Diversity of *Alternaria alternata* a common destructive pathogen of *Eichhornia crassipes* in Egypt and its potential use in biological control

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Alternaria alternata is a pathogen of the Waterhyacinth (*Eichhornia crassipes*), which is an important weed in the Delta of the Nile. We conducted a survey of this pathogen at different sites in the Delta and isolated 400 strains. The colonization frequency of the pathogen was more than 85% in all tested sites. Isolate A was the most common isolate, while isolates from 50 test sites clustered into four main groups. The diversity of *A. alternata* within these clusters was affected by pH, TE and chemical oxygen demand. Pathogenicity testing revealed that *A. alternata* strain A, was the most destructive. Host-range testing determined that the majority of Waterhyacinth plants tested are not highly susceptible to the pathogen and thus may show some resistant. Observation over three years indicated that with the exception of *P. stratiotes* and *C. alopecuroides*, the major economic crops, weeds and ornamental plants surrounding water courses with diseased Waterhyacinth showed no signs of infection by *A. alternata*. Application of the fungus crude toxins to tested plants increased the host range to include *Ipomoea tricolor*, *Nymphaea lotus* and *Lemna gibba* in addition to Waterhyacinth. We also found that a toxin plus fungal combination applied to tested plants, enhanced the degree of susceptibility but not the hosts range. Formulation of spores of *A. alternata* strain A in oil emulsion significantly improved the effectiveness of the pathogen on Waterhyacinth. Greater values of disease incidence (80), disease severity (95) and necrotic leaf area (98.5) were observed at the end of the experiment. Waterhyacinth was susceptible to the fungus at all growth stages tested. Susceptibility range decreased in sequence: leaf stages 11-14> leaf stages 5–9 > leaf stages 2–4. Thus, *A. alternata* has potential as a biocontrol agent of Waterhyacinth and its toxins may be used as a herbicide.

Key words**:** *Alternaria alternata*, diversity, host range, Nile Delta, oil emulsion, toxins.

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Introduction

Alternaria alternata (Fr.) Keissler is a cosmopolitan fungus and has been isolated from almost all habitats (Ellis, 1971; Domsch *et al*., 1980; Farr *et al*., 1989; EL-Morsy, 1999, 2000; EL-Morsy *et al*., 2000; Guo *et al*., 2004). This facultative pathogen has been also isolated from diseased Waterhyacinth worldwide. The fungus induces disease symptoms (spots and lesions) mainly on leaves and less severely on stolons and finally leads to complete death of the plant. The fungus has been described as a pathogen of *Eichhornia crassipes* (Mart.) Solms (Waterhyacinth) in Australia (Galbraith and Hayward, 1984), Egypt (Elwakil *et al*., 1989; Shabana *et al*., 1995; EL-Morsy, 2004), Bangladesh (Bardur-ud-Din, 1978) and India (Aneja and Singh, 1989).

Eichhornia crassipes is one of the world's most important aquatic weeds. This noxious weed spreads by vegetative reproduction (Sculthrope, 1971). It is known that in 8 months, only 10 plants can multiply to a population of more than 600,000. Its uncontrollable growth rate and ability to infest a wide range of freshwater habitats have created enormous environmental problems (Elwakil *et al*., 1990; Charudattan, 1996; Honmura and Miyauchi, 1998). Thus, its control is an ongoing problem and biocontrol methods are needed.

The use of bioherbicides to control weeds has gained major prominence. Bioherbicides mainly utilize endemic pathogens that are destructive to the weed and are usually applied in massive doses at vulnerable stages of host growth (Daniel *et al*., 1973). There is however, current interest in the use of plant pathogenic microbes for biological control of weeds. Inert solid carriers, alginate granules, invert emulsions and oil-in-water emulsions have all been considered as vessels for mycoherbicides as they reduce or eliminate the dew requirement for fungal colonization (Greaves, 1996; Green *et al*., 1997; Shabana, 1997b; Hanspeter and Genevieve, 1998; Greaves *et al*., 1998). The oil emulsion may provide a favourable microenvironment around the spore during the infection process, either by retention of the water present in the emulsion or by inducing an exogenous supply of water, possibly from the leaf tissue (Shabana, 1997b; Greaves *et al*., 1998). The intensity of spore infection of *A. cassiae* Jurair & Khan on *Cassia obtusifolia* L. and *A. crassa* (Sacc.) Rands on *Datura stramonium* L. was enhanced when applied in an invert emulsion (Amsellem *et al*., 1990, 1991). Amsellem *et al*. (1991) suggested that by retaining water, the invert emulsion may cause cuticular damage and could also suppress the plant's elicited responses to infection. In addition, the oil may also attach the spores more strongly to the leaf surface, as was found with *A. cassiae* spores applied in emulsified oils (Bannon *et al*., 1990).

Alternaria alternata has been evaluated as a non-efficient biocontrol agent (Bardur-ud-Din, 1978; Aneja and Singh, 1989). *Alternaria eichhorniae*

Nag Raj is known to infest Waterhyacinth and have a very narrow host range (Nag Raj and Ponnappa, 1970; Shabana *et al*., 1995) comparing to *A. alternata* a plurivorus species with several pathotypes and saprotrophic strains (Ellis, 1971). *Alternaria eichhorniae* has been extensively studied for biocontrol (Nag Raj and Ponnappa, 1970; Elwakil *et al*., 1990; Shabana, 1997a,b, 2000; Mohan Babu et al., 2002). Recently**,** *A. alternata* was used in the biological control of Waterhyacinth without effect on plants of economic and ecological importance (Mohan Babua *et al*., 2002, 2003a,b,c).

One potential approach to control weeds is to use phytotoxins or their derivatives for direct application to the noxious plant (Boyette and Abbas, 1995; Milat and Blein, 1995; Evidente *et al*., 1998; Auld and McRae, 1999). The potential use of toxins in aquatic weed control has not been sufficiently investigated. *Alternaria alternata* produces a red metabolite in potato dextrose broth, which was found to be toxic to Waterhyacinth (Mohan Babu *et al*., 2003 c,d).

The aim of this work was to determine the diversity and host specificity of *A. alternata* pathotypes in the Nile delta of Egypt in order to evaluate their potential use in the biocontrol of Waterhyacinth. The potential use of toxins produced by the pathogen in biocontrol are also assessed.

Materials and methods

Survey and isolation methods

Waterhyacinth is the most prominent water pests of the Egyptian aquatic system. Its heaviest infestations are found in Nile Delta in waterways, reservoirs, irrigation and drainage systems, furrows and northern lakes where the flow rate of water is low. This noxious weed forms dense stands and ultimately covers the surface of the slow flowing waterways, reservoirs, irrigation and drainage systems, and furrows.

