Potential of fungal pathogens for biological control of water hyacinth

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Abstract

A survey of fungal pathogens of water hyacinth were under taken in lakes Victoria and Kyoga and River Nile in Uganda and several potential isolates including *Alternaria eichhorniae* (Nag Raj and Ponnappa), *Cercospora* sp. and *Acremonium zonatum* were identified. Isolates of *Cercospora* sp. and *A. eichhorniae* were evaluated for their effectiveness on water hyacinth plants in the screen house. The disease incidence and severity progressed with time in both isolates although there was no ($p>0.05$) significant difference between the isolates, and overall disease incidence and severity was not ($p>0.05$) significant. However, *Alternaria eichhorniae* caused higher disease incidence than *Cercospora* sp. in the first and second week after inoculation while *Cercospora* sp. caused higher disease severity than *A. eichhorniae* after fourteen days. Both pathogens caused ($p>0.05$) significant reduction in overall plant fresh weight and no effects on number of living leaves and number of daughter plants. The disease infection also increased with increasing conidia concentration and as time progressed, and was significantly ($p<0.01$) lower at the lower conidia doses. Host range tests on 9 cultivated plant species showed that sorghum was highly susceptible to *Cercospora* sp. while *A. eichhorniae* did not cause disease symptoms on any of the plants. This study suggests that both pathogens have potential for biological control of water hyacinth, but A. *eichhorniae* is safer to use than *Cercospora* sp.

Keywords: Fungal pathogens, water hyacinth control

Introduction

Water hyacinth is a serious aquatic weed problem worldwide and has also been rated as the world's worst weed (Holm *et al.,* 1977). It occurs in tropical regions between latitudes 40^0N and 45^0S and has been spread from its area of origin in Brazilian Amazonia by man as an ornamental. The weed was reported in Africa during the ancient Egyptian times between 1879 and 1892 (Gopal and Sharma, 1981), and its detrimental effects on the livelihood of people have been reviewed by Gopal (1987) and Pieterse *et al.,* (1996).

Successful control of the water hyacinth has been carried out using two weevils, *Neochetina eichhorniae* Warner and *N. bruchi* Haustache that have already established in various countries (Julien, 1992). The use of weevils was adopted in Uganda in 1993 through importation of both species from the International Institute of Tropical Agriculture, Benin station. The weevils are now widely distributed throughout the range of water hyacinth infestations and have contributed to the decline of the weed problem in most locations (Ogwang and Molo, 1999). Efforts have been made to integrate weevils with different weed control measures to improve on the control strategy. Studies conducted in the United States indicate increased effectiveness of the weevils when used in combination with herbicides (Charudattan, 1986).

The potential of fungal pathogens as myco-herbicides for integration in biological control of water hyacinth has also been extensively investigated. In Egypt, promising fungal strains attacking water hyacinth have been identified (Sabana *et al.,* 1995;

Elwakil *et al.,* 1988). In East Africa, adequate knowledge about potential pathogens of water hyacinth is still lacking. Surveys have been conducted in Kenya and several pathogens of water hyacinth have been identified, but their potential role have not been fully investigated (KARI, 1997). Similar surveys have been initiated in Uganda. This study's aim was to identify and evaluate potential pathogens associated with water hyacinth in Uganda and determine their host range on cultivated plant species.

Materials and methods

Surveys and isolation of fungi

Surveys were conducted in Lakes Victoria and Kyoga and River Nile by boat during July 2000 and May 2001. Water hyacinth plants in weed mats were examined for disease symptoms like necrotic spots, leaf lesions, browning and wilting. The leaves showing disease symptoms were collected from three sampling points per site, and for each disease symptom, 3-5 leaves were collected. The leaves were put in large envelopes and taken to the laboratory for isolation of pathogens. Four infected tissues (about 5mm diameter) were cut from each leaf and surface sterilized using 70% alcohol. They were plated on PDA and isolates were pure cultured on 25% strength PDA.

The culture of each isolate was made into a strong suspension containing $1.2x10^7$ conidia/ml using distilled water. The suspensions were applied on water hyacinth plants grown in plastic buckets filled with 5 litres of tap water using hand sprayer, until the foliage was fully wetted. The plants were covered with polythene bags for 48 hours to maintain humidity and observed for 10 days for appearance of disease symptoms on the leaves. The isolates, which proved pathogenic were rated on the basis of severity of the disease symptoms as follows: + = small and few spots, light infection; ++ = small and slightly numerous spots, moderate infection; and +++ = many large spots, severe infection. Samples of pathogenic isolates were sent to CABI Bio-science, UK for identification.

