DISTRIBUTION OF THE WATER HYACINTH [*Eichhornia crassipes* (Mart.) Solms.], ITS CARPET CHARACTERISTICS, SOME OF ITS DISEASES AND PESTS IN THE WINAM GULF OF LAKE VICTORIA.

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN PLANT PATHOLOGY OF MASENO UNIVERSITY

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ABSTRACT

The occurrence of the Water hyacinth (*Eichhornia crassipes* [Mart.] Solms) in the Winam gulf has created numerous negative attributes that make its control an urgent priority. Little has been reported about its carpet characters and the occurrence of the natural enemies that are known to occur with it in other water bodies. Since these natural enemies presented the most ideal source of viable biocontrol agents, investigations meant to establish their occurrence and distribution were desirable. Experiments were set up between 1995 and 2000 in selected beaches within the Winam gulf, aimed at establishing the distribution pattern, origin, carpet characteristics, disease types, disease causative agents and their suitability for use as biocontrol agents.

Plants meant for the identification exercises were collected from 6 locations, and the characteristics exhibited by their roots, stems, leaves and inflorescence compared to those already described as *Eichhornia crassipes* [Mart.] Solms-Laubach. The entry point of the water hyacinth was confirmed by conducting an investigation exercise at the Rusinga channel. In order to have a better understanding of the seasonal changes in the carpet sizes, a surveillance program that lasted four years was conducted twice a year (between June 1995 and 1999 November) at Kisumu, Kobala, Homa-bay and Luanda-nyamasaria. The distribution of water hyacinth growth forms was determined by counting the number of each growth form that appeared out of 100 plants collected from Kisat bay, Dunga beach, Luanda Kotieno, Osodo bay, Nyakach bay and Sori bay. Carpet connectivity calculated as the difference in pressure when weights were added on a mesh wire measuring 0.434 m^2 until the carpet submerged and the pressure on the same carpet that was required to submerge it after a complete disconnection. The standing population density was determined by counting the number of plants found within quadrants measuring $1m^2$ located at Dunga beach, Kisumu pier, Kusa, Kobala and Kendu bay, while the biomass density was determined when oven dried plant materials collected from quadrants

measuring 1 m^2 were weighed. The plant mass and rhizome length measurements were correlated, while the population density was correlated to biomass density within the same carpet. Water hyacinth plants showing disease symptoms whose causative agents were unknown were collected from the Winam gulf and brought to the Laboratory for isolation purposes. Potato Dextrose Agar (PDA) cultures of each pathogen were prepared and the disease causative organism isolated. Pathogenicity trials were conducted in accordance to Koch's postulates.

When the counts for growth forms were complete, the larger growth form occurred 45.0% while the medium and small occurred at 34% and 21%. There was no correlation between the rhizome measurements and the fresh weight, but the correlation between the population density and biomass was highly significant with $r^2 = 0.9202$. Six genera of filamentous fungi together with an unidentified number of bacterial forms found occurring with the water hyacinth in the Winam gulf. The isolated species include; *Myrothecium roridium, Acremonium zonatum, Rhizoctonia solani, Fusarium* sp., *Cercospora* sp. and *Alternaria* sp.

The results obtained from this study have confirmed the occurrence of more than six water hyacinth phytopathogens that can be developed into mycoherbicides. They have shown that the water hyacinth distribution in the Winam gulf is seasonal, adopts secluded bays and mostly exhibit the large growth form. It is now clear that carpets measuring $0.434m^2$ in size that have a connectivity of 288.4 Pa (Pascal) are able to support a weight of 12.6 kg. only, with any additional weight causing them to submerge. The distribution of plant mass in the Winam gulf is normal except in locations that are subjected to external factors.

This study has provided a reference point from which any future biocontrol exercise or any scientific project designing a harvester machine for use in the Winam gulf can take off.

CHAPTER ONE

1.0 INTRODUCTION

1.1 The Original range of the water hyacinth.

Eichhornia crassipes (Mart) Solms-Laubach is a hydrophytic angiosperm plant, which belongs to the monocot family Pontederiaceae. The genus *Eichhornia* together with the genera *Heteranthera, Hydrothrix, Pontederia* and *Reussia* comprise the family pontederiaceae (Bennett, 1967). The family Pontederiaceae is placed in the series Coronarieae, together with the families- Roxburghiaceae, Liliaceae, Philydraceae, Xyrideae, Mayacaceae, Commelinaceae and Rapateaceae in the Bentham and Hooker system of classification.

The genus *Eichhornia* contains six species; *E. azurea* (Swartz.) Kunth., *E. diversifolia* (Vahl.) Urban., *E. natans* (Beauvois) Solms., *E. paradoxa* (Martius) Solms and *E. paniculata* (Sprengel) Solms and *E. crassipes* (Mart) Solms-Laubach. Among the members of this genus, *E. crassipes* is the only aquatic species. It consists of a free-floating plant that is buoyed by bladder-like inflated leaf petioles. The leaf-blades are kidney shaped and somewhat rounded. *E. crassipes* exhibits the rare genetic phenomenon of polymorphism and tristly (Barret and Forno, 1982). Within the genus *Eichhornia,* it is only the species *E. crassipes* that has become a problematic aquatic weed, the other members of this genus are rooted, either where the water is shallow or near the shore. Some of the trailing vine like species e.g., *E. azurea* and *Reussia rotundifolia* may extend over the water surface several meters from the shoreline (Bennett, 1970). Massive colonies build up when *E. crassipes* is introduced into new areas conducive for its proliferation (Charudattan, 1994). Within a short time large biomass piles up in these new areas.

The center of origin of *E. crassipes* is the Amazonian, Brazil, with natural spread to other areas of the South American continent. In its native range the water hyacinth is harmless and typically occurs in seasonally natural environments rather than in irrigation canals and large permanent lakes, as is the case in E. Africa. Man introduced the species to North and Central America, Caribbean, Asia, and Africa (Barret and Forno, 1982). From such

introductions, *E. crassipes* has slowly spread as a noxious weed in many lakes ponds and rivers in different countries throughout the world. In India for example, it has become the most persistent and troublesome waterweed (Gopal, 1987). In its range of the neo-tropics, *E. crassipes* has become the main aquatic weed in all dams or natural water bodies where the hydro biological regime has been interfered with by the activities of man, and the level of nutrients in the water has been increased.

1.2 Spread of *E. crassipes* into the Winam-gulf of L. Victoria.

The water hyacinth has spread to almost all the major East African lakes like; Kyoga, Naivasha and Victoria, thereby creating an urgent need for research that may lead to its management and control. The three riparian Governments of Kenya, Uganda and Tanzania therefore employed various control measures to curb this menace. Some of these efforts including a biocontrol exercise using the *Neochetina* spp. and other mechanical methods are being effected to date. In 1989 the water hyacinth was noticed as a threat to bio-diversity and economic survival of the Lake Kyoga (Uganda), where thick floating carpets had covered a large part of the lake creating numerous problems to the people who live around that lake.

Even though the origin of the water hyacinth infestations in the L. Kyoga, L. Naivasha, and that of many other water bodies in E. Africa have not been determined, however that of the L. Victoria has now been determined as being via the river Kagera. Until 1990 *E. crassipes* was not known to cause any major problems in L. Victoria, its occurrence being first reported in Uganda around December of 1989 when patches of the waterweed were still confined along the shoreline west of Entebbe (Twongo, 1998). As mentioned above the river Kagera was later confirmed to be the source of the water hyacinth (Twongo 1998). In 1989 the invasion was reported to be rapidly spreading along the shores of the lake Victoria in Uganda (Twongo, 1998). The invasion and spread seemed to have been aided by the interaction between the diurnal land and sea breeze that oscillated the weed propagules (viable units) to and from the shoreline and the prevailing southerly/south easterly winds, which propelled them along the shoreline (Twongo, 1998). By 1990 pockets of water hyacinth were already scattered along one half of the shores of L. Victoria in Uganda

(Twongo, 1991) and by 1992 the weed occurred in almost all-suitable shoreline environments in the Ugandan waters (Twongo, 1998). Water hyacinth infestations in Mwanza-Tanzania were reported in 1990 and in the Kenyan waters of the lake in 1992 (Twongo, 1993).

The river Kagera (Uganda) is the only major primary producer of viable units into the L. Victoria (Twongo, 1998). This river has been observed to produce a daily average of 0.8ha of the weed into the lake in the form of viable units that float down the river and ultimately reach the lake (Twongo, 1998). Violent wave action close to the mouth of the river Kagera largely fragments these viable units (Twongo, 1998).

1.3 Reproduction methods of the Water hyacinth

This weed is known to reproduce mainly by a vegetative method known as clonal propagation; rarely does it reproduce by seed production. As the stolons grow, a new plant is formed at its tip such that in a matter of days the parent plant is surrounded by several offspring's. Holms *et al.* (1969) found that when all the conditions suitable for growth were fulfilled, two single parents were surrounded by 300 offspring's in 23 days and by 1200 in 4 months.

Although clonal propagation has been noted as the main means of reproduction, sexual reproduction does occur in seasonal habitats where the conditions are most suited for it to occur. Holms *et al.* (1969) observed that a single inflorescence could produce as many as 20 flowers, each of which was able to produce around 3000 - 4000 seeds. The seeds could remain viable for more than 15 years in the bottom soil of shallow waters. Such seeds could only germinate when the water level receded to the 3 - 4 cm level, a level very common along the shoreline of the L. Victoria.

In order to have a better understanding of this rapid increase in population, different workers have tried to establish if a mathematical relationship may exist between time and plant number. According to Gopal (1987), the growth rate of a water hyacinth population is not density dependent because mats are known to rapidly grow into dense and thick strong carpets that can support a big weight.

Assuming that the given population is growing over a certain period geometrically at a uniform growth rate, the mean daily increment in the number or biomass of the given population can be calculated using the equation;

$$N_o X_t = N_t$$

Where; N_o and N_t are the number of plants growing in the beginning and after a time t (days), respectively, X is the geometric rate of daily increment.

Though the water hyacinth flowers profusely, earlier workers dealing with its biology were unable to observe its seeds or seedlings in nature, thus leading them to assume that this plant was unable to reproduce sexually. Mature fruits, seeds and seedlings have been reported from Africa, Japan, Argentina, Uruguay, Guyana, Trinidad and many other countries during field surveys (Gopal, 1987). Big variations in seed production are known to occur due to the mode of fruit development i.e. whether they are developed in or out of water, level of humidity and the temperature. Maximum fruiting of water hyacinth plants can only occur at a relative humidity greater than 90% and when the temperature range is between 22.5° C and 35.0° C (Gopal, 1987).

The water hyacinth is a self or insect pollinated plant. It shows a very high degree of self-incompatibility during pollination (Barret, 1979). Fresh pollen grains collected from a flower exhibit between 80 to 100 percent viability that decreases slightly within a few days. Controlled studies show that the time of the day and temperature affect the germination of pollen grains and the whole process of seed setting (Gopal, 1987).

Floral and pollen trimorphism, and the fact that most water hyacinth populations are monomorphic or dimorphic and the relatively less frequent seed production behaviour have raised a great interest in the breeding system that it exhibits (Barrett, 1979). Seed production in nature has no barriers and water hyacinth populations throughout the world produce seeds in very good numbers. It is important to note that the seedlings produced by these plants are not frequently observed in the field (Gopal, 1987).

Sexual reproduction during the general life cycle of the water hyacinth is very important even though its significance as a method of reproduction is limited by the poor conditions for seed germination or seedling survival that occur in nature (Gopal, 1987). This fact clearly provides a possible answer why finding water hyacinth seedlings within water bodies is not very common. The seeds that are produced by this plant require a process of drying before germination may begin (Gopal, 1987).

1.4 Distribution of the water hyacinth.

The water hyacinth restricts its distribution from the equator to 38° N and 38° S. Within these latitudes a very wide range of climatic conditions exist (Gopal, 1987). Carpets occur at temperatures ranging from as low as 1°C during the winter in the north most latitudes to 40°C during the summer within the drier tropical regions.

The optimum temperature for growth of water hyacinth plants in the Lake Victoria is between 25°C and 27.5°C (Oguya, 1998). Water hyacinth plants are subject to large or small diurnal and seasonal variations in temperatures within the different regions that they are found (Gopal, 1987). Wherever the water hyacinth occurs, it occurs in association with a large variety of aquatic and marsh plants (Gopal, 1987).

The water hyacinth infestation in most parts of the world is either an invader or in some cases a pioneer of the existing community of free-floating macrophytes (Gopal, 1987). On some river banks like the; Nile, Kyoga and within lakes such as Kyoga and Naivasha, the water hyacinth invasion has been noted to be mostly limited to free spaces that occur within the indigenous plant species found in these water bodies (Twongo, 1998). It is able to penetrate to a small extent the carpets of *Vossia* and *Echinochloa* (Gopal, 1987). The rapid development of a dense water hyacinth cover soon results in the death of submerged plants due to a non-availability of light. If the water within the lake or river is eutrophic, then the weed growth may be far more rapid and if there is any increase in density, the plant height will increase due to the development of long petioled leaves within the carpet (Gopal, 1987).

In the L. Victoria (Uganda), a study conducted by Twongo (1998) has shown that the water hyacinth is essentially a shoreline problem created by mobile mats that must have shelter from strong wind/waves and nutrients in order to expand and maintain their large sizes. This fact probably explains why the water hyacinth cover in the L. Victoria (Uganda) has never exceeded a size of 0.5 km^2 at any given time in comparison to the 29000 km^2 that is the size of the entire portion. Few studies of a similar type have been conducted in the Winam gulf (L. Victoria).

1.5 Physical characters of water hyacinth carpets.

Information available in literature about the type of charpet characters in the Winam gulf is very limited, thus creating a gap of the unknown. Considering that mechanical control of the water hyacinth in the Winam gulf is one of the viable control options, it is important that more efficient water hyacinth harvesting methods that can result from equipment design based on a better undestanding of these physical characteristics should be adopted.

Current literature is restricted to description of physical characters like plant dimensions, plant components, specific gravity and biomass of water hyacinth sample populations in other water bodies (Petrell, 1990). These physical characters though studied in other water bodies do not include studies on the interrelationships that may exist between plant dimenshions and plant components in the Winam gulf. In order to have an understanding of these interrelationships, physical features such as areal density, plant mass distribution, rhizome length, mat buoyancy and carpet connectivity need to be fully understood (Petrell, 1990.

Water hyacinth physical characteristics vary a lot and are dependent on habitat, season, plant age and plant size. Physical characters greatly affect harvesting machine performances and if known may become useful during the design of machines that are used to clear water hyacinth infestations (Petrell, 1990). For instance, the number and shape of the rhizomes and roots that are the principal plant parts projecting into the water in a carpet, influence the viscous forces generated when a harvester machine is towing a part of the carpet. The biomass density, in combination with harvesting speed and width, determines

the capacity of a harvester and the power that it requires to convey or transport a given load. Plant mass greatly affects inertial forces that are encountered in a towing operation. Mat buoyancy, the force that is required to submerge a given area of the water hyacinth carpet, is useful for the design of conveyors, booms and containers, because plants within the carpet under certain conditions can roll under such devices. Complete information on the degree of connectivity through leaf and stolons entanglements is useful when a mat is to be separated into smaller units for towing or lifting from the water surface (Petrell, 1990).

1.6 Some problems attributed to the water hyacinth in the Winam-gulf.

The water hyacinth invasion of the Winam gulf has created significant social, economical and environmental impacts that largely remain unquantified (Ochiel *et al.*, 2001). The limnology of the lake and associated bio-diversity are on the decline. *E. crassipes* has truly become a problematic aquatic weed throughout the L. Victoria, where most of the problems created and damages caused are far much greater than that attributed to any other aquatic weed weather floating or submerged. Some weeds known to be invasive in the Winam-gulf include; *Salvinia molesta, Pistia statoites, Vossia cusipidator, Cyperus papyrus, Hydrilla* sp. and several unidentified members of the graminiae family (Twongo, 1998). The overall economic cost as a result of the water hyacinth invasion in the Winam gulf has not been quantified (Ochiel *et al.*, 2001).

The water hyacinth has been noted to form dense impenetrable mats that have always become an impediment to boat traffic in many bays and beaches throughout the gulf. Boat traffic in the Kisumu-pier, Homa-bay, Kusa, Nyakwere, Kobala, Sori, Luanda-nyamasaria, Luanda k'otieno, Sio-port among others have been blocked at one time or the other by these weeds, the particular time of the blockage being dependent on the wind direction and other climatic factors.

The macrophyte recovery process has been greatly interfered with as a result of the invasion by thick dense mats of water hyacinth (Chitamwebwa, 1991). The macrophyte recovery process is therefore much reduced in areas colonized by these "moving Islands" (Chitamwebwa, 1991).

Fishing as a major economic activity on L. Victoria has been adversely affected by the water hyacinth invasion. The fish caught is either exported or consumed locally as food for human beings. The Kshs. 5.8 billion fishing in the Winam gulf industry has experienced difficulties following the invasion by the water hyacinth (Ochiel *et al.*, 2001). Available reports indicate that there is a general reduction in the fish catch, consequently, the communities living along the lakeshores have a reduced protein source in their diet (Harley, in press), as a result of the reduction in fish catches. The thick mats of floating weeds deplete the dissolved oxygen in the areas that they cover to such an extent that fish are killed and eutrophication may occur (Timmer and Weldon, 1967; Holms, 1969). The invasion of the lake by the water hyacinth has badly affected the fishing industry with a net result that thousands of jobs have been lost, hence negatively affecting the Kenyan economy.

Incidence of diseases such as skin rash, cough, malaria, encephalitis, schistosomiasis and river blindness among others are reported to be on the increase even though vital epidemiological data is lacking (Ochiel *et al.*, 2001). These high reported incidences of water and vector borne diseases is attributed to an increase in the population of vectors that transmit these diseases as a result of the enhancement on their breeding grounds, where they breed en mass.

These on and off invasion have adversely affected some of the irrigation and water treatment plants adjacent to the lake. For example in 1999, Kisumu town recorded a reduction of 25% in its water supply due to the water hyacinth (Ochiel et al., 2001). Other towns on the shores of L. Victoria which have water-works or water retaining operations by the lake sides e.g. West Kano irrigation project, the Owen Falls hydroelectric Power Station at Jinja (Uganda) and the Sewage treatment plant in Homa-bay have been under threat from these floating weeds from time to time. In the most recent case, the Uganda Government has been using mechanical removing machines to clear these weeds, while the Kenya government has contracted a private company Aquarius Unlimited to shred these floating carpets.

Loss of water due to evapotranspiration of the open water surface is also a notable problem caused by this waterweed (Benton *et al.*, 1978). Evapotranspiration of a retained water body has previously been recorded as a big problem in the Nile River. Hamdoun and Tigani in 1997 noted that the Nile lost seven thousand million cubic meters or approximately 1/10 of the total yield of the Nile per year due to evapotranspiration by *E. crassipes.*

The problems caused by the water hyacinth to the people who live around the lake region are a clear indication that there is an urgent need for a control strategy to be put in place. Consequently the extent of the spread and damage caused by the weed on L. Victoria should be established and if possible such spread be reduced to minimal.

