Occurrence and diversity of fungal pathogens associated with water hyacinth and their potential as biocontrol agents in the Rift Valley of Ethiopia

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ABSTRACT
Water hyacinth poses serious socio-economic and environmental problems in Ethiopia. To integrate fungal pathogens into water hyacinth management, a survey was conducted in the Rift Valley of Ethiopia. Based on morphological characterization and DNA sequencing, 25 fungal species were identified that belong to nine genera. Alternaria tenuissima, A. alternata, Aspergillus niger, Phoma sp., Curvularia trifoli, Mucor fragilis, M. racemosus, A. fumigatus, Fusarium oxysporum, and F. equiseti were the most common fungi detected. However, their occurrence was influenced by water wave action, temperature, season, and altitude. Among the fungal pathogens, A. alternata, A. tenuissima, F. oxysporum, F. equiseti, and Neofusicoccum parvum were highly pathogenic to water hyacinth. Alternaria alternata and A. tenuissima did not cause disease symptoms on ecologically important plant species (e.g. Noug, Tef, and Coffee). Application of the fungal pathogens on water hyacinth plants also showed 11%–67%, 22%–72%, 15%–55%, and 12%–50% reduction in fresh weight, dry weight, plant height, and root length of water hyacinth, respectively. This study suggests that fungal species have the potential to control water hyacinth biologically and provides baseline data for biological control efforts in the future.

1. Introduction
Water hyacinth [Eichornia crassipes (Mart.) Solms] is one of the most noxious aquatic weeds in the world. The high growth rate of the plant and its ability to infest a wide range of freshwater habitats causes detrimental impacts on fisheries and related commercial activities, access to clean water, hydropower generation, irrigation, navigation along water courses and tourism. The impacts are most pronounced in developing countries, where human activities and livelihoods are closely linked with water systems (Bateman 2001). In Ethiopia, water hyacinth (Pontederiaceae) was reported from Koka Lake and the Awash River about 60 years ago (Stroud 1994; Firehun et al. 2013). Since then, it has manifested itself on a large scale in many water bodies, including rivers, lakes, reservoirs, irrigation supplies and drainage systems (Stroud 1994; Rezene 2005; Taye et al. 2009). Various methods to control water hyacinth include manual and mechanical clearing and in some places chemical control. However, the use of herbicides to control water hyacinth is only effective in the short term anywhere in the world (Borokoni & Babalola 2012; Dagni et al. 2012). Biological control of water hyacinth, which involves the use of natural enemies (i.e. insects and fungal pathogens) has been reported to be the most economical and sustainable method of control (Firehun et al. 2013). Fungal pathogens have gained acceptance as a practical, safe, environmentally friendly weed management method in agro-ecosystems worldwide (Charudattan 2001) although their use has not been adopted in Ethiopia. Recommendations have been made to integrate fungal pathogens with insects to improve biological control of water hyacinth due to the insect feeding damage facilitating pathogenic infections of water hyacinth leaves (Martinez & Gutierrez 2001).

Several fungal pathogens have been reported to attack water hyacinth worldwide (Shabana et al. 1995a, 1995b, 1997, 2000; Charudattan 2001). Various strains in the genera Acremonium, Alternaria, Cercospora, and Myrothecium have been studied intensively as biocontrol agents and shown to be effective under experimental conditions (Shabana et al. 1995a, 1995b, 1997, 2000; Charudattan 2001; Martinez & Gutierrez 2001; Mohan et al. 2003; Praveena & Naseema 2004). One fungal species, Cercospora piaropi, originally described as C. rodmanii (Conway 1976) and patented by the University of Florida (Conway et al. 1978) was developed into a bioherbicide by Abbott Laboratories (Chicago, IL) for water hyacinth management. In Africa, several pathogenic fungi that attack water hyacinth offer great potential to be developed in to mycoherbicides (Bateman 2001). For example, in Egypt,
Alternaria eichholii was developed into a mycoherbi-cide for the control of water hyacinth (Shabana 2005).

In an effort to integrate fungal pathogens into water hyacinth management, this paper investigates the diversity of fungal pathogens associated with water hyacinth in Ethiopia, their impact on the growth of water hyacinth plants and their safety on important plant species.

2. Materials and methods

2.1. Survey and isolation of fungi associated with water hyacinth

Diseased water hyacinth leaves (showing browning, wilting, yellowing, spots, blights, or combinations thereof) were collected from water bodies (in and along Aba-Samuel Dam, Lake Bishoftu, Lake Koka, Lake Beseka, Koka Dam, Awash Dam, Lake Ellen, Lake Elle-toke, Wonji-Shoa sugar estate, and Metahara sugar estate) in the Ethiopian Rift Valley from 2009 to 2011. Diseased leaf specimens were taken to the laboratory and stored at 4°C in a refrigerator. Small sections of the diseased tissue (2–4 mm²) were cut from the margins of necrotic or chlorotic lesions and surface-disinfected for one minute in 10% sodium hypochlorite. The tissue pieces were rinsed three times with sterile water and four tissue pieces were plated on potato dextrose agar (PDA; Merck KGaA, Germany) in each Petri dish and incubated at 25°C. The Petri dishes were checked for fungal growth after 2–4 days and thereafter on a daily basis for 20–30 days. Pure colonies of the isolates were grown by single-spore or hyphal-tip techniques (Martinez & Charudattan 1998). The isolates were stored on agar slants at 6°C in a refrigerator. The frequency of occurrence of the isolates were rated as very frequent (>20%), frequent (10%–20%) and infrequent (<10%) according to El-Morsy (2004).

