The cultures were then send to the International Mycological Institute of CABI Biosciences (UK) for confirmation and identification to species level.

3.1.3 Fungal growth study.

The isolated fungal strains were grown on four different media. These were Potato Dextrose Agar (PDA), PDA plus yeast (5g of dry yeast per litre of medium), Sabourand Dextrose Agar (SDA) and SDA plus yeast (5g/l of dry yeast) to determine the best growth medium on which to carry out mass culturing. The media were prepared as described in section 3.1.2 above. However in the PDA plus yeast and SDA plus yeast media 5 gms of dry yeast were added to the mixture before autoclaving. For the Sabourand Dextrose Agar, 62 gms of the dehydrate powder dissolved in 1 litre of distilled water.

Single spore isolation was then carried out on the different media. This was replicated three times for each strain in each medium and incubated at 26±2°C. The radial fungal growth was measured using a caliper after every 3 days. The fungal strains were also observed under the microscope for number of spores produced per unit area to determine the medium under which the fungi sporulate faster.

3.2 Pathogenicity tests for fungal isolates on water hyacinth.

3.2.1 Single leaf infectivity

Detached fresh and uniform leaves of water hyacinth were placed one per 14-cm petri dish. Each dish was lined with moist filter paper. Three scratch marks (1-2 mm long) were made on each leaf using a sterilized inoculation needle. A small mass of fungal

mycelia and spores from the fungal cultures was applied on the scratched part of each leaf using an inoculation needle. The control leaf had scratch marks made but no fungus applied. The petri dishes were covered and the leaves incubated at 24±2°C for 24 hrs then uncovered. Fungal growth and disease symptoms were noted after every three days for a total of 9 days. Pathogenic response was rated according to the width of the lesion as follows: ++ >5mm (Highly pathogenic), +5-3mm (Pathogenic), ->3mm (Non-pathogenic).

3.2.2 Whole plant pathogenicity tests (glasshouse tests).

Pathogenicity of isolated fungi was determined on whole plants. Plants of uniform size, age and health were selected for the study. The various fungal isolates were grown on PDA medium supplemented with 0.05% yeast extract. Individual water hyacinth plants were placed in plastic pots lined with polythene bags containing 500 ml of tap water. The plants were then arranged on plastic trays (four plants per tray) which were in turn arranged in two glasshouses measuring 2.5 x 4 meters. Leaves and petioles of the unwounded water hyacinth plants were first sprayed with sterilized water. They were then inoculated with spore/mycelial suspensions from each fungal isolate at dilutions of 2.5% (w/v) in 0.2% Tween-20 in distilled water. A low-pressure atomizer sprayer was used to achieve even application on the plants. Two-week-old cultures of the fungal inoculum were used. Control plants were sprayed with sterilized water and placed under the same conditions.

The experiment was conducted in a Randomized Complete Design (RCD) with 22 treatments and four replicates. Placing other trays vertically in between to avoid cross-contamination separated the trays with different treatments. The plants were then covered with polythene bags for 48 hrs to maintain high relative humidity (RH). The treated plants were observed daily for the appearance of disease symptoms. Data collection commenced immediately disease symptoms appeared and continued weekly over a six week period.

Plants with disease symptoms were recorded and scored to give disease incidence and severity. Also new leaf turnover was measured. The disease incidence (DI) was taken as the percentage number of leaves on the plant that exhibited disease symptoms. This was measured by observing all the leaves of the inoculated plant in each pot and calculated as percentage of the total number of leaves on the plant. Disease severity (DS) was assessed using the numerical rating scale developed by Freeman and Charudattan (1984) (Fig 3).

Disease severity was recorded for specific leaves (4 per replicate) that had been earlier tagged. The new leaf turnover was taken as the number of young leaves produced by each plant per week. This was recorded to verify the notion that the water hyacinth plants show increased proliferation when inoculated with disease agents so as to compensate for disease pressure. Disease severity data was arcsine square root transformed and together with disease incidence data was subjected to a two way Analysis of Variance (ANOVA) to determine the most pathogenic fungal isolates. Where treatment effects were significant, means were separated using Student-Newman-Keuls multiple range test (SNK) (Snedecor and Cochran, 1989). Fungi which proved to be non-pathogenic to water hyacinth were discarded. International Mycological Institute (IMI) carried out confirmation on the identity of pathogenic strains of CABI Biosciences, UK. The two most pathogenic strains were mass cultured and used for trials integrating the fungi and weevils.

Figure 3. The progression of disease damage and damage rating scale used in assessing disease severity.

3.3 Effect of combined fungal pathogens and *Neochetina eichhorniae* on water hyacinth

The above study was repeated using water hyacinth plants that had been previously fed on by the weevils to determine if integrating fungi and the weevils proved more effective in controlling the weed than only fungi or the weevils alone. A Randomized Complete Design was used with four treatments replicated four times. The treatments were; (i). Weevils plus fungal Strain (A), (ii). Weevils plus fungal Strain (B), (iii). Weevils alone, and (iv). Control (neither weevil nor fungi). The fungal strain (A) was obtained from *Alternaria alternata* isolate while fungal strain (B) was obtained from the new *Fusarium* isolate as the two proved to be the most pathogenic to water hyacinth.

