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Full Length Research Paper

Exploration of fungal pathogens associated with water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laubach) in Ethiopia

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Indigenous fungi found associated with water hyacinth were identified and evaluated for their biocontrol potential against the weed. During the study, a series of laboratory and lath-house experiments were conducted and 19 fungal species were identified. Among these, nine species with better virulence were selected based on preliminary test. These included *Alternaria alternata, Alternaria geophila, Ascochyta chartarum, Cochliobolus carbonum, Epicoccum nigrum, Fusarium chlamydosporium, Fusarium equiseti, Pythium ultimum* and *Stemphylium vesicarum.* Accordingly, pathogencity test was carried out in RCBD design with three replications to select the best candidates and five of them were found to be virulent to water hyacinth with disease severity ranging from 4.33 to 5.67 in 1 to 6 disease severity rating scale. The five promising candidates were *A. alternata, A. chartarum, F. chlamydosporium, F. equiseti* and *P. ultimum.* The highest disease severity 5.67 was recorded by *P. ultimum* while the least severity with 4.33 was recorded by *A. chartarum*. Based on the current findings, we concluded that, *A. alternata, A. chartarum, F. chlamydosporium, F. equiseti* and *P. ultimum* could be used as effective biocontrol agents against water hyacinth following performance evaluation under natural environmental conditions and their host specificity test.

Key words: Biocontrol, indigenous fungi, virulent, pathogenecity.

INTRODUCTION

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae) is a perennial, herbaceous, aquatic plant. It is widely recognized as the world's worst aquatic weed. It poses serious socioeconomic and environmental problems on millions of people in riparian communities (Howard and Matindi, 2003). The weed obstructs electricity generation, irrigation, navigation, and fishing; increases evapo-transpiration resulting in water loss; increase cost of crop production; provides habitat for vectors of malaria and bilharzias; harbours poisonous snakes; causes skin rashes; and can host agents of amoebic dysentery and typhoid. In general the weed causes enormous socioeconomic problems, the magnitude and extent of which are unquantified (George et al., 1998). In Ethiopia also, water hyacinth is considered as a constraint on the development of the country (Senayit et al., 2004).

Several authors have tried to put an approximate figure to the economic consequences caused due to water hyacinths problem specifically on utilization of water for irrigation. According to Gopal (1987) the annual water loss through evapo-transpiration due to water hyacinth in Sudan would be enough to irrigate more than 400 ha of land. Similarly, Firehun et al. (2007) reported that the weed causes 393,660 to 2,945,160 m^3 water loss at Wonji-Shoa, which could irrigate 31.12 to 232.84 ha of

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land in any cropping season.

Increasing concern about the financial costs and environmental pollution associated with herbicidal control measures and their limited effectiveness has led to growing interest in the use of biological control (Julien et al., 2001). Surveys for natural enemies of water hyacinth that may be used as biological control agents began in 1962 and continued up until recently (Center et al., 2001). Many pathogens gave promising results as biological control agents of water hyacinth in different countries. Among them are *Uredo eichhorniae*, suitable as a classical biocontrol agent and *Acremonium zonatum, Alternaria eichhorniae, A. alternata, Cercospora piaropi*, *Myrothecium roridum*, and *Rhizoctonia solani,* which are widely distributed in different continents, as bioherbicides. Other less widely distributed pathogens include notably species of *Bipolaris, Drechslera,* and *Fusarium,* which may hold promise, but further studies are needed to confirm their usefulness (Charudattan et al., 1985; Shabana et al., 1995).

Attempts have been made to identify potential biological agents in Ethiopia. Earlier studies indicate that some fungal as well as insect bioagents are available in the country (Stroud, 1991). For instance, a survey carried out in the Gambella region (1970's) revealed that fungus *Cercospora rodmanii* affected water hyacinth by about 5 to 15% in place of infestation (Stroud, 1991). Further high aphids infestations of about 25 to 30 aphids per plant were recorded (Stroud, 1991). Another survey by the same author, showed aphids and mite infestations on water hyacinth in Awash River gorge area (Stroud, 1991). During a preliminary survey conducted by Firehun et al. (2006) at Wonji-Shoa Sugar Estate water reservoirs, a fungal pathogen, *Fusarium equiseti* (Corda) Sacc. was found to infect water hyacinth. The damage caused by the fungus in the field as well as in green house on the weed was promising. This fungus could be considered as a candidate for bio control following host specificity assessment.