2.2. Characterization and identification of water hyacinth fungi

Fungal isolates were obtained from agar slants using a cork borer (5-mm diameter) and transferred onto freshly prepared PDA media amended with streptomycin sulfate in 80-mm Petri dishes. The plates containing each isolate were arranged in a completely randomized design, with each isolate replicated four times. Colony diameter of each isolate was measured daily on a daily basis for seven consecutive days. The colony appearance and color on PDA in the Petri dishes were recorded for three days after incubation using the Munsell soil color charts (1994). The fungal isolates were characterized morphologically under a microscope based on their mycelium, fruiting structures and spores and identified according to Ainsworth et al. (1973) and Barnett and Hunter (1972).

To confirm the identity of fungal isolates, molecular characterization was conducted at the Farming Systems Ecology Group Laboratory, Wageningen University, Netherlands. DNA was extracted by using FastDNA® Spin Kit (Q-Biogene, Germany). Electrophoresis and nano-drop measurements were conducted to determine purity, concentration and integrity of isolated DNA. Polymerase chain reaction (PCR) was performed in a total volume of 25 µl containing Nuclease free water, PCR MM Promega, ITS1 primer, ITS4 Primer and target DNA. The mixture was incubated in a Thermocycler (Spectrum 48 real Time, Germany). The intertranscribed spacer (ITS) regions including ITS1, 5.8S, and ITS2 regions of fungal ribosomal DNA were amplified with primers ITS1 and ITS4 (White et al. 1990) with an initial denaturing step at 94°C for five minutes followed by 30 cycles of denaturation at 94°C for two minutes, annealing at 57°C for one minute and elongation at 72°C for two minutes. The PCR amplicons were purified by using MiniElute PCR purification kit (Qiagen, Germany) using a microcentrifuge. The purified PCR products (10 µl) were directly sequenced by using an automated sequencer. The nucleotide sequences were aligned by using a nucleotide-blast program (MegaBlast) in the Greonomics Laboratory of Wageningen University and the results showed a close phylogenetic match with other known isolates of the same taxon.

2.3. Pathogenicity tests

Healthy water hyacinth plants (collected from Wonji-Shoa irrigation and drainage canals) were grown in plastic buckets (50 cm diameter and 40 cm height) filled with water and were acclimatized for 15 days inside a lathhouse. Mycelia or spores from 10 days old laboratory cultures grown on PDA in Petri dishes were obtained aseptically and blended with 10 ml of sterile distilled water. The blended mycelium and spore suspension were diluted to give 1×10⁶ propagule/ml (El-Morsy 2004) and 20 ml of the suspension was applied on to water hyacinth leaves by using hand sprayers. Young leaves of water hyacinth plants were rubbed with carborundum (a universally used abrasive) and painted with Tween 80 to ensure penetration and subsequent infection by mycelia and spores. Water hyacinth leaves were also rubbed with carborundum and sprayed with sterile distilled water and used as controls. The inoculated and control plants were covered with clear plastic bags for two days to maintain a high relative humidity (Shabana et al. 1995a).

After inoculation, the buckets containing the water hyacinth plants were arranged in a completely randomized design and treatments were replicated.
three times. Plants were examined for disease symptom expression five days after inoculation for five weeks. Disease symptom expression on the water hyacinth leaves was rated on a severity scale of 0–5 (Waipara et al. 2006). The experiment was repeated three times.

The isolates with high disease severity (DS) (scores 3–5) were screened for their effect on water hyacinth plant growth. Twenty milliliter of suspensions containing 10^6 conidia/ml were applied to water hyacinth leaves. To estimate disease incidence (DI), the number of infected leaves per the total number of leaves was counted after 1 and 4 weeks after inoculation (WAI), while disease symptom on each leaf was rated on a scale of 0–9 (Freeman & Charudattan 1984). Values for individual leaves were summed and averaged to derive DS to determine DS for a whole plant. Fresh and dry weights of plants were recorded 4 WAI. The experiment was repeated three times. Data on the DI, DS and plant biomass was analyzed using SAS Version 9.1 (SAS Institute 2008). To achieve normality, percentage data were arcsine transformed before analysis.

### 2.4. Safety of water hyacinth fungi to plants

The highly pathogenic isolates (scores 3–5) were screened for their safety by exposing them to a selection of aquatic weeds and economically important crop species. The crops were selected based on their economical importance and their production in the lowland areas of the Ethiopian Rift Valley. The crops included; cereals (wheat, barley, sorghum, tef, and maize); vegetables (onion, garlic, pepper, cabbage, tomato, radish, carrot and cucumber); pulses (haricot bean, soy bean, lentil, chickpea, faba bean, field pea); oil crops (sesame, noug, safflower); fiber crop (cotton); spices (fenugreek, cumin seeds and mustard); sugar crop (sugarcane); and stimulants (coffee).

