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Microbial Inhibition to Water Hyacinth (*Eichhornia Crassipes* [Martius] Solms-Laubach) in Abu Zirk Marsh by Using Selected Fungi

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ABSTRACT

Native fungi associated with water hyacinth have been identified and evaluated the potential of biological control against waterhyacinth in Abu zirk marsh (south of Iraq).Twelve species of fungi associated with different parts of the waterhyacinth (leaf, petiole) were isolated under sterile conditions in the laboratory.These fungi belong to genera Ascomycota (*Alternaria alternate, Stemphyllium* sp,,*Aspergillus niger,A.flavus,A.ostianus,A.candidus,A.fumigatus,Penicillumfumiculosum.Trichodermharzianum,T. lignorum*,Basidiomycota (*Rhizoctonia solani)* Zygomycota(*Rhizopus stolinifer*) environmental parameters such as temperature, relative humidity, pwere measured in field and in laboratory,the total occurrence of fungi reached to (146 colony *)A. alternata* and *R.solani* was more frequency *R.solani* showed the severity of disease 91.8%,and ability to change in the wet weight, number of leaves and the height of plant.Thus,evaluated *R. solani* more effective and severe plant death. And can be developed as an effective biological control.

Keywords: *Mycoherbicide, water hyacinth, Native bioagent, Abu zirk marsh*

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Introduction

Water hyacinth was found originally from the Amazon basin, and its entry into Africa,Asia,Australia,and North America has been facilitated through human activities,is belongs to the family Pontederiaceae, monocotyledon and aquatic macrophyte, free floating with beautiful clusters of violet and yellow flowers. Various methods to control water hyacinth include manual, mechanical,chemical and Biological control [1],which involves the use of natural enemies (insects and fungal pathogens)[2].Fungal pathogens have gained acceptance as a practical, safe environmentally friendly. 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. First appeared of Eichhornia *crassipes* in Iraq was in the mid-1980s, in Baghdad, after then spread to the Diyala river and parts of the Tigris river and attacked marshes, Including Abu Zirk marsh, which exposed to the invasion of water hyacinth in 2016.The amount of area infection with water hyacinth plants was about 2265875 $Km^2$and thus the percentage reached to 18% [3].The rapid and intensive growth of the water hyacinth plant has caused environmental and economic damage in the marsh. The main objective of this study is development of an effective bio-control strategy for water hyacinth by using fungal pathogens as bioagents, in Abu Zirk marsh by isolating and diagnosing the native fungi associated with the water hyacinth and its pathogenic capacity. The article is the first study in the marshes of southern Iraq.

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Materials and Method

Area Study: Abu Zirk marsh is located in Al-Islah District (50 km) east of Al-Nasiriya city. They were located between latitude lines (33°00'-31°40') N, and between longitude lines(45°10'-48°14') E. The marshes are located in a natural depression of an estimated size120 million cubic meters, and the marsh is filled with grasses of reed, papyrus and run through by a stream of a head called Shatt Abu Lahya Figur(1)

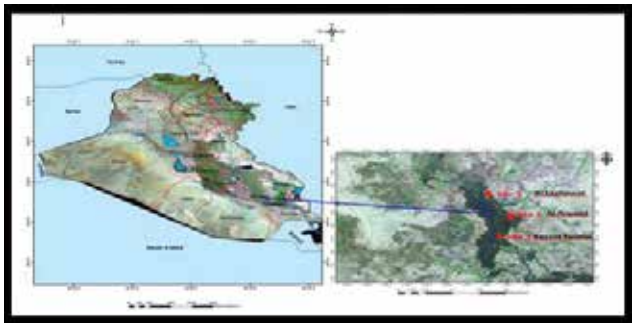

Figure 1: Abu zirk marsh (Google Earth, 2018)