Individual healthy water hyacinth plants were placed in plastic pots lined with polythene bags containing 500 ml of tap water. The plants were then placed in trays and arranged in the glasshouses as described in section 3.2.2. Leaves and petioles of all the water hyacinth plants were first sprayed with sterilized water. For the first three treatments the weevils were introduced to the potted plants a week before infection with the pathogenic fungal isolates to allow for enough weevil feeding scars to serve as entry points for the fungal spores. A total of three weevils, two females and one male were introduced per plant.

After a week the plants in the three treatments were then inoculated with a spore/mycelial suspension of each fungus diluted to 2.5% (w/v) in 0.2% Tween-20 in distilled water using low-pressure atomizers. All fungi were grown on PDA medium supplemented with 0.05% yeast extract. Two-week-old cultures of the fungi were used. Control plants were sprayed with sterilized water and placed under the same conditions. The plants were then covered with polythene bags for 48 hrs to maintain high relative humidity (RH).

The disease incidence and severity and new leaf turn over and number of weevils per plant were noted weekly over a period of six weeks for each treatment. The data was then subjected to Analysis of Variance and means separated, as described in section 3.2.2, so as to compare the different treatments.

CHAPTER 4

4 Results

4.1 Introduction

The survey revealed that the water hyacinth plant population both in Lake Victoria and Lake Naivasha had declined considerably. In Lake Victoria, the plants showed heavy damage by the *Neochetina* weevils, while the cause of reduction in Lake Naivasha water hyacinth infestation was due to reduced water levels. In some cases a stretch of up to 5 m of water hyacinth plants were rooted in mud at the dried shores of the lake.

At all sites sampled disease symptoms such as leaf spot, leaf blight, leaf dieback, lesions on leaf and petiole with yellow halo on the edges, leaf necrosis and lesions with necrotic centres were observed. Most of the disease symptoms appeared on the leaf lamina and petioles with few occurring on the stolons or daughter plants.

It was noted that water hyacinth plants are now growing in association with other plants at both Lakes. In Lake Victoria, wetland plant species such as papyrus (*Cyperus papyrus*) and hippo grass (*Vossia cuspidata*) could be seen growing a float decaying water hyacinth mats. The population of hippo grass and papyrus was high at the edge of the lake. Other sites, especially on the southern end of the gulf in Nyando District, mats of up to >10 m from the shore line could be seen. At a few other sites on the same lake, water hyacinth was also growing in association with water lettuce (*Pistia stratiotes*) and *Azolla* sp. In Lake Naivasha vast mats of papyrus were growing along the shore on the northern end. On the southern end, water hyacinth was found growing in association with Azolla sp. and *Salvinia molesta.*

4.2 Occurrence and distribution of pathogenic fungi in Lakes Victoria and Naivasha.

4.2.1 Incidence of fungal pathogens on water hyacinth in Lakes Victoria and Naivasha.

A total of 12 fungal species were isolated from diseased plant parts collected during the first survey i.e., December 1999 whereas 17 fungal species were isolated from the second survey in May 2000. Majority of the fungi did not sporulate on PDA media and therefore identification to species level was not possible at NARC-Muguga. The identification and confirmation of specimens from the International Mycological Institute of CABI Biosciences U.K showed that there were twenty-two different fungal species isolated from both study areas. In Lake Naivasha a total of 5 fungal species were isolated from the first survey whereas 7 fungal species were isolated during the second survey (Table 2). In Lake Victoria, a total of 13 and 18 fungal species were isolated from samples collected during the first and second survey respectively (Table 3).

Table 2. Fungi associated with diseased water hyacinth in Lake Naivasha.

Fungi Species/Genera isolated from Lake Naivasha	
Dry season Survey	Wet Season survey
Alternaria alternata (Fr.) Keissi. Agg.	*Alternaria alternata* (Fr.) Keissi. Agg.
Nigrospora sacchari (speg.) E.W. Mason.	*Ascomycetous Rhizoctonia*
Pellionella sp.	*Phoma tropica*
Phoma sorghina (Sacc.) Boerema, Dorenb. & Kesteren.	*Alternaria eichhorniae*
Fusarium equiseti (Corda) Sace. Teleomorph: *Gibberella*	*Helminthosporium* sp.
	Fusarium equiseti (Corda) Sace. Teleomorph: *Gibberella*
	Fusarium pallidoroseum (Cooke) Sacc.

Table 3. Fungi associated with diseased water hyacinth in Lake Victoria.

Fungi Species/Genera isolated from Lake Victoria	
Dry season Survey	Wet Season survey
Alternaria alternata (Fr.) Keissi. Agg.	*Alternaria alternata* (Fr.) Keissi. Agg.
Cladosporium cladosporloides (Fresen.) G.A. de Vries.	*Ascomycetous Rhizoctonia*
Agg*intricans* Wollenw.	*Melanospora* sp.
Phoma cf *chrysanthemicola* Hollós.	*Phoma tropica*
Fusarium pallidoroseum (Cooke) Sacc.	*Glomerella cingulata*
Nigrospora sacchari (speg.) E.W. Mason.	*Spegazzinia tessarthra*
Nigrospora sphearica (Sacc.)E.W. Mason.	*Alternatia eichhorniae*
Khuskia Oryzae H.J. Huds.	*Alternaria*** sp.
Nigrospora oryzae (Berk. & Broome).	*Ascochyta* sect. *Ascochytella*
Pellionella sp.	*Helminthosporium* sp.
Phoma sp.	*Bispora* sp.
Phoma sorghina (Sacc.) Boerema, Dorenb. & Kesteren.	*Nigrospora sacchari* (speg.) E.W. Mason.
**Fusarium* sp.	*Khuskia Oryzae* H.J. Huds.
	Nigrospora oryzae (Berk. & Broome).
	Fusarium equiseti (Corda) Sace. Teleomorph: *Gibberella*
	Phoma cf *chrysanthemicola* Hollós.
	Fusarium pallidoroseum (Cooke) Sacc.