Five seeds of each crop species, five plantlets of each aquatic weed species (water pennywort, water lettuce, typha and water hyacinth) and five single bud sets of sugarcane cuttings were planted in pots filled with sterilized soil and each plant species were replicated six times. The plants were thinned at three leaf stage to one plant and acclimatized in a lath-house for five days. The plants were sprayed with 20 ml suspension having 1×10^6 conidia/ml concentration of the respective fungal isolate.

In each pot, the various plant species were also left untreated as controls with each control replicated six times. After seven days, all plants were examined for disease symptoms such as ovate leaf spot with a brownish center, necrotic foliar spot, browning and leaf blight. The isolates that showed symptoms were further re-isolated and tested to demonstrate Koch’s postulate. The experiment was repeated three times.

### 2.5. Fungal species dominance in relation to water and environmental factors

Community analysis was used to relate fungal species dominance to water and environmental factors. DS was estimated for each water hyacinth leaf in 4-m² quadrats at monthly intervals from 2009 to 2011. Water quality and environmental conditions including temperature, wave action, pH, N, P, EC, rainfall and altitude were analyzed. DS data were transformed using arcsine transformation. DS, fungal species, water quality and environmental data were subjected to multivariate analysis of variance (MANOVA). Multivariate regression analysis was used to quantify associations of groups of response variables (species and DS) and independent variables (water quality and environmental variables). All analyses were done on the Statistical Analysis System using multivariate general linear model procedure.

Finally, Canoco, a Fortran program version 4.5 (Ter Braak 2003) was used to determine the association of fungal species with water quality and environmental conditions.

### 3. Results and discussion

#### 3.1. Morphological and molecular characteristics and fungal species identity

In total, 25 isolates sporulated within 9 days of incubation and colonies formed different colors on PDA. Some isolates produced red pigment with reddish grey and dark reddish grey mycelia (Figure 1; Table 1). Colony growth varied with different isolates ranging from 0.5 to 2.5 cm/day and there were significant differences (P < 0.05) in radial colony growth among the isolates (Table 1). The isolates were categorized as fast growing (>1.5 cm/day) [for isolate 1, 9 and 21], intermediate

![Figure 1. The fungal isolates #1–25 colony appearance and color in the Rift Valley water bodies of Ethiopia grown in PDA.](image-url)
(1.0–1.49 cm/day for isolate 2, 13, 15, 16 and 23), and slow-growing (<1.0 cm/day) [for isolate 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 14, 16, 17, 18, 19, 20 22, 24, and 25].

All fungal isolates, except isolate 11, showed clear septation of mycelia after seven days of incubation on PDA, whereas isolates 4, 5, and 6 showed both horizontal and vertical macroconidial septation. The growth habit of isolates 4, 5, and 6 were stretched, cottony or raised. The observed differences among the isolates in morphological characteristics in this study suggest that these isolate are a diverse assemblage of species.

The DNA banding of each isolates ranged between 500 and 700 bp (Figure 2). However, nano-drop measurements of the diluted DNA of isolate 5 and 12 revealed no abnormalities, i.e. a

| Isolate | Mean growth rate per day (cm) | Front plate color | Back plate color | Surface appearance |
|---------|-------------------------------|-------------------|------------------|-------------------|
| 1       | 1.465                         | Black             | Yellow           | Stretched         |
| 2       | 1.075                         | Yellow green      | Reddish gold     | Cottony           |
| 3       | 0.696                         | White with red    | Light red        | Cottony           |
| 4       | 0.662                         | Grey              | Black            | Cottony           |
| 5       | 0.701                         | Dark gray         | Olive brown      | Stretched         |
| 6       | 0.666                         | Pale brown        | Gray             | Stretched         |
| 7       | 0.715                         | Light gray        | Very pale brown  | Stretched         |
| 8       | 0.687                         | Reddish gray      | Black            | Cottony           |
| 9       | 1.594                         | Light gray        | Pale yellow      | Stretched         |
| 10      | 0.664                         | Light gray        | Pale yellow      | Stretched         |
| 11      | 0.654                         | Greenish gray     | Yellow           | Raised            |
| 12      | 0.768                         | Light gray        | Yellow           | Stretched         |
| 13      | 1.012                         | White             | Gray             | Cottony           |
| 14      | 0.471                         | Dark gray         | Gray             | Stretched         |
| 15      | 1.302                         | Greenish gray     | Pale yellow      | Stretched         |
| 16      | 1.004                         | Greenish gray     | Brownish yellow  | Stretched         |
| 17      | 0.712                         | Reddish gray with red circle | Black with red circle | Stretched         |
| 18      | 0.671                         | White             | Pale yellow      | Cottony           |
| 19      | 0.708                         | Light brownish gray| Gray            | Stretched         |
| 20      | 0.700                         | Dark reddish gray with red circle | Black with red circle | Stretched         |
| 21      | 2.456                         | Grayish brown     | Dark brown       | Stretched         |
| 22      | 0.633                         | Olive yellow gray circle | Very dark gray  | Stretched         |
| 23      | 1.232                         | White             | Yellow           | Cottony           |
| 24      | 0.776                         | White             | Pale yellow      | Cottony           |
| 25      | 0.662                         | White             | Brownish yellow  | Stretched         |

Figure 2. Validation of PCR using fungal primers ITS1–ITS4 for isolates # 4–25. As a positive control DNA from isolate # 3 was used. Amplicon size is between 500 and 700 bp.
ratio of A260/A230 > 1.8. MegaBlast result of the respective fungal isolate indicated that both the forward and reverse primers identified the fungal isolates at the species or genus level with <3% gap and most of the isolates were identified at about a 99% precision level. Differentiation of some isolates based on phenotypic differences, besides being unclear in some solutions, was supported by the DNA sequencing data.