Sampling Collected and Isolation Pathogen: Diseased water hyacinth leaves (showing browning, wilting, yellowing, spots, blights, or combinations thereof) were collected from water bodies in three sites within Abu zirk marsh this site called (1) Al-laghawat, site (2) Al-Rramid, site (3) Sayyid yashua. Infected leaves were collected in sterilized plastic container. Isolation of the pathogen followed the standard procedure on water hyacinth dextrose agar (WHDA) medium. The constituents were as follows (Water hyacinth leaves-200.0 g, Dextrose-15.0 g, Agar-agar- 20.0 g, Distilled water- 1000.0 ml, pH-5.6. Water hyacinth leaves (200 g) were washed in running tap water and then in distilled water. These were boiled for 15-20 min in 500 ml distilled water and filtered through thin gauze cloth for the collection of extract. The rest of the procedure was similar to the PDA pre-paration) [4]. The infected leaves were washed in running tap water to remove soil particles. The infected portions of leaf tissue were from the margins of necrotic or lesions cut into 3-mm pieces by using a sterile scalpel and surface-disinfected for one minute in 10% sodium hypochlorite. The tissue pieces were rinsed three times with sterile water and dried with sterile blotting-pape. Four tissue pieces were done on (WHDA) enriched with chloramphenicol (0.1 mg/l) in order to avoid bacterial growth, pieces were aseptically transferred to the inoculated plates were incubated at 25C° for 3-5 days**.** The fungal colonies were sub cultured individually by using a cork borer (5-mm diameter) and transferred on to freshly prepared PDA media amended with in chloramphenicol (0.1 mg/l) in 80-mm Petri dishes. The plates containing each isolate were arranged in a completely randomized design. The Petri dishes were checked for fungal growth after 4 -7 days and thereafter on a daily basis for 20–30 days. This action was repeated three time until pure cultures were obtained pure colonies of the isolates were grown by single-spore or hyphal-tip techniques. The isolates were stored on agar slants in 6 C° in a refrigerator. Microscopic observation of each isolate was done with an optical microscope at a magnification of40× and fungal isolates were characterized morphologically under a microscope based on their mycelium, fruiting structures and spores and identified according reference to the key of [5], The frequency of occurrence of the isolates were rated as very frequent (>20%), frequent (10%–20%) and infrequent (<10%) according to [6].

Pathogenicity test: Young healthy leaves of water hyacinth were washed under running tap water and then wiped with cotton swab dipped in 70% alcohol. The surface of leaves was injurey by pricking with a sterilized needle. One mycelial disc of 7days old culture was placed on both injured and healthy leaf each, and then covered these leaves with moist sterilized cotton. After these leaves were placed in a sterilized petri dish. At the bottom of the petri dish, sterilized moist filter paper was kept. Plates were incubated at 25 C °. After verifying the disease on the health water hyacinth leaf, the disease was tested on the growing water hyacinth in plastic container measuring (45 cm, 35cm, 20 cm), was kept in Marsh Research Center, Thiqar University. And fill a soil from the same sites was weighed with a kilogram of weight with field water 10 liters, and provided with an oxygen compressors throughout the study period, plants were placed in a plastic container, one plant homogeneous in weight and size as three replicates for each plant. After 7days of acclimate follow the growth Characterizes had taken such as wet weight/gm, total length of plant/cm, total length of root/cm, numbers of leaves/cm) addition to the daily monitoring of air and water temperatures and the relative humidity ratio. Corn oil emulsions were prepared with 35% v:v corn oil amended with 15% lecithin and 5% Tween 20, was diluted in sterile distilled water containing the spores (at a concentration of $1x10^4$ spores/ml in the final oil-water emulsion) [7], 20 ml of the suspension was applied on to water hyacinth leaves by using hand sprayers. The various plant species were also left untreated as controls. Plants were rated for disease symptoms including leaf spots, leaf lesions, and leaf death, and identification of the severity of the pathogenicity of the selected fungus such as *Alternaria alternata* and *Rhizoctonia solani*.