Infected leaves were collected from the main irrigation canals of the Nile Delta between 2002-2004. Fifty sites were investigated in eight provinces of the Delta as follows: 10 from Damietta (1-10) and Dakhalia (11-20), and five from Kafr EL-sheik (21-25), Albehira (26-30), Algharbeia (31-35), Alsharkia (36-40), Almonofia (41-45) and Alkaluobia (46-50) respectively (Fig. 1). Samples were placed in clean plastic bags, brought to the laboratory, and stored at 4ºC, until processing within 48 hours. Isolation of pathogenic species follow (Photita *et al*., 2004). Stored leaves were scrubbed under running water to remove surface debris, dissected into small segments; approximately 1 × 1 cm,

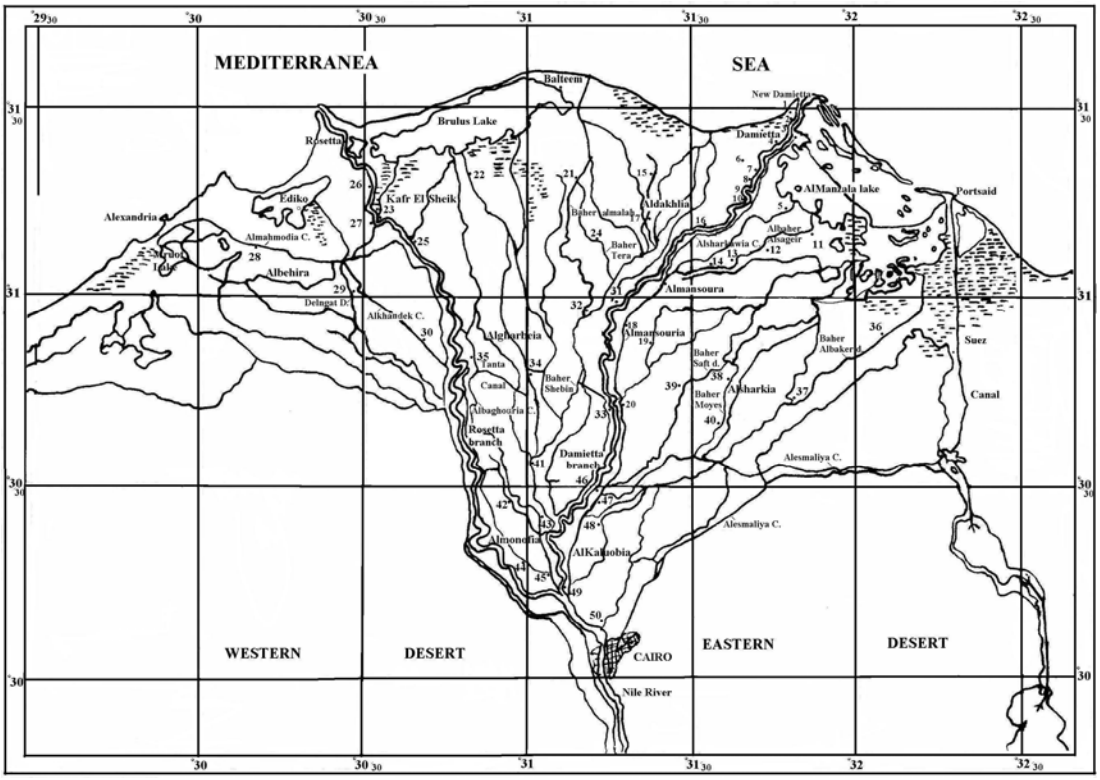

Fig. 1. Map of the irrigation system of the Egyptian Delta of the River Nile showing sample sites. Damietta (1, New Damietta; 2, Kafer Elbateik; 3, Elshoara; 4, Ewlad hamam; 5, Elbasarta; 6, Kafer Alghab; 7, Meet Abou-ghaleb; 8, Faraskour; 9, Meet Elkholy; 10, Shermsah), Aldakhlia (11, Albosrat; 12, Algonina; 13, Dekrinis; 14, Tanah; 15, Alsherka; 16, Sherbin; 17, Belkas; 18, Aga; 19, Alsemblawine; 20, Meet Ghamer), Kafr Elsheik (21,Alhamoul; 22, Sedi Salem; 23, Foa; 24, Beyla; 25, Desouk), Albehira (26, Edfina; 27, Almahmodia; 28, Kafr Aldawar; 29, Aldelngat; 30, Etai Albaroude), Algharbia (31, Samanoude; 32, Almahala; 33, Zifta; 34, Tanta; 35, Kafr Alziat), Alsharkia (36, Baher Albaker; 37, Faqouse; 38, Kafr Saker; 39,Deyarb negm; 40, Hehya), Almonoufia (41, Shebin Alkoum; 42, Monouf; 43, Albaghou; 44, Ashmoun; 45, Shatanouf), Alkaluobia (46, Kafr Shoker; 47, Banha; 48, Toukh; 49, Alkanater Alkhayria; 50,Kaluob).

and surface-sterilized by sequential immersion in 5% hypochlorite for 1-2 minutes followed by 75% ethanol for 30 seconds. Surface sterilised segments (4 segments / plate) were selected, plated out on water agar amended with 0.5 gm streptomycin, 2000 units penicillin G per plate and 0.3 g/L Rose Bengal. Ten plates were used for each plant part. The plates were then incubated at 27ºC for 7-15 days. Developing hyphal tips and / or spores were picked up aseptically and inoculated on to potato-dextrose agar (PDA). Developing colonies were then picked up and subcultured on PDA. Approximately 400

isolates of *A. alternata* were made. The isolates are stored and maintained in slants of PDA, in an incubator at the Department of Botany, Mansoura University. Based up on morphology, the number of isolates was reduced to ten.

Water characteristics

Fifteen water samples were collected throughout the study period in cleaned, sterilized, 1L screw cap glass bottles. Examination of samples was conducted immediately following return to the laboratory. Dissolved oxygen (Azide modification method) and chemical oxygen demand were measured as described in standard methods for the examination of water and wastewater (American Public Health Association, 1992). Temperature measurements were made *in situ*. Conductivity and pH of water were measured by Corning pH-meter 215 and Corning® TDS-60 Sensor respectively.

Pathogenicity testing

Healthy Waterhyacinth plants were collected from natural infestations in New Damietta region and maintained in a sterilized greenhouse. For the inoculation procedure, plants were kept in plastic dishes (12 cm × 9 cm) filled with water, 3 plants per dish. A 2-mm plug of fungal mycelium was plated on PDA broth for 10 days under aseptic conditions and incubated on a laboratory bench at 26ºC ± 2. Mycelium and, if present, spores were harvested, rinsed with sterile distilled water and blended aseptically with distilled water (1:1 w/v). The resulting mycelium suspension was diluted to give 1×10^6 propagules/ml. The plant leaves were wounded manually by removing the cuticle layer with an empty pen cover and painted with Tween 80. For comparison of pathogenicity, each suspension was then liberally applied to the surface of water hyacinth plants using a hand-sprayer. Results were recorded two and four weeks after spraying.