Both morphological and molecular analyses showed that the fungal isolates belonged to nine genera (Table 2). Of these fungi, A. tenuissima (23.5%) and A. alternata (26.5%) were the most prevalent. Among the fungal pathogens, A. alternata, A. tenuissima, Phoma sp., Alternaria sp., F. oxysporum, and F. equiseti were the most common species reported as pathogens of water hyacinth. Alternaria alternata has been described as a pathogen of water hyacinth in Australia (Galbraith 1987), Egypt (Shabana et al. 1995a, b; El-Morsy 2004; El-Morsy et al. 2006), Bangladesh (Bardur-ud-Din 1978) and India (Aneja & Singh 1989; Mohan Babu et al. 2002 2003). Research conducted in India and Egypt also indicated that this fungus has potential as a biocontrol agent of water hyacinth and its toxins may also be used as a herbicide (Mohan Babu et al. 2002; El-Morsy et al. 2006). Despite the occurrence of several fungal species on water hyacinth in Ethiopia, C. trifolii, M. fragilis, M. racemosus, A. fumigatus, Botryosphaeria sp., and N. parvum have not been previously isolated from water hyacinth.

3.2. Pathogenicity to water hyacinth

Among the various fungal isolates, ten were pathogenic to water hyacinth, while six were found to be severely pathogenic (showing >70% severity) to water hyacinth in the present study (Table 3). The disease symptoms occurred 8–14 days after inoculation. As the symptoms progressed, they coalesced and covered a larger surface area of the leaves within 5 WAI, while other isolates showed restricted spread. The pathogenic fungal pathogens observed in the present study have also been reported from other countries (Barreto et al. 2000; Charudattan 2001; El-Morsy 2004; Ray 2006). Although the fungal species N. parvum was reported for the first time as pathogenic to water hyacinth, it also attacks Eucalyptus spp. and Tibouchina spp. (Pavlic et al. 2007; Heath et al. 2011).

3.3. Effect of fungal pathogens on water hyacinth plant growth

The pathogenic fungi showed significant differences in the impact on the growth of water hyacinth plants (Table 3). Severe reduction in plant height (48%–55%), root length (45%–50%), fresh (53%–67%) and dry weight (60%–72%) was recorded due to infection by A. alternata, N. parvum and A. tenuissima, compared to small reductions in plant height (15%–18%), fresh (11%–15%) and dry weight (20%–23%) due to M. fragilis, P. macrostomata, and C. trifolii, respectively. Meanwhile fungi such as Alternaria sp., F. equiseti, and F. oxysporum showed low to moderate reduction (26%–50%). There were negative and significant associations (r = −0.93) between DS and dry weight, growth rate as well as other growth parameters (Table 3). These findings indicate that some fungal species may be useful in water hyacinth management since they affect the growth of water hyacinth plants. The reduction in plant height and biomass following exposure to fungal pathogens suggests that the number of reproductive and vegetative propagules of water hyacinth and the doubling time of the plant would be prolonged. In other studies, Shabana et al. (1995b) and

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**Table 2. List of fungal pathogens isolated from water hyacinth, their symptoms and frequencies of occurrence in the Rift Valley water bodies of Ethiopia (2009–2011).**

| Isolates | Fungal pathogens | Symptoms and infected plant part | Frequency (%) |
|----------|------------------|-------------------------------|---------------|
| 1        | Aspergillus niger VanTiegh | Leaf spot on leaf | 14.5          |
| 2 & 21   | Aspergillus flavus Link ex Fr. | Leaf spot on leaf | 8.9           |
| 3        | Phoma sp.          | Blight on leaf and petiole     | 10.4          |
| 4        | Alternaria sp.     | Leaf spot on leaf and petiole  | 17.8          |
| 5 & 7    | Alternaria tenuissima (Nees ex Fr.) Wiltshire | Leaf spot on leaf and petiole | 23.5          |
| 6        | Alternaria alternata (Fr.) Keisler | Leaf spot on leaf and petiole | 26.2          |
| 8        | Curvularia trifolii (Kaufman) Boedijn | Leaf spot on leaf | 11.2          |
| 9, 19, & 24 | Mucor fragilis Bull. | Blighting on leaves | 11.6          |
| 10       | Mucor racemosus Fres. | Blighting on leaves | 14.2          |
| 11       | Pencillium sp.     | Zonate leaf spot on leaves     | 6.7           |
| 12       | Phoma macrostomata | Blight on leaf and petiole     | 7.3           |
| 13       | Neofusicoccum parvum | Leaf spot on leaf and petiole | 7.3           |
| 14       | Aspergillus oxyze (Ahibburg) E. Cohn | Leaf spot on leaf | 11.0          |
| 15       | Aspergillus fumigatus Fresenius | Leaf spot on leaf | 12.2          |
| 16       | Fusarium equiseti (Corda) Saccardo | Leaf spot on leaf | 12.3          |
| 17       | Phoma sp.          | Blighting on leaves            | 7.4           |
| 18       | Fusarium oxysporum Schlechtendal | Leaf spot on leaf | 13.8          |
| 20       | Botryosphaeria sp. | Zonate leaf spot on leaves     | 6.5           |
| 22       | Phoma sp.          | Blighting on leaves            | 7.4           |
| 23       | Phoma sp.          | Blighting on leaves            | 7.4           |
| 25       | Phoma sp.          | Blight on leaves and petiole   | 7.4           |