In spite of the wide spread infestation of water hyacinth in the most economically important water bodies of the country, efforts directed towards finding out possible antagonistic fungal isolates are insufficient. In Ethiopia, pesticides have not been used extensively for controlling pests and the possibility of finding potential antagonistic microbes (fungi) that may be used in integrated management of this weed is enormous. Therefore, the present study was undertaken with the following specific objectives to find the native fungal antagonists associated with water hyacinth and to evaluate their bio control potential against the weed in Ethiopia.

MATERIALS AND METHODS

Description of the study area

Field surveys were conducted in 2008 in some water bodies having connection with the Awash River including Abasamuel Dam, Koka Dam, Lake Ellen, Wonji-Shoa Sugar Estate and Awash River near Koka Town (Figure 1). Abasamuel Dam is located near Akaki at 8°52' N longitude, 38°04' E latitude and at an altitude of 1900 masl. According to information given by the local people this dam has been used by a significant number of local people for irrigation, fishing and as drinking water for cattle. Farmers in the area reported that foreign inhabitants residing near the dam introduced water hyacinth to the dam 30-40 years ago. Lake Ellen is located at 08°23' N longitude, 38°59' E latitude and at an altitude of 1700 masl, 8 km west of Alem Tena town in Dugda Bora District. Koka Dam is located at 08°26' N longitude and 39°10' E latitude at an altitude of 1589 masl, is one of a few man-made reservoirs formed on the Awash River Southeast of Addis Ababa in the Rift Valley. Wonji-Shoa Sugar Estate is located in the central part of the main East African Rift Valley at 8°30` to 8°35` longitude and 39°20` latitude, at an altitude of 1540 masl. It is situated at about 107 km southeast of Addis Ababa.

The economic importance of these water bodies is significant in the areas of hydroelectric power generation, irrigation and transportation. They have paramount importance as the major source for domestic and industrial irrigation water supply for the respective areas. In addition, they are a "natural laboratory" for education and scientific research. On the other hand, the fisheries located in these water bodies support over 3,000 families through commercial and subsistence fishing activities and many more in processing, distribution and marketing centers (Abebe and Geheb, 2003).

Occurrence and incidence of diseases associated with water hyacinth in the field

Assessment of fungal diseases associated with water hyacinth was made on the different water bodies mentioned earlier. The disease assessment was made based on visual observation of symptoms (browning, wilting, yellowing, spots, blights, disease of whole plant, mixed symptoms, etc.) and number of plants showing each category of these symptoms was recorded. During the assessment, 10 quadrats were thrown systematically in x (criss-cross) fashion and the number of healthy and diseased plants was counted and disease incidence was determined. The number of diseased plants was expressed as percentage of the total number of plants in each quadrat following the formula of Wheeler (1977):

$$\text{Disease incidence} = \frac{\text{No of diseased plants}}{\text{Total no. of plants inspected}} \times 100$$

In addition, severities of the different foliar disease symptoms were determined by estimating the percent of plant tissue affected by the disease as used by modified Naseema et al. (2001) disease severity rating scale (Table 1).

Samples of water hyacinth plants showing each type of symptom were collected, tagged and pressed between thick bundles of news paper in a standard plant press and transported to Ethiopian Sugar Development Agency Research Directorate (ESDARD) Plant Protection Laboratory on the same date of collection. Finally, all quantitative data such as type of symptom on diseased plants, disease incidence and disease severity on water hyacinth were analyzed using SPSS software (1996).