Formulation

The blended fungal mycelium suspension was diluted 1:4 (v/v) with 1.33% (w/v) sodium alginate in distilled water. This mixture was dripped into 0.25 mol $CaCl_2$ to form gel beads of 3-5mm. The beads were then sifted and air-dried to yield pellets. The dried pellets were ground and the resulting powder contained about 1×10^6 propagules/g. Alginate pellets were also prepared for the control experiment. The fungal suspension emulsion

comprised 0.5 g (1% w/v) mycelium-alginate-powder, 75 ml (15% v/v) corn oil, 15 ml (4% v/v) of an emulsifier soybean lecithin and 500 ml (80%) water (Shabana, 1996). For comparison of pathogenicity, a suspension of fungal propagules was then liberally applied to the surface of plants in the field using a hand-sprayer. A control experiment was carried out simultaneously using formulation without fungal propagules and water.

Host range test

The non-weed plant species included in the test were selected on the basis of their economic and ecological importance in addition to their relation to the target plant (Nag Raj and Ponnappa, 1970; Martinez Jimenez and Gutierrez Lopez, 2001). Healthy aquatic weeds such as *Lemna gibba* L., *Typha domingensis* Pers., *Pistia stratiotes* L., *Potamogeton nodosus* Poir., *Nymphaea lotus*, *Cyperus alopecuroides* L. and *Phragmitis australis* were also included and collected from the field. Other plants were grown in pots filled with soil treated with methyl bromide to kill any fungi present. For aquatic plants soil was replaced with water. Each pot contained two plants. Each experiment was replicated three times with a control.

Isolation of crude toxin

To obtain crude toxins, the fungus was grown on potato dextrose broth (PDB) for 20 days. The fungus metabolites were collected, filtered and then evaporated till dry. The residue was then scraped off, incorporated into an alginate (1:4 w/v) formulation and dripped into 0.25 mol $CaCl_2$ to form gel beads. Produced beads were then ground and diluted in sterile water at 1 mg/ml. The suspensions were then liberally applied to the surface of plants in the field using a hand-sprayer without injuring the leaves. A control experiment was carried out simultaneously using formulation without toxin. The tests were repeated three times to verify the results.

Toxin-fungus application.

Both formulated fungus and toxins were applied using hand sprayer to the surface of healthy plants. The toxin formulation applied 48 hours before the addition of fungus formulation and repeated three times. A control experiment was also carried out.

Disease assessment

Plants were rated for disease symptoms including leaf spots with a brownish centre, leaf lesions, and leaf death after 15, 30 and 60 days. The impact of the pathogens was determined by counting the number of leaves infected per total number of leaves present (disease incidence). Disease severity was determined for each leaf by visual rating of disease symptoms for each individual using a scale of 0–6 (0 = no disease; 1= 0–5%; 2 = 6 – 25%; 3 = 26 – 75%; 4 = 76 – 95%; 5 > 95% of leaf surface with necrosis; 6 = leaf dead). The total necrotic leaf area was calculated as a percentage using the formula (2.5 × n1 + 15 × n2 + 50 × n3 + 85 × n4 + 97.5 × n5+100 × n6) / N where nx is the number of leaves with rating x and N is the total number of leaves treated. At 2 weeks after inoculation, the leaves of each plant sprayed with the spore suspension were counted and visually rated individually for disease symptoms.

Data analysis

The colonization frequency (Cf %) was calculated from the following formula: Cf = (N1 / N2) X 100, where N1= no. of segment infected and N2 = total no. of segment observed (Hata and Futati, 1995). Correlation and analysis of variance were performed by using SPSS.10 for windows. Average and standard deviation were performed using MS EXCEL 6.

Results

Ecology

Fifty sites along the deltaic region of Egypt were investigated for the presence of Waterhyacinth plants infested with *Alternaria alternata* (Fig. 1). In addition *Acremonium*, *Drechslera*, *Curviularia*, *Fusarium*, *Phoma*, and *Ulocladium* species and *Rhizoctonia solani* were collected. Only *Alternaria alternata* were sufficiently pathogenic to be considered as a potential biological control agent.

Ten plants with leaves spots and lesions symptoms were collected from each sample site. Most plants were infected with *A. alternata* whereas the degree of infection was variable (Table 1). Not all plants with symptoms however, were infested with *A. alternata*. Plants from 19 sites (1, 2, 8, 9, 11, 16, 17, 18, 21, 25, 29, 30, 29, 30, 36, 38, 43, 44 and 49) were completely infected, whereas the infection at other sites varied between 7 to 9 plants per

Table 1. Record of isolates of *A. alternata* infecting waterhyacinth, results of pathogencity test and water characterisitics of samples representative to 50 localities along the Nile Delta of Egypt.

Site	Isolates	Number of infected plants	Colonization frequency %	Disease symptoms %	Dissolved oxygen mg/L	Chemical oxygen demand gm/L	Conductivity mm hose /cm	Temperature °C	pH
1	A	10	100	80	15.7	2.8	14.0	21	7.5
2	C	10	65	75	16.2	1.0	0.85	19.5	6.5
3	A, B	9	80		17.0	1.1	0.52	20	6.7
4	A	9	85	80	18.0	1.2	0.6	19	6.93
5	E	8	85	70	14.0	1.0	0.9	19	6.78
6	A	9	85	80	11.0	1.0	0.9	18.5	6.83
7	B	8	90	73	14.0	1.2	1.9	18	7.2
8	A	10	90	80	17.6	1.2	0.55	19	7.6
9	E	10	95	70	10.2	2.4	0.45	23	8.2
10	B	9	95	73	12.2	2.2	0.46	22	8.3
11	B, A	10	100		12.9	0.9	0.64	18	7.5
12	E	9	80	70	13.5	0.6	0.64	19	7.5
13	C	8	60	75	10.9	0.8	0.7	18.5	7.5
14	C	8	80	75	6.1	0.4	0.56	23	8.1
15	A	9	90	80	4.0	1.8	0.72	22	8.3
16	A	10	95	80	4.0	1.2	0.6	23	8.3
17	C	10	95	75	8.1	2.8	0.63	23	7.9
18	A	10	95	80	8.1	2.2	0.5	23	8.3
19	D, A	9	90		8.1	0.6	0.5	24	8.1
20	A	8	75	80	8.1	2.2	0.45	24	8.8
21	D	10	95	77	8.1	2.4	0.49	23	8.2
22	E, C	8	95		8.2	2.0	0.49	21.5	8.1
23	D	7	75	77	14.1	2.4	0.4	21.5	8.3
24	F	9	90	76	8.1	2.6	0.49	21.5	8
25	A	10	90	80	8.1	2.6	0.46	21	8.3
26	F	8	80	76	8.1	2.2	0.41	21	8.3
27	G, H	10	95		10.2	2.2	0.45	22	8.3
28	G	9	88	75	12.1	0.6	0.49	22	8.2
29	A	10	95	80	6.1	2.6	0.46	22	8.2
30	G	10	98	75	8.1	2.4	0.45	22	8.3
31	G	9	90	75	8.1	2.2	0.45	22	8.1
32	H	9	95	72	8.1	3.0	0.45	22	8.3
29	A	10	100	80	10.2	2.2	0.42	23	8.3
30	H	10	90	72	10.2	2.6	0.45	22	8.3
35	H, A	8	85		8.5	2.5	0.45	22	8.3

Table 1 continued. Record of isolates of *A. alternata* infecting waterhyacinth, results of pathogencity test and water characterisitics of samples representative to 50 localities along the Nile Delta of Egypt.