* = Frequency, is, the number of locations in which a species occurred expressed as a percentage of the total number of locations surveyed.
Table 3. Effect of potential fungal pathogens on different plant parts of water hyacinth at 4WAI

| Fungal pathogens | Fresh wt at end (g) | Relative reduction (%) | Dry wt | Relative reduction (%) | Plant height (cm) | Relative reduction (%) | Root length (cm) | Relative reduction (%) | DI 4WAI | DS 4WAI |
|------------------|---------------------|------------------------|--------|------------------------|------------------|-----------------------|------------------|-----------------------|---------|---------|
| Alternaria sp.    | 118.5 de            | 47.71                  | 7.13 de| 55.33                  | 11.63 e          | 44.09                 | 17.93 g          | 41.41                 | 88.7 cd | 73.3 c  |
| Alternaria tenuissima | 105.8 e            | 53.31                  | 6.37 e | 60.09                  | 10.77 f          | 48.22                 | 16.83 h          | 45.00                 | 91.2 b  | 74.2 c  |
| Alternaria alternata | 73.9 f            | 67.39                  | 4.93 f | 72.49                  | 9.28 g           | 55.38                 | 15.30 j          | 50.00                 | 92.2 a  | 80.4 a  |
| Curvularia trilobi | 191.7 b            | 15.40                  | 12.23 b| 23.37                  | 16.99 bc         | 18.32                 | 23.52 d          | 16.60                 | 89.1 cd | 49.5 e  |
| Mucor fragilis    | 200.4 b            | 11.56                  | 12.62 b| 20.93                  | 17.64 b          | 15.19                 | 26.71 b          | 12.71                 | 77.4 f  | 30.2 h  |
| Phoma macrostomata| 196.7 b            | 13.20                  | 13.88 b| 22.43                  | 17.51 b          | 15.82                 | 26.22 c          | 14.31                 | 89.5 c  | 40.6 f  |
| Neofusicoccum parvum | 87.9 f            | 61.21                  | 4.96 f | 68.92                  | 9.30 g           | 55.29                 | 15.61 i          | 48.99                 | 93.1 a  | 78.6 b  |
| Fusarium oxysporum| 126.5 f            | 44.17                  | 7.79 f | 51.19                  | 12.36 d          | 40.58                 | 18.12 fg         | 40.78                 | 75.6 g  | 71.4 d  |
| Fusarium oxysporum| 132.6 d            | 41.48                  | 7.85 d | 50.81                  | 12.19de          | 41.39                 | 18.30 f          | 40.20                 | 87.7 d  | 70.4 d  |
| Botryosphaeria sp. | 173.6 c            | 23.10                  | 13.30 c| 35.28                  | 16.56 c          | 20.38                 | 23.84 e          | 22.09                 | 83.9 cd | 34.7 g  |

Uninoculated 226.6 a 15.96 a 20.80 a 30.60 a 0.0 h 0.0i 118.5 de 47.71 7.13 de 55.33 11.63 e 44.09 17.93 g 41.41 88.7 cd 73.3 c

Means within a column for each week followed by the same letter are not significantly different according to Duncan’s multiple range test;

***WA = weeks after inoculation;

**** = DI = disease incidence (percentage of leaves infected); DS = disease severity (percentage severity of infection);

***** = DS and DI was determined for each leaf on a scale of 0–9, where 0 = healthy, and 9 = 100% diseased and finally the DS grouped in to five groups, where “N” no significant damage or infection; “Mild,” <25% of infection; “Low Moderate” 26%–50% of infection; “High Moderate”, 51%–75% of infection and “Severe,” >75% of infection.

3.4. Safety of water hyacinth fungi to plants

Among the fungal species, F. oxysporum was pathogenic to a wide range of plant species, including cabbage, papyrus, mustard, chickepa, faba bean, pea, lentil, fenugreek, sesame and pepper (Table 4). Meanwhile, F. equiseti was pathogenic to tomato, cabbage, haricot bean, soy bean, mustard and pepper. N. parvum was pathogenic to carrot and tomato. The

Table 4. Risk assessment of fungal morphotypes proved to be pathogenic to water hyacinth