Comparative evaluation studies of fungal isolates

Two isolates of *Cercospora* sp. and *Alternaria eichhorniae* were selected and 3-weeks old cultures were made into suspensions containing $1.2x10^7$ conidia/ml in distilled water plus Tween 80. The suspensions were applied on water hyacinth plants grown in plastic buckets and the plants were maintained in the screen house as described in the previous experiment. Disease incidence was measured weekly for 4 weeks by counting the number of infected leaves on each plant and expressing as percentage of infected leaves. The disease severity was also measured by scoring lesion development on each leaf on a scale 0-9 according to Charudattan *et al.,*(1985). Control plants were sprayed with sterile distilled water only. All treatments were replicated three times.

The plants in each bucket were also drained of excess water and weighed individually, and the number of living leaves and number of daughter plants produced by each plant in each treatment were also counted.

In a separate experiment, 3-weeks old cultures of each isolate were made into suspensions containing 1.2 x 10^7, 1.2 x 10^6, 1.2 x 10^5 and 1.2 x 10^4 conidia/ml using distilled water and Tween 80. The respective concentrations were applied on the water hyacinth plants grown in plastic buckets and the plants were maintained as described in the previous experiment. The diseased infection caused by each conidial concentration was estimated weekly for 4 weeks after inoculation. The treatments were replicated three times

Effect of isolates on cultivated plant species

Seeds of 8 cultivated plant species were planted in plastic bowls (30 cm diameter) in the screen house in 1000g of heat sterilized soil media supplemented with 10g of N.P.K. fertilizer. After sprouting, the seedlings were thinned to leave 6 plants per bowl. Banana suckers were also planted singly in polythene bags and left to establish for three weeks. About 5mm diameter agar blocks of inoculum of each isolate of *A. eichhorrniae* and *Cercospora* sp. were obtained from 3 weeks old cultures grown on PDA and placed on the leaves of 2 weeks old seedlings and banana plants. Some leaves were inoculated with sterile agar blocks as control. The treated plants were covered with transparent polythene bags and maintained as described in the previous experiment. They were observed one week after inoculation and weekly thereafter for 4 weeks for susceptibility to the diseases.

Results

Identification of fungal pathogens

A total of six fungal pathogens were isolated from water hyacinth, of which *Alternaria eichhorniae, Cercospora* sp. and *Acremonium zonatum* were found to be highly pathogenic (Table 1.). They caused necrotic spots, which enlarged to various sizes on the leaves of water hyacinth plants and symptoms appeared between 5 and 6 days after inoculation. Other isolates, which proved pathogenic, are still awaiting identification.

Table 1. List of fungal pathogens collected and disease reaction to water hyacinth plants in the screen house

Isolate	**Scientific name**	**Disease Reaction**
MP- N-001	*Cercospora* sp.	+++
KA-N-001	Awaiting identification	+
NA-V-001	*Alternaria eichhorniae*	+++
G-V-002	Awaiting identification	+
MA-V-012	Awaiting identification	+
DR-V-004	*Acremonium zonatum*	+++

Disease infection of *A. eichhorniae,* and *Cercospora* sp. applied as suspensions of the isolates on water hyacinth plants are presented in Figures 1 and 2. Both pathogens caused substantial disease infection during the study period and the infected plants

showed clear necrotic disease symptoms on the leaves, which progressed with time, and no disease symptoms were observed on the control plants.

Alternaria eichhorniae caused higher disease incidence than *Cercospora* sp.. in the third week of inoculation (Figure 1), although there was no ($p>0.05$) significant difference in disease incidence between these isolates. After 28 days, the disease incidence increased to over 80% and was the same for both isolates. Overall there was no significant difference in disease incidence between the isolates. *A. eichhorniae* instead caused lower disease severity than *Cercospora* sp. after 21 days (Figure 2), but the disease severity increased to maximum score 1.6 after 28 days and were the same for both isolates. By this time, infected leaves of some water hyacinth plants were beginning to die. The overall disease severity caused by the isolates was however not significant.