**Isolate with unusual conidia, may represent a new taxon. DNA analysis to confirm its identity is being undertaken at IMI.

4.3 Characteristics of some common fungi found in association with water hyacinth.

Disease symptoms were diverse but most plants showed leaf die-back and general necrosis of the leaf. Plants infected with *Alternaria* sp showed brown lesions that coalesced to form dark blotches and yellowing of the leaves (Plate 1). At an advanced stage of the disease the leaf started to die off from the edges. Plants inoculated with *Fusarium* sp. showed small brown spots on leaf lamina also appearing at the under side of the leaf, the leaves were yellowing and were also drying from the apex (Plate 2). Other disease symptoms observed necrotic zonate lesions caused by *Acremonium zonatum* (Plate 3) and leaf die-back caused by an unidentified fungus (Plate 4).

Plate 1: Leaves showing symptoms caused by *Alternaria* sp.

Plate 2: Water hyacinth leaf showing disease symptoms caused by *Fusarium* sp.

Plate 3: Characteristic concentric leaf zonation caused by *Acremonium* sp.

Plate 4: Leaf dieback caused by fungal infection

4.3.1 *Alternaria alternata*

Disease caused by *Alternaria alternata* manifested itself as leaf spots and blotches usually involving the older leaves. Lesions were found to vary in size ranging from minute, brown necrotic flecks to well developed spots. Larger lesions were observed as being oval to elliptical running parallel to the longitudinal axis of the leaf, brown to dark brown in the centre and dark brown to brownish black at the margin surrounded by a faint yellow halo. Several leaf spots sometimes occurred on a single leaf-blade occasionally coalescing to form large irregular blotches covering the entire leaf. Sometimes spots occurred on the petioles. *In vitro* culture of *Alternaria alternata* in Potato Dextrose Agar showed aerial mycelia that were dark gray or olivaceous black in colour (Plate 5). The culture did not color the PDA medium. Conidiophores arose singly or in small groups, were simple or branched, straight or flexous, sometimes geniculate, pale to mid olivaceous or golden brown, smooth, up to 50 µm long, 3-6 µm thick with 1 or several conidial scars (Plate 6).

4.3.2 *Alternaria eichhorniae*

This pathogenic fungi showed similar disease symptoms as that of *Alternaria alternata.*. Growth on Potato Dextrose Agar (PDA) was fairly fast being, 40-50 mm diameter colony in 6 days and filling a 90 mm petri dish in 9 days. The mycelium was found to be aerial, velvety to cottony in the middle, changing colour from 'shell pink' to 'vinaceous Purple' and 'Dull Indian Purple'. It was often indented slightly at the edge with effuse cottony mycelium rising to a height of 6 mm. *Conidiophores* emerged from the stomata of the host in bundles of 4-8, unbranched or branched, erect and golden-brown to brown in colour. Conidia were in chains of 3-4, ovate-obclavate obpyriform, with 4-10 transverse septa and 1-4 longitudinal septa and are yellow to golden-yellow. The symptoms found on water hyacinth in Lakes Victoria and Naivasha were similar to those described by Nagaraj and Ponnapa (1970).

4.3.3 *Acremonium zonatum*

The fungus caused distinct necrotic zonate lesions with dark and light brown or brownish-gray concentric bands with the centres of the spots tending to fall away and leave holes. The spreading lesions were most noticeable on the upper laminar surface. On the lower surface, the area directly

under the spot sometimes had a sparse layer of white mycelium. Cultures on PDA were creamy-white to slightly pinkish, felted to somewhat floccose; reverse colourless at first but becoming somewhat brownish-tinged with age. *Conidiophores* were erect and conidia formed singly at the apices of the conidiophore cells often becoming aggregated into dense slimy heads. Conidia were simple and colurless.

4.3.4 *Fusarium* sp.

Plants infected with the new taxa of *Fusarium* caused reddish brown spots on water hyacinth leaves that started on the margin. After some time the leaves started to whither from the upper surface. In culture the fungus was initially peach to buff but later becoming brown (Plate 7). Conidia at first were sparse and produced from simple lateral phialides on aerial mycelium. After about 14 days conidia were more abundant with production of compact penicillately branched conidiophores. The conidia were falcate with a well-developed pedicellate foot cell and attenuated apical cell that was bent inwards, exaggerating the normal curvature of the spore (Plate 8).

4.3.5 *Myrothecium roridum*

This fungus caused a striking teardrop-shaped leafspot (up to 1 x 2.5 cm), rounded on the side towards the petiole that tapered to a narrow point in the direction of the lamina tip. The older leafspots appeared necrotic with dark brown margins and a centre covered with discrete white conidial masses.

4.3.6 *Phoma sorghina*

Colonies were extremely variable, usually with a fluffy to dense aerial mycelium which was grey-green to olivaceous or darker in colour, but with very characteristic white to salmon pink tinges or areas with the reverse often reddish. Conidia were 4-5 x 2-2.5 μ and ellipsoid. Chlamydospores were single-celled or *Alternaria*-like were variable. The cultural characteristics were easily recognizable..