1.7 Efforts to control the water hyacinth in the Winam-gulf and L. Victoria in general.

The Governments of Kenya, Uganda and Tanzania have instituted biological control of the water hyacinth in the L. Victoria as a whole. This is an undertaking that is still ongoing through the auspice of the Lake Victoria Environment Management Program (LVEMP). The various scientific organizations of these three riparian countries jointly introduced the *Neochetina* species (i.e. *N. bruchi* and *N. eichhorniae*) in 1996. Success in achieving meaningful control has been achieved in several areas where there were weed problems, while in other areas such success is yet to be realized. These three E. African countries at different parts of the lake where urgent clearance is needed have employed mechanical removal methods. In the Kenyan waters for example an American company 'Aquarius Unlimited' was awarded the contract to clear the infestations in the Winam-gulf under the supervision of the Ministry of Environment and natural resources and LVEMP. Work aimed at clearing this infestation is still in progress, though sometimes being slowed by limited finance, and the emergence of other invasive weed species in the various localities in which work had been going on.

The method of manual removal of these weeds from the shoreline has been adopted now and then by the local communities and fishermen who clear these beaches under the

guidance of beach leaders. The affected communities team up to clear all the weeds that have encroached in their fish-landing waterfront. The weed is dumped on to the shoreline where it dries up in the warm sunshine.

Little attempts if any seems to have been made using fungi or bacteria in a classical or a tactical program in the control of the weed within the L. Victoria and the Winam gulf for that matter.

1.8 The significance of this study.

Current knowledge pertaining to the types of phytopathogens occurring in the Winam-gulf of L. Victoria or which may have co migrated with the water hyacinth into this range is very limited. Therefore any future scientific project aimed at controlling the spread of this waterweed relying entirely on bacteria or fungi as biocontrol agents would be greatly hampered or affected by this inadequacy of information. It is important to note that the mat characteristics of water hyacinth in the Winam gulf for that matter are unknown, thus making the design and selection of harvester machines or equipment for use during bioherbicidal applications difficult.

It is imperative therefore that before any biocontrol program is to be instituted in the Winam-gulf, the catalogue of all the fungi, Insects and bacteria pathogenic and found occurring in the Winam-gulf must be well known and properly identified. The subsequent selections of suitable candidate agents need to make full use of those agents that have been associated with the water hyacinth in the Winam gulf.

Similar importance should be given to any information gathered pertaining to their distribution and carpet characters. The mode of distribution of the fungal pathogens in E. Africa can help us to determine the origin of a water hyacinth infestation in any lake in E. Africa or in any part of the world (Charudattan, 1991). Therefore a complete knowledge of the mode and distribution pattern, together with having a complete information on the occurrence of these fungal pathogens in the Winam gulf, will go along way in filling a big gap of the unknown.

A complete knowledge of the life history, biology and the carpet characters of the water hyacinth in the Winam gulf is important before any successful control strategy is to be employed. This is because of the fact that more efficient water hyacinth harvesting methods can only result from equipment design based on a better understanding of physical characteristics of the mats that affect machine performances. Similarly better biocontrol results can only be obtained with a better understanding of the physical characteristics of the weed carpet.

1.9 The objectives of this study.

1.9.1 General objective.

To study the carpet characters of *E. crassipes,* outline its distribution, document its diseases and the disease causative agents with an aim of determining their distribution and hence recommend their suitability as biocontrol agents in the Winam-gulf.

1.9.2 Specific objectives

1. To study the distribution and origin of the water hyacinth sample population in the Winam-gulf.
2. To study the constitution and carpet characteristics of the water hyacinth mats in the Winam gulf.
3. To study the water hyacinth diseases and their causative agents using the "Host approach".
4. To study the occurrence, distribution of disease causative agents, conduct virulence tests and determine their suitability for use as potential biocontrol agents in the Winam gulf.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Some known natural enemies of the water hyacinth

Previous investigations on the natural enemies of the water hyacinth have been conducted in different parts of the world. Each part having its own lists different from those of the other regions (Bennet and Zwolfer, 1968). In India for example, where the water hyacinth is also an introduced species, many organisms found occurring with the water hyacinth are those organisms that either co migrated with it or those that seem to have moved from other indigenous plants and hence are not host specific (Bennet and Zwolfer, 1968).

Even though much information pertaining to the types and forms of natural enemies of the water hyacinth is available in current literature, little is available pertaining to the forms that occur with this weed in the Winam gulf. Much is known about the occurrence of these natural enemies with their host in different countries of the world. The identities and biology of some of these virulent pathogenic organisms have already been determined by surveys already conducted in several different parts of the world. It is important to note that the aim of such surveys is restricted mainly to determining whether or not these natural enemies occurring in those areas showed sufficient controlling potentials that warrant detailed investigations on their biology, virulence and host specificity. If any promising species were to be encountered, host specificity tests for the purpose of selection for introduction in other areas are undertaken. The result of such work is volumes and volumes of literature all trying to outline the biology and life history of the water hyacinth pathogens.

Several organisms that are able to cause disease and meaningful damage to the water hyacinth are known to exist. This includes several different species of fungi, insects and other animals.

2.1.1 Some fungal pathogens of *E. crassipes*.

Fungal phytopathogens known to cause serious damage to the water hyacinth, and found occurring on the water hyacinth in different continents include hyphomycetes fungal forms like; *Bipolaris* sp., *Helminthosporium* sp., *Acremonium zonatum, Myrothecium roridium, Rhizoctonia solani, Cercospora piaropi, Cercospora rodmanii, Alternaria alternata, Alternaria eichhorniae* and *Uredo eichhorniae* among others. The fungi *Uredo eichhorniae* is known to occur only within the water hyacinth's original home range of the Amazonian Brazil. It has never been observed to occur outside its host's original range. Since the weed is known to migrate from one continent to another through the agency of man, it is important to have an understanding as to why the weed rarely co migrates with *U. eichhorniae* to its adventive ranges. The other above-mentioned pathogens are found occurring on their host in several different countries (Charudattan, 1991).

2.1.1.1 The *Cercospora* spp.

The fungal pathogen *Cercospora rodmanii* (Conway) was discovered in 1973 in Florida. *C. rodmanii* causes a destructive disease of the water hyacinth (Conway, 1976). It incites a debilitating leaf spot-disease causing the leaf to die back from the tip (Conway, 1976). Severely infected plants become chlorotic and stressed. In advanced stages of the disease, root deterioration occurs (Conway, 1976). With the spread of the disease, the plant population begins to decline, and open water appears where previously there had been dense stands of *E. crassipes*. Small clusters of plants gradually sink to the bottom. This type of disease progression may take several weeks or months (Freeman and Charudattan, 1978). *C. rodmanii* is originally from the native range of *E. crassipes,* where it has been observed to cause minimal damage to the water hyacinth. It has successfully undergone host-specificity test in Florida (Charudattan *et al*, 1985). Scientific studies to determine its suitability for use as a biocontrol agent have been conducted by scientists in several different parts of the world, where it has been found to be a suitable agent for introduction in biological control exercises. Conway *et al.* (1978) observed that *C. rodmanii* is pathogenic only to water hyacinth and therefore concluded that it was only host specific to the water hyacinth.

Disease progressive of the *Cercospora* disease has been observed to only occur when conditions favor disease development (Conway *et al.*, 1977). Since *E. crassipes* is one of the most productive aquatic plants in the world, it is able to control the biocontrol efficiency of *Cercospora* sp. The efficacy of the *Cercospora* sp. in this case being related to the growth rate of the water hyacinth (Conway *et al.*, 1978). Conway *et al.* observed that under the conditions favorable for growth of the water hyacinth, only one new leaf was produced every 5-6 days and the water hyacinth was thus capable of out-growing the *Cercospora* disease. Yet when conditions were present that favored disease development and limit leaf production to less than one leaf for every 3 weeks, the *Cercospora* disease could limit the plant growth such that they would be unable to produce new leaves. Under these circumstances the plants could then become debilitated and die back due to the effects of the disease, unless conditions become less favorable for the disease (Charudattan *et al.*, 1978). Determination of the relationship between the disease and host growth rate at different nutrient levels tested the above results. This was accompanied by measuring plant growth, disease incidence, and disease severity, and calculating the level of disease stress and the rates of disease progress required to kill water hyacinth in different situations. Major reductions of between 20-90% in host growth rates were seen, as measured by the weekly increments of green leaves due to the disease (Charudattan *et al.*, 1985). Higher reductions in growth rates occurred on the lower nutrient levels, but when the nutrient concentration was most favorable for growth, water hyacinth grew at a rate faster than the rate at which the disease progressed to newer leaves.

The results which were obtained by Charudattan *et al.* (1985) indicated that for practical levels of control of the water hyacinth by *C. rodmanii*, the fungus should only be used under conditions that support only low to moderate host growth rates, a condition which is not feasible under field conditions. Alternatively for better results, the bio-control efficacy of the fungus should be somehow improved. These could be done in such a way that for example the disease could be established only on host population at severe levels by multiple applications of innoculum when the water-hyacinth was in its early phase of seasonal growth, or the fungal innoculum could be combined with other biotic or abiotic

agents capable of retarding host growth, such as with pathogenic insects or sub lethal rates of a chemical herbicides. A combination of insects (water hyacinth weevils) and *C. rodmanii* infections yielded 98% control of water hyacinth in an experimental field test study in Florida (USA), hence confirming the potential of this integrated approach (Charudattan, 1985).

2.1.2 Other fungal pathogens of *E. crassipes*

Many other water-hyacinth phytopathogens have been successfully isolated from several different parts of the world. Some of these fungal pathogens include the fungi *Bipolaris* sp., *Helminthosporium* sp., *Acremonium zonatum*, *Ureds eichhorniae*, *Myrothecium roridium, Rhizoctonia solani, Alternaria alternata* and *Alternaria eichhorniae* among others. The biology and host-specificity's of these phytopathogens are as follows:

2.1.2.1 *Acremonium zonatum*

The fungus *Acremonium zonatum* (Sawada) Gams [= *Cephalosporium zonatum* Sawada] belongs to the fungal form class hyphomycetes. It is the causative agent of the zonate leaf spot disease of the water hyacinth. It was first isolated by Rintz in 1973, and further evaluated as a biocontrol agent by Martyn and Freeman in 1978. *A. zonatum* is a facultative parasite, known to co-migrate with its host to various regions of the world (Charudattan, 1991). *A. zonatum* causes an easily identifiable necrotic leaf spot disease that is characterized by the spreading of lesions most noticeable on the upper laminar surface of the leaf. On the lower surface, which is normally sheltered from the direct sunlight a sparse layer of white mycelial growth may be seen. Each spot may be small (2mm diameter) to large (3> cm in diameter). The spots may coalesce to cover the entire lamina. The zonate pattern may not be evident in new infections when most spots are small. The infection is favored by the presence of high humidity. Severe spotting and death of infected plants may occur if the attack is severe.

Acremonium zonatum has shown promising results against the control of water hyacinth (Charudattan, 1994). It is widely distributed in all areas where the water hyacinth is found. This fungus has been reported to occur with the water hyacinth in many countries located in Central America, South America and Asia.

2.1.2.2. *Alternaria* sp.

Two species of the genus *Alternaria* i.e.; A. *eichhorniae* and *A. alternata* have been reported to occur on the water hyacinth in different countries like Australia, Bangladesh, Egypt, India, Mexico and South Africa. *A. eichhorniae* is known to be a host specific pathogen of the water hyacinth, and has been shown to have good potentials as a bioherbicide (Charudattan, 1994). A. *alternata* a species closely related to *A. eichhorniae,* is an opportunistically weak parasite that is known to cause minimal damage to the water hyacinth, while *A. eichhorniae* is a more virulent pathogen is host specific only to the water hyacinth (Charudattan, 1994).

2.1.2.3. *Myrothecium roridium.*

The fungus *Myrothecium roridium* Tode. ex. Fries, causes a striking, teardrop shaped leaf spot disease of the water hyacinth. The teardrops may be about 1-5 cm in size. The lesions are normally rounded on the side towards the petiole, tapering to a narrow point in the direction of the laminar tip. As the disease develops the older leaf spots appear necrotic with dark brown margins, while the center of the spot gets covered with discrete white conidial masses (Charudattan, 1994). Occurrence of the water hyacinth with this pathogen has been reported in many different countries including India, Mexico, Malaysia and Australia (Charudattan, 1994).

2.1.2.4 *Bipolaris* sp. and *Helminthosporium* sp.

Bipolaris sp. and *Helminthosporium* sp. are two very closely related genera. Several species of the genus *Bipolaris* and *Helminthosporium* have been reported to be pathogenic to the water hyacinth in many different parts of the world (Charudattan, 1994). One particular species *B. maydis* (=*B. stenospila*) was reported to cause a severe leaf blight of the water hyacinth in the Dominican Republic (Charudattan, 1994). Some isolates when tested were found to be pathogenic to sugarcane, rice and Bermuda grass and therefore such species may not be safe for use as bioherbicide agents. More studies need to be done to ascertain their pathogenicity, safety and efficacy as bioherbicide candidate agents for use in the control of the water hyacinth (Charudattan, 1994).

2.1.2.5. *Rhizoctonia solani.*

The symptoms of the *Rhizoctonia* disease are similar in appearance to damage by chemical desiccant type of chemical herbicides (Charudattan, 1994). They are characterized by the presence of irregular, necrotic spots and broad lesions. Unlike the damage due to a chemical application, these brown necrotic areas are usually surrounded by thin water soaked margins of dark brown color. This pathogen has been reported to occur with the water hyacinth in different parts of the world, including, the south-eastern United States, Brazil, Mexico, Panama, Puerto Rico, India, Malaysia and Indonesia (Charudattan, 1994). *R. solani* is reported to be a very virulent and destructive pathogen, capable of killing large amounts of water hyacinth biomass in a rapid and complete manner (Charudattan, 1994).

2.1.2.6. *Uredo eichhorniae.*

Uredo eichhorniae is a rust fungus found only in S. America, the original home of the water hyacinth. The occurrence of this pathogen only inside the native range of the water hyacinth and not outside this range is to be expected on the basis of ecological theory of host parasite co-evolution (Charudattan, 1994). This fungus is rarely found outside the host range of Argentina and Brazil, it is a suitable classical biocontrol agent (Charudattan, 1994).

2.1.3 Bacterial Pathogens.

Various bacterial forms from the species belonging the genera *Xanthomonas* and *Erwinia* have been reported to be pathogenic to the water hyacinth (Charudattan, 1994). They are known to cause a destructive disease to the water hyacinth known as bacterial halo. The disease symptoms attributed to these two bacterial genera are similar in symptomatology. The halo symptoms are restricted mainly on leaf or stem areas that have insect feeding scars (Charudattan, 1994). Other bacterial species like; *Azotobacter chroococcum, Azospirillium lipoferum, Pseudomonas* sp. and *Rhodospirillum* sp. are known to be pathogenic to the water hyacinth (Gopal, 1987). Water hyacinth leaves or stems damaged by adults of the *Neochetina* weevils are known to be very susceptible to infection by these bacterial species. Not much is known about the association between the bacterial pathogens

and the insect attack scars caused by the water hyacinth weevils. It has been confirmed that major attack by these bacterial species is aided by the insect feeding scars (Charudattan, 1994). The occurrence of a condition characterized by a chlorotic halo surrounding the weevil feeding spots has been observed in Florida (USA), Brazil, Mexico and Venezuela (Charudattan, 1994).

2.1.4 Some insect pests

Insect pathogens known to attack this weed in different parts of the world include *Neochetina bruchi* Hulst. Curculionidae Bagoini, *Neochetina eichhorniae* Hulst Curculionidae Bagoini, *Sameoides albigutalis (= Epipagis albigutalis)* Hmps. (Pyralidae: Pyraustinae), *Orthogalumna terebrantis (=Leptogalumna), Acigona ignitalis* Hmps. (Pyralidae Crambinae), *Cornops logicorne* (Bruner Acrididae Cyrtacanthacridinae), *Thrypticus,* sp., *Arzama densa* Wlk. (Lepidoptera, Noctuidae), *Cornops scudderi* (Orthoptera, Locustidae). *Samea multiplicalis* Guenee (Lepidoptera, Pyralidae) and *Spenophorous pontederiae* Chttn. (Coleoptera, Curculionidae) among others. The description of some of them is as follows.

2.1.4.1 The *Neochetina* spp.

The adults of *Neochetina bruchi* are nocturnal in habit. They feed preferentially on the narrow upper third of the petiole and on the upper surface of the lamina where they remove the epidermal layer and some of the underlying cells to form small sub-circular scars. The maximum ovipositor is 8.5 eggs/female/day and eggs are usually placed in the 2nd and 3rd layer of aerenchymatous cells in the middle third of older bulbous petioles (De Loach and Cordo, 1976a). Eggs of *N. bruchi* develop at lower temperature than those of *N. eichhorniae*. The minimum development time for *N. bruchi* also occurs at a lower temperature. During the day the adults usually hide among the ligules, or inside young, rolled leaves in buds near the base of the plant (De Loach and Cordo, 1976a). Newly hatched larvae tunnel towards the base of petioles and into the crown where they excavate small pockets that occasionally become continuous. Fully-grown larvae leave the crown and pupate under water making a cocoon of root hairs (De Loach and Cordo, 1976a). Three

generations have been observed in the native range in Argentina (De Loach and Cordo, 1976b).

Tests of *N. bruchi* by the United states Department of agriculture (USDA) and the Indian Institute of Agriculture (IIHR) on 107 different plants from 52 families representing a wide range of terrestrial and aquatic species indicated that *N. bruchi* feeds preferentially on pontederiaceae family members and specifically the water hyacinth (Harley, 1990). *N. bruchi* laid one or several eggs on 22 species of plant. Oviposition was even more restricted than adult feeding and in five replications of a test in which 50 weevils were caged on plants for seven days, the total number of eggs laid was less than four on all plants except *E. azurea* (14) and *E. crassipes* (749). A small number of larva fed on *R. rotundifolia*, *E. azurea* and *P. lanceolata* but mortality was much greater than on water hyacinth and larvae rarely developed to the pupal stage. Larvae emerged from eggs laid on *Amaryluss* spp., *Tradescantia fluiminensis*, *L. sativa*, *Vallisnaria* sp. and *Trapa bispinosa* but all died within 3 days. Half-grown larvae died when inserted into these plants confirming that they are unsuitable as host plants. Mature larvae of *N. bruchi* pupate under water in chambers made from the root hairs, as this weevil could not pupate, and complete its life cycle on any terrestrial plant or aquatic plants rooted in the substrate. *N. bruchi* can feed and reproduce only on water hyacinth, rarely on two other species of the Pontederiaceae.