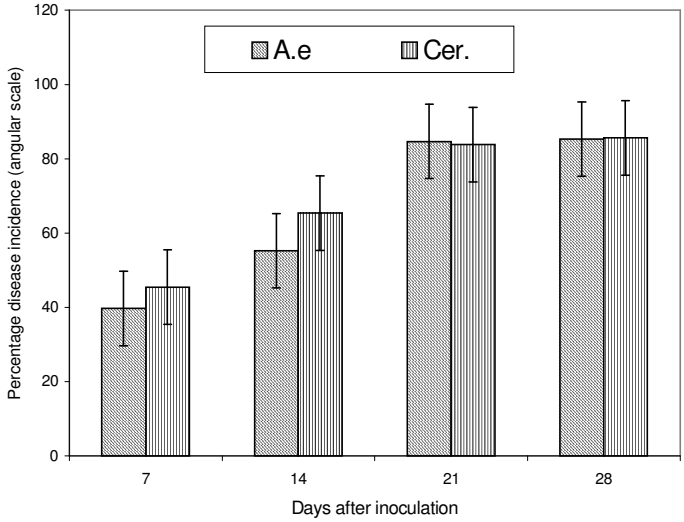

Figure 1. Disease incidence after inoculation of water hyacinth plants with isolates at conidia concentration $1.2x10^7$ conidia ml^{-1}

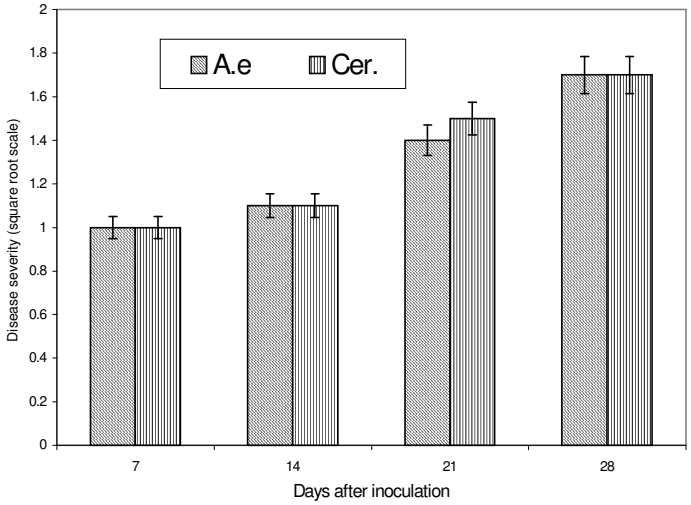

Figure 2. Disease severity after inoculation of water hyacinth plants with isolates at conidia concentration $1.2x10^7$ conidia ml-1

The effects of isolates on growth characteristics of inoculated water hyacinth plants are presented in Table 2 and Figure 3. The plants treated with isolates showed significant ($p<0.05$) reduction in overall fresh weight and no significant effects on number of living leaves and number of daughter plants (Table 2). The fitted regression lines of mean plant weight versus time after inoculation showed linear relationships in all treatments, and regression analysis showed significant ($p<0.05$) decrease in fresh plant weight with time in inoculated compared to the control plants (Figure 3), and there was no ($p>0.05$) significant difference in plant weight between the treated plants.

Table 2. Mean plant weight, number of living leaves and number of daughter plants after inoculation at conidia concentration $1.2x10^7$ conidia/ml

Isolate	Plant Weight (g)	Number of living leaves	Number of daughter plants
A. eichhorniae	190.7^b	6.7^a	1.0^a
Cercospora sp.	294.6^a	6.8^a	1.0^a
Control	320.1^a	6.9^a	1.0^a

a, b: Means bearing the same superscripts are not significantly different at P= 0.05 (Tukey's test)

When the plants were inoculated with isolates at four conidia densities, the disease infection increased with increasing conidia concentration (Figures 4 and 5). There was no significant ($p>0.05$) difference in disease incidence of the isolates at the first two doses, but it varied significantly ($p<0.05$) at the last two conidia concentrations (Figure 4). A similar trend was observed for disease severity. There was no significant ($p>0.05$) difference in disease severity at the first two doses, although the disease severity varied significantly ($p<0.05$) at the last two conidia concentrations (Figure 5).