Laboratory activities

Studies were jointly conducted at ESDARD and Ambo Plant

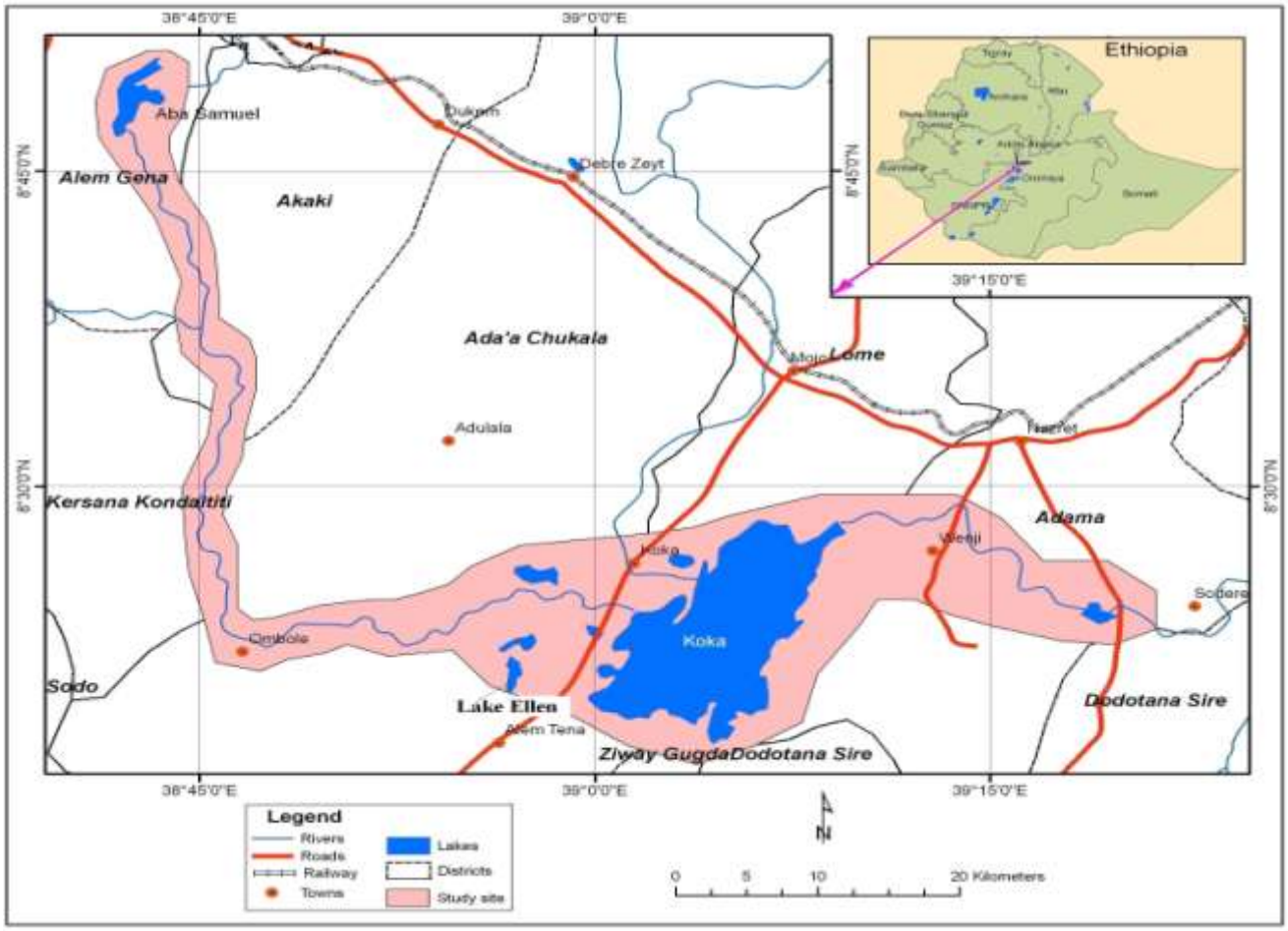

Figure 1. Map of study areas (Lake Abasamuel, Lake Ellen, Lake Koka , some parts of Awash River and its catchments).

Table 1. Modified Naseema et al. (2001) disease severity rating scale.

Disease severity rating scale	Type of symptom produced/ symptom description
1	No symptom
2	1-9% symptom developed around the pin pricked area only
3	10% of the leaf area showing yellowing or browning
4	11-25% of the leaf area showing yellowing or browning
5	26-50% of the leaf area including petiole showing symptom
6	51-100% of the leaf area including petiole showing symptom to complete drying of the plant

Protection Research Centre (APPRC) laboratories. Studies like isolation, maintenance and pathogenicity trials were accomplished at Crop Protection Laboratory, ESDARD while mass production of the fungal inoculum was done at APPRC.

Isolation, maintenance and storage of fungal isolates and fungal inoculation of water hyacinth were done using standard laboratory procedures (Dingra and Sinclair, 1995). Fungi associated with diseased water hyacinth plants were isolated using tissue plating method (Dingra and Sinclair, 1995; Martinez and Charudattan, 1998). Fungi were identified according to descriptions provided by Barnett and Hunter (1972) and the potential fungal species were grown in ependor tube and sent for further confirmation to the Department of Phytomedicine, Humboldt University of Berlin, Germany.