Neochetina eichhorniae resembles *Neochetina bruchi* in appearance, life history and behavior. Its maximum oviposition is 7.3 eggs/female/day. Eggs are usually placed just underneath the epidermal layer in the central leaf in the tissue at the base of other leaves or in the ligules. Mature larvae pupate underwater in a cocoon amongst root hairs (De Loach and Cordo, 1976a). The effects of *N. eichhorniae* on the water hyacinth are similar to those of *N. bruchi*. *N. eichhorniae* laid more eggs and its larvae develops faster than that of *N. eichhorniae*. *N. bruchi* killed water hyacinth plants in the laboratory in Argentina sooner than did *N. eichhorniae* (De Loach and Cordo, 1976a). *N. bruchi* was more abundant in spring and summer whereas *N. eichhorniae* was more abundant in autumn and winter (De Loach and Cordo, 1976a).

2.1.4.2 Natural spread of the *Neochetina* spp.

In the USA, Sudan and India *N. bruchi* is known to have spread extensively throughout these regions where it was introduced in a bid to control water hyacinth infestations. In the areas mentioned above, the water hyacinth is known to have attained very high population densities before control exercises were initiated. When the *Neochetina* sp. were introduced in these areas for the control purposes, they are not known to have attacked any other plant, thus showing that these species were host specific in the areas where they were introduced (Harley, 1990).

There is no doubt that *N. bruchi* is restricted to attacking the water hyacinth only and that it is suitable for introduction into several regions within Eastern Africa, which are infested with this weed without any risk or damage to any other plant species (KARI, 1998). The insect weevil species *N. bruchi* and *N. eichhorniae* have therefore been confirmed to be highly host specific on *E. crassipes* only (Harley, 1990).

2.1.4.3 Complementary activity of *N. bruchi* and *N. eichhorniae*.

Neochetina bruchi and *Neochetina eichhorniae* complement the action of each other and are able to co-exist successfully with each other if they are introduced into any country for biological control of water hyacinth (De Loach and Cordo, 1976a). This has been vindicated by the success of these weevils in controlling water hyacinth in USA and Sudan (Harley, 1990).

In the USA, Durden and Center (1986) identified characteristics of a water-hyacinth infestation in a state of decline due to attack by *Neochetina* sp. Adult feeding had destroyed over 35% of lamina area in some cases. This caused desiccation of the leaves that then curled in response. The petioles became thin and spindly. Submerged, waterlogged plant material pulled the shoot apices below the surface. Holes appeared in the mat, which were caused by the submergence of entire patches of plants. Areas of newest growth dropped out first, which reflected the lesser ability of smaller plants to survive attack. The canopy became very open such that water was visible among the plants. Plant

structure became much more uniform as did leaf size and shape. Basically *Neochetina* colonization first reduces plant-size, and then decreases water coverage. Provided the weevils were well distributed over the infestation, control may be achieved in about three years after introduction of these weevils (Durden and Center, 1986).

2.1.4.4 Biology of *Sameodes albiguttalis* [= *Epipagis albiguttalis*].

Sameodes albiguttalis is a moth from the center of origin of the water hyacinth, where it is susceptible to heavy attacks by parasites and pathogens (De Loach and Cordo, 1978). Its action on the water hyacinth during a control exercise is complementary to the action of the weevils. It concentrates the attack on tender, bulbous plant of water hyacinth. It has been found to be host specific only on water hyacinth (Bennett, 1970). The female moths lay an average of 300 eggs each, the eggs usually being laid in the injury spots on the leaves of the water hyacinth. Laboratory studies indicate that it has the capacity to increase 150 times in each generation. The larvae feed inside the petiole buds and bulbous-type petioles.

Attacks by *S. albiguttalis* on water hyacinth may be heavy but is sporadic due to its preference for tender, often bulbous plants. *S. albiguttalis* discriminates between the different growth forms of water hyacinth (Center, 1984) . Consequently moths dispersed from areas of water hyacinth where the plant form was unfavorable and concentrated in areas where it was favorable, resulting in a patchy distribution. Young larvae are unable to enter leaves with a hard cuticle and attack is predominately on young plants with bulbous petioles found in area of low plant density, but may also occur on lush larger plants. *S. albiguttalis* was more active during cooler months (Center, 1984), although higher populations were found in southern Florida during spring and summer than during autumn and winter. The reverse was true in Northern Florida. Established populations persisted throughout Florida in spite of a very cold winter in northern regions (Center, 1984). *S. albiguttalis* has been found to be highly host-specific on water hyacinth (Center, 1984).

2.1.4.5 *Cornops logicorne* (Bruner) (Acrididae, Cyrtacanthacridinae)

Specimens of this insect species have been collected from different parts of the world, like Trinidad, Guyana, Surinam and Brazil among others. It has also been isolated form other species of the pontederiaceae like *E. azurea* (Brazil and Guyana) and *Reussia* in Brazil (Bennett *et al*, 1968). Specimen collected attacking water hyacinth carpets from various different places have been identified to be *Cornops longicorne.* The species *C. longicorne* differs from the species *Cornops aquaticum* Bruner, which has also been reported to be pathogenic to the water hyacinth. The difference in this case being evidenced by some minor characters only. Details pertaining to the biology of these two species have been clearly outlined by different authorities including Bennett & Zwolfer (1968). Silveira-Guido noted that these insect species fed readily on the epidermis of the leaf and petiole of *E. crassipes* and *E. azurea* but rarely did they feed on any other member of the pontederiaceae species.

Eggs are laid within the leaf petiole in a chamber made by the ovipositor of the female (Bennet *et al.*, 1968). The opening of the chamber is above the water level. The elongate yellowish eggs that are normally surrounded by a creamy sticky froth are usually laid in two overlapping rows. The remaining upper part of the chamber is normally filled with a creamy froth that darkens later. The young nymphs feed on the epidermis of the leaf whereas the large nymphs and the adults eat the other leaf tissue as well and will also feed on the petioles (Bennet *et al.*, 1968). Detailed feeding tests and ovipositor preferences and limitations of these species in the Winam gulf would be a prerequisite before the possibility of future introduction is considered.

The nymphs as well as the adults are known to be subject to predaceous spiders and the predacious grasshopper *Phlugis teres* (DeGeer). Eggs are known to be attacked by the curious weevil, *Ludovix fasciata* Gyll. and Hymenopterous egg parasites (Bennet *et al.*, 1968).

2.1.4.6 *Thrypticus* sp. (Diptera: Dolichopodidae)

The larvae of *Thrypticus* sp. are known to bear small tunnels in the bases of the petioles below the water level. These tunnels are open at either end. The attacked plants are recognized by the small-blackened tunnel orifices. These tunnels sometimes may be up to 30 or more on one stem, depending on the intensity of the attack (Bennett *et al*, 1968). The two openings of each tunnel are usually on the same horizontal plane and the older tunnels usually are readily traced by the darkening of the tissue of the neighboring cells.

2.1.4.7 Other insects and mites known to attack the water hyacinth.

Several species belonging to different genera of insects have been found, which are pathogenic to the water hyacinth. These forms have been found mainly among the Lepidoptera, Coleoptera, Orthoptera, Blattaria, Hemioptera and Diptera.

The efficacy and suitability for use of the species mentioned above are yet to be tested in the Winam gulf.

2.2 Options available for biocontrol of the water hyacinth in the Winam gulf and the L. Victoria in general.

Biocontrol is one of the cheapest, self-sustaining long-term control methods of the water hyacinth. Here the plants own diseases are used to control its spread. The introduction of natural enemies known to be pathogenic to the target weed is done, thus activating diseases. For the diseases to achieve any meaningful control of the weed, a susceptible host, a virulent pathogen and favorable environmental conditions are essential components.

The concepts of plant disease and epidermiology are well documented in the articles by Quimbly (1982), Holcomb (1982), Barret *et al* (1982) and Leonard (1982). Each of these articles has attempted to review the fundamentals of plant pathology as related to biological control of weeds with the use of plant pathogens. Genetic variability and spatial distribution of the target weed population are the key host-related factors in disease development. Spatial distribution may restrict disease spread if the target weed has a patchy distribution

or geographical barriers separate populations. Since the usual target weeds of classical biological control is a dominant widespread weed forming dense infestations in pastures or rangeland habitats, spatial distribution may not be a limitation to disease in some host pathogen systems. Similarly, in cultivated habitats; targeted weeds for biological control are usually dominant and relatively uniformly distributed. If the populations of the target weed are grouped in favorable habitats or widely separated from one another, man could aid spread of the disease. Genetic variability does occur within natural plant populations, and weed populations are often described as highly variable. Asexual reproduction, with its associate genetic homogeneity, has also been positively linked to the successful biological weed control (Burdon, 1985).

Favorable environmental conditions are also essential for disease development, since environmental deficiencies like the absence of free moisture and inhibitory temperatures along with lack of sufficient innoculum and innate resistance of the host population all constrain disease development.

In the Winam gulf, suitable host specific and virulent fungal and bacterial pathogens have not been identified. They must therefore be identified in fulfillment of the standard procedures proposed by Harris (1971). Such candidate agents must have been successfully tested either in other parts of the world or within E. Africa for host specificity, pathogenicity and suitability for use in the Winam-gulf.

It is now clear that in the Winam gulf, the water hyacinth having created numerous negative attributes has fulfilled the first criteria recommended by Harris (1971). Most of the information available in the literature pertaining to the occurrence and distribution of water hyacinth phytopathogens originated from surveys and observations conducted in other water bodies located in different parts of the world. For any meaningful success in the control of the water hyacinth to be achieved in the Winam gulf, suitable candidate agents for use, which must be host specific and originally from the native range of *E. crassipes* or which may have co-migrated with the water hyacinth into this adventive range should be adopted.

Research specifically directed on the biological control of the water hyacinth began in 1961. For many years a scientific journal has been dedicated only for reporting studies of the water hyacinth and the environment around it. Today one or more species have been introduced into 21 countries (Julien, 1987), all this being as a result of these research work. The net result of all these amount of work is that the water hyacinth is under control in many different countries and various infestations in many other countries have been reduced.

Various biological control agents have been discovered and described within the native range of the water hyacinth and outside its native range. Several of these agents are known to be host-specific only to the water hyacinth (Harley, 1990). It is therefore correct to state that; "Research proven agents are available for introduction into other countries where the water hyacinth is a problem". The success rate of bio-control method of water hyacinth in other parts of the world is very high in most situations where it is an introduced weed. (Harley, 1990).

In order for biocontrol in the Winam-gulf to achieve any meaningful success, the standard procedures used in classical control exercises in other water bodies should be employed. Harris (1971) outlined some of these procedures, which are applicable for insect, nematode and microbial plant pathogens, which are due for use in a control exercise.

The following steps can summarize the procedures recommended by Harris (1971);

(1) Determination of the suitability of the weed for classical biological control.

(2) Conducting surveys for suitable plant pathogens in the target weeds native range.

(3) Study of the ecology of potentially suitable (effective) plant pathogens.

(4) Evaluation of the host specificity of selected plant pathogens.

(5) Introduction and establishment of selected plant pathogens into the new habitat

(6) Evaluation of the effect of the biocontrol agent on the target weed population.

It is important to note that Hasan (1980) and Schroeder (1983) have reviewed specific procedures for plant pathogenic agents in classical biological weed control exercises at length.

2.2.1 Suitability of the weed for classical biological control.

Most biological control programs in the past were under taken only as a last resort because conventional control methods could not be applied, had failed or could be uneconomical (Schroeder, 1983). However prior to the initiation of a classical biocontrol project, a careful analysis of the suitability of the target weed should be conducted (Schroeder, 1983). The ideal target weed should be an aggressive introduced species that infects large areas of marginal lands, such as rangeland, pastures and large water bodies. Additionally the target weed should be of a low economic value to all segments of the society.

2.2.2 Surveys for suitable plant pathogens.

This is the discovery phase of the biocontrol exercise, in which a comprehensive literature survey to gather all the information on the occurrence and distribution of the target weed and its associated disease in its native and introduced ranges should be carried out. These literature surveys provide the basis for selection of possible candidate to biocontrol agents and the selection for areas for foreign exploration to enhance the opportunities to collect pathogens. During such surveys as stated by Hasan (1988) and Templeton *et al* (1981) it should be noted that all kinds of pathogens including virus, bacteria, fungi, nematodes and others that adversely affect the growth and reproduction rate of the target weed may be considered as classical biological weed control agents.

Different types of rust fungi have been the agents of choice exclusively in most classical biological weed control programs. Rust fungi are often damaging to their hosts. They are wind disseminated and usually have a very high degree of host specialization.

2.2.3 Potentially suitable (effective) plant pathogens.

The choice or selection of a suitable biocontrol agent is based on two criteria i.e. efficacy and safety (Schroeder, 1983). However in most classical biological weed projects

especially with insects, the emphasis has been on the selection of "safe" biocontrol agents with little or no evaluation of potential effectiveness of a prospective biocontrol agent prior to introduction. For the purpose of selecting the most effective pathogen Harris (1971) and Goeden (1983) have proposed various different mechanisms including a scoring system that can be used to determine the relative potential effectiveness of insects prior to introduction. These approaches are based primarily on the biology and ecology of the agent and not on the impact (real or potential) of the target weed. The difficulties of making realistic experimental estimates of effectiveness have been discussed at length by Wapshere (1985). As such these difficulties highlight our dilemma when we try to determine the effectiveness of a given plant pathogen.

It is important to note that a detailed efficacy evaluation is difficult to conduct in the field, and as such these evaluations most often must be conducted under quarantined controlled laboratory conditions for a better evaluation to be conducted. Research within controlled facilities has its own limitations (e.g. the plants morphology and anatomy of controlled-environment grown plants may differ from field-grown plants). The advantages of being able to evaluate effects of temperature, fertility, soil moisture, humidity, light and plant competition carry more weight when you compare the use of the green house to conduct the evaluations and conducting the evaluations in the field, where you may be able to get only one opportunity per year to conduct field trials (Watson, 1991).

The success of most classical biological weed control insect agents has been due not to direct mortality of the target weed caused by the insects, but due to the increased relative competitiveness of associated plant species or increased susceptibility of the stressed weed to adverse environmental conditions (Watson, 1991). A similar picture can clearly be painted to the L. Victoria weed problem where the infestation by the water hyacinth seems to be on the decline as a result of the re-emergence of other weed species.

2.2.4 Evaluation of the host specificity of selected plant pathogens

Rigorous host testing is essential to ensure that a prospective exotic pathogen will not damage beneficial plants in the country of proposed introduction and into neighboring

countries (Hasan, 1983), Extensive Pre-introduction investigations must be conducted on the pathogen to see if it is host specific only to its host, and it will cause no danger to other plants or animals. Candidate agents meant for introduction in North America are normally screened in Europe or within specialized quarantine facilities at the Agriculture Canada Research Station in Regina, Saskatchewan; at the United States Department of Agriculture (USDA) quarantine facility at Fort Detrick in Fredrick, Maryland; at the quarantine facility at the University of Florida, Gainesville; or within the containment facility of the biopesticide research laboratory of Macdonald College of McGill University, Ste-de-Bellevue, Quebec (Watson, 1991).

All studies with exotic organisms in Kenya must be carried out in quarantine conditions, and they must be in a facility approved by the government of the republic of Kenya. In Kenya such a facility is based at Muguga, under the control of the Kenya Plant Health Inspection Services (KEPHIS). The KEPHIS quarantine Laboratory is specifically designed to prevent the escape of plant pathogens that have unconfirmed host range and virulence during scientific studies (Ochiel *et al,* 2001).

To confirm the host range of any candidate pathogen, infectivity tests require to be conducted under controlled conditions that are optimum for infection and disease development (Hasan, 1980). It is important to note that the results obtained from environmentally controlled chambers or green houses may often be different from those obtained in the actual field range. Therefore the host ranges for pathogens should be artificially expanded under artificial growth conditions, which suggest more realistic test procedures and care in the interpretation of test results (Watson, 1991).

The potential risk of damage to desirable plant species by an exotic plant pathogen species, which has been introduced for biological control purposes, has been a topic of discussion by many plant pathologists. Numerous research articles and reviews have attempted to explain the potential danger this risk carries. All pathogens employed therefore must be host specific to their host only (Watson, 1991).

2.2.5 Introduction and establishment of selected plant pathogens into the new habitat.

After an exotic pathogen species has been found to be a potentially safe and effective biocontrol agent, a detailed proposal is released by the researcher to the authorities, who would review it and make recommendations. In the USA such proposals are given to the Working group on Biological Control of Weeds (WGBCW) that was formed in 1957 WGBCW, who review and make recommendations, the approval for the release of any exotic pathogen, is made by the federal and state or provincial Department officials (Watson, 1991). In Canada the Animal Plant Health Inspection Service (APHIS) or Agriculture Canada (AG) does these approvals (Watson, 1991), while in Kenya, the Kenya Agricultural Research Institute (KARI) does the approvals.

Once this approval for release has been obtained from the authorities the liberation of the natural enemy is released at pre-selected, relatively undisturbed sites where the target weed occurs at relatively high densities (Goeden, 1977). Since climate exerts a profound effect on the disease development, the location of release sites and timing of releases to coincide with suitable environmental conditions are extremely important. Similarly, care must be taken to ensure that the agents released are free from their own natural enemies such as hyperparasites (Hasan, 1980). The release phase requires the cooperation of extension workers, research scientists and landowners in the area where the weed is a problem. Infestations of the target weed need not be located and the release site must be maintained for some time at a site where there is minimal disturbance. Many attempts to establish insects as biocontrol agents of weeds have failed because of disturbance and a slow rate of establishment of the insects (Hasan, 1980).

2.2.6 Evaluation of the effect of the biocontrol agent on the target weed population.

Once the agent is released as a control agent, it may get established within this weed population or not. The evaluation stage is therefore the final stage in which determination is done on the weeds performance, to see if the population of the released biocontrol agent is increasing or decreasing and to determine if the agent is having an effect on the target weed population. The evaluation studies are not essential to the success of a project, even though

they certainly have a bearing on the implementation and success of future biocontrol projects (Watson, 1991).

During the evaluation, qualitative and quantitative records of the status of the target weed population before and after introduction of a biocontrol agent are essential, in order for the evaluation the project as a whole. Different methods are normally employed to monitor the density of both the target weed and the introduced pathogen. Actual counts, life table analysis, computer simulation models and photographic records can contribute to the accurate assessment of the success or failure of a biocontrol project (Watson, 1991).