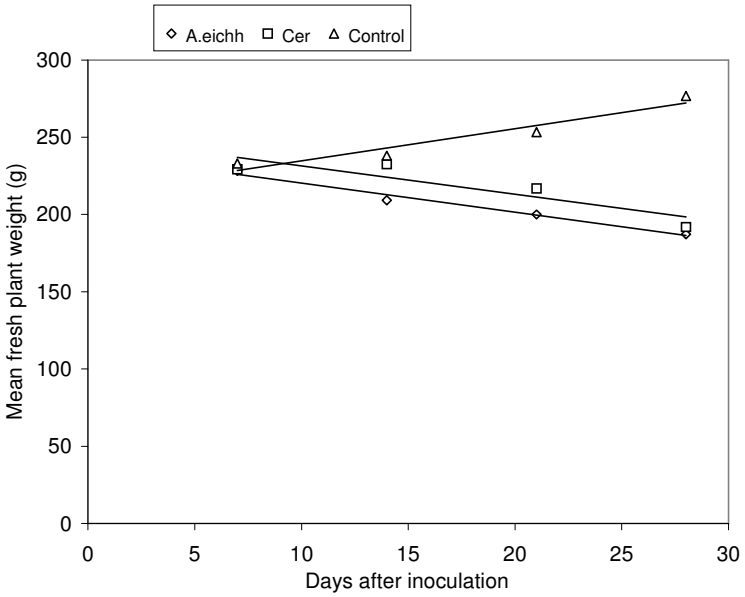

Figure 3. Regression relationship of fresh plant weight and time after inoculation

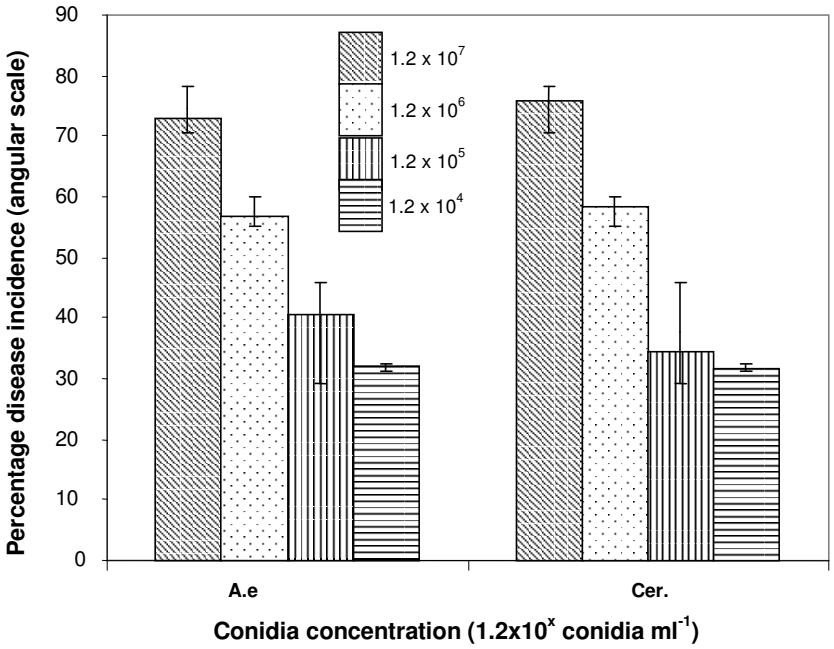

Figure 4. Dose-disease incidence relationship of isolates on water hyacinth plants 28 days after inoculation at four conidia doses

The disease infection at all conidia concentrations also increased with time after inoculation (Tables 3 and 4). As time progressed, the weekly disease incidence did not vary ($p>0.05$) significantly at the first conidia concentration, but at lower concentrations, there was ($p<0.05$) significant difference in disease incidence of the isolates (Table 3). The weekly disease severity also did not vary significantly ($p>0.05$) at the first conidia concentration, but at lower concentrations the disease severity varied ($p<0.001$) significantly (Table 4).