Plate 5. Fungal plate culture of *Alternaria alternata*

Plate 6. Spores of *Alternaria alternata* (Magnification X200)

Plate 7. Fungal plate culture of *Fusarium** sp.

Plate 8. Spores of *Fusarium** sp. (magnification x 200)

4.4 Pathogenicity of fungal species on water hyacinth

4.4.1 Fungi from Lake Naivasha

A total of five fungal species isolated from Lake Naivasha during the first survey were tested for pathogenicity. The five represented four families/classes namely Hyporaceae, Hyphomycetes, Mycophaerellaceae and Aleurioporae. Of the five, *Alternaria alternata* was the most common species, occurring in all sites sampled, followed by *Fusarium equisetti* which occurred in two sites namely Loldia and Fishermans Camp. *Phoma sorghina*, *Pellionella sp.* and *Nigrospora sacchari* were the least common each appearing only at one site.

Pathogenic response was rated according to the width of the lesion as follows: ++ >5mm (highly pathogenic), +5-3mm (pathogenic), ->3mm (non-pathogenic). *Alternaria alternata* and *Fusarium equisetti* proved to be highly pathogenic, *Pellionella* sp. was slightly pathogenic whereas *Nigrospora sacchari* showed no pathogenicity to water hyacinth under laboratory conditions (Table 4).

Table 4. Fungal species isolated from Lake Naivasha and their pathogenicity

Site	**Family/Class*/Order****	**Species**	**Pathogenicity (++/+/-)**
Loldia	Hypocreaceae	*Fusarium equiseti*	++
	Hyphomycetes*	*Alternaria alternata*	++
	Mycosphaerellaceae	*Phoma sorghina*	++
Fisherman's camp	Hyphomycetes*	*Alternaria alternata*	++
	Hypocreaceae	*Fusarium equisetti*	++
	Aleurioporae	*Pellionella* sp.	+
		Nigrospora sacchari	-
Sher agencies	Hyphomycetes*	*Alternaria alternata*	++

Key: ++ - Highly Pathogenic, + - Pathogenic,- - Non-pathogenic

4.4.2 Fungi from Lake Victoria

Lake Victoria had wider species diversity than Lake Naivasha. In Lake Victoria, *Phoma sorghina* was the most common species followed by *Alternaria alternata*, which occurred in three of the sites sampled namely, Kisumu Police Pier, Kisumu Yatch Cub and Sango Rota Beach. *Fusarium pallidoseumi* and *F. Equisetti* where in presented only at two sites whereas *Pellionella, Phoma cf chysantemicola, Fusarium* and *Nigrospora sacchari* were present at one site only.

All thirteen species identified isolated from Lake Victoria during the first survey were tested for pathogenicity against water hyacinth under laboratory conditions. Majority (69.2%) of fungal species isolated from Lake Victoria were found to be pathogenic to water hyacinth. *Alternaria alternata, Fusarium equisetti, Phoma sorghina* and the new species of *Fusarium* showed high pathogenicity to water hyacinth. *Pellionella* sp., *Fusarium pallidoseum* and *Phoma cf. chysantemicola, Phoma* sp and *Khuskia oryzae* were slighltly pathogenic while *Cladosporium clasporloides, Nigrospora sacchari, Nigrospora sphaerica* and *Nigrospora oryzae* were non-pathogenic (Table 5).

Table 5. Fungal species isolated from Lake Victoria and their pathogenicity

Site	Family	Species	Pathogenicity (++/+/-)
Kisumu Police Pier	Hyphomycetes*	*Alternaria alternata*	++
		Pellionella sp.	+
	Mycosphaerellaceae	*Phoma sorghina*	++
Kisumu Tourist Resort	Hypocreaceae	*Fusarium** sp.	++
	Hypocreaceae	*Fusarium pallidoseum*	+
	Mycosphaerellaceae	*Phoma sorghina*	++
Kisumu Yatch Club	Hyphomycetes*	*Alternaria alternata*	++
	Mycosphaerellaceae	*Phoma sorghina*	++
	Mycosphaerellaceae	*Phoma cf. Chysantemicola*	+
	Mycosphaerellaceae	*Cladosporium clasporloides*	-
Karungu Bay	Hypocreaceae	*Nigrospora sacchari*	-
		Nigrospora oryzae	-
	Hypocreaceae	*Fusarium pallidoroseum*	+
		Fusarium equisetti	++
		Khuskia oryzae	+
		Nigrospora sphaerica	-
Sango Rota Beach	Hyphomycetes*	*Alternaria alternata*	++
		Fusarium equisetti	++
	Hypocreaceae	*Phoma sorghina*	++
	Mycosphaerellaceae	*Phoma* sp.	+

Key: ++ - Highly Pathogenic, + - Pathogenic,- - Non-pathogenic

4.5 Pathogenic effects of selected fungal species on *Eichhornia crassipes*

4.5.1 Lesion size

The results showed that under suitable conditions (25°C, 75% R.H), a number of the isolated fungi caused lesions on healthy water hyacinth leaves and were therefore pathogenic. No lesion growth was recorded on the control leaves where the leaves remained healthy. The lesion size differed significantly between isolates. At the end of the 9 day experiment, lesion growth in *Phoma sorghina* was 1.27 cm which was significantly higher than all other fungal species at p=0.05 significance level (Table 6). This was followed by *Alternaria alternata* (0.87 cm) then *Fusarium equiseti* (0.64 cm). The new *Fusarium sp* had the least lesion growth of 0.59 cm. Minimal lesion growth was recorded within the first 3 days after infection.