2.3 The mycoherbicidal tactic of biocontrol of aquatic weeds

The mycoherbicidal approach was first introduced by Daniel *et al.*, (1973), who demonstrated that an endemic (i.e. native) pathogen might be rendered completely destructive to its weed host by applying a massive dose of innoculum at a particular susceptible stage of weed growth. The application of an inundative dose of innoculum and its proper timing would shorten the lag period for innoculum build-up and pathogen distribution, essential for natural epiphytotics. To be successful in this approach it must be possible to produce abundant and durable innoculum 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. The fungus would be applied annually shortly after the weeds emergence when conditions for disease were favorable (Charudattan, 1991). The spore innoculum for this purpose was to be raised in artificial media, harvested, and prepared in a manner to withstand storage and handling. It was to be applied like a chemical herbicide. Thus, the in vitro culturing of the pathogen to obtain large quantities of innoculum and the inundative application of the innoculum to achieve rapid epidemic buildup and a high level of disease are two distinctive aspects of the mycoherbicidal concept.

Since this initial definition of the concept, the term mycoherbicide has been redefined as "Plant pathogenic fungi developed and used in the inundative strategy to control weeds in the way chemical herbicides are." Or in other words, " Living products that control specific

weeds in agriculture as effectively as chemicals". Other ways of defining it include; "The use of a pathogen in product form" and "An application technique similar to the chemical tactic". The features of the mycoherbicide formulations require that they be treated as pesticides and therefore be subject to regulations governing pesticides rather than those covering biological control materials (Charudattan, 1991). The regulatory requirements for these mycoherbicides dictate that these products conform to certain performance and safety standards and therefore they be standardized at least with respect to each innoculum batch. Prof. R. Charudattan of the University of Florida has successfully perfected various methods for preparation of different mycoherbicide formulations from different fungal species, which can be employed in the control of water hyacinth infestation. Other workers have also developed different bioherbicides formulations suitable for use in the control of different water hyacinth infestation throughout the world.

Mycoherbicide formulation for *C. rodmanii,* prepared for use in the control of the water hyacinth has been done successfully by Conway *et al.* (1978). Conway *et al* outlined it as a simple process, which utilizes surface culture of mycelium in Roux bottles followed by homogenization of the mycelium. Presently, different mycoherbicide formulations are in existence at the Institute of food and Agricultural Sciences of the University of Florida at Gainesville, Florida (Charudattan, 1994).

2.3.1 Some accomplishments of the mycoherbicidal. approach.

Even though different mycoherbicide formulations are known to be in existence, the cases of two seem to stand out as the most outstanding, formulations that are used commercially in the United States of America. These formulations are namely DeVine and COLLEGO. These two mycoherbicides are used to control milk vine (stranglervine), *Morrenia odorata*, in citrus groves of Florida and the northern joint vetch, *Aeschynomene virginica,* in rice and soybean fields of Arkansas and the neighboring states (Watson, 1991). DeVine a mycoherbicide that is marketed by Abbott Laboratories based in the USA, was the first ever-registered mycoherbicide formulation (Charudattan, 1991).

2.3.2 The choice of an agent for mycoherbicide development.

The choice of a biological agent that can control a water hyacinth infestation within a given water body in any part of the world has been a subject of discussion, considering that the water hyacinth has many known natural enemies. Different workers maintain opposite views on which is the best candidate of choice.

One school of thought fully supports the use of insects; the biology and life history of some of these insect agents have been outlined by Julien (1987) and Harley (1990), while the others propose bacteria, fungi and other organisms. Julien (1987), Harley (1990) and Ochiel (2001) seem to maintain the view that the *Neochetina* spp. i.e. *Neochetina bruchi* and *Neochetina eichhorniae* are the best candidates of choice that can be used to control water hyacinth infestations throughout the tropics. Charudattan (1994) maintains the opinion that fungal and bacterial agents if employed to control water hyacinth infestations may lead to meaningful control. The successful use of fungal and bacterial agents to control water hyacinth infestations has been reported by many workers from different countries, for example in 1976, Conway reported that the water hyacinth could be controlled biologically using *Cercospora rodmanii*. Other fungal agents with similar virulence that are pathogenic to the water hyacinth have been reported.

In Kenya few research expeditions have ever been conducted in order to fully document the water hyacinth phytopathogen flora within this country. This inadequacy of information seems to have seriously hampered the development and eventual employment of the mycoherbicidal tactic to control the water hyacinth. Mwende *et al* (2001) while working in the lake Naivasha have shown that the water hyacinth phytopathogen flora is made up of more than 12 fungal species. A complete knowledge of other water hyacinth phytopathogens that occur in Kenya and the Winam gulf for that matter may provide a catalogue from which the best fungal or bacterial agents of choice can be selected.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Positive identification of the host.

Plant specimens meant for this exercise were collected in March 1995. After collection each was labeled before being transported to the Laboratory. The specimen number, place and the part of the lake where it was collected are shown on table 3.1. These locations were chosen because they had very high population densities at the time when the collections were conducted, thus making the chances of finding complete-plants (i.e. with entire morphological and anatomical structures such as roots, stem, flowers and complete inflorescence) high. Only complete plants with normal structures were desirable otherwise any abnormalities that may have existed would not have been recognized, thus leading to a possible incorrect inference.

The characteristics that were exhibited by the major structures were compared to those already described under the species *Eichhornia crassipes* (Mart) Solms-Laubach. An accurate identification and description of the host was necessary since this study was based on the " host approach " with plant pathogens. If the specific identity of the host plant was uncertain, then the genus may have provided an adequate point of reference.

Before the identification process began, the plants were placed in 6-labeled plastic pots having a height of 16.5 cm and having a diameter of approximately 50cm. Water was maintained in these plastic pots in order to keep these plants alive during the entire exercise i.e. transportation to the laboratory and identification. Each pot was labeled according to the place of origin. The pots were therefore labeled as specimen 1,2,3,4,5 and 6 respectively (Plate 3.1).

Table 3.1 Showing specimen numbers, Place and the part of the lake where it was collected.

Specimen No.	Place collected		Collection point
	Location	District	
1	Dunga	Kisumu	Shoreline
2	Sio-port	Busia,	Shoreline
3	Luanda-k'otieno,	Bondo	Shoreline
4	Kusa-Nyakach	Nyando	Shoreline
5	Sori-bay	Suba	Shoreline
6	Muhuru-bay	Suba	Shoreline

Plate 3.1 The selected specimens from 6 different locations

Key showing the locations from which the specimen were collected.
Dunga (Kisumu) - specimen – 1
Sio-port (Busia) - specimen – 2
Luanda-k'otieno (Bondo)-specimen – 3
Kusa (Nyakach) - specimen – 4
Sori-bay (Suba) - specimen – 5
Muhuru-bay (Suba) - specimen – 6

The morphology of each individual plant's body parts were thus described and used to determine its generic and specific identity. The specimens collected as earlier mentioned originated from six locations of the Winam gulf that were separated by large tracts of open lake. The locations were the samples were collected were; Dunga (Kisumu)- specimen – 1, Sio-port (Busia)- specimen – 2, Luanda-k'otieno (Bondo)-specimen – 3, Kusa (Nyakach)-specimen – 4, Sori-bay (Suba)-specimen – 5 and Muhuru-bay (Suba) - specimen – 6.

3.2 The level of infestation and distribution of the water hyacinth in the Winam gulf.

The selected locations were those locations along the shoreline and those within the open waters that could be surveyed using boats or observations carried out along the shoreline.

Three types of experiments were conducted all of which were aimed at;

- Determining the origin of infestation.
- Determination of the weed distribution pattern in the Winam gulf.
- Determination of the mat characteristics and genetic morphological forms.

3.2.1 Determining the origin of infestation.

The Rusinga Channel was surveyed during this exercise; it was selected due to the fact that it provided the highest chances of being the entry point.

3.2.1.1 Survey of the Rusinga channel.

The Rusinga channel presented the most suitable location from were explorations for the entry points could be conducted; hence surveys were conducted in locations within this channel. The Mogari islands, Ulugi point, Mbita point, the Naya bay, Olambwe bay, Mirunda bay and the Uyoma point were surveyed.

Three survey expeditions aimed at confirming whether or not the above supposition was true or false were conducted, each originated from the Mogari Island at 1400hrs. They were initiated when the wind direction favored carpet movement into the gulf.

Each expedition passed through the Rusinga channel (next to the Sentinel Island) and proceeded to the Olambwe bay finally ending at the Homa bay pier. Even though the

Rusinga channel was assumed to have higher chances of being the entry point before this study, little seem to have been done to confirm this assumption scientifically.

3.3 Determination of the weed distribution pattern in the Winam gulf.

The bays surveyed during this study included Kisumu (Dunga), Kobala, and Homa bay and Luanda–nyamasaria. These areas were mainly selected due to the fact that they had the highest level of infestation at the times of study. In case the water hyacinth infestations were permanent in these locations, then the carpet size was supposed to be the same throughout the year. If the opposite were true then the water hyacinth carpet size at any of these beaches or bays would fluctuate from time to time, depending on the prevailing environmental factors such as wind direction and wave action among other factors.

To determine which of the suppositions mentioned above was true, a surveillance program was initiated over a period of 3 years (1995 – 1999), during which the approximate number of hectares covered by the water hyacinth in the four selected locations was recorded two times a year (i.e. June and November). Points determined as the approximate limits of infestations were extrapolated onto a map and the approximate size of the carpet in hectare was estimated on a map that was drawn to scale. The change in carpet sizes were recorded two times in one year, the first being in June, while the other in November of the same year.

Water hyacinth mats were divided into two categories depending on whether they were mostly drifting from one point to another or whether they were stationed in one place. If they were stationary then they were referred to as the "Stationary fringes", while those that were able to move were referred to as the "Mobile fringes".

3.4 Determination of the mat characteristics and the distribution of growth form in the Winam gulf.

These studies were conducted in June 1996 at Kisat-bay (Kisumu), Dunga (Kisumu), Luanda-k'otieno (Bondo), Kusa (Nyakach), Kobala (Rachuonyo), Kendu bay (Rachuonyo) and Sori-bay. These locations where selected because they had a high population density.

The major attributes that were considered were measured on the same carpet. These included;

- Distribution of growth forms.
- Connectivity and mat buoyancy.
- Standing population density
- Measurements of rhizome length, leaf length and plant mass.

3.4.1 Distribution of growth forms.

One hundred plants were randomly picked within square quadrates measuring about $1m^2$ located at; Kisat-bay (Kisumu), Dunga (Kisumu), Luanda-k'otieno (Bondo), Kusa (Nyakach), Kobala (Rachuonyo) and Sori-bay. These square quadrants were secluded using a rope and four pegs that were placed at the four corners of the square area. Mature plants within these secluded areas that showed the small, medium and large growth forms of the water hyacinth were randomly collected. Plants falling within each category were sampled and then counted.

The average of all the plants collected from the selected beaches within the Winam gulf was later expressed in percentage of occurrence of each of the growth forms. The abundance of these growth forms was represented as being abundant, not abundant or rare and most abundant, depending on how the growth forms of these plants were found occurring when 100 plants were analyzed. Each experiment was replicated three times at the locations mentioned above.

3.4.2. Connectivity

Connectivity was measured based on the principle that as it increases on a mat, more weight/ pressure is required to submerge a plant within the mat. This is because the plant is supported in part by the rest of the mat. It was expected therefore that without connectivity, each water hyacinth plant in a mat would be free floating and supported by buoyancy.

A rigid wire mesh measuring 62cm x 70cm in size, weights and a scale were used to determine this attribute. The mesh steel frame was placed on top of an undisturbed mat in the lake at the Kisumu pier where the water was stagnant. Weights were added onto the surface of the mess wire until the surface submerged under the water and the weight of submergence recorded. The mat under the frame was disconnected from the surrounding plants by cutting all the attachments such as the leaves and stolons, and weights added on the mesh surface until it was able to submerge. The same experiment was replicated four more times within the Winam gulf. These locations were Dunga beach, Kobala beach, Kusa beach and Kendu bay.

Connectivity in each case was calculated as the difference between the pressures P_1 and Pa_2 that was required to submerge the connected and disconnected mats at the specified locations.

Connectivity was calculated using the equation;

$$C = Pa_1 - Pa_2$$

Where;

Pa_1 = the pressure in Pascal required to submerge the connected mat

$$= \frac{\text{Force required to submerge connected mats (N)}}{\text{Area } (m^2)}$$

While,

Pa_2 = the pressure in Pascal required to submerge the disconnected mat.

$$= \frac{\text{Force required to submerge disconnected mats (N)}}{\text{Area } (m^2)}$$

And,

C = the connectivity (Pascal).

The force in Newton that was being exerted on the weed carpet by the loads added on the mesh wire was calculated using the formula;

Force = Mass (kg) x acceleration due to gravity (m/s^2)

= (Mass x 9.8) N

The weight (W) needed to submerge the connected and disconnected carpet was calculated as the sum of the weight of mesh wire measuring 0.62m x 0.70m and that of the load added on to it until the carpet got submerged, i.e.

$$W = L + Me.$$

Where;

W = Weight needed to submerge mats covering an area of about 0.434 m^2.

And

L = The Load required to submerge connected mat (Kg).

While;

Me = Mesh weight =1.4644 Kg.

3.4.3 Mat buoyancy.

Mat buoyancy equaled the pressure (Pa_2) required to submerge the mat under the frame when the disconnection was completed. It was the pressure that submerge the mat under the mesh frame when all the main attachments to the carpet such as the stolons, leaves, rhizome and roots attached to the carpet were disconnected by physically cutting them using a scissors. It was determined on the same carpet under the frame from which the carpet connectivity was derived.

3.4.4 Population density.

The estimated population density of the given water hyacinth population was determined by counting the number of plants found within a quadrant measuring $1m^2$ in size located in the Kisumu pier.

Four wooden pegs and a rope were used to secure the selected spot within the Kisumu pier, from where all the enclosed plants were counted. The count obtained when expressed per square meter was the population density.

This count was expressed using the formula;

$$\text{Pop density} = \frac{\text{Number of w/hyacinth plants counted}}{m^2}.$$

The same experiment was replicated a total of three times, each being mounted at Dunga beach, Kusa, Kobala and Kendu bay. The mean of these counts when calculated expressed the mean population density for the Winam gulf.

3.4.5 Biomass density.

Four wooden pegs and a rope were used to secure a quadrant measuring 1 m^2 at the Kisumu pier. All the water hyacinth plants that were enclosed within this area, were collected, weighed and oven dried in the laboratory at Maseno University. The weight thus obtained when these plants had just been collected was the wet weight, while the weight after oven drying represented the dry weight.

When the dry weight was expressed per square meter, it designated the biomass density for the Kisumu pier.
This variable was expressed as;

$$\text{Biomass density} = \frac{\text{Weight of dried w/h material}}{m^2}$$

The same experiment was replicated at the Dunga beach, Kusa, Kobala and Kendu bay. The mean count for all the five locations expressed the biomass density for the Winam gulf.

3.4.5.1 Correlation of population density and biomass density.

The data obtained for biomass density and that of population density from the same locations were correlated, and a scatter graph drawn to determine if there was any relationship existing between these two attributes in the Winam gulf.

3.4.6 Measurements of rhizome length, leaf length and plant mass

The aim of measuring these weed characters was to determine their usefulness to predict any relationship that may exist. In order to determine the measurements for rhizome length, leaf length and leaf length, 15 plants were randomly picked from quadrates measuring 1 m^2 within the lake at the Kisumu pier. Using a measuring tape, the rhizome length, leaf length and root length were measured in centimeters.

After measuring these attributes, the weight of each plant was determined using an electronic weighing machine driven by a car battery. These measurements were recorded in a table that depicted the disparity in this location. The same experiment was replicated at the Dunga beach, Kisumu pier, Kusa and Kobala.

3.4.6.1. Correlation of plant mass to rhizome length.

The measurements obtained for rhizome length (centimeters) and the weight of the given plant (in kilograms), were plotted on a graph. The scatter graph produsedwas used to determine if there existed any relationship between these two attributes in the Winam gulf. Data collected from the Kisumu pier, Dunga beach, Kusa beach and Kobala beach were used during this comparison.

3.4.7 Distribution of mass within the water hyacinth.

In order to determine the distribution of plant mass within the Winam gulf, thirty plants were sampled from quadrants measuring $1m^2$ in size from mats located at the Kisumu pier. Each plant was individually weighed to determine its weight in kilograms.

Using a classification system (Table 3.2) adopted from Petrell *et al* (1991), the plants were aggregated in six mass class categories depending on their weight. Plants weighing less than 0.1kg were placed in class 0, while those that had a weight more than 0.6 kg. were placed in class 5. Other categories ranged in between these two categories.

Table 3.2 Plant mass classes used to characterize water hyacinth mats in the Winam-gulf of L. Victoria (adopted from Petrell et al., 1991).

Class	Plant mass (kg.)
0	< 0.1
1	(0.1-0.2)
2	(0.2-0.3)
3	(0.3-0.4)
4	(0.4-0.6)
5	>0.6

Sampled plants were distinguished into the six mass class intervals that are illustrated in table 3.2. Four histograms were than drawn to illustrate these contrasting distribution of mass in the Winam gulf.

3.5 Diseases of the water hyacinth and the determination of their causative agents.

3.5.1 Isolation of the phytopathogens.

Plant parts that had visible signs on their leaves/stems i.e. necrosis, lesions, rots, and browning were collected from the Winam gulf. After the collection process was completed, each specimen was categorized according to the necrotic symptoms that it exhibited. Each infected plant part was cut into $1mm^2$ pieces, surface sterilized in 0.5% NaOCl for 2 minutes and thoroughly rinsed in sterile distilled water. Sterile portions were transferred to PDA (Potato dextrose agar) plates that were kept in the laboratory at room temperature ($\cong$ 25° C) for any fungal growth present. Any emergent fungal growth was transferred to new PDA media. A similar isolation method of isolation employed by Charudattan (1973) and Ponnappa (1970) was able to produce more than 32 fungal isolates able to grow on PDA and 6 unidentified bacterial pathogens that were able to grow on NA.

Pure cultures were obtained from these blooming mycelial growths by transferring emergent mycelia to fresh PDA plates. Pure cultures obtained were allowed to sporulate after which microscopic examinations were initiated to determine the characteristics of these blooming mycelia. The type of hyphae, conidiophores and conidium produced by each isolate was taken into account. The characteristics exhibited by their reproductive apparatus and vegetative plant bodies among other structures were recorded. All the PDA isolates were stored under strict sterile conditions for future use. Identification keys adopted from Olga Fastinova (1986) were employed to reach the correct systematic position of each isolate.

3.5.2 Pathogenicity trials.

Pathogenicity trials were conducted at the botany laboratory (Maseno University) in conformity to Koch's postulations. Plants meant for this exercise were kept in plastic buckets of 30-cm diameter, filled with tap water. Pure innoculum was transferred onto healthy leaves/stem at the areas that appeared clean and free of any visible disease

symptoms. The innoculum used for this purpose consisted of a drop of dense suspension made up of fungal spores and mycelium. Four leaves were inoculated using these innoculum made from each of the suspected fungus. Each leaf was inoculated at two spots, one on each surface. Control plants were treated in a similar manner except that they were inoculated with a drop of sterile distilled water. After the inoculation process was complete, each plant and its bucket was covered with a plastic bag in order to maintain the high level of humidity that is a requirement for the disease establishment. Each experiment was replicated three times. The disease progression was observed over a period of four weeks and a system adopted from Charudattan (1973), used to express the pathogenicity of each isolate.