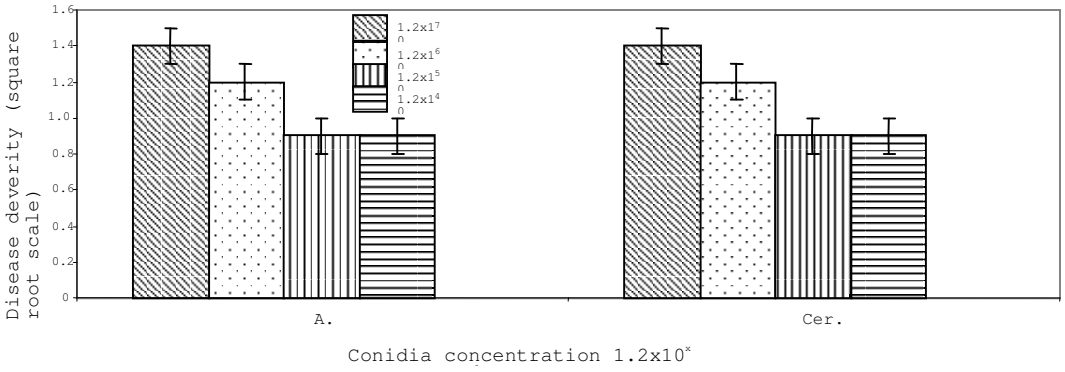

Figure 5. Dose-disease severity relationship of isolates on water hyacinth plants 28 days after inoculation at four conidia doses

Table 3. Mean percentage of disease incidences of isolates at four conidia densities

Conidia Density	Isolate	Days after inoculation			
($1.2x10^x$)		7	14	21	28
$1.2x10^7$	*A. eichhorniae*	44.9^{ab}	69.2^a	84.9^a	93.3^a
	Cercospora sp.	51.5^a	73.3^a	83.2^a	94.6^a
$1.2x10^6$	*A. eichhorniae*	28.0^{bcd}	55.7^b	68.2^b	75.1^b
	Cercospora sp.	35.3^{abc}	51.2^b	70.1^b	76.8^b
$1.2x10^5$	*A. eichhorniae*	20.8^{cd}	41.7^c	42.2^c	57.1^c
	Cercospora sp.	16.1^{cd}	37.8^c	40.0^{cd}	44.5^d
$1.2x10^4$	*A. eichhorniae*	13.5^d	37.6^c	36.0^{cd}	40.5^d
	Cercospora sp.	12.5^d	37.6^c	34.6^d	41.2^d

a, b, c, d: For each monitoring date, means bearing the same superscripts are not significantly different at P= 0.05 (Tukey's test)

Table 4. Mean disease severity of isolates at four conidia densities

Conidia Density	Isolate	Days after inoculation			
($1.2x10^x$)		7	14	21	28
$1.2x10^7$	*A. eichhorniae*	1.1^a	1.2^a	1.5^a	1.7^a
	Cercospora sp.	1.1^a	1.2^a	1.4^{ab}	1.7^a
$1.2x10^6$	*A. eichhorniae*	0.9^{ab}	1.1^{ab}	1.3^{bc}	1.5^b
	Cercospora sp.	1.0^a	1.1^{ab}	1.2^{cd}	1.5^b
$1.2x10^5$	*A. eichhorniae*	0.4^b	1.0^{bcd}	1.1^d	1.2^c
	Cercospora sp.	0.4^b	1.0^{bcd}	1.2^{cd}	1.2^c
$1.2x10^4$	*A. eichhorniae*	0.4^b	0.9^{cd}	1.1^d	1.2^c
	Cercospora sp.	0.4^b	0.1^{cd}	1.1^d	1.1^c

a, b, c, d: For each monitoring date, means bearing the same superscripts are not significantly different at P= 0.05 (Tukey's test)

Effect of isolates on cultivated plant species

The results of host specificity tests of the two isolates are presented in Table 5. The disease symptoms caused by *Cercospora* sp. appeared on sorghum leaves after 3 days of inoculation. The leaf necrosis increased with time and after 7 days, some of the infected leaves had died. There were however no apparent disease symptoms on the plant species treated with *A. eichhorniae*.

Table 5. Susceptibility of some cultivated plant species to isolates of *A. eichhorniae* and *Cercospora* sp.