Pathogenicity test of fungal isolates

Pathogenicity test for fungal species was performed by preparing spore concentration of approximately 10^9 spores ml^{-1} (Firehun et al., 2006) using counting chamber method as used by Dhingra and Sinclair (1995). Spore suspension from each isolate was obtained by adding 50 ml water with 0.05% Tween 20 onto the spores of each fungal isolate and by mixing properly using sterilized triangular glass spatula. The amount of fungal spores was based on the adjustment made on the number of spores when one gram of the

Table 2. Intensity of different disease symptoms on water hyacinth in different Awash catchment water bodies in the Rift Valley 2008 crop season.

Water bodies	Number of sample areas surveyed	Mean disease incidence on water hyacinth plant (%)[1]	Disease severity (%)
Abasamuel	44	21.93	16.00 (4.00)[2]
Afer Gidib	10	16.50	13.10 (4.00)
Awash River	18	3.94	22.00 (4.00)
Bate Gurmame	12	17.00	12.00 (4.00)
Dodo Wedera	44	15.61	20.00 (4.00)
Ellen	33	34.40	30.00 (5.00)
Ellen Golode	15	25.24	27.00 (5.00)
Koka Dam	10	15.37	12.50 (4.00)
Melka Hida	15	29.10	28.00 (5.00)
Sire Robi	32	21.55	25.50 (5.00)
Tere	24	8.98	15.00 (4.00)
Mean	23	19.06	

[1]Mean incidence was expressed as percent of plants showing disease symptoms; per average of 8 m^2 per sample area considered [2] Numbers in bracket indicates disease severity in 1 to 6 scale.

spore powder was dissolved in 10 ml of sterilized distilled water. Spore concentration of 1 x 10^9 spores/ml was established for each isolate and then inoculated to 20 healthy young water hyacinth leaves. For facilitating penetration of the spores, young leaves of the water hyacinth plants were rubbed with carborundum and then were washed-off with sterile distilled water (Firehun et al., 2006). Plants with the same number of leaves were also abraded with carborundum and rinsed with sterilized distilled water and were kept at saturation to be used as control. Sterilized camel hair brush was used for each isolate to inoculate the spore suspension on the target leaves and to avoid unnecessary spore drift to the next treatment. Thus the spores were gently rubbed onto healthy water hyacinth plants.

After inoculation, the ten treatments along with the uninoculated control plants were arranged in randomized complete block design (RCBD) with three replications each. Five days after inoculation, isolates were rated for their ability to cause disease based on the presence or absence of disease symptoms, such as leaf spot, chlorosis, and necrotic blight. Disease severity was assessed using 1 to 6 scale. The plants were maintained for 4 weeks, to obtain maximum expression of disease symptoms. Isolates rated at 4 to 6 were considered highly virulent and aggressive and therefore potential bio-control agents.

Data on fungal genera or species encountered, frequency of each species, frequency of species found pathogenic for water hyacinth, incidence, severity, type of symptom inflicted on water hyacinth by each isolate, biomass, plant height and number of flowers aborted were collected a week after inoculation. Biomass, plant height and number of flowers were recorded because the reduction in these parameters clearly revealed the ability of the fungi to control the weed. Mean comparisons were made using Duncan's Multiple Range Test (DMRT). Analyses of variances (ANOVA) of disease incidence, biomass, plant height and number of flowers aborted were also performed using SAS computer software (SAS Institute, 1999).

Moreover, relative percent reduction of water hyacinth plant height was calculated using the following formula (Robert and James, 1991):

$$R = \frac{Yp - Yt}{Yp} X 100$$

Where, R is relative percent reduction, Yp is water hyacinth height from the control pot and Yt is water hyacinth height from pots of other treatments.

Similarly, relative percent reduction of water hyacinth biomass was calculated as follows:

$$I = \frac{A_p - A_t}{A_p} \times 100$$

Where, I is relative reduction increment, A_p is water hyacinth biomass from control pot and A_t is water hyacinth biomass from the treated pots with different fungal isolates.

RESULTS AND DISCUSSION

Occurrence and incidence of disease associated with water hyacinth in the assessed areas

Two distinct disease symptoms: leaf spot and leaf blight were observed in the field. Leaf spot showed the highest frequency of (57.04%) followed by leaf blight (42.95%). Infected water hyacinth leaves showed necrotic type spots, zonate leaf spots and blight symptoms with varying severities. Similarly, Charudattan (2001) summarized different fungal disease symptoms found in association with water hyacinth as zonate leaf spot, discrete necrotic foliar spots, necrotic spots and blighting on the leaves.