3.6 The occurrence and distribution of the diseases of the water hyacinth and their causative agents in the Winam-gulf.

Field sites with severe water hyacinth infestations were selected for this purpose because the probability of finding infected plants and the frequency of disease incidences could be better studied in such heavily infested sites than in locations where the infestation were sparse.

One hundred plants that showed disease symptoms were randomly picked from the fish-landing site at Kendu bay, and sorted according symptoms that they exhibited. The mean number of plants showing each disease symptom was expressed as a percentage. This experiment was replicated at Homa bay, Kusa beach and Kisat bay and a histogram drawn to express the distribution in the Winam gulf.

CHAPTER FOUR

4.0 RESULTS

4.1 Identification of the host plant.

This aquatic weed is a monocotyledonous, angiosperm plant belonging to the monocot family pontederiaceae. Some members from the genus *Eichhornia* have similarities to those of the species *E. crassipes.* A comparison of the collected plants meant for identification to those described under the species *Eichhornia crassipes* (Mart) Solms-Laubach indicate that this plant is the same to the one already described. A summary of the identification and description of the major structure is as follows;

4.1.2 Summary of the Identification and description process.

The results obtained during the analysis of plants collected (table 4.1), for descriptions are summarized below;

(a) Habit: Aquatic plant, consisting of free-floating plants, buoyed by bladder-like inflated Leaf petioles; found freely floating in the lake waters. Some of the growth forms were large while others were of the small and medium types.

(b) Root: Adventitious type, consisting of a long and feathery structure.

(c) Stem: Free floating, bright green, consisting of a semi-succulent thick rhizome, which is under the water surface. It is branched with a creeping stem (stolon) attached to it. At the end of the stolon is attached a clone.

(d) Leaf: Plant exhibits heterophylly, leaf petioles inflated and bladder-like, the leaf-blade is kidney shaped and somewhat rounded consisting of three types of leaves,

(a) The float leaf

(b) The canopy leaf

(c) Intermediate leaf type

(a) **The float leaves;** these leaves had short expanded petioles of chlorophyllous tissue. They had a small leaf blade and were held to the stem at an angle of 30° from the water surface.

(b) **Canopy leaves;** these leaves had an elongated, unexpended petiole and a much larger leaf blade in comparison to the float leaves. They were more upright and were held at angles of between 80-90° from the water surface.

(c) **Intermediate leaf type;** these leaves seemed to be of an intermediate type, with characteristic intermediate to the two above categories.

(d) Inflorescence: Spike-like, with up to 20 flowers. No seeds seen.

(e) Flower: Consists of 6 petals, the upper one is marked with blue and yellow. 6 stamens seen with a 3-segmented ovary.

(f) Fruit: Not found

(h) Classification and identification:

Monocotyledons - (1) Venation parallel

(2) Flowers trimerous

Coronarieae – (1) Inner perianth petaloid

(2) Ovary superior

Pontederiaceae– (1) Inflorescence usually of a scapiferous racemose type.

(2) Perianth in two whorls.

(3) Stamens in two whorls and epipetalous

(4) Gynoecium 2-5 locular and placentation axile.

Eichhornia crassipes

(1) Plants are aquatic, found free floating

(2) Plants exhibit genetic polymorphism, shoot axis sympodial, leaves not whorled, stamens 6, Ovary trilocular, ovules numerous

(3) Flowers zygomorphic, perianth lobes connate forming a tube, stamens inserted on perianth tube at different levels, anthers sub equal, dorsifixed, opening longitudinally same level, anthers equal

(4) Vegetative reproduction is the main method of reproduction, mainly by clonal propagation

Other specimen collected from the areas such as Sio-port, Luanda-k'otieno in Bondo District, and Muhuru-bay beach in Suba District confirmed that the plant was *Eichhornia crassipes* (Mart) Solms-Laubach. These plant specimens though collected from different sites had similar characters and therefore the identification to the generic level was the same in all the cases.

4.2. The origin of the water hyacinth into the Winam gulf

The water hyacinth invaded Winam gulf through the Rusinga channel. The Rusinga channel is the water channel that separates the Kenyan Island of Rusinga (Suba district) and the mainland at Naya bay (Bondo District) just off the pier at Luanda K'otieno (Plate 4.1). Several small islands occur within this particular channel, these include small islands like; Mogari Islands, Wahondo Island, Sentinel Island and Chamarungo island among others. All the major boats entering into the Winam gulf or leaving the Winam gulf communicate through the Rusinga channel when entering or leaving, since it is the only communication route open to the Winam gulf.

The current water hyacinth invasion of the Winam gulf was accomplished through this channel. The invasion process being enhanced by the small and large units of floating water hyacinth carpets that drifted into the gulf when the wind direction favored mass movement towards the inside of the gulf. These carpets then settle in quiet and isolated beaches like Luanda–nyamasaria (Olambwe bay), Mirunda bay and Homa bay (Ruri bay), that are directly facing the Rusinga channel. It is from beaches located within these bays that the water hyacinth seems to have later drifted to the remainder of the Winam gulf. The water hyacinth finally settled in new habitats where mixed vegetations were formed (Plate 4.3). This was dependent on whether the beach or bay under consideration is secluded or not. After the invasion of the given bay the water hyacinth was able to remain for some period only to drift out when the wind direction favored mass movement out of the given beach or bay.

4.3. Level of infestation and distribution of the water hyacinth in the Winam-gulf.

The level of infestation in the Winam-gulf is not constant throughout the year. It is in a dynamic state dependent on the season, environment and climatic factors. Within all the beaches that this surveillance program was conducted there was continuous change in carpet size during different times of the year. The estimated carpet cover in the months of June and November between 1995 and 1999 in the four beaches that this study was conducted are shown in table 4.1.

At one time of the year the water hyacinth was resident in one end of the gulf, but it migrated to other parts of the lake when the season and wind direction changed. The changes are more exemplified by the increases or reductions of the approximate total area of the water surface covered by water hyacinth carpets within the four selected locations. An analysis of the data obtained between 1995 and 1999 for the months of June and November indicated that the changes were not significant at $P \leq 0.05$ (Appendix 9). Seasonal changes measured between these three years also showed no significant differences at $P \leq 0.05$.

The water hyacinth in the Winam gulf thrives more in isolated lagoons and not in the open waters (Plate 4.2).

Plate 4.1 A view of the Rusinga channel from Luanda K'otieno.

Plate 4.2 A view of the open waters showing no water hyacinth infestation at Kendu bay.

Table 4.1 Approximate changes in sizes of water hyacinth carpets in selected beaches from 1995 – 1999.

Location/month	95	97	99	Mean	S.E
Kisumu					
June	2	10	10	7.33	2.66
Nov	2	2	2	2.00	0.00
Kobala					
June	2	9	9	6.66	2.33
Nov	2	9	9	6.66	2.33
Homa-bay					
June	2	2	2	2.00	2.00
Nov	2	12	12	8.66	3.33
Luanda –nya					
June	2	2	2	3.33	1.33
Nov	3	8	9	6.66	1.85

The water hyacinth cover in the Winam gulf comprises of two types of carpet forms i.e.

- Stationary fringe.
- Mobile mats.

The stationary fringe comprises mostly of water hyacinth mats that are mainly restricted to the lakeshore. Such fringes are to be were found localized in beaches at Kusa, Kendu-bay, Sikri, Matoso, K'onyango-ngira, Dunga, and Sio-port among others. The wind and water currents propel about the mobile mats the net result being translocation from place to place. As earlier mentioned the distribution of the mobile mats in the Winam-gulf is controlled by seasonal storms and prevailing winds that induce mass movement of the waterweed from one point to the next. The new arrivals attach themselves to other floating or attached vegetation within the shoreline (Plate 4.4).

Plate 4.3 Shoreline vegetation consisting of water hyacinth and other grasses at Luanda - nyamasaria.

Plate 4.4 Water hyacinth vegetation onto which floating water hyacinth attach before they get established (Sori beach next to the pier)

4.4 The constitution and carpet characters of the water hyacinth mats in the Winam gulf.

4.4.1 Water hyacinth growth forms.

When the counting of plants exhibiting the three growth forms i.e. small, medium and large were completed, it became clear that the water hyacinth carpet in the Winam gulf consists of plants that exhibit a majority of the large growth form.

The large growth form is the most widely occurring growth form of the water hyacinth within the Winam-gulf. About 45 % of all the plants that were counted exhibited the large growth form, while 34% and 21% exhibited the medium and small growth forms respectively.

4.4.2. Carpet characteristics.

4.4.2.1 Connectivity and mat buoyancy.

When the mesh wire was placed on top of the weed carpet and weights added on to it, the mean weight that the carpet was able to support before it could submerge was 12.770 Kg. (Table 4.2). This was before the entangled stolons and petioles below it were disconnected. When the separations were successfully done, the water hyacinth carpets were able to support a mean weight of 11.740 Kg. (Table 4.2), which is equivalent to a force of 115.05N. The mean mat buoyancy of the water hyacinth mats in the Winam-gulf was therefore calculated to be 265.1Pa. The mean connectivity water hyacinth mats in the Winam gulf was equal to 23.1Pa (N/m^2) kg. The differences in weight between connected and disconnected carpets were signicant at $P \leq 0.05$. Connected water hyacinth mats covering 0.62m x 0.70m (0.434 m^2) of the Winam-gulf was able to sustain a mean pressure of 288.4 Pa (Pascal) before it could submerge. Any additional pressure will cause the connected carpet to sink into the water (Table 4.2).

The mat buoyancy i.e. the pressure that was required to submerge the mat under the mesh frame when all the main attachments to the carpet such as the stolons, leaves, rhizomes and roots were disconnected by cutting had a mean value of 265.1 Pa. Disconnected carpets

having a dimension of 0.62m x 0.70m (0.434 m^2), the weed carpets were able to submerge and sink when the weight added exceeded a mean of 11.740 Kg (table-4.2).

Table 4.2. Showing the weights W_1 and W_2, means and carpet onnectivity of water hyacinth in five beaches in the Winam gulf.

Location	W_1	W_2	Mean	$W_1 - W_2$	Pa	S.E.	Significance
Kisumu pier	12.75	11.50	12.125	1.25	28.2	0.6250	*
Dunga beach	13.5	12.50	13.000	1.00	22.6	0.5000	*
Kobala beach	12.0	10.90	11.250	1.10	24.8	0.7500	*
Kusa beach	12.5	11.20	12.000	1.30	29.3	0.5000	*
Kendu bay	13.1	12.60	12.850	1.50	11.24	0.2500	*

W_1 the weight in kg required to submerge the entangled carpet.
W_2 the weight in kg required to submerge the disconnected carpet
Pa is the connectivity in Pascal
S.E. is the Standard error.
S.D. is the Standard deviation
* Significant at $P \leq 0.05$

4.4.2.2 Correlation of plant mass to rhizome length.

The measurements of the rhizome length and the wet weight varied from location to location that the measurements were done. The rhizome length measurements in each case was determined by several factors such as; Plant health, the location in terms of nutrients availability, wave action, harvesting exercises and the activity of biological agents. In these experiments rhizome length measurements ranged between 9cm and 13cm, while the plant wet weight varied between 0.09kg to 0.8kg. Four least square equations were obtained when scatter graphs were drawn to depict the relationship between these two variables at each location, i.e. $r^2 = 0.0066$, $r^2 = 0.0053$, $r^2 = 0.6021$and $r^2 = 0.4034$. Correlation of these variables for plants that were collected from Kobala beach (Osodo bay) seemed to show a minimal correlation i.e. $r^2 = 0.6021$ (Fig 4.2). Correlation of the variables for plants collected from Kisumu pier and Dunga beach showed no correlation i.e. $r^2 = 0.0066$ and $r^2 = 0.0053$, while those collected from Kusa beach showed a minimal correlation i.e. $r^2 = 0.4034$ (Fig 4.1).

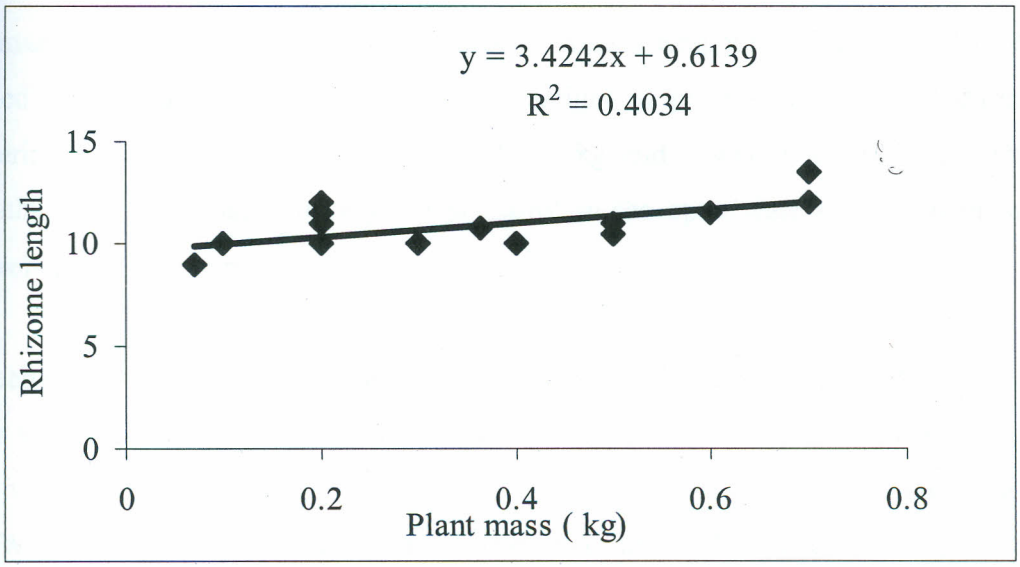


Figure 4.1 Correlation of plant mass with the rhizome length of plants collected from Kusa beach (Nyakach bay).

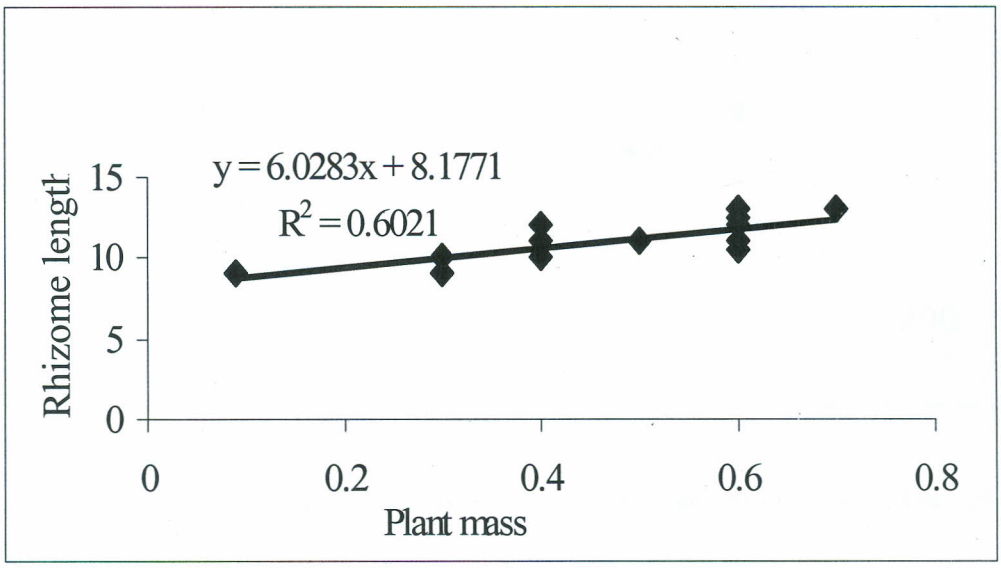


Figure 4.2 Correlation of plant mass with the rhizome length of plants collected from Kobala beach (Osodo bay)

4.4.2.3. Estimated population density.

Biomass density in these experiments varied between a weight of 2.5 kg/m^2 and 10 kg/m^2. it varied from location to location. The wet weight of plant materials used during these experiment varied between a weight of 47.35 kg and a weight of 71.5 kg.. The mean population density varied between 0 plants/m^2 in the open waters and 156 plants/m^2 in densely populated carpets.

A scatter diagram plotted produced a least square equation i.e. $r^2 = 0.9202$ (Figure 4.3). This least square equation obtained indicated the existence of a close relationship between these two variables in the Winam gulf. It appears that 92% of the changes in biomass density in the Winam gulf can be accounted for by the changes in population density (Figure4.3).

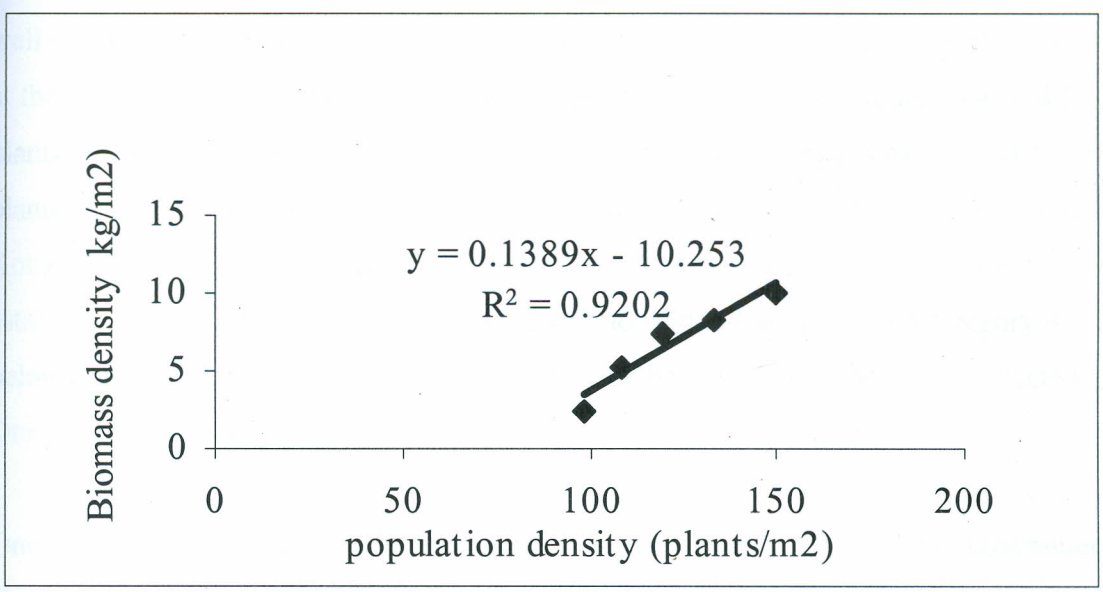


Figure 4.3 Correlation of the population density and the biomass density of the water hyacinth in the Winam gulf.