Table 6. Mean lesion size caused by different fungi on test water hyacinth leaves under laboratory conditions

Isolate	Mean lesion size (cm)
Phoma sorghina	$1.27^{a} \pm 0.54$
Alternaria alternata	$0.87^{b} \pm 0.44$
Fusarium equiseti	$0.64^{bc} \pm 0.59$
*Fusarium***	$0.59^{cd} \pm 0.21$
Control	0.00

CV- 54.53; R-Square - 0.58 SNK test at Alpha=0.05: Key: *- New fungal taxa.
Means with the same letter are not significantly different

4.5.2 Disease Incidence

At the end of the six weeks the highest disease incidence per plant was recorded in plants inoculated with *Alternaria alternata* (50.5%) (Table 7). This was significantly higher than that of the new *Fusarium* sp (40.6%) (p=0.05) which was rated second. *Fusarium equiseti* and *Phoma sorghina* did not differ significantly from each other and had an incidence of 33.5% and 30.9% respectively. The control plants had a disease incidence significantly lower than all other treatments (19.8%).

Table 7. Mean disease incidence caused by selected fungi

Isolate	Mean percentage incidence
Alternaria alternata	50.5 ± 3.62^{a}
*Fusarium** sp.	40.6 ± 3.65^{b}
Fusarium equisetti	33.5 ± 4.37^{c}
Phoma sorghina	30.9 ± 3.26^{c}
Control	19.8 ± 2.8^{d}

CV- 24.61; R-Square - 0.827 SNK test at Alpha=0.05 Key: *- New fungal taxa

Means with the same letter are not significantly different

4.3.3 Disease severity

Disease severity was highest in plants inoculated with *Fusarium** sp. with a lesion leaf cover of 27.9% (Table 8). *Alternaria alternata* also caused a relatively high severity of 15.4%. Severity by *Fusarium equisetti* and *Phoma sorghina* was almost equal measuring 6.1 and 6.6 percent respectively. Though the differences in severity appeared variable, they were not significantly different statistically (p=0.05). Most isolates showed minimum lesion growth during the first two weeks. Control plants gave the lowest disease severity of 3.2%

Table 8. Mean disease severity for selected fungi

Isolate	Mean severity
*Fusarium** sp.	27.9^{a}
Fusarium equisetti	6.1^{a}
Alternaria alternata	15.4^{a}
Phoma sorghina	6.6^{a}
Control	3.2^{b}

R-Square - 0.941; CV- 133.4 SNK test at Alpha=0.05 Key: *- New fungal taxa

Means with the same letter are not significantly different.

4.5.4 Relationship between disease incidence and disease severity

A Pearson Correlation Analysis showed that disease incidence and severity were positively correlated with a Pearson Correlation Coefficient of 0.93 ($r^2 = 0.8702$) which was significant at p=0.05 (Figure 4). This indicates that at the concentration of 2.5% w/v of mycelial/spore suspension in 0.2% Tween-20 solution fungi that caused a high incidence or dispersal of disease were more virulent or caused more severe disease on water hyacinth over the six-week period of data collection.

4.6 Effect of fungal pathogens on plant growth and development

4.6.1 Effect on growth/leaf generation

A Pearson Correlation Analysis for the parameters new leaf turnover, disease incidence and disease severity showed that correlation between new leaf turnover and disease severity was significant at 0.05 significance level and that the two had a strong negative correlation (r=-0.99) (Fig 5). Correlation between the new leaf turnover and disease incidence was also significant and negative (r =-0.92) as shown in Figure 6. This shows that increase in disease severity and disease incidence caused a significant reduction in the generation of new leaves by the inoculated plants.

Fig. 7 shows that for the fungi *Alternatia alternata, Fusarium* and *Phoma sorghina* the number of new leaves generated increased on the second week then declined peaking again during the 4th week before declining steadily during the following weeks. However, plants inoculated with *Fusarium equisetti* showed an initial decline in number of new leaves generated and the number did not vary much over the six-week period.

4.6 Effect of integrated control on water hyacinth

4.7.1 Disease incidence

The integrated control study showed that there was a marked increase in disease incidence when *Neochetina* weevils were integrated with the pathogen *Alternaria alternata* and the new *Fusarium* sp. Table 9 gives the mean disease incidence for the different treatments. Integrating with *Neochetina* weevils increased the incidence of disease on the leaves by 14.6% from 46.3% in plants infected with the new *Fusarium* alone to 53.1% in plants infected with *Fusarium* and *Neochetina* weevils. An increase of 31.1% from 40.9% in plants infected with *Alternaria alternata* alone to 53.6% in plants with both *Alternaria alternata* and *Neochetina* weevils.