Site	Isolates	Number of infected plants	Colonization frequency %	Disesse symptoms %	Dissolved oxygen mg/L	Chemical oxygen demand gm/L	Conductivity mm hose /cm	Temperature °C	pH
36	H, A	10	95		12.1	2.0	0.49	22	8.2
37	I	9	99	n	10.2	2.0	0.45	22	8.1
38	A	10	100	80	8.1	2.4	0.45	22	8.3
39	I, C	9	95		14.1	2.2	0.45	23	8.0
40	A	9	95	80	14.2	2.1	0.45	23	8.2
41	J	9	97	n	14.0	2.1	0.45	23	8.2
42	C	9	96	75	14.1	2.0	0.45	23	8.2
43	A	10	100	80	13.1	2.0	0.44	23	8.0
44	J, A	10	95		13.4	2.0	0.44	23	8.2
45	A	8	99	80	13.6	1.9	0.44	23	8.2
46	B, A	9	90	73	16.0	1.9	0.44	23	8.3
47	B	9	90	73	15.4	1.8	0.44	23	7.5
48	A, C	9	90	80	15.3	1.8	0.44	23	7.5
49	A, D	10	100	80	14.0	1.8	0.44	23	7.5
50	B	9	85	73	14.5	1.7	0.44	23	8.1
Average		**9.18**	**90.34**		**11.36**	**1.85**	**0.81**	**21.74**	**7.94**
Sd		**0.80**	**8.76**		**3.55**	**0.67**	**1.92**	**1.61**	**0.51**

site. The colonization frequency of *A. alternata* ranged of 85 to 100% at all localities (Table 1). The most common and destructive isolates were Strain A with the highest colonization frequency at sites 1, 38, 43 and 49.

The degree of cohesiveness between studied habitats is shown in figure 2. Analysis based on plants infected with *A. alternata* and the colonization frequency showed that the 50 infested sites separated into four distinct branches. The first cluster consisted of 17 localities (6, 50, 4, 5, 35, 25, 34, 8, 47, 48, 15, 31, 46, 19, 24, 7 and 28) representing sites in most of investigated Provinces, with the exception of Almonoufia Province (Fig. 2). A second cluster comprised 25 localities found ing all Provinces. Cluster 3 included six localities, whereas Cluster 4 comprised only two localities (2, 13) from Damietta and Aldakhlia Provinces.

The main water characteristics of tested habitats are illustrated in Table 1. The distribution of *A. alternata* was significantly correlated with COD, TE and pH of water, and not significantly correlated with DO and EC (Table 2).

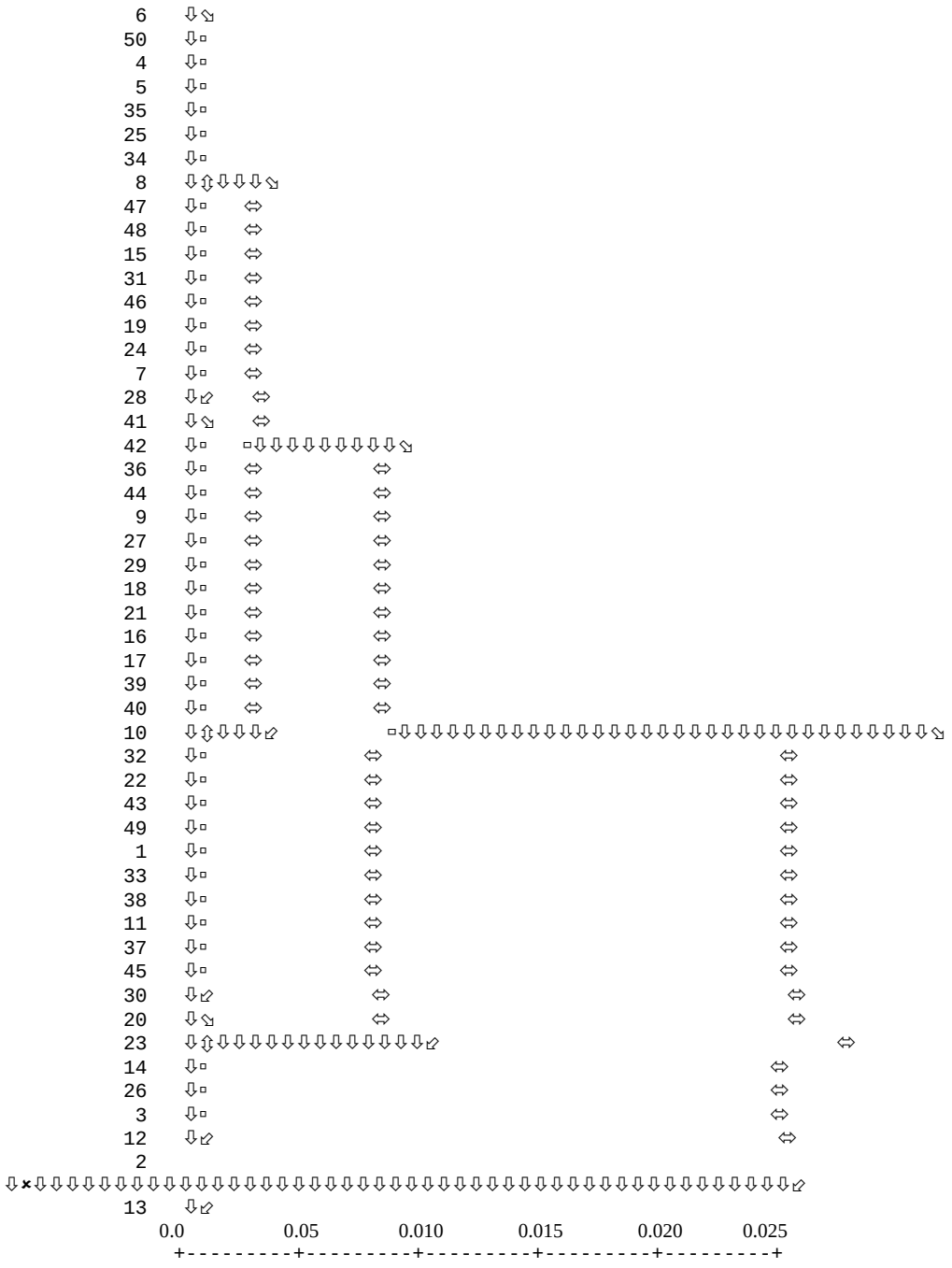

Fig. 2. Dendrogram using average linkage between groups. It is based on no. of plants infected with *Alternaria alternata* and its colonization frequency. Numbers at the branch nodes represent the studied habitats as described in Fig. 1.

Table 2. Analysis of variance and correlation between *A. alternata* Cf % and water variables.