The highest mean incidence of diseased water hyacinth plants (> 25%) was observed at Lake Ellen, Ellen Golode, and Melka Hida water bodies, while the lowest disease incidence (< 9%) was encountered at Awash near Koka town and Tere (Upper Koka) water bodies (Table 2). A survey carried out in the Gambella region in the 1970's also revealed that the fungal diseases affected water hyacinth by 5 to 15% at the place of infestation (Stroud, 1991). Field survey conducted by Evans and Reed (2001) along the Napo River in Ecuador and along the Ucayali River around the port of Pucallpa revealed the association of three groups of fungal pathogens with

(a) (b) (c)

Figure 2. Plates showing diseased *Eichhornia crassipes* plants pattern due to different fungal species. (a) Necrotic leaf spot; (b) Zonate leaf spot; and (c) blighting symptoms all on *E. crassipes* leaves.

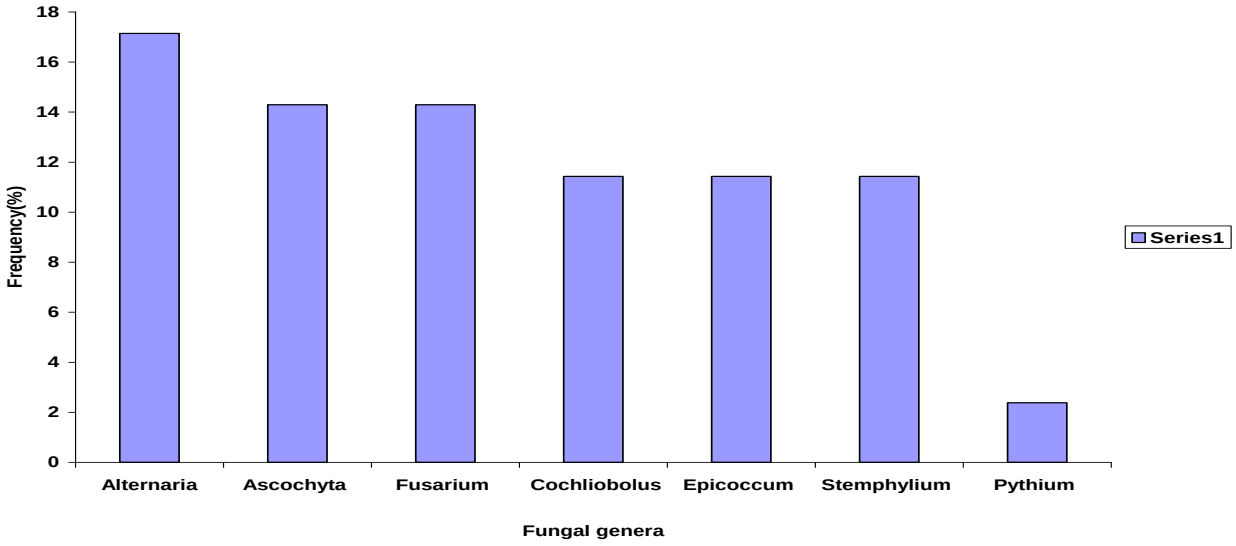

Figure 3. Frequency of fungal genera associated with water hyacinth leaves.

water hyacinth based on their symptoms: those producing blighting on still green leaf tissues, those producing necrotic leaf spots and zonate spot.

The disease prevalence in association with water hyacinth indicated that different disease symptoms were prevalent on water hyacinth. Leaf spots and blight were the only frequently observed disease symptoms (Figure 2). Leaf spot showed the highest frequency of (57.04%) followed by leaf blight (42.95%). Infected water hyacinth leaves showed necrotic type spots, zonate leaf spots and blight symptoms with varying severities.

Similarly, Charudattan (2001) summarized different fungal disease symptoms found in association with water hyacinth as zonate leaf spot, discrete necrotic foliar spots, necrotic spots and blighting on the leaves.