| Family | Species and common name | ALLAL | ALNSP | ALLTE | FUSOX | FUSEQ | NEOPA |
|--------|-------------------------|-------|-------|-------|-------|-------|-------|
| Apioaceae | Daucus carota L. cv. unknown; Carrot | –     | –     | –     | –     | –     | +     |
| Araceae | Hydrocotyle verticillata L. Water pennywort | –     | –     | –     | –     | –     | –     |
| Asteraceae | Guizotia abyssinica cv. Vogera; Noug | ++   | +     | ++     | ++     | ++     | ++     |
| Brassicaceae | Raphanus sativus L. cv. unknown; Radish | –     | –     | –     | –     | –     | –     |
| Chenopodiaceae | Ipomea batata (L.) Lam. cv unknown; Sweet potato | –     | –     | –     | –     | –     | –     |
| Compositae | Cynara cardunculus var. scolymus; Artichoke | –     | –     | –     | –     | –     | –     |
| Cruciferae | Brassica napus L. cv. unknown; Cabbage | –     | –     | –     | –     | –     | –     |
| Labiatae | Salvia farinacea Benth. | –     | –     | –     | –     | –     | –     |
| Liliaceae | Allium sativum L. | +     | +     | ++     | ++     | ++     | ++     |
| Malvacceae | Gossypium hirsutum L. cv. Arba; Cotton | –     | –     | –     | –     | –     | –     |
| Poaceae | Hordeum vulgare L. cv. Desta; Barley | –     | –     | –     | –     | –     | –     |
| Rubiaceae | Coffea arabica L. cv. Gisha, Coffee | –     | –     | –     | –     | –     | –     |
| Solanaceae | Lycopersicon esculentum Mill. cv. Melka-Shola; Tomato | –     | –     | –     | –     | –     | –     |
| Typhaceae | Typha orientalis Roscoe | –     | –     | –     | –     | –     | –     |
| Umbelliferae | Cuminum cymiferum cv. unknown; Cumin seed | –     | –     | –     | –     | –     | –     |

*Plants of economic importance; **Plants ecologically related to water hyacinth; ***Plants reported susceptible to test fungi; ****Plants ecologically important; Bayer Code: ALLAL = Alternaria alternata, ALLTE = Alternaria tenuissima, ALNSP = Alternaria sp., FUSOX = Fusarium equiseti, FUSEQ = F. oxysporum and NEOPA = Neofusicoccum parvum.

Disease reaction from leaf inoculation; – no reaction, + slight, ++ moderate, +++ severe leaf necrosis.

Shabana (2005) also reported reductions in water hyacinth growth and reproduction due to infection by fungal pathogens, reinforcing the potential for pathogenic fungi to play an important role in weed management. Damage by pathogenic fungi to water hyacinth plants results in rotting of the lower petioles, waterlogging of the crown and gradual sinking of the plant (De Jong & de Voogd 2003) leaving the water surface clear of the weed.
most susceptible crops were sesame, tomato, fenugreek, pepper, haricot bean and mustard while cotton, sweet potato, sugarcane, cumin, maize, wheat and radish were not infected by the fungal pathogens. Similarly, A. alternata, A. tenuissima, F. oxysporum and N. parvum showed severe leaf necrosis on water lettuce. Re-isolation of the fungi from all symptomatic plants confirmed the occurrence of the respective fungus as the causative agent of the disease symptoms. The lack of susceptibility of plants inoculated with A. alternata, A. tenuissima, and A. tenuissima sp. suggests that some plant species may be immune to these pathogens.

In another study, Rhomela et al. (1999) reported that A. alternata did not infect lettuce, soybean, common bean, winged bean, mung bean, string bean, banana, and rice but caused some disease on cabbage, radish and okra. Similarly, in this study, A. alternata did not infect any of the plant species except water hyacinth and water lettuce, which indicate their safety against plants. However, it has been described as a worldwide pathogen of water hyacinth (El-Morsy et al. 2006).

Both F. equiseti and F. oxysporum were able to produce moderate to severe leaf necrosis on 13 out of the 29 plant species tested. The host range of F. equiseti and F. oxysporum on economically important plant species have been adequately described (Rahim & Tawfig 1984; Jamil & Rajagopal 1986; Taye et al. 2009). Conversely, in India, a risk assessment study revealed that F. equiseti was not pathogenic to any of the crop plants tested except amaranthus (Naseema et al. 2001). The susceptibility of plant species to F. equiseti in the present study may therefore be attributed to the occurrence of different strains of the pathogen and would preclude their use as biocontrol agents of water hyacinth.

### 3.5. Fungal species dominance in relation to water and environmental factors

MANOVA indicated that the occurrence of fungal pathogens was significantly influenced by wave action, ambient temperature, season (survey month) and altitude (Table 5). Multivariate regression analysis indicated that fungal pathogens and their extent of occurrence are significantly associated with water quality and environmental variables. Among the water quality and environmental factors, regression analysis indicated that altitude and survey month had a positive and significant influence on the occurrence of fungal pathogens.

### Table 5. Multivariate analysis of variance using "wilks" test.

| Parameters | DF | F-Value | Pr (>F) | Significance |
|------------|----|---------|---------|--------------|
| Altitude   | 1  | 9.61    | 0.001   | ""           |
| pH         | 1  | 3.07    | 0.079   | ""           |
| Month      | 1  | 69.54   | <2.2e-16| ""           |
| Rainfall   | 1  | 30.64   | 3.79e-08| ""           |
| Temperature| 1  | 78.35   | <2.2e-16| ""           |
| EC         | 1  | 1.03    | 0.871   | ""           |
| Wave       | 1  | 5.82    | 0.015   | ""           |
| N          | 35 | 2.50    | 4.05e-06| ""           |
| P          | 1  | 0.11    | 0.736   | ""           |

*EC = electric conductivity; N = nitrogen; P = phosphorous.

Significance codes: 0.001***; 0.01**; 0.05*; 0.1+.
fungal pathogens occurrence and level of severity. Similarly, a unit change in altitude and month resulted in an increase in extent of DS by 0.45% and 2.93%, respectively.