4.4.2.4. Plant mass distribution

Plant mass distribution indicated normal distribution in some locations where there was little interference by external factors and the mats studied in these areas appeared to be well established in the various areas where data was collected, However the mean values of plant

mass differed from one collection point to the next (Figures 4.4, 4.5, 4.6, & 4.7). In all the four observational spots (i.e. Kisat, Dunga, Kobala & Kusa), plant specimen of mass class–4 category dominanted.

One set of data that was obtained from plants collected from Kusa beach seemed to indicate abnormal distribution of plant mass in the given area (Fig 4.7). Here plants belonging to two mass class intervals dominated. The reason for this abnormal growth seemed to be the activity of the *Neochetina* spp. Several plants that had evidence of insect and bacterial attack were visibly seen among the sample population collected for examination. Two classes dominated the group of plant that was collected at this particular location; these were the plants of the mass classes 1 and 4. The whole weed population at this location in Kusa seemed to be under attack by these insects, which possibly led to this uneven distribution of plant mass within population. At the Kisumu pier the water hyacinth mats appeared to be well established, and the plants were larger and probably of the same age as those growing at the Dunga beach. Plants of class-4 dominated both these areas (Figures 4.4 & 4.5). The plants sampled for observation from the Kisumu pier and Dunga area seemed to be old plants and the collection consisted of larger plants in comparison to plants sampled from Kobala beach that consisted mostly of small plants, which seemed to be very well established. Many members of this population belonged to the mass-class category 4. Plants belonging to the mass class-4 dominated the number of plants that were collected from Dunga beach (Fig 4.5).

One population of plants from the Nyakach-bay, Kusa beach (Fig 4.7) had unsymmetrical mass distribution and had more than one class dominating the mass of plants collected. The reason for such distribution may have been due to the activity of the *Neochetina* spp. that appeared to be very active. The feeding scars of the two species of insect weevil could be seen on the leaves of most plants, while the adults of both the species *N. bruchi* and *N. eichhorniae* were found residing on the affected plants, thus confirming the source of the unsymmetrical distribution as being attributed to them. The histograms (Figures 4.4 – 4.7) outlining the distribution of mass within each of these locations illustrated that the water

hyacinth sizes can differ greatly from one location to another and even differing within a given location if the population is not a well-established one.

Plants having a mean mass between 0.4 kg - 0.6 kg formed the majority of plants that were sampled and weighed from Kisumu pier, Dunga beach and Kobala beach. Plants weighing below 0.1kg i.e. (class-1) formed a minority in comparison to those that weighed between 0.1kg and 0.4 kg, which were placed in the classes 1, 2, & 3 that formed a majority. Small plants that weighed less than 0.1 kg and heavy plants that weighed more than 0.7 kg formed a minority of the plants that were collected from all the four locations.

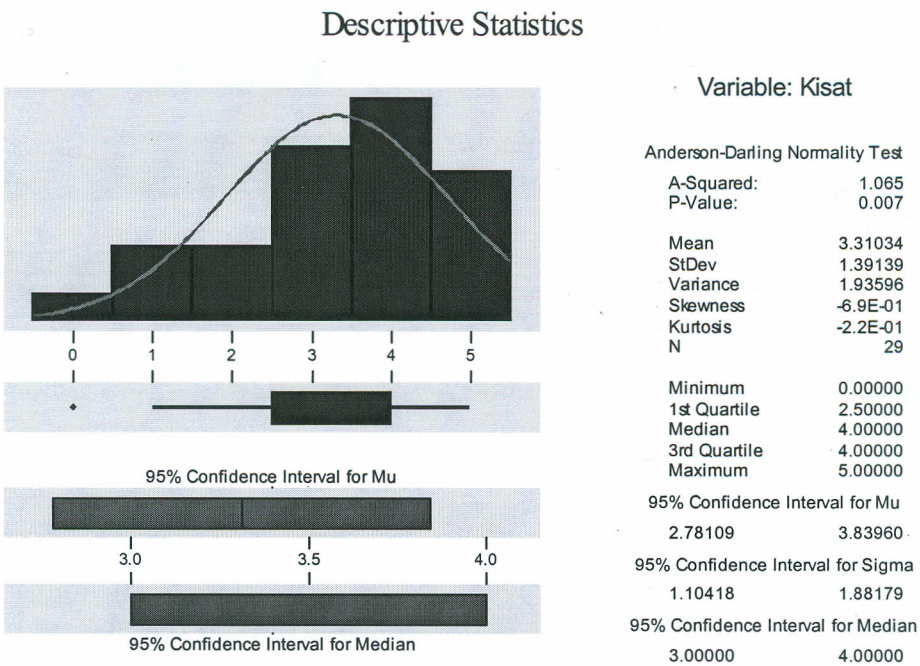
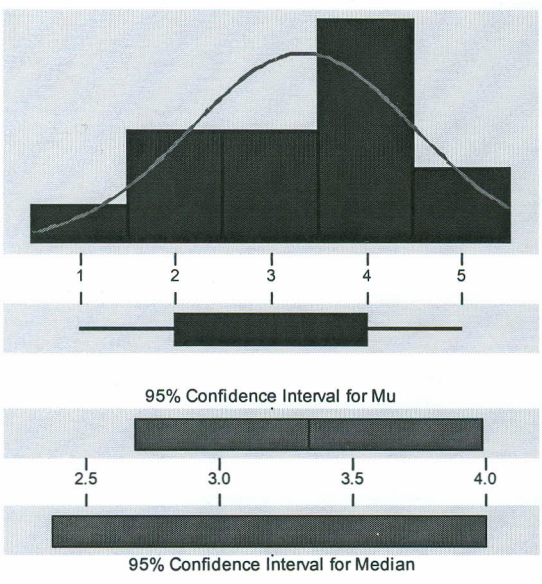


Figure 4.4 Showing a well established and an almost normally distributed plant mass at the Kisat bay next to the Kisumu pier (class-4 dominated)

Classification key

Class - 0 = < 0.1 kg
Class –1 = (0.1- 0.2) kg
Class –2 = (0.2 – 0.3) kg
Class –3 = (0.3 -0.4) kg
Class –4 = (0.4– 0.6) kg
Class –5 = 0.6 kg<



Variable: Dunga

Anderson-Darling Normality Test	
A-Squared:	0.673
P-Value:	0.063
Mean	3.33333
StDev	1.17514
Variance	1.38095
Skewness	-4.5E-01
Kurtosis	-5.6E-01
N	15
Minimum	1.00000
1st Quartile	2.00000
Median	4.00000
3rd Quartile	4.00000
Maximum	5.00000

95% Confidence Interval for Mu	
2.68256	3.98410
95% Confidence Interval for Sigma	
0.86035	1.85331
95% Confidence Interval for Median	
2.37351	4.00000

Figure 4.5 Showing a well established and an almost normally distributed plant mass at Dunga beach (class 4 dominated)

Classification key

Class - 0 = < 0.1 kg
Class –1 = (0.1- 0.2) kg
Class –2 = (0.2 – 0.3) kg
Class –3 = (0.3 -0.4) kg
Class –4 = (0.4– 0.6) kg
Class –5 = 0.6 kg<

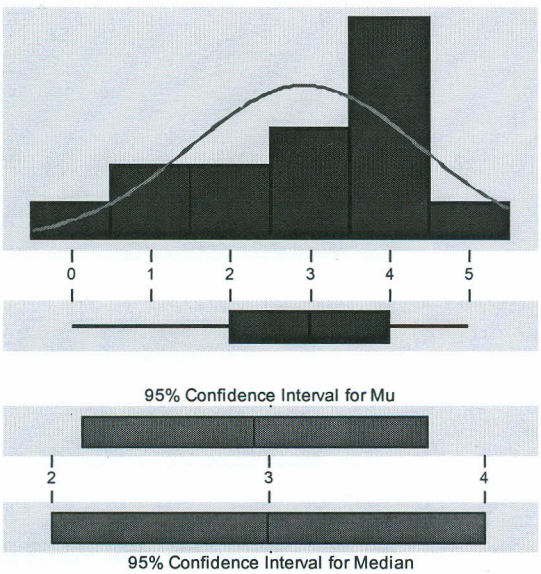
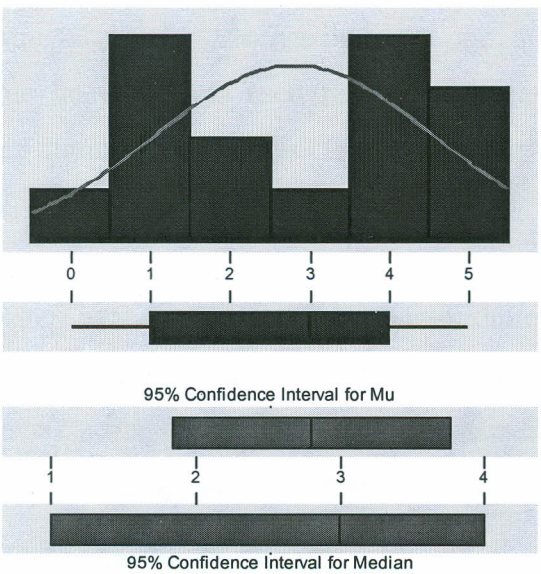


Figure 4.6 Showing a well established and an almost normally distributed plant mass of plants from Kobala beach Rachuonyo (class 4 dominated).

Classification key

Class - 0 = < 0.1 kg
Class –1 = (0.1- 0.2) kg
Class –2 = (0.2 – 0.3) kg
Class –3 = (0.3 -0.4) kg
Class –4 = (0.4– 0.6) kg
Class –5 = 0.6 kg<

Descriptive Statistics



Variable: Kusa

Anderson-Darling Normality Test	
A-Squared:	0.714
P-Value:	0.049
Mean	2.80000
StDev	1.74028
Variance	3.02857
Skewness	-1.2E-01
Kurtosis	-1.57858
N	15
Minimum	0.00000
1st Quartile	1.00000
Median	3.00000
3rd Quartile	4.00000
Maximum	5.00000

95% Confidence Interval for Mu	
1.83627	3.76373
95% Confidence Interval for Sigma	
1.27410	2.74459
95% Confidence Interval for Median	
1.00000	4.00000

Figure 4.7 Showing abnormal distribution of plant mass at Kusa beach, Nyakach bay (classes 1 & 4 dominated).

Classification key

Class - 0 = < 0.1 kg
Class –1 = (0.1- 0.2) kg
Class –2 = (0.2 – 0.3) kg
Class –3 = (0.3 -0.4) kg
Class –4 = (0.4– 0.6) kg
Class –5 = 0.6 kg<

4.5 Water hyacinth diseases found in the Winam- gulf.

During this study, more than seven water hyacinth diseases were encountered within the different locations in the Winam gulf where these surveys were conducted. Out of these diseases, six disease causative agents were isolated and their association to the disease confirmed using Koch's postulation.

To distinguish the difference between the different diseases encountered, information available literature was used for the purpose of comparison. Further information was obtained during a personal visit to the laboratory of Prof. R. Charudattan of the University of Florida, Gainesville (USA).

The water hyacinth diseases diagnosed during this study included those caused by; *Rhizoctonia*, *Acremonium*, *Myrothecium*, *Alternaria*, *Cercospora* and *Fusarium.* The organisms causing a strange disease that appeared to be associated to the insect feeding scars of the *Neochetina* sp. was not determined.

4.5.1. The Foliar blight disease.

Infected leaves collected for the purpose of identification, in this case had a lot of similarities in appearance to damage possibly attributed to a desiccant type of chemical herbicide (Plates 4.5 & 4.6). Two plants with leaves characterized by irregular, necrotic spots with broad lesions and from which a *Rhizoctonia* sp. was isolated were collected at the Kisumu bay, next to the pier (Plate 4.6).

The damage attributed to this fungus however, unlike chemical damage had brown necrotic areas that were surrounded by noticeable, thin water-soaked margins of darker brown color, the color being much darker than the rest of the necrosis. Damage to the plants due to this disease was restricted to the leaves, never were they encountered on any part of the affected plants. Plants showing symptoms of the foliar blight disease were sighted at Kusa and Kendu-bay along the Winam gulf.

Plate 4.5 Water hyacinth leaf-showing damage attributed to *R. solani*.

Plate 4.6 Water hyacinth leaves showing damage attributed to *R. solani* (Note that this damage is similar to damage by a chemical herbicide).

4.5.1.2. Mycelial characters of the foliar blight disease isolate.

PDA cultures obtained after 7 days indicated that this fungus is a filamentous fungus, producing hyphae that were able to grow so rapidly in the petri dishes. They covered the entire surface of the agar media on which they were germinating. The hyphae produced were of two types; i.e. the straight long and the short types. Generally, these mycelia had a raised elevation only being limited by the lid of the petri dishes. They had a characteristic white color that changed to brown.

The texture of the fungal colonies was rough and appeared to be made up of cylindrical strands of highly branched hyphae. These hyphae were septate, and contained cytoplasmic interruptions within them. The interruptions had regular intervals throughout the entire length of the vegetative hyphae. The hyphae had multinuclear cells, with the mycelia appearing as aggregates of 'threads' having right-angled turns. White sclerotia that turned brown were formed by these PDA cultures. Sclerotia thus formed seemed to originate in such a way that the fungal hyphae appeared to form multiple anastosomes that looked like ball like structures.

Throughout the observation period, only sterile mycelia were observed within these cultures, no spores or spore-producing structures could be obtained. Within the same period, the only mode of reproduction observed in these PDA cultures seemed to be only the random fragmentation of hyphae.

Pathogenicity experiments conducted in conformity with Koch's postulations (Plates 4.9, 4.10. 4.11 & 4.12) confirmed the association between the causative fungus and the water hyacinth. Based on the results obtained in this experiments, the pathogen causing this disease was inferred to be possibly one of the isolates of *Rhizoctonia solani* Kuhn. The identification key for systematics of fungus belonging to form-order Mycelia sterilia adopted from Olga F. (1986) i.e. Identification key-5, was used for the purpose of establishing the systematic position of this isolate. The fact that the hyphae produced in

Plate 4.7 Water hyacinth plants inoculated with *R. solani* on 30-6-99.

Plate 4.8 Water hyacinth plants inoculated with *R. solani* on 30-6-99.

Plate 4.9 The Control experiment showing the original plant covered with a polythene plastic bag to maintain a high level of humidity.

Plate 4.10 Water hyacinth leaf-exhibiting symptoms of the *R. solani* disease after it had been inoculated with an inculum of *R. solani*

PDA culture by this fungal isolate produced no conidia throughout the observation period, made it clear that this fungus was only capable of producing sterile mycelia consisting of hyphae that could not produce any conidium. Any filamentous imperfect fungus unable to produce conidium could be a member of the form order Mycelia sterilia, the form-order in which the two well known genera are *Rhizoctonia* and *Sclerotium.*

Since these hyphae formed were sterile hence forming no conidium or conidia producing structures, it was therefore desirable to include this isolate under a type genus of the form order mycelia sterilia, possibly a member of the genera *Rhizoctonia* or *Sclerotium*. But since the hyphae formed multi cellular cells that branched at right angles, then it became increasingly clear that this fungus was a species of *Rhizoctonia* and more so a member of the species *R. solani*.

4.5.2 The zonate leaf spot disease

The zonate leaf spot disease was frequently sighted during this study. It was sighted in almost all areas that were visited including; Kisumu, Kusa, Kobala, Kendu-bay, Homa-bay, Sori-bay, Mbita-point, Mfangano island, Takawiri island and Muhuru-bay among the many other places where surveyed.

4.5.2.1 The symptoms

The disease was mainly foliar and was identified by the occurrence of zoned necrotic spots characterized by spreading lesions that appeared as concentric rings most noticeable on the upper laminar surface of the leaves (Plate 4.11 & 4.12). After growing for about 2-3 weeks, the plants that were brought to the laboratory (which has shady conditions) from the field exhibited white mycelial growth on both the upper and lower surfaces of the leaves. When the leaves were left to grow for a few more days the mycelial growth covered a large part of the leaf surface (Plate 4.13). The zonate leaf spots ranged in size from 2 mm to 30 mm or even covering the entire leaf whenever these spots coalesced to form large scars (Plate 4.12). An epiphytotic as a result of this pathogen was seen in progress at Kusa beach.

Plate 4.11 Water hyacinth plant growing in the field. (Note the leaf showing the zonate disease of *A. zonatum)*

Plate 4.12 A leaf showing the zonate disease of *A. zonatum*

4.5.2.2 Mycelial characters of the zonate leaf spot disease isolate.

The PDA cultures that were obtained sporulated after a period of about five to seven days at room temperature on the laboratory desk. These cultures formed colonies that grew relatively slowly. They appeared to be of a light cream color that appeared to be felted and fluffy. The mycelia were hyaline and very fine and consisted of hyphae that showed branching and septation.

When the hyphae produced were subjected to a close scrutiny under the microscope the conidiophores seemed to be formed by lateral simple or branched phialides that appeared narrow and elongated slowly tapering towards the apex. Conidia formed by these mycelia were unicellular, hyaline and were produced at the orifices of the phialides. The conidia formed appeared to form head-like clusters at the apices of the phialides or remained in chains before getting scattered. When the mycelia on the observation slides were spread, conidial masses appeared scattered all over the observation area of the slide.

The association between the water hyacinth and the zonate leaf spot disease causative organism was confirmed after successful completion of pathogenicity experiments in conformity with Koch's postulations.

The occurrence of the zonate spots on the leaves, the light creamy hyaline mycelium, the occurrence of branched phialides that were narrow and elongated strengthens the need for the inclusion of this fungus under the genus *Acremonium*. The occurrence of unicellular conidia in head-like structures that remained in chain further reinforced the inference that this fungus was actually a member of the genus *Acremonium*. The fact that the phialides formed were able to produce phialospores that appeared to arise singly on the hyphae, further strengthened the inference that this was a species from the genus *Acremonium*.