Fungal species isolated from diseased water hyacinth leaves

Identification result of fungal isolates by the Department of Phytomedicine, Humboldt University of Berlin, Germany, showed that different fungal genera with variable frequency were observed on water hyacinth leaves (Figure 3). The fungal genera, associated with water hyacinth leaves, with high frequency were *Alternaria* (17.14%), *Ascochyta* (14.29%), *Fusarium* (14.29%), *Cochliobolus* (11.43%), *Epicoccum* (11.43%) and *Stemphylium* (11.43%), while the least frequent fungal pathogen was *Pythium* (2.38%) (Figure 3).

These fungal genera were reported on water hyacinth by different authors. For instance two hundred fungal isolates belonging to several genera were isolated from diseased water hyacinth plants in Egypt (Shabana et al., 1995). These included three isolates of *Alternaria* spp. from the Dakahlia Governorate. The isolates were associated with a severe leaf blight that spread rapidly from disease foci (Shabana et al., 1995). Fusarium species like *Fusarium equiseti* (Corda) Saccardo, *Fusarium moniliforme* Sheldon were isolated from infected samples in the Sudan, India and Ethiopia

Table 3. Level of disease intensity and effects of fungal flora on water hyacinth.

Treatment	Disease incidence (%) 15 DAI*	Disease severity (1-6 scale)	Height (cm)	Relative reduction (%)	Shoot dry weight(gm)	Relative reduction (%)	No. of flowers aborted
Alternaria alternata	93.00^{s}	4.67^{c}	7.22^{n}	42.73	7.50^{f}	58.08	25.33^{b}
Alternaria geophila	57.67^{v}	3.00^{g}	11.86^{r}	5.99	15.37^{b}	14.06	15.33^{d}
Ascochyta chartarum	81.00^{u}	4.33^{d}	9.67^{o}	23.35	12.16^{d}	31.99	16.33^{d}
Cochliobolus carbonum	54.67^{w}	3.33f	11.67^{r}	7.49	13.21^{cd}	26.14	12.00^{ef}
Epicoccum Nigrum	59.33^{v}	3.33f	10.22^{o}	18.93	13.86^{c}	22.48	11.33^{f}
Fusarium chlamydosporum	86.67^{t}	5.33^{b}	5.77^{l}	54.27	10.27^{e}	42.54	28.33^{a}
Fusarium equiseti	86.67^{t}	4.67^{c}	6.23^{lm}	50.60	11.46^{d}	35.93	20.33^{c}
Pythium ultimum	87.33^{t}	5.67^{a}	6.76^{mn}	46.42	8.03^{f}	55.08	19.67^{c}
Stemphylium vesicarium	50.33^{x}	3.33^{f}	11.77^{r}	6.70	16.20^{b}	9.40	14.33^{de}
uninoculated plants	4.33^{z}	2.00^{h}	12.61^{r}	0.00	17.88^{a}	0.00	4.00^{g}
SE	2.80	0.31	0.95		1.15		2.95
CV (%)	7.30	12.4	17.50		15.80		30.60

[1]Means within a column followed by the same letter are not significantly different at p ≤ 0.05 according to DMRT [*]Incidence of disease15 days after inoculation.

(Barreto and Evans, 1996; Firehun et al., 2006). *Stemphylium vesicarium* and *Pythium* spp were originally reported as potential pathogens from USA though detail datum about their pathogenicity remains absent (Barreto and Evans, 1996).

Pathogenicity test

Analysis of the pathogenicity test result indicated that most of the fungal species were pathogenic to water hyacinth. Moreover, the result showed that there were variations among the isolates in the extent of damage or virulence. Intensity of disease due to isolates (both severity and incidence) on water hyacinth recorded fifteen days after inoculation varied significantly (p ≤ 0.05) among the treatments (Table 3). The highest disease incidence (93.00%) with the severity of 4.67 in 1-6 disease severity rating scale was recorded on plants treated with *A. alternata*. This was followed by disease incidence of 87.67% and severity of 5.67 on plants treated with *Pythium ultimum*. *Fusarium chlamydosporum*, *Fusarium equiseti* and *Ascochyta chartarum* were also the potential pathogenic isolates which scored between 4.33 to 5.33 severity and 81.00 to 86.67% disease incidence respectively. So, the above fungi species which had a severity score of greater than four in 1-6 severity rating scale were considered promising. However, *A. geophila, E. nigrum, C. carbonum* and *S. vesicarium* showed relatively mild virulent reaction in this test.