Statistical analysis: $P<0.05$ was chosen as a level of significance for all analyses. SPSS version 17.0 was used for all statistical tests (t- test)

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Results and Discussion

Isolation of Fungi: Twelve fungal isolated from different parts in water hyacinth (leaves and petioel). These fungi belong to three genera. The common genera was Ascomycetes (*Alternaria alternata*, *Stemphyllium* sp**,** *Aspergillus niger, A.flavus, A.ostianus, A. candidus, A. fumigatus, Penicillum fumiculosum. Trichoderma harzianum, T. lignorum* the second genera was *Basidiomycota* (*Rhizoctonia solani***),** and third genera was zygomycota (*Rhizopus stolinifer).* The total colony reached to (146 colony).Table (1)showed that *Rhizoctonia solani* was more frequency(61.49%),also *Alternaria alternate* reached to, (60%),but the frequency of *Penicillum fumiculosum* (28.6%), *Trichoderma harzianum* (12.87%), *T. lignorum, Rhizopus stolinifer* reached to (8.58%),,*Aspergillus niger, A.ostianus*(5.7%), *A. flavus, A. candidus*, *A. fumigatus* and *Stemphyllium* sp reached to (4.2%). For tests and evaluated of severity disease and determined as biocontrol agents waterhyacinth. The statistical methods showed significant differenced were found between the *A. alternata* (leaves), (petiol) and *R.solani* (leaves), (petiol).

Table 1: Isolated fungal species associated with water hyacinth in Abu zirk marsh

Fungal species	S (1) Number		S (2) Number		S (3) Number		Total Colony	Frequency %
	P	L	P	L	P	L		
Alternaria alternate	4	10	2	12	5	9	42	60 %*
Rhizoctonia solani	6	10	4	9	3	11	43	61.49%*
Penicillum fumiculosum	2	5	1	3	2	7	20	28.6%*
Aspergillus niger	0	1	0	1	1	1	4	5.7%*
A.fumigatus	0	1	0	1	0	1	3	4.2%
A.candidus	0	1	0	0	1	1	3	4.2%
A.flavus	0	1	0	1	0	1	3	4.2%
A. ostianus	0	1	1	1	0	1	4	5.7%
Trichoderma lignorum	1	2	0	1	1	1	6	.58%*8
Trichoderma harzianum	1	2	1	1	1	3	9	*% 12.87
Stemphyllium sp	0	1	0	1	0	1	3	4.2%*
Rhizopus stolinife	1	1	0	1	1	2	6	8.58%
Total	15	36	9	32	15	29	146	
L)leaves,(p), Petoile, (S)site, (0)No recored, (*) significant p <0.05)								

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The total occurrence of fungi colony reached to (146 colony).These are widely distributed taxa,. A alternata has been described as a pathogen of water hyacinth in Bangladesh Australia, India and Egypt [8] in the present study have also been reported from El-Morsy screened 22 fungal isolates for their ability to infect waterhyacinth out of which Alternaria alternata, showed 79%, tissue death after four weeks post inoculation[6]. In the present study, Rhizoctonia solani has increased its susceptible of diseases severity to the water hyacinth compared with the rest of the accompanying fungi. [9].

Pathogenecity test: Table (2) showed, the growth development of water hyacinth.The same wet weight was selected in all treatments,with air temperature 40 °C and relative humidity 17%. The other growth parameters were different between the three treatments (1, 2, 3). The total plant height in treatments (1,2,3),were (34 cm,35 cm, 30 cm), root length (23 cm, 20 cm, 16 cm),Numbers of healthy leaves were (60, 57, 40) and the total chlorophyll concentration was(35, 32, 35) respectively in the present study have also been reported from Yirefu [7]. The statistical method showed that significant differences between treatment.