	Variables	SS	df	MS	F	PC	Sig.
Colonization frequency	DO	107.14	12	8.93	0.65	-0.113	0.434
	COD	9.54	12	0.80	2.40	0.446**	0.001
	EC	98.61	12	8.22	0.15	0.112	0.437
	TE	45.24	12	3.77	1.64	0.402**	0.004
	PH	6.37	12	0.53	3.01	0.372**	0.008

Cf % = colonization frequency, SS = sum-of-squares, df = degree of freedom, MS = mean-square, F = variance ratio, PC = Pearson Correlation, Sig. = significance.
** = Correlation is significant at the 0.01 level (2-tailed).

Pathogenicity testing

Ten isolates of *A. alternata* were tested for their ability to infect water hyacinth plants *in vitro*. Table 1 illustrates also that all isolates, except isolates, I and J are able to infect the plant and produce disease symptoms. Disease started as small necrotic spots and developed into a leaf blight that entirely covered the whole leaf after a maximum of four weeks following incubation. Thus, owing to its dominancy and destructive nature (disease symptoms= 80%), Strain (A) was chosen for further study.

Host range

Table 3 provides data on 79 economic or ecologically importance weeds and ornamental plants in close proximity to the infested Waterhyacinth plants. With the exception of *Pistia stratiotes* and *Cyperus alopecuroides* these plants were not infected by *A. alternata*, even though they were either irrigated by water from canals with Waterhyacinth or grew along the banks or amongst Waterhyacinth. Twenty-eight plant species representing 20 families were selected for host range testing (Table 4) and only *Pistia stratiotes* and *Cyperus alopecuroides* were susceptible to strain A of *A. alternata*. Table 4 also illustrates that when crude toxin from *A. alternata* is applied as a spray to the plants, the number of plants susceptible to Strain A increased and included *Ipomoea tricolor* Forsk, *Nymphaea lotus* L. and *Lemna gibba* L. with disease severity of 50%, 50% and 40% respectively. *Pistia stratiotes* (50%), and *Cyperus alopecuroides* (45%) were also infested. The disease severity increased when both toxin and fungus were combined together for *Pistia*

Table 3. Economic plants and common weeds in close proximity to Waterhyacinth not affected by *Alternaria alternata*.

Species		Species		Species	
MONOCOTYLEDONS		*C. linifolia* L.	cb	*Pisidium guagava* L.	ec
Cyperaceae		***Convolvulaceae***		***Nymphaeaceae***	
Cyperus rotundus L.	W	*Ipomoea batatas* (L.) Lam	ec	*Nymphaea lotus L.*	aw
C. alopecuroides L. *	cb	***Brassicaceae***		**Moraceae**	
Lemnaceae		*Brassica oleraceae var. capitata* L.	ec	*Morus alba*	ec
Lemna gibba L.	aw	*Brassica oleraceae var. botrytis* L.	ec	*M. nigra*	ec
Lileaceae		*B. rapa* L.	ec	*Ficus sycomorus*	ec
Allium cepa L.	ec	*B. tournefortii* L.	w	***Pedaliaceae***	
Allium Sativum L.	ec	*Eruca sativa* L.	ec	*Sesamum indicum* L.	
Palmae		*Rhaphanus sitivus* L.	ec	***Primulaceae***	
Phoenix dactylifera L.	ec	***Cucurbitaceae***		*Anagalis arvensis*	w
Poaceae		*Cucurbita pepo* L.	ec	***Polygonaceae***	
Zea mays L.	ec	*Cucumis melo var. chito* L.	ec	*Polygonum equisetiforme*	w
Oryza sativa L.	ec	*C. sativus* L.	ec	*P. salicifolium*	w
Saccharum officinarum L.	ec	*Citrullus vulgaris*	ec	*P. senegalenese*	w
S. spontaneum VAR. aegyptiacum L.	cb	*Lagenaria vulgaris*	ec	*Rumex dentatus*	ec
Phragmitis australis (cav.) Trin. Ex Steud.	aw	***Euphorbiaceae***		***Portulacaceae***	
Polygonum monospeliensis L.	aw	*Ricinus communis* L.	ec	*Portulaca oleraceae*	w
Triticum aestricum L.	ec	***Fabaceae***		***Salicaceae***	
Hordeum vulgare L.	ec	*Phaseolus vulgaris* L.	ec	*Salix baylonica*	cb
Echinochloa staginum L.	a'w	*Vicia fabae* L.	ec	*S. subserrata*	cb
Arundo donax	cb	*Pisum sativum* L.	ec	*S. tetrasperma*	cb
Cynodon dactylon	w	*Lupinus tremis* Forsk.	ec	*Populus alba*	cb
Typhaceae		*Trifolium alexandrinum* L.	ec	***Solanaceae***	
Typha domingensis Pers.	cb	*Trigonella foenum-graecum* L.	ec	*Capsicum annuum L.*	ec
DICOTYLEDONS		***Labiatae***		*Lycopersicon esculentum* Mill.	ec
Apiaceae		*Mentha piperata*	ec	*Solanum tuberosum* L.	ec
Daucus carota L.	ec	*M. microphyla*	w	*S. melongena* L.	ec
Araceae		***Linaeae***		*S. nigrum* L.	w
Pistia stratiotes L.*		*Linum usitatissimum*	ec	*Datura strammonium*	w
Casuarinaceae		***Malvaceae***		***Tiliaceae***	
Casuarina stricta L.	w	*Gossypium barbadense* L.	ec	*Corchorus olitorius*	ec
Compositae		*Hibisicus esculentus* L.	ec	***Verbenaceae***	
Lactuca sativa L.	ec	*Malva parviflora* L.	w	*Lantana camara*	or
Cichorium endivia	w	***Myrtaceae***		*Duranta plumieri*	or
Helianthus annuus L.	ec	*Eucalyptus rostrata* L.	or	***Chenopodiaceae***	
Conyza dioscoridis L.	cb	*E. citridora* L.	or	*Spinacea oleracea* L.	

aw, aquatic weed; cb; canal banks; ec, economic; or, ornamental; w, weed; * infected

Table 4. Host-specificity range of *A. alternata* based on percentage disease severity.

Plant species	Family	Strain A	Toxin	Toxin + Strain A
Daucus carota L.	*Apiaceae*	—		
Pistia stratiotes L.	*Araceae*	+	50%	65%
Raphanus sativus L.	*Brassicaceae*	—		
Spinacea oleracea L.	*Chenopodiaceae*	—		
Sorghum vulgare Pers.		—		
Cyperus alopecuroides L.	*Cyperaceae*	+	45%	55%
Helianthus annuus L.	*Compositae*	—		
Cucurbita pepo Mill.	*Cucurbitaceae*	—		
Cucumis sativus L.		—		
Ricinus communis L.	*Euphorbiaceae*	—		
Phaseolus vulgaris L.	*Fabaciea*	—		
Allium cepa L.	*Lileaceae*	—		
Allium sativum L.		—		
Gossypium barbadense L.	*Malvaceae*	—		
Hibisicus esculentus L.		—		
Zea mays L.	*Poaceae*	—		
Oryza sativa L.		—		
Phragmitis australis		—		
Capsicum annuum L.	*Solanaceae*	—		
Lycopersicon esculentum Mill.		—		
Solanum tuberosum L.		—		
Duranta plumieri	*Verbenaceae*	—		
Potamogeton nodosus Poir.	*Potamogetonaceae*	—		
Ipomoea tricolor Forsk	*Convelvulaceae*	—	50%	60%
Ipomoea batatas (L.) Lam		—		
Nymphaea lotus L.	*Nymphaeaceae*	—	50%	64%
Lemna gibba L.	*lemnaceae*	—	40%	60%
Typha domingensis Pers.	*Typhaceae*	—		

—, nill; +, positive; A, strain A of *A. alternata*.

stratiotes (65%), *Cyperus alopecuroides* (55%), *Ipomoea tricolor* (60%), *Nymphaea lotus* (64%) and *Lemna gibba* (60%).