A Canonical Correspondence Analysis (CCA) was used to ordinate the fungal species dominance percentage data in relation to the environmental and water variables (Ter Braak 2003). The first two ordination axes explained 71.4% of the total variance in the weighted averages for dominance of the fungal pathogens with respect to the environmental variables. Several of the most frequent fungal pathogens such as A. alternata, A. tenuissima, Alternaria sp., F. oxysporum, N. parvum, and Botryosphaeria sp. were located near the origin of the ordination diagram (Figure 3) indicating their ability to thrive under very diverse agro-ecosystems. In agreement with this finding, Firehun et al. (2013) reported that A. alternata, A. tenuissima, Alternaria sp., and F. oxysporum, have a wide geographical distribution and produce virulent toxins. Their wide distribution in the Rift Valley of Ethiopia would also ensure the potential to use the fungal pathogens as a mycoherbicide and avoid the quarantine issues associated with exotic pathogens.

In the CCA biplot (Ter Braak & Prentice 1988) the length of arrows indicates the importance of the factors (longer arrows = more important). Accordingly, among the variables considered, wave action, temperature and altitude were more important, followed by N content, survey month and pH than EC, which had a short arrow. These results are in agreement with previous findings where the patterns of fungal abundance were influenced by most of the above-mentioned factors either positively or negatively (Mohan et al. 2003; El-Morsy 2004; El-Morsy et al. 2006; Dагo et al. 2010).

4. Conclusion

Based on the analysis of data on pathogenicity, host-range, and association with environmental and water factors, A. alternata, A. tenuissima, and Alternaria spp. hold promise as possible biocontrol agents of water hyacinth. Infection by these fungal pathogens resulted in reductions in fresh weight, dry weight, plant height, and root length. They did not cause disease symptoms when inoculated onto plants of economical and ecological importance in Ethiopia, demonstrating they do not pose a risk to these tested plants.

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References

Ainsworth GC, Sparrow FK, Sussman AS. 1973. The fungi. Vol. IVA. New York: Academic Press; p. 621.

Aneja RR, Singh R. 1989. Alternaria alternata (Fr) Kissler, a pathogen of water hyacinth with biocontrol potential. Trop Pest Manage. 35:354–356.

Badur-ud-Din AA. 1978. Control of aquatic weeds. Second annual report, project No. FG-Pa-271. Lahore: University of the Punjab; p. 61.

Barnett HL, Hunter BB. 1972. Illustrated genera of imperfect fungi. Minneapolis: Burgess Publishing Company; p. 241.

Barreto RW, Charudattan R, Pomella A, Hanada R. 2000. Biological control of neotropical aquatic weeds with fungi. Crop Prot. 19:697–703.

Bateman R. 2001. IMPECCA; an international, collaborative program to investigate the development of mycoherbicide for use against water hyacinth in Africa. In: Julien MH, Hill MP, Jianqing D, editors. Proceedings of the second meeting of global working group for the biological and integrated control of water hyacinth, ACIAR proceeding number 102; 2000 October 9–12; Beijing, China; p. 57–61.

Borokoni T, Babalola F. 2012. Management of invasive plant species in Nigeria through economic exploitation: lessons from other countries. Manag Biol Invasion. 3:45–55.

Charudattan R. 2001. Biological control of weeds by means of plant pathogens: significance for integrated weed management in modern agro-ecology. Biol Control. 46:229–260.

Conway KE. 1976. Cercospora rodmani a new pathogen of water hyacinth with biological control potential. Can J Bot. 54:1079–1083.

Conway KE, Freeman TE, Charudattan R. 1978. Inventors; Abbott Laboratories, assignee. 27 June 1978. Method and composition for controlling water hyacinth. U.S. patent 4,097,261.

Dагo K, Lahlali R, Diourte M, Jijakli H. 2010. Effect of temperature and water activity on spore germination and mycelial growth of three fungal biocontrol agents against water hyacinth (Eichhornia crassipes). Applied Microb. 110:521–528.

Dагo K, Lahlali R, Diourte M, Haissam J. 2012. Fungi occurring on waterhyacinth (Eichhornia crassipes [Martius] Solms-Laubach) in Niger River in Mali and their evaluation as Mycoherbicides. J Aquat Plant Manage. 50:25–32.

De Jong MD, De Voogd WB. 2003. inventors; Biological Farming Group, Wageningen University, assignee. 18 September 2003. Novel mycoherbicides for biological control of aquatic weeds such as water hyacinth and water lettuce. European patent, EPO-DG1 (102).

El-Morsy ME. 2004. Evaluation of microfungi for biological control of water hyacinth in Egypt. Fungal Diver. 16:35–51.

El-Morsy ME, El-Dohlob SM, Hyde KD. 2006. Diversity of Alternaria alternata a common destructive pathogen of...
Eichhornia crassipes in Egypt and its potential use in biological control. Fungal Diver. 23:139–158.

Firehun Y, Struik PC, Lantinga EA, Taye T. 2013. Joint use of insects and fungal pathogens in the management of water hyacinth (Eichhornia crassipes): perspectives for Ethiopia. J Aquat Plant Manage. 51:109–121

Freeman TE, Charudattan R. 1984. Cercospora rodmanii Conway, a potential biocontrol agent. Gainesville, Fla Agric Exp Stn Technol Bull. 842:18.