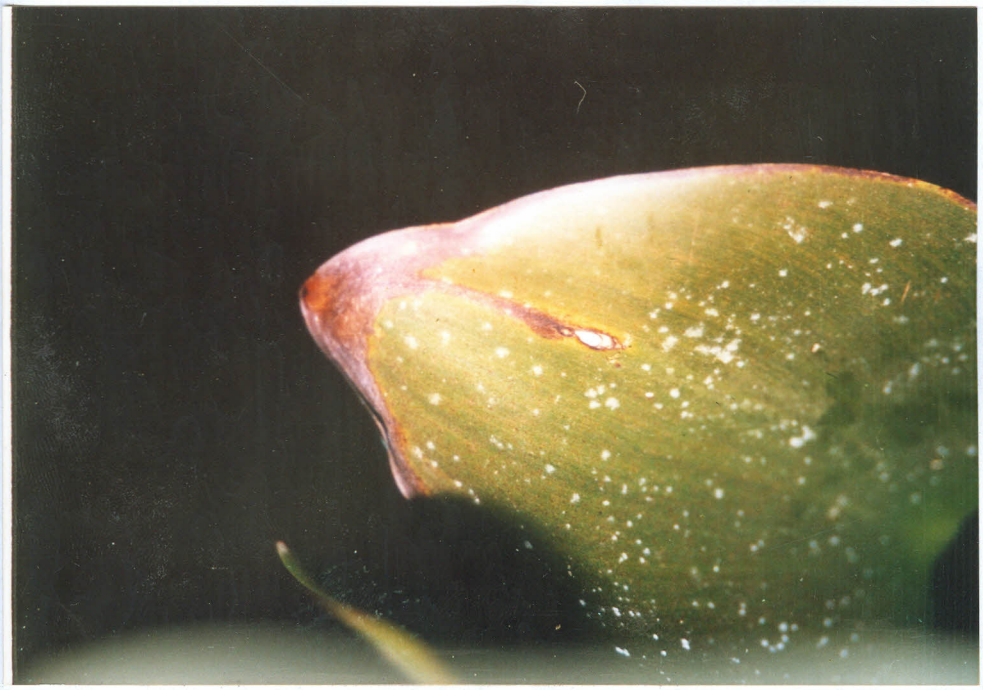


Plate 4.13 Water hyacinth leaf exhibiting white mycelial growth on the upper side due to *A. zonatum*

4.5.3 The necrotic leaf spot disease.

The necrotic leaf spot disease was sighted in several places visited during these surveys. This included; Kendu-bay, Kusa, Kobala, Homa-bay and Kisumu among others. In several of the areas where it was found in occurrence, it was noticeable that this pathogen is very virulent and could possibly be able to cause an epiphytotic so long as the environmental conditions were suitable for its development.

4.5.3.1 The symptoms.

Leaves of infected plants showed a teardrop shaped type of infection, which had an average length of between 1cm up to 5cm for larger infections (Plates 4.14 & 4.15). The spots produced were somewhat rounded on the side towards the petiole, finally tapering to a narrow point towards the direction of the laminar tip. The older leaf spots appeared necrotic and had dark brown margins (Plates 4.14 & 4.15). The center of the older leaf spots had visible whitish structures, which were later confirmed to be conidial masses. Similar spots were reported by Ponnappa (1970). When the conidial masses produced were later transferred to new agar plates they were able to germinate to produce mycelia that were similar in appearance and morphology to those obtained from the leaf postules.

4.5.3.2 Mycelial characters of the necrotic leaf spot isolate.

The mycelium obtained from PDA cultures of infected plant parts had a white hyaline mycelium with dark green sporodochia being formed within the petri dishes in which the cultures were grown. These sporodochia were convex and seemed to mutually fuse, finally appearing to change color with maturity from dark green to black.

The conidiophores were straight and seemed to branch once or twice. They were terminated by bundles of narrow clavate phialides in-groups of approximately 3 – 8 and 10 – 12. Bundles of phialides of single conidiophores were seen. They were closely attached to each other in such a manner that they formed continuous palisades. The conidia were formed at the apices of phialides, in masses associated and enveloped by a black slime. They were cylindrical with rounded apices and had two to three fat drops. They were in clusters that seemed to adopt a spherical to pyramidal shape.

Plate 4.14 Water hyacinth plant growing in the field (note; the leaf showing the necrotic leaf spot disease symptoms)

Plate 4.15 Water hyacinth leaf showing symptoms of the necrotic leaf spot disease.

The association between the host and the disease causative agent was confirmed in conformity with Koch's postulates. After examination of the various characters of the hyphae, mycelium and disease symptoms, it became evident that the fungus under examination was a species of the genus *Myrothecium roridium* (Tode) ex. Fr.,. This inference was deduced using the identification key–2 adopted from Olga F., (1986). Due to the fact that the hyphae were filamentous, aseptate and formed convex sporodochia that bore unicellular conidia, the above inference seamed to be correct.

4.5.4 The *Alternaria* leaf spot disease

4.5.4.1 The symptoms

Disease symptoms similar to those already described by Charudattan (1994), as being due to *Alternaria* sp. were sighted within the Winam gulf. Such sites include; the Kisumu pier and Kusa beach among others. The infection in each case was mainly foliar (Plate 4.16).

The infected leaves showed spotting signs on them, the leaf spots in each case appearing as darker spots that appeared to become necrotic on leaves that looked older. The spots had a characteristic dark green appearance with their color changing from green to yellow and finally changing to brown as the infections spread on to cover the entire leaf. The *Alternaria* disease seemed to be more manifested in older leaves than in younger leaves, which seemed to be more resistant to the infection.

4.5.4.2 Mycelial characters of the *Alternaria* leaf spot isolate.

PDA cultures obtained after culturing diseased water hyacinth leaves for more than five days indicated that the causative organism responsible for this disease was filamentous. These hyphae were septate and branched. The vegetative mycelium was partly hyaline and partly dark brown in appearance as was evidenced by the aerial mycelium. The dark brown color of the mycelium was later confirmed to be the color of the conidia, which had a dark brown color. The conidia were produced by conidiophores that were densely segmented and showed less branching.

Plate 4.16 A leaf from which the *Alternaria* isolate was isolated.

Conidia formed in these cultures were apically multicellular and club-shaped (dictyospores), they were formed in long chains that were mostly unbranched. They were brown, smooth, spiny and typically alternarioidous. Only the terminal conidia in the chain were globose without a beak. The conidia had two types of septae that ran horizontally and longitudinally across the conidia.

The association between the water hyacinth and the disease causative agent of this leaf spot disease was confirmed by conducting pathogenicity trials. These trials were conducted in conformity with Koch's postulations. The disease causative organism when isolated from the diseased parts of the inoculated leaves was confirmed to be similar to the ones that were obtained from the PDA cultures.

The fungus causing this disease was later confirmed to be a species belonging to the form genera *Alternaria* (Nees) Wiltshire. It possibly belongs to the form-species *Alternaria alternata* Fr. Kessler, a species assigned to the above genera. The fact that this isolate produced conidia in long chains, that were mostly elongated, narrow and had apical beaks that were unbranched (dictyosporous), further confirmed its systematic position as *A. alternata.*

4.5.5 The *Cercospora* leaf spot disease.

4.5.5.1 The symptoms.

Plants showing symptoms of the Cercospora disease were found occurring in several places. The pathogen was isolated from Kusa, Kendu-bay and the Kisumu pier. The most imminent symptoms on the leaves were the yellowing of the leaf occasioned with the presence of numerous small sunken brown lesions or necrotic spots that were seen on their lamina and petioles. The centers of the leaf spots appeared to have characteristic pale centers that were surrounded by darker regions (Plate 4.17).

Plate 4.17 Infected water hyacinth leaf from which a *Cercospora* isolate was obtained.

Occasionally on a few leaves, these spots appeared to have a teardrop shape similar to the symptoms attributed to the necrotic leaf spot of *Myrothecium* sp., hence creating a little confusion. However those spots attributed to the *Cercospora* sp. leaf spot disease would coalesce as the leaf matured, thus causing the entire leaf to turn necrotic and senescent.

4.5.5.2 Mycelial characters of the *Cercospora* isolate.

PDA cultures obtained from infected leaf parts, showed mycelium made up of multicellular, septate and branched hyphae. Conidia were inversely clavate i.e. rounded at the base and tapering towards the apex. The conidium was generally 4 - 5 septate, the septation being horizontal only. The color of the conidium produced by this isolate was light brown in color. The conidia were produced singly at the end of short vertical conidiophores.

The conidiophores in this case were brown in color, small, unbranched, septate and appeared to be somewhat thicker than the rest of the vegetative hyphae that were located on the same mycelium.

Pathogenicity experiments conducted in conformity with Koch's postulations confirmed the association between the water hyacinth and this pathogen. Based on the structure of its mycelial, conidial and the symptoms it exhibits the fungus causing this disease was systematically confirmed to belong to the genus *Cercospora.* Isolate might actually be the species *C. piaropi* Tharp., the fact that this fungus was able to form a mycelium in PDA culture with a conidiophore and conidium of brown or dark colors reinforces this inference. The fact that it also formed; conidiophores that were geniculate and conidia that were clavate further confirms the need for it inclusion in the species *C. piaropi* Tharp., and not *C. rodmanii.*

4.5.6 The stem necrosis disease.

4.5.6.1 The symptoms

The symptoms of the stem necrosis disease appeared as dark colored infection or necrosis on the stems of the affected plants (Plate 4.18). Surveys conducted during these studies showed that several plants from different observation sites had the symptoms of this disease these included; the Kisumu-pier, Kusa and Kendu-bay among others.

4.5.6.2 Mycelial characters of the stem necrosis disease isolate.

The mycelia formed in PDA were rich and cottony, with light pink-colored mycelia, which seemed to rise up the surface of the cultivation petri dish. The under side of the petri dish had a characteristic brown–pink color. The conidiophores produced were more or less branched, with elongated phialides that gave rise to conidia. The conidia were produced in a fine cushion shaped sporodochia. The cultures also seemed to form two types of conidia namely the microconidia and the macroconidia

Macroconidia; They were multicellular and falcate-shaped
Microconidia; They were unicellular, ellipsoidal, and oval in shape and were produced in slimy heads at the apex of the conidiophore.

Stiff spherical structures believed to be sclerotia were seen within PDA cultures. Pathogenicity experiments conducted in conformity with Koch's postulations confirmed the association between the water hyacinth and this stem necrosis isolate.

The fungus causing this necrosis of the stem was identified to be a species of the genus *Fusarium* Link. This is a genus with several species that are widely distributed in several different habitats (Olga F, 1986). They may be found occurring saprophytically in the soil or living as parasites on other higher plants and animals. The identification and eventual placing of this fungus to the form genus *Fusarium,* was completed using an identification key-2, adopted from Olga F. (1986).

Plate 4.18 Water hyacinth stem from which a *Fusarium* isolate was obtained.

The occurrence of unicellular microconidia and the multicellular macroconidia that were crescent shaped, strengthens the need for the inclusion this fungus in the form genus *Fusarium.*

4.5.7 The necrotic halo disease.

Several leaves showing halo symptoms, many of which had characteristic occurrence of a chlorotic halo that seemed to surround the weevil feeding spots were encountered during these surveys. Most of the infected plants had the weevil feeding scars appearing together with the halo symptoms at the same spot (Plates 4.19, 4.20 & 4.21).

As the halo disease progressed, it was able to cover an entire region of the leaf /stem onto which insect feeding scars were present. Such regions eventually became damaged, finally breaking up at the areas that were most heavily affected by the disease, thereby causing the affected parts of the stem/leaf to finally dry up.

Not much is known about the relationship between the insects attacking the water hyacinth and the bacterial forms causing this halo disease (Charudattan, 1995). Water hyacinth leaves showing insect attack scars that appear together with the necrotic halo symptoms were comparatively the most prevalent diseases of the water hyacinth in the Winam gulf.

When the mean counts for each disease was calculated for 100 infected plants collected within the Winam gulf, 57% of the leaves collected showed symptoms of this particular disease (Fig 4.8). Due to this high frequency of the bacterial disease symptoms throughout the Winam gulf, the *Neochetina* spp. that are the main vectors of this disease seem to have successfully spread to all the Winam gulf. These weevil species were introduced by the Kenya Agricultural Research institute (KARI). The causative agents of the halo disease of the water hyacinth are known to belong to species from the genera *Xanthomonas* and *Erwinia* (Charudattan, 1994). These bacterial pathogens are known to be potentially useful as biocontrol agents

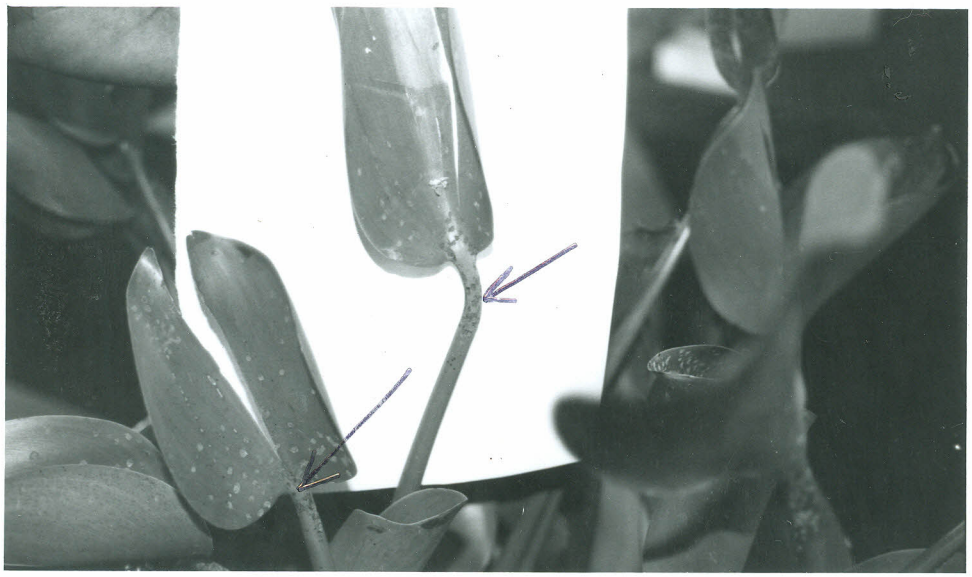


Plate 4.19 Water hyacinth leaves showing halo symptoms located on insect feeding scars.

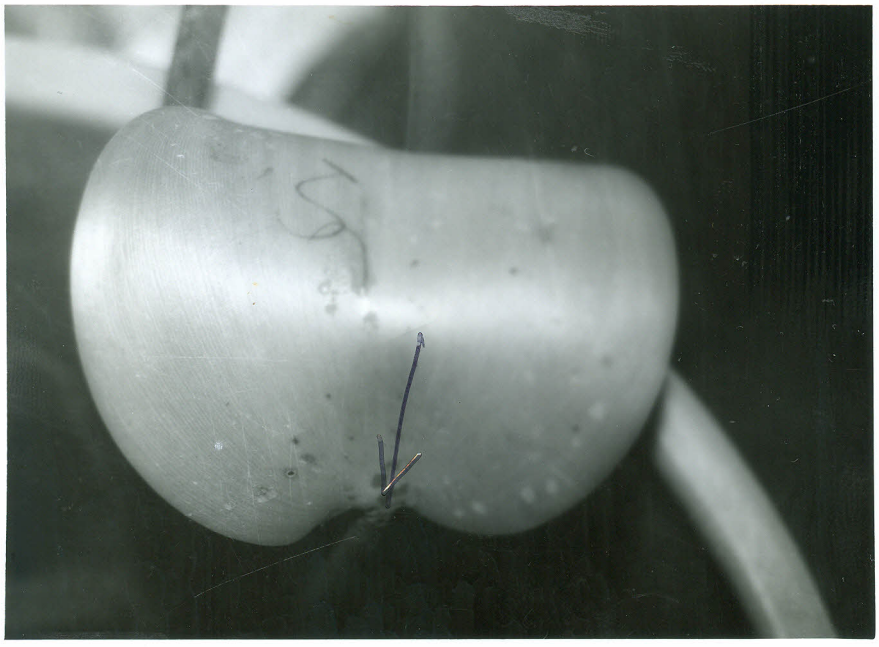


Plate 4.20 Insect feeding scars occurring together with halo symptoms on a leaf.

Plate 4.21 Water hyacinth leaves with insect feeding scars.

4.6 The Pathogenicity of the pathogens isolated from the Winam gulf.

After an observation period of 4 weeks, four pathogens proved to be highly pathogenic to the water hyacinth (Table 4.3). The degree of damage to the water hyacinth was different from one individual pathogen to the other; this disparity in pathogenicity was expressed using a scoring system adopted from Charudattan (1973).

Out of the six confirmed fungal isolates, the *Acremonium* isolate appeared to be very virulent, while the isolates of *Alternaria* sp. and *Fusarium* sp. appeared to be less virulent during laboratory trials (Table 4.3). The *Acremonium* isolate was the most well distributed fungal pathogen associated with the water hyacinth in the Winam gulf. The *Myrothecium* isolate that was also virulent was the second most distributed foliar disease.

Table 4.3 Fungi/ bacteria pathogenic to *E. crassipes* in the Winam-gulf.

Organism	Degree of Pathogenicity	Number Of isolates
Acremonium zonatum	+++	1
Myrothecium roridium	+++	1
Rhizoctonia solani	+++	1
Fusarium sp.	++	1
Cercospora sp.	+++	1
Alternaria sp.	++	1
Unidentified bacteria	+++	None

++ = Spreading but not extensive necrosis on the leaves.
+++ = Spreading and extensive necrosis, resulting in further spread of the disease to non-inoculated leaves.
(Note; Method of expressing pathogenicity adopted from Charudattan (1973)

4.7 Relative abundance and frequency of occurrence of the water hyacinth diseases in the Winam gulf.

A comparison of the relative abundance of the water hyacinth disease symptoms on the leaves/stems at four selected locations indicated that the plants with leaves/stems showing symptoms of chlorotic halo attributed to bacteria were the most abundant water hyacinth disease symptom in the Winam gulf. When the mean difference in the distribution of water hyacinth diseases in the Winam gulf was analyzed the difference in disparity in the distribution between these diseases symptoms was highly significant at $P \geq 0.05$. The disease symptom attributed to *Acremonium* sp. was very frequently sighted throughout the beaches where these studies were conducted. Other disease symptoms like those are attributed to *Cercospora* sp. *Fusarium* sp., *Alternaria* sp. and *Rhizoctonia* sp. were also sighted during these field studies.

After the counting process was completed, and the mean number of leaves showing disease symptoms expressed per 100 leaves in the different observation locations (Table 4.4), a histogram was drawn to express the differences between the means of these counts outlined the disparity that exists in distribution of these water hyacinth diseases symptoms (Figure 4.8). The mean counts obtained for the diseases attributed to bacterial pathogen associated to the *Neochetina* spp. were 57% of all the counts. This high percentage of occurrence of the chlorotic halo may be used to illustrate the success of the *Neochetina* spp. known to facilitate the infection and spread of this particular disease.

More than 20% of all the diseased leaves that were counted showed the *Acremonium* disease symptom, 13.5% showed the *Myrothecium* disease symptom, while the *Cercospora*, *Alternaria,* and *Rhizoctonia* the *Fusarium* disease occurred in 4.5%, 3%, 0.25% and 1.25% of all the counted disease symptoms respectively (Fig 4.8).

Table 4.4 Showing the mean counts of disease symptoms.