Formulation and field application

Figure 3 Illustrates that all growth stages of Waterhyacinth tested were susceptible to *A. alternata*. The younger plants were slightly more susceptible than older ones. Disease incidence, disease severity and necrotic leaf area increased and reach their maximum after 60 days. The greatest values for

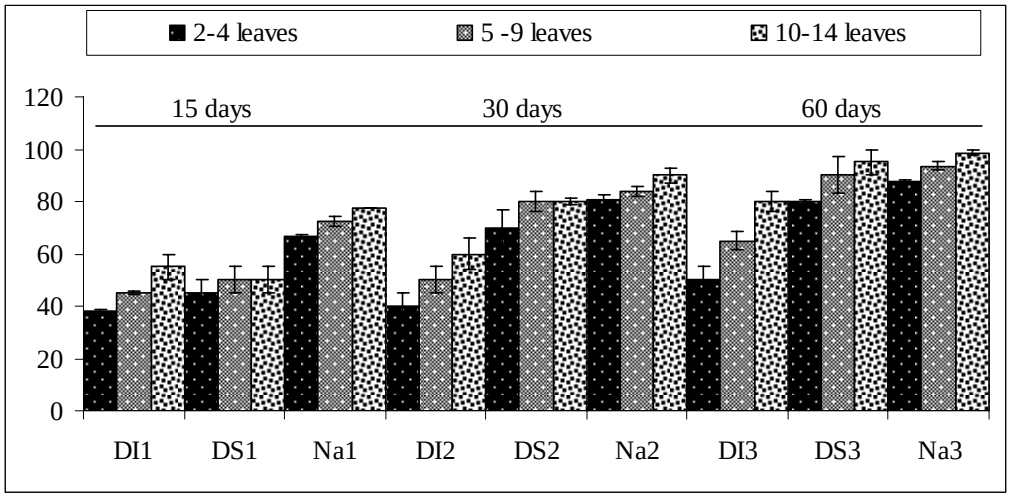

Figure 3. Influence of fungal suspension on the growth of three ages of waterhyacinth in field condition. DI, disease incidence; DS, disease severity; Na, necrotic area.

disease incidence, disease severity and necrotic leaf area were 80, 95 and 98.5% respectively on plants with 11-14 leaves, following 60 days of infection. Plants with 5-9 leaves were more vulnerable than plants with 2-4 leaves (Fig. 3).

Discussion

Ecology

Four-hundred isolates of *Alternaria alternata* were isolated from different plant regions along the Nile delta. *Alternaria alternata* has been described as a worldwide pathogen of Waterhyacinth (Aneja and Singh, 1989, Bardur-ud-Din, 1978; EL-Morsy, 2004; Elwakil *et al*., 1989; Galbraith and Hayward, 1984; Mohan Babu *et al*., 2002, 2003c,d; Nag Raj and Ponnappa, 1970; Shabana *et al*., 1995). The fungus showed colonisation frequencies of greater than 85 % in all localities tested and isolate A was the most common isolate. Based on colonisation frequency the sites clustered into four main groups. The diversity of *A. alternata* within these clusters was affected by chemical oxygen demand, water temperature and pH. Most fungi have a wide pH tolerance and hence it was believed that pH would not be a decisive factor in fungal distribution (Lund, 1934). On the contrary, Tyler (1989) reported that

any appreciable changes in pH may affect the availability of nutrients and thus govern species diversity.

Pathogenicity testing

As a result of pathogenicity testing and on the basis of disease severity, *A. alternata* strain A was found to be the most destructive. *Alternaria alternata* has previously been evaluated to be a non-efficient biocontrol agent (Bardur-ud-Din, 1978; Aneja and Singh, 1989; EL-Morsy, 2004) because it is a plurivorus species with several pathotypes. Nevertheless, *A. alternata* may be capable of being a biological control agent of weeds (Shabana *et al*., 1995). *Alternaria alternata* has recently been used in India in the biocontrol of Waterhyacinth without effecting plants of economic and ecological importance in close proximity (Mohan Babua *et al*., 2002, 2003a,b,c) and so was tested in this study. Strain (A) produces typical brown necrotic lesions on the leaves of Waterhyacinth causing extensive defoliation.

Host specificity

Host specificity testing schemes have been developed for assessing the safety of non-target host plants against pathogens (Wapshere, 1974; Charudattan, 1989). Host-range testing determined that the majority of plants tested are not susceptible to *Alternaria alternata*. *Alternaria eichhorniae*, which has worldwide use as a biocontrol agent of Waterhycinth was reported to infect *Spinacea oleracea*, *Cucumis sativus*, *Curcurbita pepo*, *Helianthus annuus*, *Ricinnus communis*, *Daucus carota*, *Allium cepa*, *Raphanus sativus*, *Phaseolus vulgaris*, *Ficus carica* and *Lycopersicon esculentum* (Nag Raj and Ponnappa 1970). None of these plants was infected by Strain (A) of *A. alternata*. Assays have been developed for assessing the safety of non-target host plants against pathogens (Morris *et al*., 1999; Jimenez and Lopez, 2001; Charudattan, 1996 Mohan Babu *et al*., 2002) and was used in this study against 29 plant species. With the exception of *Pistia stratiotes* and *Cyperus alopecuroides*, none of the plants tested was infected by *Alternaria alternata*.

***Crude toxins application*.**

Application of the crude toxins from *Alternaria alternata* increased the number of infected plants**.** The crude toxin also caused disease to *Pistia stratiotes*, *Cyperus alopecuroides*, *Ipomoea tricolor*, *Nymphaea lotus* and *Lemna gibba*. Similar bioherbicidal activity from *A. alternata* toxin has been

observed against *Eichhornia crassipes*, *Pistia stratiotes*, *Nymphaea nouchali Burm.* and *Nymphaea stellata* (Mohan Babua *et al*., 2003c). Thus, toxins are not only toxic to plants that are infested by the fungus. Toxin and fungal combinations further enhanced the degree of susceptibility, but did not increase the number of susceptible hosts. Indeed, the combined action of the toxin and the saprobic organisms ultimately leads to partial or complete destruction of the affected parts (Wolpert *et al*., 1995). It was also reported that phytotoxins are not specific to their host, when a host plant is weakened by the action of a toxin; saprobic fungi and bacteria invade the necrotic region and hasten the process of decomposition (Wheeler and Luke, 1963). The genus *Alternaria* has been reported to produce toxins that have bioherbicidal properties (Kohmoto *et al*., 1995; Ohra *et al*., 1995; Otani *et al*., 1998; Saxena and Pandey, 2000; Mohan Babu *et al*., 2003c). Thus, purified toxins of *A. alternata*, may be used as a broad-spectrum herbicide to control Waterhyacinth, and other floating aquatic weeds.