Table 2: Growth characters of water hyacinth as a control

Growth characters	Control		
	Container 1	Container 2	Container 3
Air temperature	40	40	40
Relative humidity	17	17	17
wet weight gm./m^2	105*	105*	105*
Plant height/cm	34	35	30
Root length/cm	23	20	16
Numbers of leaves in plant/cm^2	*60	*57	*40
Total Chlorophyll	35	32	*35
(*) significant *p <0.05*			

Table (3) showed that *R. solani* was more effected with treatment *A.alternata* in plant growth. The wet weight was deceased to 85gm/cm when comparative with control (treatment 3). The statistical method showed that significant difference between these treatment, also plant height was 10 cm,but the numbers of infections leaves were increased to 53/m^2 when compared with control,in the same time the chlorophyll concentration was decreased to 2,but the disease severity was increased to 92.98% also the statistical method showed that significant differences between these treatment.

Table 3: Growth characters of water hyacinth after treatment with *Alternaria alternata* and *Rhizoctonia solani*

Growth characters	Treatment		
	1	2	3
Air temperature ċ	40	40	40
Relative humidity	17	17	17
wet weight gm/cm^2	*94	*85	*120
Plant height/cm	15	10	37
Root length/cm	23	19	17
Numbers of infected leaves/m^2	*52	*53	3
Total Chlorophyll	5	2	*33
disease Severity **DS%**	86	*92.98	*7.5
treatment (1) *A. alternata* suspension, treatment (2) *R. solani* suspension, treatment (3) control (*) significant $p < 0.05$			

We have focused on two fungal pathogens (*Alternaria alternata* and *Rhizoctonia* solani) isolated from diseased hyacinths to develop them as effective agents in biological control. According to [10], the effect of mycoherbicide on water hyacinth can be determined based on its ability to limit host plant development, fewer living, leaves, and more death leaves on individual plants, rather than producing new clones. Analysis of the disease test results indicated that most fungal species effect on water hyacinth. The results showed that there were differences between isolates in the severity of the disease on the water hyacinth were recorded after 6 weeks of treatment in vitro. The severity of the disease for both *Alternaria alternata* and *Rhizoctonia* solani has played an important role in reducing biomass (wet weigh) and plant growth number of leaves, daughters plant. *Alternaria alternata* and *Rhizoctonia* solani as pathogens that will lead to a decrease in plant productivity of biomass. On the other hand, lead to major changes in the physiological characters such as low carbohydrate and chlorophyll and changes in water content [11]. Refer that this fungus caused disintegration of original water hyacinth mats into smaller mats, stunted growth, decline in water hyacinth biomass, reduced flowering potential, reduced daughter plants production and finally rotting of the petioles followed by sinking.

Infected water hyacinth leaves showed necrotic type spots, zonate leaf spots and blight symptoms with varying severities,

Histopathology of plant tissue: Figure (2,3) showed the infection of water hyacienth by treatment with *R.solani* and *A. alternata* and also the penetration of both fungi the epidermis of the upper surface and the lower surface of the leaf through the stomata and penetration of tissue petiole led to damage in the tissues of the palisade layer, and the stability of the fungi in the aerenchyma tissue of the leaf and tissues petiole,these infection with pathogenic fungi causes change in the size spongy layer compared to control,The Transmission Electron Microscopy (TEM) also demonstrates the stability of the fungus hyphae of *Rhizoctonia solani* and spores of *Alternaria alternata*