Family/Species	Common name	Disease reaction	
		A. eich.	*Cercospora*
Fabaceae			
Glycine max (L.) Merr.	Soybean	-	-
Vigna unguiculata (L.) Walp	Cowpea	-	-
Arachis hypogaea (L.)	Peanut	-	-
Pedaliaceae			
Sesamum indicum (L.)	Sesame	-	-
Poaceae			
Zea mays (L.)	Maize	-	-
Oryza sativa (L.)	Rice	-	-
Eleusine indica (L.)	Finger millet	-	-
Sorghum valgare (L.)	Sorghum	-	+
Musasea			
Musa sp.	Banana	-	-

Discussion

This is the first report of an inventory of water hyacinth pathogens in Uganda. A total of six fungal pathogens were collected from lakes Victoria, Kyoga and River Nile, but only *A. eichhorniae*, *Cercospora* sp. and *Acremonium zonatum* were found to be highly pathogenic. These pathogens have previously been reported in other countries (Nag Raj and Ponnappa, 1970; Conway, 1976; Abdel Rahim and Tawfig, 1985; Elwakil *et al.,* 1988; Morris, 1990) and they are considered as potential myco-herbicide agents for integration in biological control of water hyacinth.

Comparative studies of efficacy conducted between *A. eichhorniae* and C*ercospora* sp. showed that both isolates were highly pathogenic to water hyacinth and the infected plants showed clear disease symptoms. The pathogens however, did not cause mortality to the water hyacinth plants after 28 days of inoculation, but exhibited a reduction in fresh weight of plants, low number of living leaves and few daughter plants. This observation confirms the findings of Shabana *et al.,* (1995) with different strains of *A. eichhorniae*. The reduction in plant weight was presumably attributed to the severe stress caused by the pathogens to the plants, which affected the ability of the mature plants to produce fresh leaves and daughter plants. The inability of the isolates to kill the plants during the study period was possibly due to the reduced virulence of the isolates caused by repeated sub-culturing, which was carried out five times during single spore isolation. Loss of virulence and changes in some growth characteristics has been observed after repeated sub-culturing of pathogens (Nyvall and Hu, 1997).

The highest conidia dose of $1.2x10^7$ was the most effective in causing the disease infection in water hyacinth plants and disease infection declined with reducing conidia concentrations. A positive linear relationship between inoculum concentration and disease infection has also been reported for other potential myco herbicide agents (Walker and Tilley, 1997). At low conidia doses, the disease infection also occurred. The susceptibility of water hyacinth plants even at low conidial doses may be attributed to the physiological state and age of the plants. These pathogens that can cause disease infection at even low conidia doses are considered potentially most promising agents for myco-herbicide development (Spotts and Cerventes, 1991).

Sorghum was found to be highly susceptible to *Cercospora* sp. when plants were inoculated using agar blocks containing the mycelia. The necrosis appeared on the plants 3 days after inoculation. *Cercospora* disease has been reported in many plant species and this isolate could therefore be closely related to *Cercospora sorghi* which has been reported in sorghum (Mansuetus, 1995). Sorghum is a very important staple food crop in Eastern and South Western Uganda. The usefulness of *Cercospora* sp. to control water hyacinth has however been demonstrated in the field (Charudattan *et al.,* 1985). Therefore this pathogen could still be developed for restricted application in the water environment where water hyacinth occurs.

The plants treated with *A. eichhorniae* did not show any disease symptoms. The lack of susceptibility of cultivated plants tested in this experiment to *A. eichhorrniae* confirms the findings of Nag Raj and Ponnappa, (1970) and (Shabana *et al.,* 1995) and suggests that the pathogen has a narrow host range. The level of host specificity exhibited by *A. eichhorniae* however makes evaluation of this fungus as a biological control agent more promising than *Cercospora* sp.

Conclusions and recommendations

Studies conducted indicate two fungal pathogens, *A. eichhorniae* and *Cercospora* sp. that occur on water hyacinth in Uganda are effective in causing disease infection in water hyacinth plants, and higher disease infection was caused by application of high concentration of conidia. The attack of the pathogens resulted in reduced plant weight as time progressed. There was also a higher level of host specificity exhibited by *A. eichhorniae*. This study suggests that *A. eichhorniae* is safer to use than *Cercospora* sp. to control water hyacinth. Control of water hyacinth would therefore be improved by integrating these pathogens with weevils. However, adequate knowledge on the interactions of weevils with the pathogens needs to be determined for effective integration.