Formulation and field application

Formulation is recognized as a way to increase both efficiency of application and efficacy of the control agent (Greaves *et al*., 1998; Evans and Reeder, 2001). Oil emulsion formulations, in particular, may reduce dew requirements (Greaves, 1996; Shabana, 1997b; Greaves *et al*., 1998) and the number of spores required (Egley and Boyette, 1995). In this study the data revealed that all growth stages of Waterhyacinth tested were susceptible to *A. alternata* strain (A). All disease parameters had higher values towards the end of the experiment. Thus, formulation of spores of *A. alternata* strain A in a vegetable oil emulsion significantly improved the effectiveness of the pathogen. The older plants were slightly more vulnerable than younger ones. Similar results were reported in India (Mohan Babu *et al*., 2003c). Thus, the susceptibility of plants is as follows: plants with 11-14 leaves> plants with 5-9 leaves> plants with 2-4 keaves.

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References

American Public Health Association, American Water Works Association & Water Pollution Control Federation (1992). Standard Methods for the Examination of Water and Waste

Water. American Public Health Association Office, 1015 Fifteenth st. NW Washington, DC 20005.

Amsellem, Z., Sharon, A. and Gressel, J. (1991). Abolition of selectivity of two mycoherbicidal organisms and enhanced virulence of a virulent fungi by an invert emulsion. Phytopathology 81: 985–988.

Amsellem, Z., Sharon, A., Gressel, J.and Quimby Jr., P.C. (1990). Complete abolition of high inoculum threshold of two mycoherbicides (*Alternaria cassiae* and *A. crassa*) when applied in invert emulsion. Phytopathology 80: 925–929.

Aneja, K.R. and Singh, K. (1989). *Alternaria alternata* (Fr.) Keissler a pathogen of waterhyacinth with biocontrol potential. Tropical Pest Management 35: 354-356.

Auld, B.A. and McRae, C. (1999). Emerging technologies in plant protection-bioherbicides. In: Proceedings of New Zealand Plant Protection Society (NZPPS), New Zealand: 1–4.

Bannon, J.S., White, J.C., Long, D., Riley, J.A., Baragona, J., Atkins, M. and Crowley, R.H. (1990). Bioherbicide technology: an industrial perspective. In: *Microbes and Microbial Products as Herbicides*, (ed. R.E. Hoagland), ACS Symposium Series, American Chemical Society, Washington DC 439: 305–319.

Bardur-ud-Din, A.A. (1978). *Control of aquatic weeds.* Second Annual report, Project N0. FG-Pa-271. University of the Punjab. Lahore, Pakistan.

Boyette, C.D. and Abbas, H.K. (1995). Weed control with Mycoherbicides and Phytotoxins. A nontraditional application of allelopathy. In: *Allelopathy*: *Organisms, Processes, and Applications*, (eds. K.M. Inderjit, M. Dakshini and F.A. Einhellig): 280–299.

Charudattan R., 1996. Biological control of weeds. An international overview. In: V. SICONBIOL (Simposio de Controle Biologico), Foz do Iguacu, Brazil, June 9–14, pp. 270–282.

Charudattan, R. (1989). Assessment of efficacy of mycoherbicide candidates. In: *Proceedings of the VII International Symposium on Biological Control of Weeds*, (ed. E.S. Derfose,). Institute Sperimentale per la Patologia Vegetale, Ministero dell' Agricoltura e delle Foreste, Rome : 455–464.

Charudattan, R. (1996). Biological control of weeds. An international overview. In: *Simposio de Controle Biologico*, (ed. V. Siconbiol), Foz do Iguacu, Brazil : 270–282.

Daniel, J.T., Templeton, G.E., Smith, Jr. R.J. and Fox, W.T. (1973). Biological control of northern joint vetch in rice with an endemic fungal disease. Weed Science 21: 303–307.

Domsch, K.H., Gams, W and Anderson, T.H. (1980). *Compendium of Soil Fungi*. Academic Press, New York.

Egley, G.H., Boyette, C.D. (1995). Water-corn-oil emulsion enhances conidia germination and mycoherbicidal activity of *Colletotrichum truncatum*. Weed Science 43: 312–317.

Ellis, M.B. (1971). *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew. UK.

EL-Morsy, E.M. (1999). Microfungi isolated from the ectorhizosphere-rhizoplane zone of different halophytic plants from the Red Sea Coast of Egypt. Mycologia 91: 228-236.

EL-Morsy, E.M. (2000). Fungi isolated from the endorhizosphere of halophytic plants from the Red Sea Coast of Egypt. Fungal Diversity 5: 43-54.

EL-Morsy, E.M. (2004). Evaluation of microfungi for the biological control of water hyacinth in Egypt. Fungal Diversity 16: 35-51.

EL-Morsy, E.M., Serag, M.S., Zahran, J.A.T. and Rashed, I.G. (2000). The occurrence of microfungi in the ectorhizosphere-rhizoplane zone of some selected macrophytes from the Nile Delta of Egypt. Bulletin of the Faculty of Science, Assiut University 2 (2-D): 15-26.

Elwakil M.A., Sadik E.A., Fayzalla E.A. and Shabana Y.M. (1990). Biological control of waterhyacinth with fungal plant pathogens in Egypt. In: *Proceedings of the VIII*

International Symposium on Biological Control of Weeds. Lincoln University, Canterbury, New Zealand :483–497.

Elwakil, M.A., Fayzalla, E.A., Sadik, E.A. and Shabana, Y.M. (1989). Biological control of water hyacinth with fungal plant pathogens in Egypt. In: *Proceedings of the 7th International Symposium on Biological Control of Weeds* (ed. E.S. Delfosse) Rome, Italy.

Evans, H.C. and Reeder, R.H. (200). Fungi associated with *Eichhornia crassipes* (water hyacinth) in the Upper Amazon basin and prospects for biological control. In: Proceedings of the Second IOBC Global Working Group Meeting for the Biological and Integrated Control of Water Hyacinth, (eds. Hill, M.P. and Julien, M.H.), Beijing, China, 197pp.

Evidente, A., Capasso, R., Cutignano, A., Taglialatela-Scafati, O., Vurro, M., Chiara Zonno, M. and Motta, A. (1998). Ascaullitoxin, a phytotoxic bis-aminoacid N-glucoside from *Ascochyta caulina*. Phytochemistry 48: 1131–1137.

Farr, D.F., Bills, G.F., Chamuris, G.P. and Rossman, A.Y. (1989). Fungi on Plant and Plant Products in the United States. APS Press, St. Paul. MN, USA.