Table 9. Disease incidence for different treatments integration experiment

Treatment	**Mean percentage incidence**
*Fusarium** sp. only	46.33 ± 2.84^{ba}
Alternaria alternata only	40.90 ± 3.02^{b}
*Fusarium** sp. + *Neochetina*	53.10 ± 2.54^{a}
A. alternata + Neochetina	53.63 ± 3.45^{a}

R-Square- 0.618, CV - 24.59, Alpha = 0.05

4.7.2 Disease severity

The results show that disease severity was higher in plants where the weevils were integrated with pathogens than in plants treated with the pathogen alone. However the severity was higher in plants treated with new *Fusarium* sp. than in plants treated with *Alternaria alternata.* Table 10 gives the mean severity for each treatment.

There was an increase of 76.0% in disease severity in plants infected with *Fusarium* and *Neochetina* weevils (21.6) over those infected with *Fusarium* only. An increase of 16.1% was recorded in plants in which *Alternaria alternata* was integrated with *Neochetina* weevils (11.3) over those infected with *Alternaria alternata* alone.

Table 10. Disease severity for different treatments integration experiment

Treatment	**Mean percentage severity**
*Fusarium** sp. only	12.27 ± 4.29^{b}
Alternaria alternata only	9.73 ± 3.46^{b}
*Fusarium** sp. + *Neochetina*	21.6 ± 4.83^{a}
A. alternata + *Neochetina*	11.3 ± 2.80^{b}

R-sqaure - 0.682, C.V - 101.38, Alpha = 0.05

CHAPTER 5

5.0 Discussion, Conclusions and Recommendations.

5.1 Discussion

5.1.1 Fungi associated with diseased water hyacinth in Lake Naivasha and Lake Victoria

In developing an integrated management system for water hyacinth in Lake Victoria, it is important to remember that these packages could be site specific. Integrated management programs largely depend on the hydrological and nutrient status of the system, the extent of the infestation, the climate of the area and the usage of the water body. To minimize some of the problems associated with the introduction of control agents such as lack of specificity and poor establishment rates to name a few, it is important to first survey for local or indigenous biological control agents.

Isolation of fungi from diseased water hyacinth plants in Lake Victoria and Lake Naivasha revealed the occurrence of several fungal species most of which have been isolated from water hyacinth species in water bodies elsewhere (Shabana *et al.*, 1995; Evans and Reeder, 2001). However it is important to note that from the present study two new species were isolated, one of *Fusarium* and the other of *Alternaria.* Though DNA analysis to confirm their identity is yet to be undertaken, it is possible that these fungi are only present within the two lakes.

There was an increase in the number of fungal species isolated from the survey carried out during the rainy season over that carried out during the dry season. A total of ten additional species were isolated from the rainy season survey than were isolated during the dry season. This may be attributed to the fact that during the rainy season temperature and relative humidity were more favourable for fungal growth, than during the dry season. Studies conducted on development and growth of fungi support this finding (Dhingra and Sinclair, 1995).

The field results in both Lake Naivasha and Lake Victoria showed that the naturally induced severity of disease in the field relatively low especially on plants that showed no weevil damage. Where disease symptoms were present, they occurred mainly on the leaf lamina and petioles with few occurring on the stolons. None were observed on the rhizome. Disease symptoms on the

petioles were higher where *Neochetina* larval damage was evident. This may be attributed to the fact that larval tunneling on the petiole created openings for the penetration of fungal spores (Charudattan, 1986). The low natural levels of disease severity may be due to the fact that water hyacinth leaves are known to produce phenolic compounds that give resistance to fungal disease (Martyn and Cordy 1983). It could mean that though the fungi were pathogenic to water hyacinth, the inoculum densities in the field were too low to cause severe disease.

The most common diseases observed in the field during the survey included the leaf spot caused by A*lternaria* species, the *Rhizoctonia* leaf blight caused by *Rhizoctonia solani* and the zonate leaf spot caused by *Acremonium zonatum.* However it was noted that *Acremonium zonatum* was found only on the southern shoreline of Lake Victoria and was not observed in Lake Naivasha. The mite *Orthogalumna terebrantis* that is though to be a carrier of this fungus (Charudattan, 1986) was found along this shoreline and tunnelling damage caused by the mite was apparent in this region.

5.1.2 Pathogenicity of isolated fungi against water hyacinth

Laboratory tests showed that the majority of the fungi isolated (over 75%) were moderate to highly pathogenic to water hyacinth. This shows great potential for development of a mycoherbicide for the control of water hyacinth in Kenya. Though majority of the fungi isolated in this study are well known, from literature, to have a wide host-range a few such as *Alternaria eichhorniae* that are thought to be specific (Shabana *et al.,* 1995).

This study recorded the occurrence of *A. alternata* as the most common fungus found in association with water hyacinth in Lake Victoria and Lake Naivasha. Pathogenicity tests carried out show that the species is highly pathogenic to water hyacinth causing up to 53% disease incidence six weeks after application. This is in agreement with a study carried out in India where a strain of *Alternaria alternata* was found to be highly virulent to water hyacinth (Aneja and Singh 1989). However, it is important to note that host range tests, which were beyond the scope of this study are necessary before the fungus can be applied in the field.

Alternaria eichhorniae was isolated and evaluated as a bioherbicide for water hyacinth in Egypt by Shabana *et al.* (1995). Their study showed that the fungus produced 93% leaf necrosis and an 81% decrease in the fresh weight 2 months after application. It was concluded that the fungus might be useful for water hyacinth control. Evaluation of *Alternaria alternata* along side *A. eichhorniae* indicated that *A.alternata* was also pathogenic to water hyacinth but was less virulent.