Galbraith JC. 1987. The pathogenicity of an Australian isolate of Acremonium zonatum to water hyacinth, and its relationship with the biological control agent, Neochetina eichhorniae. Aust J Agric Res. 38:219–229.

Heath RN, Roux J, Slippers B, Drenth A, Pennycook SR, Martínez JM, Charudattan R. 1998. Survey and evaluation of aquatic weeds. Crop Prot. 17:27–34.

Heath RN, Roux J, Slippers B, Drenth A, Pennycook SR, Martínez JM, Gutierrez EL. 2001. Host range of Cercospora piaropi and Acremonium zonatum, potential fungal biocontrol agents for water hyacinth in Mexico. Phytoparasitica. 29(2):175–177.

Mohan Babu R, Sajeena A, Seetharaman K. 2003. Bioassay of the potentiality of Alternaria alternata (Fr.) Keissler as a bioherbicide to control water hyacinth and other aquatic weeds. Crop Prot. 22:1005–1013.

Mohan Babu R, Sajeena A, Seetharaman K, Vidhyasekeran P, Rangasamy P, Som Prakash M, Senthil Raja AK, Biji R. 2002. Host range of Alternaria alternata—a potential fungal biocontrol agents for water hyacinth in India. Crop Prot. 21:1083–1085.

Munsell Soil Color Charts. 1994. Munsell color. New Windsor: Macbeth Division of Kollmorgen Instruments Corporation.

Naseema A, Praveena R, Balakrishnan S, Peethambaran CK. 2001. Management of water hyacinth [Eichhornia crassipes (Mart.) Solms] with fungal pathogens. Weeds 1:263–268.

Pavlic D, Slippers B, Coutinho TA, Wingfield MJ. 2007. Botryosphaeriaceae occurring on native Syzygium cordatum in South Africa and their potential threat to Eucalyptus. Plant Pathol. 56:624–636.

Praveena R, Naseema A. 2004. Fungi occurring on water hyacinth [Eichhornia crassipes (Mart.) Solms] in Kerala. J Trop Agri. 42(1–2):21–23.

Rahim AM, Tawfiq S. 1984. Pathogenicity of fungi and bacteria from Sudan to water hyacinth. Weed Res. 24:233–238.

Ray P. 2006. Management of water hyacinth employing some insects and fungi. PhD Thesis, Jabalpur: R.D. University.

Rezene F. 2005. Water hyacinth (Eichhornia crassipes): A review of its weed status in Ethiopia. Arem. 6:105–111.

Rhomela F, Masangkay M, Mabpayad O, Timothy CP, Alan KW. 1999. Host range of Alternaria alternata causing leaf blight of Sphenoeola zeylanica. Can J Bot. 77:103–112.

SAS Institute. 2008. SAS Version 9.1, 2008 © 2007-2008. Cary, NC: SAS Institute.

Shabana YM. 2005. The use of oil emulsions for improving the efficacy of Alternaria eichhorniae as a mycoherbicide for water hyacinth (Eichhornia crassipes). Biol Control. 32:78–89.

Shabana YM, Baka ZAM, Abdel-Fattah GM. 1997. Alternaria eichhorniae, a biological control agent for water hyacinth: mycoherbicidal formulation and physiological and ultrastructural host responses. Eur J Plant Path. 103:99–111.

Shabana YM, Charudattan R, Elwakil MA. 1995a. Identification, pathogenicity, and safety of Alternaria eichhorniae from Egypt as a bioherbicidal agent for water hyacinth. Biol Control 5:123–135.

Shabana YM, Charudattan R, Elwakil MA. 1995b. Evaluation of Alternaria eichhorniae as a bioherbicide for water hyacinth (Eichhornia crassipes) in greenhouse trials. Biol Control 5:136–144.

Shabana YM, Elwakil MA, Charudattan R. 2000. Effect of media, light and pH on growth and spore production by Alternaria eichhorniae, a mycoherbicidal agent for water hyacinth. J Plant Dis Prot. 107:617–626.

Streoud A. 1994. Water hyacinth (Eichhornia crassipes [Mart.] Solms) in Ethiopia. In: Rezene F. (ed.). Proc. 9th Ann. Conf. EWSC 9-10 April 1991, Addis Ababa, Ethiopia; p. 7–16.

Taye T, Rezene F, Firehun Y, Derje T, Tamado T. 2009. Review invasive weed research in Ethiopia. In: Abrahm T, editor. Increasing crop production through improved plant protection: Vol. 2. Plant Prot. Soc. Eth., Addis Ababa, Ethiopia; p. 381–407.

Ter Braak CJF. 2003. Program CANOCO Version 4.5A (1988-2003) Biometrics – quantitative methods in the life and earth sciences. the Netherlands: Plant Research International, Wageningen University and Research Centre Wageningen.

Ter Braak CJF, Prentice IC. 1988. A theory of gradient analysis. Adv Ecol Res. 18:271–317.

Waipara NW, Bourdôt GW, A Hurrell GA. 2006. Sclerotinia sclerotiorum shows potential for controlling water lettuce, alligator weed and wandering jew. New Zealand Plant Prot. 59:23–27.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols. A guide to methods and applications. New York: Academic Press; p. 315–522.