Disease type	Mean counts	S.D.
Acremonium	20.50	3.109
Myrothecium	13.50	1.732
Rhizoctonia	0.250	0.500
Cercospora	4.500	1.291
Fusarium	1.250	0.957
Alternaria	3.000	0.816
Chlorotic halo	57.00	4.163

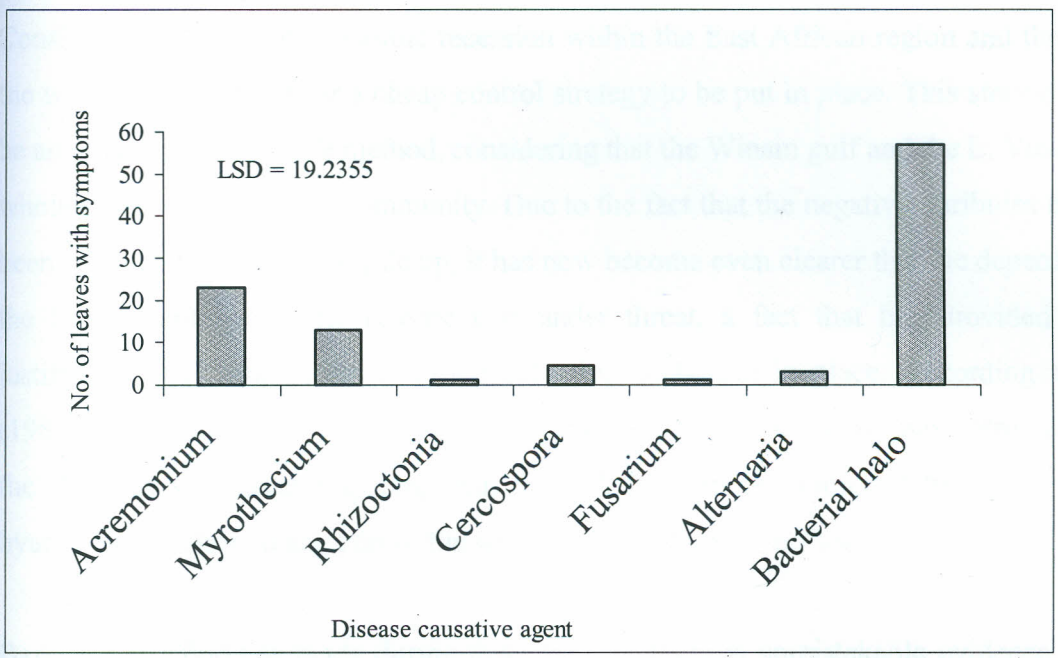


Figure 4.8 Histogram showing the distribution of water hyacinth diseases symptoms in the Winam gulf.

CHAPTER 5

5.0 GENERAL DISCUSSIONS.

The water hyacinth has established itself in the Winam gulf. Its occurrence within this adventive range has created numerous negative and a few positive attributes that have not been fully quantified. Some of these problems are very serious, thus implying that the control of this weed is urgent, necessary and important.

Considering the current economic recession within the East African region and throughout the world, there is need for a cheap control strategy to be put in place. This strategy should be an environmentally safe method, considering that the Winam gulf and the L. Victoria as a whole support a very large community. Due to the fact that the negative attributes that have been sited have continued to pile up, it has now become even clearer that the dependency of the local people on water resources is under threat, a fact that has provided a good justification for a long-term control mechanism to be put in place. According to Julien (1987), Charudattan (1994), Harley (1990), Ochiel (2001) and many biocontrol scientists, the cheapest self sustaining long term method that can be employed to control a water hyacinth infestation in any part of the world is the biological method.

Based on the findings made during this research, there is unmistakable evidence that has confirmed the occurrence of more than six virulent phytopathogens of the water hyacinth in the Winam gulf. More research is desirable to confirm their safety, virulence and efficacy within the Winam gulf. These organisms seem to be ideal mycoherbicidal development agents since all are known to be pathogenic to the water hyacinth in other water bodies other than the lake Victoria. The six confirmed in this case being; *Rhizoctonia solani, Myrothecium roridium, Acremonium zonatum, Alternaria* sp., *Fusarium* sp. and *Cercospora* sp.. Even though Charudattan (1994), Harley (1990), Julien (1987), Watson (1990) and other workers have reported the occurrence of these organisms with the water hyacinth in other water bodies, very little is known about their occurrence with their host in any other East African water body. Mwende *et al* (2001) have reported the occurrence of *Phoma*

sorghina, Alternaria alternata, Fusarium sp, *Fusarium equisetti, pellionella* sp., *Khuskia oryzae, Phoma chrysantemicola, Nigrospora sacchari,* and *Cladosporium cladosporloides* in Lake Naivasha. Little information exists pertaining to the organisms that are associated with the water hyacinth in the Winam gulf.

The difficulties encountered during the systematics of these six water hyacinth isolates from the Winam gulf can be better pointed out by the cases of *Rhizoctonia solani* and *Acremonium zonatum*. The form *Rhizoctonia solani* was placed in the form-order mycelia sterilia that is known to posses more than 30 form genera, of which *Rhizoctonia* and *Sclerotium* are the two well known and widely distributed genera. The similarities in their mycelia and the fact that both form sclerotia in culture create problems during their systematics. A close study of the sclerotia formed and the branching exhibited by *R. solani* distinguishes this two genera. *R. solani* can further be distinguished by the specific characters that it forms when in its perfect stage; a Basidiomycete fungus *Aquathanatephorus pendulus* [=*Thanatephorus cucumeris*]. The identification of the *Acremonium zonatum* (Gams) the pathogen that causes the debilitating zonate leaf spot disease also showed complications during its systematics. It is important to note that in many occasions during the systematics of the form genus *Acremonium,* complications are known arise as a result of the highly variable conidia that it produces and the similarities that *Acremonium* has with the genera *Verticillium* and *Fusarium,* who both produce micro conidia in the same way as *Acremonium,* thus creating the difficulties in systematics. The form-order Moniliales into which the fungus *Myrothecium roridium* was placed is a large form-order sub-divided into four distinct form-families including the families; Moniliaceae, Dematiaceae, Stilbaceae and Tuberculariaceae. Allocation of the PDA isolate isolated during this studies and later inferred as *M. roridium* to the form-family Tuberculariaceae and species *M. roridium,* was reinforced by the fact that this particular isolate had conidia that were light colored, and had conidiophores that formed flat to cushion like structures known as sporodochia. According to Olga Fastinova (1986) an organism showing those characteristics is placed under the species *M. roridium.* Two species placed in the form genus *Alternaria* (i.e. A. *alternata* and A. *eichhorniae*) have been reported by other workers to be pathogenic to the water hyacinth in different countries such as Australia, Bangladesh,

Egypt, India and South Africa among others. According to Charudattan the pathogenicity and disease symptoms of each these two species of *Alternaria* are more or less similar.

Senescence of a water hyacinth carpet as a result of the effect of a fungal pathogen has been reported by workers investigating water hyacinth pathogens, the case of *Cercospora* sp. reported by Charudattan (1994) is the most outstanding example of this type of accelerated carpet senescence as a results of the *Cercospora* leaf spot disease. According to Charudattan (1995), this type of disease progression eventually spreads across other water hyacinth plants causing large areas of biomass to become brown and necrotic. No such conditions have been reported in the Winam gulf, though it is expected that under such conditions the effected water hyacinth plants would become stressed, loose their ability to regenerate and finally become water logged only to sink down to the bottom of the Winam gulf. Two species of the form genus *Cercospora* are reported to be able to cause this type of senescence these include; *C. rodmanii* and *C. piaropi.* The species *C. piaropi* has been reported by other workers to occur more often with its host in other continent, unlike *C. rodmanii* that is mostly found occurring with its host within its original range. According to Olga Fastinova (1986), over 40 species of the genus *Fusarium* occur in different parts of the world. On the basis of their conidial stages this genus can be divided into 12 sections. These 12 stages can be used to delimit the various specific types within this otherwise big genus. Even though the *Fusarium* isolate obtained during this study was not identified beyond the generic level, the evidence made available after a complete study of the PDA culture it produced clearly supported the assumption that this was an isolate of *Fusarium.* The fact that PDA isolate was able to form two types of conidia, i.e. the macroconidia and microconidia further reinforced this inference.

Currently the *Neochetina* spp. are the only biological agents in the Winam gulf (Ochiel, 2001). These biocontrol agents were introduced by the KARI (Kenya Agricultural Research Institute) in order to control the proliferation of the water hyacinth in the Winam gulf and other parts of the L. Victoria (Kenya). These two closely related species seem to have spread to almost all the major parts of the Winam gulf (Ochiel *et al,* 2001) the effect of their spread has been felt with a net result that currently large chunks of water hyacinth carpets

have been eliminated from many areas where they were once a threat (Ochiel *et al,* 2001). The well distributed feeding scars or injury spots attributed to the *Neochetina* spp. that are encountered whenever individual plants are subjected to close a scrutiny attests to this fact. The free spread of the water hyacinth within the Winam gulf has been checked by the introduction of the *Neochetina* spp..

The success story of this biological control exercise using insect species have portrayed how a properly coordinated biological control exercise may produce good results. Even though there are other control exercises in progress (mechanical removal using a harvester machine and manual removal), it seems there is a need for conducting more research on how these six phytopathogens that have been isolated during this study can be utilized. The probability of achieving meaningful success when these agents are employed seems to be high, according to the findings of Charudattan (1994) in Florida. He observed that a properly formulated mycoherbicide of *A. zonatum* when produced and used in the control of the water hyacinth, success was possible only if the formulation was properly constituted such that it was able to overcome all the conditions that support the growth of the host.

The assumption that the fungal parasite *A. zonatum* is the most suitable fungal agent seems to be correct, more so because this research has confirmed that it is the most widely occurring phytopathogen amongst the six that were isolated. It appears to be the most suitable candidate agent for use in the biological control of the water hyacinth in the Winam gulf, the virulence it has shown to the water hyacinth during pathogenicity trials attests to this fact. Other pathogenic fungi like *Penicillium oxilicum, curvulaira lunata, Aspergillus niger* and *Trichoderma viride* reported by other workers to be pathogenic to the water hyacinth in other countries need to be isolated in the Winam gulf. The biological control of the water hyacinth in the Winam gulf using fungal and bacterial agents isolated from this adventive range appear to be more feasible than before. The good prospects for this success being more emphasized in this research project than by any other.

There is need for the disease causative agents that cause chlorotic halo to Identified. Future searches for water hyacinth pathogens in the Winam gulf should highlight their identities,

life history and pathogenicity. Even though bacterial forms including; *Erwinia* sp., *Xanthomonas* sp., *Azotobacter chroococcum, Azospirillium lipoferum, Pseudomonas* sp. and *Rhodospirillum* sp. have been reported to be associated with the water hyacinth in different countries throughout the world, Charudattan (1994) reported that only *Erwinia* sp. and *Xanthomonas* sp. are able to cause the halo disease of the water hyacinth. There are more than one species from the genera *Erwinia* and *Xanthomonas* that are pathogenic to the water hyacinth (Charudattan, 1994). Since there have been few or no reports pertaining to the occurrence and distribution of bacterial pathogens in the Winam gulf, it seems there is a need for these pathogens to be isolated more so because information existing in scientific literature pertaining to their distribution in the whole world is still incomplete. This therefore implies that much information in this area of biological science seems to be unknown. Filling this gap with knowledge will facilitate further work on biological control of the water hyacinth in the Winam gulf by use of these unknown and known bacterial pathogens.

It has become apparent that the water hyacinth adopts shoreline environments that are generally sheltered from violent offshore winds and wave action. In the Winam gulf, the water hyacinth prefers flat or gently sloping shores (rarely deeper than 5 meters) with soft muddy bottom rich in organic matter, such as the case in the shoreline extending from Nyakach to Kendu-bay. Twongo (1998) while working in L. Victoria (Ugandan) made similar observations when he noticed that Uganda had the most suitable shoreline environment for water hyacinth establishment. In the Winam gulf these types of shorelines with a lot of organic matter deposited along their shores while being generally very well sheltered from violent weed action emanating from the open lake are also plenty. Clearly this natural cover from wind action and external interference need to be maintained if the given bay or beach had to retain its water hyacinth infestation (Twongo, 1998). If such an infestation were to be subjected to any form of external wind action emanating from the open lake, then the weeds located within it would get blown out of the water onto the dry shoreline from where they would get dried by the strong sunlight in a matter of hours.

The cover of the mobile water hyacinth mat fluctuates from time to time depending on whether the bay or beach in question produces or stores the weed. For example the cover of

the mat in the Kisumu-bay changes between a situation when the bay is completely covered and a time when it is clear of water hyacinth mat (Table 4.2). In many bays within which this study was conducted, small batches of plants were continuously being blown by the wind into or out of the bay into the open waters, finally the new arrivals in the bay would get attached to other floating or attached vegetation along the shoreline. These attachments assist the pioneer weeds in holding on to the shores, where otherwise the wind would sweep them so that they would dry along the sides of the lakeshore. Weeds growing within such shores also act as a grazing ground for cattle and other livestock. This was very common around Karungu-bay and Sori-bay. Marked reduction on the level of infestation was seen in some of the grazed spots. The grazing animals consisting mainly of cows and goats grazed along the shoreline and very rarely did they venture into the lake waters.

In the Winam gulf water hyacinth cover can be categorized into two; i.e. stationary fringe and mobile fringe. According to Twongo (1998) the stationary fringes in the Lake Victoria (Uganda) were able to develop rapidly in the earlier stages of the water hyacinth invasion, this was due to the fact that when wind and wave action translocated the medium sized and large mats that got anchored and hence resulted in the creation of the stationary fringes. Large portions of the stationary mats eventually floated away leading to the instant establishment of other stationary fringes along the shore, as a result of these translocations. The stationary fringes formed in the Winam gulf act as sources of subsequent water hyacinth infestation of other parts of the Winam-gulf. According to Twongo (1998) the first wave of stationary fringes in the L. Victoria (Uganda) seemed to have stabilized in 1996, field observations done around the same period in the Winam gulf indicated that these first stationary fringes were mostly found on sheltered bays and beaches such as Kisumu, Kobala, Kusa, Senye, Kendu-bay, Homa- bay, Luanda-nyamasaria, Sio-port and K'onyango bay. Due to the fact that the stationary fringes were able to attach themselves to the shoreline vegetation, a stiff competition for space and nutrients between the water hyacinth and these indigenous species resulted, thus resulting in an ecological succession process that pitted the water hyacinth against mostly the members of the family graminiae. In Uganda, a similar process was reported by Twongo (1998) to have originated along the shoreline around 1992-1993. This process seemed to have continued in Uganda up to April 1998 when *Vossia*

cuspidator appeared to occupy over 70% of the linear length of the stationary fringe, thereby reducing the effective cover of the water hyacinth in these fringes to an estimated 600ha. The source of the mobile fringes in the Winam gulf was the shredding of water hyacinth plants off the stationary fringes, especially at places where rivers and small streams flow into the lake. Water hyacinth growth appeared to be prolific in areas that also had a lot of nutrients. The mobile fringe is the source of infestation in the Winam-gulf via the Rusinga channel, through which weed carpets are blown into the Winam-gulf. Large carpets emanating from the open lake float into the Winam gulf in a continuous manner that is dependent on the wind direction. All the efforts aimed at controlling future infestations of the Winam gulf should take into account the need for controlling all the subsequent re-infestation especially by the continuous supply of new mobile fringes from the open lake waters into the gulf via the Rusinga channel. The surface area covered by the water hyacinth in the Winam gulf is on the decline and the population of other aquatic weeds like Vossia *cuspidator, Cyperus papyrus, Phragmites* sp., *Typha* sp., *Aponogeton* sp., and *Polygonum* sp. has suddenly increased. This sudden increase in population requires a comprehensive study the may help to determine the factors leading to their proliferation and identity.

The information gained when the two variables (i.e. rhizome length and plant mass) of the water hyacinth were correlated indicates that the relationship between these two factors within the Winam gulf is very poor. Therefore it is more than appropriate to assume that for the purpose of estimation of the population density or employing these two variables for any purpose in the Winam gulf would be misleading. The least square equations produced bears testimony to this assumption.

5.1.1 CONCLUSION AND RECOMMENDATION

It seems that from the information made available by this research, no accurate estimation of plant population could be obtained using data of biomass density, this fact being evidenced by the results obtained when the population density was correlated to biomass density. The relationship exhibited by these two variables of the water hyacinth is evidenced by the least square equation (i.e. $r^2 = 0.9202$) that was produced after correlation. If further analyzed this relationship could form a basis from which sample water hyacinth populations in the Winam

gulf can be estimated. A more accurate estimator of water hyacinth population densities based on standing biomass density and plant length/petiole length was observed by Tucker (1981). The method used by Tucker was found to be very accurate for plant populations that were less than 100 plants/m^2. The equation produced for this type of estimator is a non-linear, regression relation involving the logarithm of average plant length and biomass density as independent variables. Both estimators for plant populations were established for populations with short roots and hence they should be used with caution for long rooted water hyacinth plant systems. The studies conducted to determine the physical characteristics of plants that formed the carpets were done in order to provide a better understanding of the water hyacinth carpets within the Winam gulf. It was hoped that these studies if completed, would prove to be useful to weed harvester designers and scientists attempting to select suitable harvester machines for future mechanical control exercises in the Winam gulf. These studies would also be useful by providing a better understanding of how the various physical characteristics of the water hyacinth would affect mycoherbicide applications during future control expeditions.

It is important to note that during these studies the area covered was only limited to the Winam gulf of the L. Victoria (Kenya). Since the Winam gulf only covers a small part of the L. Victoria when you compare the general size of the L. Victoria in relation to the sizes of the Kenyan, Tanzanian and the Ugandan waters of Lake Victoria, this study seems to be only a tip of the iceberg. Additional surveys in other regions of the L. Victoria particularly those within the Ugandan and the Tanzanian sides of the L. Victoria are necessary and important. A comprehensive study covering the other areas of the L. Victoria is necessary so that all the factors pertaining to the incidences, distribution and impact of other pathogens of the water hyacinth found in other parts of the L. Victoria may be better understood.

5.1.2 RECOMMENDATIONS.

1. Biological control of the water hyacinth using fungal and or bacterial agents isolated from the Winam gulf is feasible and can be fully recommended as a viable option of water hyacinth control.

2. More research should be done in the Winam gulf to fill gaps existing between the distribution and possible impact attributed to the water hyacinth and all its unknown pathogens in the Winam gulf.

3. The possibility of employing; *A. zonatum, M. roridium, R. solani, Cercospora* sp., *Fusarium* sp. and *Alternaria* sp. as mycoherbicidal agents should be explored.

4. The identities, life history and pathogenicity of the entire bacterial phytopathogen flora responsible for chlorotic halo in the Winam-gulf should be determined.

5. The surface area covered by the water hyacinth in the Winam gulf (L. Victoria) is on the decline; further studies on the factors that have led to this increase or decline of the surface area are desirable.

6. It is important to note that the area covered by this survey was only limited to the Winam gulf of the Lake Victoria (Kenya). Additional surveys in other regions of the L. Victoria should also be taken up.

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