The new *Fusarium* sp. isolated from the present study also proved to be highly pathogenic to water hyacinth in laboratory tests. It caused the highest disease severity of 27.9% at 2.5% w/v in 0.2% Tween-20 solution over a period of six weeks after infection. It also caused a disease incidence of 40.6% over the same period. Considering that this species not been reported elsewhere, it would be important to study its mode of growth, sporulation and also its host specificity. Further identification to species level would enable proper characterization and comparative study on water hyacinth and other host plants.

Another study carried out in Zimbabwe by Mpofu (1995) showed that fungi in the genera *Fusarium* were pathogenic to water hyacinth. She was able to isolate three *Fusarium* sp. namely *Fusarium moniliforme, F. solani* and *F. pallidoroseum* all of which were found to be highly pathogenic to water hyacinth. She, however, reported that the fungi were not host specific, a critical consideration in the development of a biological control agent is the determination of host range (Weidemann, 1991).

The use of bioherbicides for water hyacinth control is relatively new in Kenya. Studies to determine optimal inoculum densities for control are necessary but are beyond the scope of this study. However, studies by Shabana *et al.,* (1995) showed that disease severity increased with increasing inoculum density up to 5% mycelial wet weight. In the present study 2.5% w/v in 0.2% Tween-20 solution was used. Generally, few pathogens are known that cause disease sever enough to kill water hyacinth (Charudattan *et al.,* 1990). However, certain foliar pathogens have been recorded to severely curtail the plant's growth and productivity (Charudattan *et al.,* 1990).

It is also important to determine the culture medium in which highest concentration of conidia is produced for each fungus. Dhingra and Sinclair (1995) stated that fungi grown on a rich medium are more vigorous than those grown on a nutritionally poor one. The present study was based on Potato Dextrose Agar medium in containing 5% yeast, which probably needs other comparative media studies for better performance.

In this study, the new leaf turnover/generation was strongly negatively correlated to disease incidence and severity. Diseased plants produced less leaves and the plant generally became stunted. It is necessary to determine the optimal concentration required to over-come the plant's compensatory capacity. Caunter and Mohamed (1990) reported that the water hyacinth had a high physiological capacity which allowed for diseased plants to compensate for damaged leaves with a supply of healthy young leaves that carry on the normal metabolic reactions to support further leaf production.

5.1.3 Integrated control using fungi and *Neochetina* weevils

Studies on the synergism of weevils and fungi showed a significant increase in disease severity and incidence when the two control agents were integrated. The pathogens caused accelerated decay of weevils infested plants. For plants infected with *Neochetina* weevils and *Alternaria alternata* disease incidence increased significantly by 31.1% whereas the disease severity increased marginally by 16.1% over those infected with *Alternaria alternata* alone. Plants infected *Fusarium* sp. showed a slight increase in disease incidence (14.6%) but the increase in disease severity was highly significant (76%). The weevil feeding marks as well as the tunnels produced by *Neochetina* larvae appeared to provide the fungi with entry points into the otherwise water-repellant foliage of water hyacinth. Charudattan (1986) reported that interactions between arthropods and several saprophytic and parasitic fungi and bacteria were common on arthropod damaged water hyacinth. In south Florida, the occurrence of the necrotic zonate leaf spot disease of water hyacinth was found to be related to the incidence of arthropod damage especially that of the mite *Orthogalumna terebrantis.*

5.2 Conclusion

From the present study it may be concluded that, there are several species of fungi found in association with water hyacinth in Lake Victoria and Lake Naivasha, Kenya. Majority of these are pathogenic to water hyacinth. Some of the pathogenic species isolated during this study are known not to be host specific and their use, as mycoherbicides has not been well investigated. However, there are a few fungi such as *Acremonium zonatum* and *Alternaria eichhorniae* that are known to have a narrow host range and could be considered candidates for use in controlling water hyacinth. One new fungal pathogen, *Fusarium* sp. was collected, isolated and identified for the first time on water hyacinth. Pathogenicity tests showed that the fungus was also a promising candidate against water hyacinth.

Integrated control of water hyacinth is a requirement if benefits from control agents are to be maximized. The *Neochetina* weevils released in Lake Victoria in 1997 have reduced water hyacinth population considerably. This study concludes that synergetic control of water hyacinth may be obtained by a combination of the weevil and a pathogenic fungus as was observed when the weevils and *Alternaria alternata* and *Fusarium* sp. were tested under screen house conditions.

5.3 Recommendations

The following are recommendations made from this study:

(i) More surveys and isolations are necessary so as to determine all species found locally.

(ii) DNA identification should be carried out on all promising isolates.

(iii) A detailed study on optimal growth conditions, pathogenicity and specificity should be carried out on the new *Fusarium* species.

(iv) Studies on the inoculum levels appropriate to over-come compensation by the water hyacinth plants should be conducted.

(v) Host-specificity tests should be carried out on promising isolates for water hyacinth control.

(vi) Detailed field evaluation of promising fungal species

(vii) Use of mycoherbicides is recommended a component of integrated water hyacinth management strategy for Lake Victoria.