References

Abdel Rahim, A. M., and Tawfig, S. 1985. Pathogenicity of Fungi and Bacteria from the Sudan to Water hyacinth. *Weed Research*, **24**, 233-238.

Charudattan, R. 1986. Integrated control of water hyacinth *Eichhornia crassipes* with a pathogen, insects and herbicides. *Weed Science* **34,** 26-30.

Charudattan, R., Linda, S. B., Marjan Kluepfel and Osman, Y. A. 1985. Biological control effeicacy of *Cercospora rodmanii* on water hyacinth. *Phytopathology* **75,** 1263-1269.

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

Elwakil, M. A., Sadik, E. A., Fayzalla, E. A., and Shabana, Y. M. 1988. Biological Control of Water hyacinth with Fungal Plant Pathogens in Egypt. In: *Proc. VII Int. Symp. Biol. Contr. Weeds,* 6-11 March 1988, Rome, Italy, (Delfosse, E.S ed.). 1st Sper. Patalog. Veg. (MAF) pp 483-497 (1989).

Gopal, B. 1987. Water Hyacinth. Aquatic Plant Studies 1. Elsevier, Amsterdam.
Gopal, B., and Sharma, K. P. 1981. Water hyacinth, *Eichhornia crassipes*; the most troublesome weed of the world. Hindasia Publishers, New Delhi, India, 128

Holm, L. G., Plucknett, D. L., Pancho, J. V., and Herberger, J. P. 1977. The World's Worst Weeds: Distribution and Biology. University Press, Hawaii, Honolulu, H. I. 609pp.

Julien, M. H., 1992. Biological Control of Weeds. *A World Catalogue of Agents and their Target Weeds 3rd Edition*. Commonwealth Agricultural Bureaux

KARI, 1997. Lake Victoria Environmental Management Programme: Control of Water Hyacinth and other Invasive Weeds. *Quarterly Report, Kenya.*

Mansuetus, S. B. A. 1995. Sorghum diseases in Tanzania. *African Crop Science Journal **3:** (2) 203-207.*

Morris, M. J. 1990. *Cercospora piaropi* recorded on the aquatic weed, *Eichhornia crassipes* in South Africa. *Phytopathology* **22**, 255-256.

Nag Raj, T. R., AND Ponnappa, K. M. 1970. Blight of Water hyacinth caused by *Alternaria eichhornia* sp.Nov. *Trans. Br. Mycol. Soc.* **55**, 123-130.

Nyvall, R. F., and Hu, A. 1997. Laboratory Evaluation of Indigenous North American Fungi for Biological Control of Purple Loosestrife. *Biological Control* **8**, 37-42.

Ogwang, J. A., and Molo, R. 1999. Impact studies on *Neochetina bruchi* and *Neochetina eichhorniae* in Lake Kyoga, Uganda. In: *Proc. 1st IOBC Global Working Group Meeting for the Biological and Integrated Control of Water Hyacinth, 16-19 November 1998, Harare, Zimbabwe (*Martin, P. Hill, Mic, H. Julien and Ted, D. Center Eds) pp182.

Pieterse, A. H., Mangane, A., Traore, M., Klashorst, G. U. D., and Rijn, P.J.Van.1996.

The Water Hyacinth Problem in West Africa and Proposal for Control Strategies. In: *Strategies for Water Hyacinth Control*. Report of a Panel of Experts Meeting, 11-14 September, 1995, Florida, USA,pp 215. FAO, Rome, pp47-55.

SAS (Statistical Analysis Systems, 1985). SAS User's Guide. Cary, NC.

Shabana, Y. M., Charudattan, R., and Elwakil, M. A. 1995. Identification, Pathogenicity, and Safety of *Altenaria eichhorniae* from Egypt as a Bioherbicide Agent for Water hyacinth. *Biological Control* **5**:123-135.

Spotts, R.A., and Cervantes, L. A.1991. Effect of Temperature and Wetness on Infection of Pear by *Ventura pirina* and the Rrelationship between Preharvest inoculation and storage scab. *Plant Disease* **75:** 1204-1207

Walker, H. L., and Tilley, A. M. 1997. Evaluation of an isolate *of Myrothecium verrucaria* from sicklepod *Senna obtusifolia* as a Potential Mycoherbicide Agent. *Biological Control* **10:** 104-112