Similarly, pathogencity tests of indigenous fungal pathogens on water hyacinth were conducted in different countries including Ethiopia. For example, surveys of fungal pathogens from water hyacinth infested water bodies like Lake Victoria, Lake Naivasha and Nairobi Dam in Kenya came up with 20 strains of pathogenic fungi. Pathogenicity tests were also carried out and *Cercospora, Fusarium* and *Alternaria* spp. were diagnosed as potential mycoherbicides (Mailu et al., 1998). A pathogenicity study on *F. equiseti* by Firehun et al. (2006) showed up to 98.3% infection. Naseema et al. (2001) reported 62.7% infection due to this pathogen. Martinez and Charudattan (1998) reported that *Alternaria* spp. and *Fusarium* spp. were highly virulent and severely damaged the inoculated water hyacinth leaves. Thus these two fungi were considered to be potential bioherbicide agents of water hyacinth. These authors grouped the fungal genera, such as *Cochliobolus, Epicoccum* and *Stemphylium as* mildly virulent isolates. Aneja and Srinivas (1992) recorded *F. chlamydosporium* as new potential pathogen on *E. crassipes* in India. Moreover, the potential of *A. alternata* as biocontrol agent was reported by Abbas et al. (1995). Accordingly, AAL-toxin, a product of *A. alternata*, is effective as an herbicide at low concentrations against a range of broadleaf plants, including *E. crassipes*.

There was also significant variation (p ≤ 0.05)

among fungal species in reducing plant height, shoot dry weight and the number of flower produced. The highest relative plant height reduction (54.27%) recorded was due to *F. chlamydosporum.* This was followed by *F. equisiti* which caused 50.60% relative reduction. Whereas the lowest (5.99%) reduction occurred on plants treated with *A. geophila*. This indicates that pathogenic isolates also played a great role in affecting the reproductive process of the weed. The reduced plant height following exposure to fungal pathogen suggests that the number of reproductive materials, vegetative propagules resulting in young water hyacinth would be reduced. On the other hand, *A. alternata* caused the highest biomass reduction with a maximum score of 58.08% followed by species of *P. ultimum* which caused (55.08%) relative reduction. It was observed that the number of flowers produced by water hyacinth was significantly affected by the potential pathogenic fungi having variable effects. The highest number of flower aborted (28.33) was recorded on plants treated with *F. chlamydosporium* followed by *A. alternata* (25.33), while the lowest (4.00) was observed on control plants. This also indicates that pathogenic isolates also played a great role in affecting the reproductive process of the weed. The reduced shoot dry weight following exposure to fungal pathogen suggests that the number of reproductive materials both seed and vegetative propagules resulting in young water hyacinth would be reduced. Consequently the doubling time of the plant would be prolonged.

Similarly, studies confirm that use of potential fungal pathogens would result in reduced water hyacinth biomass (Martyn and Freeman, 1978; Charudattan et al., 1985; Shabana et al., 1995). On the other hand, Shabana (1997) reported significant physiological changes in water hyacinth infected with *Alternaria* spp., such as decrease in pigments, carbohydrates, and relative water content which negatively affect the growth and development of the weed. Julian et al. (2001) also indicated that potential fungal pathogens could reduce plant growth rate and production of new leaves and new stolons. Moreover, increased severity results in rotting of the lower petioles, water logging of the crown and gradual sinking of the plant.

Disease incidence due to fungal pathogens on water hyacinth had highly significant ($p \leq 0.01$) effect and positively correlation ($r = 0.63$) with the number of flowers aborted from water hyacinth. This also indicates that the fungal pathogens played a great role in aborting the potential seed-bearing flowers. As a result, the number of seeds which could result in new infestation decreased. Disease incidence on water hyacinth plant showed highly significant ($p \leq 0.01$) and negative association ($r = -0.74$) with plant height. This indicates that the pathogens had adverse effects on the growth of water hyacinth plant.

Abbas et al. (1995) and Mailu et al. (1998) also reported similar findings that biological control agents (either insects or pathogens) caused disintegration of original water hyacinth mats into smaller mats; stunted growth; decline in water hyacinth biomass, reduced flowering potential, reduced ramet (daughter plants) production and finally rotting of the petioles followed by sinking.

Conclusion

Based on the current findings, we can conclude that, *A. alternata, A. chartarum, F. chlamydosporium, F. equiseti* and *P. ultimum* could be used as an effective bio control agent against water hyacinth following performance evaluation under natural environmental conditions and their host specificity test. Besides, integration of these fungal agents with insect bio control led to a sustainable control of this weed.

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