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7.0 Annexes

Annex 1. Data on site, water hyacinth, and diseased plant parts collected during survey.

a) Lake Victoria

Site Details			Water Hyacinth Data				Diseased plants data **(parts showing disease)**		
Loc. **(District)**	Site	Site type	S.o.G	Flow	E.o.I	A.P	Leaves	Petiole	Stolon
Kisumu	**R. Kasat**	**River**	**Large**	**<1/sq m**	**Fringing**	Hippo grass	X	X	X
	Police Pier	**Lake**	**Medium**	None	**Fringing**	Papyrus	X	X	
	Yatch Club	**Lake**	**Medium**	<1/sq m	**Fringing**	Hippo grass	X		
	Tourist Resort	**Lake**	**Medium**	None	**Scattered plants**	Papyrus, Hippo grass	X	X	
Nyando	**Sango Rota**	**Lake**	**Medium**	<1/sq m	**Clumps,** >1 sq m	Papyrus, Hippo grass' Azolla	X	X	
	Kendu Bay	**Lake**	**Large**	None	Mat	None	X	X	X
	Kusa	**Lake**	**Medium**	1-2/sq m	Clumps, >1 sq m	Water lettuce, Azolla, Hippo grass, Papyrus	X		
Migori	**Karungu Bay**	**Lake**	**Small**	1-2/sq m	Scattered plants	None	X		
Busia	**Budalangi**	**Pond**	**Medium**	1-2/sq m	Clumps, <1 sq m	Water lettuce, Papyrus, Hippo grass, Azolla	X		

Key: Loc. = Locality, S.o.G = Stage of growth, Flow. = Flowering, E.o.I = Extent of infestivity, A.P = Associated plants.

b) Lake Naivasha

Site Details			Water Hyacinth Data				Diseased plants data **(parts showing disease)**		
Loc. **(District)**	Site	Site type	S.o.G	Flow.	E.o.I	A.P	Leaves	Petiole	Stolon
Nakuru	**Loldia**	**Irrigation channel**	**Medium**	**>2/ sq m**	**Clumps,** <1 sq m	Papyrus	X	X	
	Fisherman's camp	**Lake**	**Small**	None	Fringing	Papyrus	X	X	
	Sher agencies	**Irrigation channel**	**Medium**	<1/sq m	**Clumps, <1 sq m**	Papyrus, *Salvinia*, water, lettuce, Azolla	X	X	
	Elsa mere	**Lake**	**Small**	None	Scattered plants	Papyrus	X	X	

Key: Loc. = Locality, S.o.G = Stage of growth, Flow. = Flowering, E.o.I = Extent of infestivity, A.P = Associated plants.

Annex 3: Analysis of Variance (ANOVA) Tables

Pathogenicity tests

a) Lesion size

Source	**DF**	**S.S (III)**	**M.S**	**F Value**	**Pr>F**
Isolate	20	31.345	1.568	19.79	0.0001
Replicate	1	0.0538	0.578	0.68	0.4105
Day	2	6.628	3.314	41.85	0.0001
R-Square	**C.V**	**Root MSE**	**Lesion size Mean**		
0.581	**54.537**	**0.281**	**0.516**		

Alpha=0.05

b) Disease incidence

Source	**DF**	**S.S (III)**	**M.S**	**F Value**	**Pr>F**
Isolate	3	5519.375	1839.791	20.08	0.0001
Replicate	3	574.951	191.650	2.09	0.1093
Date	5	14865.269	2973.054	32.44	0.0001
Isolate*Date	15	9195.701	613.047	6.69	0.0001
R-Square	**C.V**	**Root MSE**	**Incidence Mean**		
0.8267	**24.612**	**9.572**	**38.895**		

Alpha=0.05

c) Disease severity

Source	DF	S.S	M.S	F Value	Pr>F
Isolate	3	0.719	0.239	1.97	0.3544
Replicate	3	1.151	0.384	3.15	0.2503
Date	5	1.997	0.666	5.46	0.1587
R-Square	**C.V**	**Root MSE**	**Severity Mean (arcsine sqrt tranformed)**		
0.9407	**133.363**	**0.349**	**0.262**		

Alpha=0.05. N/B: Disease severity data was arcsine square root transformed.

d) New leaf turnover

Source	DF	S.S	M.S	F Value	Pr>F
Isolate	3	1717.860	572.620	62.05	0.0001
Replicate	3	45.005	15.002	1.63	0.1922
Date	5	908.534	181.707	19.69	0.0001
Isolate*Date	14	777.119	55.508	6.01	0.0001
R-Square	**C.V**	**Root MSE**	**Severity Mean**		
0.9407	**133.363**	**0.349**	**0.262**		

Alpha=0.05

Integrated control experiment

a) Disease incidence

Source	DF	S.S	M.S	F Value	Pr>F
Trtmt	3	3298.81	1099.60	7.73	0.0001
Rep	4	1705.20	426.30	3.00	0.0225
Date	5	12261.01	2452.20	17.24	0.0001
Trtmt*Date	15	3897.81	259.86	1.83	0.0422
R-Square	**CV**	**Root MSE**	**DI Mean**		
0.6179	**24.597**	**11.9264**	**48.488**		

b) Disease severity

Source	**DF**	**S.S**	**M.S**	**F Value**	**Pr>F**
Trtmt	3	2578.69	859.56	4.44	0.0058
Rep	4	408.72	102.18	0.53	0.7156
Date	5	32441.87	6488.37	33.51	0.0001
Trtmt*Date	15	2808.96	187.26	0.97	0.4956
R-Square	**CV**	**Root MSE**	**DI Mean**		
0.6822	**101.384**	**13.9149**	**13.725**		