Galbraith, J.C. and Hayward, A.C. (1984). *The Potential of Indigenous Microorganisms in the Biological Control of Water Hyacinth in Australia*. Final report of the Australian water Research Project. No. 80/132. Department of Resources and Energy, Canberra.

Greaves, M.P. (1996). Microbial herbicides-factors in development. In: *Crop Protection Agents from Nature*, (ed. Copping, L.G.), Royal Society of Chemistry, Cambridge, UK: 444–467.

Greaves, M.P., Holloway, P.J. and Auld, B.A. (1998). Formulation of microbial herbicides. In: *Formulation of Microbial Biopesticides*, *Beneficial Microorganisms and Nematodes*, (ed. Burges, H.D.), Chapman & Hall, London and New York; Kluwer, pp. 203–233.

Green, S., Stewart-Wade, S.M., Boland, G.J., Teshler, M.P. and Liu, S.H. (1997). In: (eds Greg, J., Bnandand Kuykendau, L.D.), *Plant-Microbe Interactions and Biological Control*. Marcel Dekker INC, New York, USA: 249–281.

Hanspeter, A.P. and Genevieve, D. (1998). The potential of Stagonospora sp. as a mycoherbicide for field Bindweed. Biocontrol Science and Technology 8, 93–101.

Hata, K. and Futati, K. (1995). Endophytic fungi associated with healthy pine needles and needles infested by the pine needle gall midge Thiocodiplosis japonensis. Canadian Journal of Botany 73: 384- 390.

Honmura, J. and Miyauchi, N. (1998). A survey on the growth of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) in Kagoshima Prefecture. Bulletin of the Faculty Agriculture, Kagoshima University 48: 7–14.

Kohmoto, K., Otani, H., and Tsuge, T. (1995). *Alternaria alternata* pathogens. In: *Pathogenesis and Host specificity in Plant Diseases*: *histological, biochemical, genetic and molecular bases* (eds. K. Kohmoto, U.S. Shin, and R.P. Shin), Eukaryotes Volume II. Pergamon, Oxford, UK,: 51–63.

Lund, A. (1934). Studies on Danish freshwater Phycomycetes and notes on their occurrence particularly relative to the hydrogen ion concentration of the water. Memoirs of the Royal Academy of Denmark, Section Science 9: 1-97.

Martinez Jimenez, M. and Gutierrez Lopez, E. (2001). Host range of *Cercospora piaropi* and *Acremonium zonatum*, potential fungal Biocontrol agents for water hyacinth in Mexico. Phytoparasitica 29: 175–177.

Milat, M.L. and Blein, J.P. (1995). *Cercospora beticola* toxins III. Purification, thin-layer and high-performance liquid chromatographic analyses. Journal of Chromatography 699: 277–283.

Mohan Babu, R., Sajeena, A. and Seetharaman, K. (2003c). Bioassay of the potentiality of *Alternaria alternata* (Fr.) keissler as a bioherbicide to control waterhyacinth and other aquatic weeds. Crop Protection 22: 1005–1013.

Mohan Babu, R., Sajeena, A., Seetharaman, K., Vidhyasekaran, P., Rangasamy, P., Som prakash, M., A. Raja, Senthil and Biji, K.R. (2002). Host range of *Alternaria alternata*—a potential fungal biocontrol agents for waterhyacinth in India. Crop Protection 21: 1083–1085.

Mohan Babu, R., Sajeena, A., Seetharaman, K., Rangasamy, P., Ebenezar, E.G. and Vidhyasekaran, P. (2003d). *Alternaria alternata* toxin detection by fluorescence derivatization and separation by high performance liquid chromatography. Phytoparasitica 31: 61–68.

Mohan Babu, R., Sajeena, A., Seetharaman, K., Vidhyasekeran, P., Rangasamy, P., Som Prakash, M., Senthil Raja, A. and Biji, K.R. (2003a). Advances in bioherbicides development—an overview. Crop Protection 22: 253–260.

Mohan Babu, R., Sajeena, A., Vidhyasekaran, P., Seetharaman, K. and Reddy, M.S. (2003b). Characterization of a phytotoxic glycoprotein produced by *Phoma eupyrena*—a pathogen on water lettuce. Phytoparasitica 31: 265–274.

Morris, M.J., Wood, A.R., Breeyen, A.D., Den, B.A., Olckers, T. and Hill, M.P. (1999). Plant pathogens and biological control of weeds in South Africa: a review of projects and progress during the last decade. Biological Control Weeds South Africa 1: 129–137.

Nag Raj, J.R. and Ponnappa, K.M. (1970). Blight of water hyacinth caused by *Alternaria alternata* sp. Nov. Trans. Br. Mycol. Soc. 55: 123–130.

Ohra, J., Morita, K., Tsujino, Y., Fujjimori, T., Goering, M., Evans, S. and Zorner, P. (1995). Production of two phytotoxic metabolites by the fungus *Alternaria cassiae*. Bioscience, Biotechnology and Biochemistry 59: 1782–1783.

Otani, H., Kohnobe, A., Kodama, M., Kohmoto, K., (1998). Production of host-specific toxin by germinating spores of *Alternaria brassicicola*. Physiol. Mol. Plant Pathol. 52: 285–295.

Photita, W., Lumyong. S., Lumyong, P., McKenzie, E.H.C. and Hyde, K.D. (2004a). Are some endophytes of *Musa acuminata* latent pathogens? Fungal Diversity 16: 131-140.

Saxena, S. and Pandey, A.K. (2000). Preliminary evaluation of fungal metabolites as natural herbicides for the management of *Lantana camera*. Indian Phytopathol. 53: 490–491.

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

Shabana, Y.M. (1997a). Formulation of *Alternaria eichhorniae*, a mycoherbicide for waterhyacinth, in invert emulsions averts dew dependence. Z. P.anzenkr. P.anzenschutz 104 (3), 231–238.

Shabana, Y.M. (1997b). Vegetable oil suspension emulsions for formulating the weed pathogen (*Alternaria eichhorniae*) to bypass dew. Z. Pflanzenkr. Pflanzenschutz 104 : 239–245.

Shabana, Y.M., Elwakil, M.A. and Charudattan, R. (2000). Effect of media, light and pH on growth and spore production by *Alternaria eichhorniae*, a mycoherbicide agent for water hyacinth. Z. Pflanzenkr. Pflanzenschutz 107: 617–626.

Tyler, G. (1989). Edaphical distribution patterns of macrofungal species in deciduous forest of south Sweden. Acta Ecologica 10: 309-326.

Wapshere, A. (1974). A strategy for evaluating the safety of organisms for biological weed control. Ann. Appl. Biol. 77: 201–211.

Wheeler, H. and Luke, H.H., 1963. Microbial toxins in plant disease. Annual Rev. Microbiol. 17: 223–242.
Wolpert, T.J., Navarre, D.A., Lorang, J.M. and Moore, D.L. (1995). Molecular interactions of victorin and oats. Canadian Journal of Botany 73 (I): S475–S482.

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