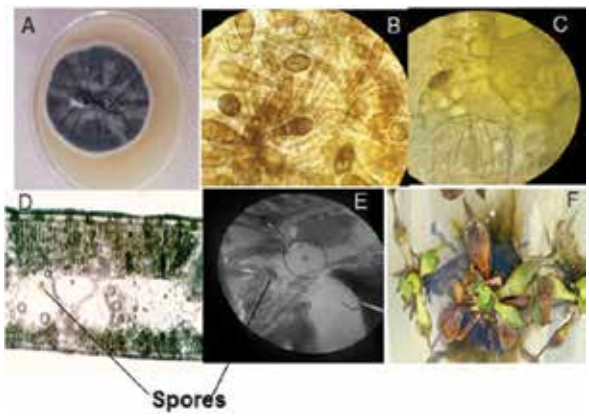

Figure 2: (A) Colony of *Alternaria alternata* on PDA (B) Conidia of *A.alternata* under light microscope (C, D) *Alternaria alternata* spores penetration the epidermis leave of water hyacinth and causing damage in the tissue of leaf (E) TEM micrograph of a spores of *A. alternata* located in the intercellular space of water hyacinth leaf (F) water hyacinth after infection with *A.alternata*.

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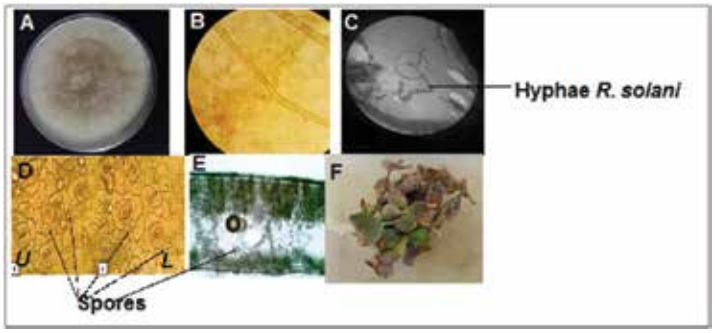

Figure 3: (A) Colony of *Rhizoctonia solani* on pDA (B) hyphae of *R.solani* under light microscope (C) TEM micrograph of a hypha of *R.solani* located in the intercellular space of water hyacinth leaf (D.E) *Rhizoctonia solani* spores penetration epidermis leave of water hyacinth and causing damage in the tissue(F)water hyacinth after infection with *Rhizoctonia solani*

Conclusion

Based on the current findings, we can conclude that, *Alternaria. alternata, Rhizoctonia solani,* could be used as an effective bio control agent against water hyacinth following performance evaluation under natural environmental conditions and their host specificity test.

Conflict of Interest: None of the authors have any conflicts of interest to declare.

Source of Funding: The research was performed independently, there is no funding, influence over study design, analysis, manuscript preparation, or scientific publication.

Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

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REFERENCES

1. Adegunloye DV, Olosunde SY, Omokanju AB. Evaluation of ratio variation of water hyacinth (*Eichhornia crassipes*) on the production of pig dung biogas. Int. J. Biol. Sci., 2013, 2: 44-48.
2. Firehun Y, Struik PC, Lantinga EA, Taye T.Joint use of insects and fungal pathogens in the management of water hyacinth (Eichhornia crassipes): perspectives for Ethiopia. J Aquat Plant Manage. 2013, 51:109–121
3. Marshlands Rehabilitation Center and wetland of Iraqi. Report, to the Ministry of Water Resources Iraq. 2018.
4. Aneja KR. *Experiments in Microbiology, Plant Pathology and Biotechnology*. 4th ed. New Delhi: New Age International Publishers. 2003.
5. Ainsworth GC, Sparrow FK, Sussman AS. The Fungi, an Advanced Treatise. Vol. IV A: A Taxonomic Review with Keys: Ascomycetes and Fungi Imperfecti. 621 S., 39 Abb., 78 Tafeln. New York-London. Academic Press Inc. 1973.
6. El-Morsy ME. Evaluation of microfungi for biological control of water hyacinth in Egypt. Fungal Diver. 2004, 16:35–51.
7. Yirefu Firehun, Struik PC, Lantingaa EA, Taye Tessema. Occurrence and diversity of fungal pathogens associated with water hyacinth and their potential as biocontrol agents in the Rift Valley of Ethiopia, Journal International of pest management, 2017 vol. 63,No.4,355-363
8. Elwakil MA, Fayzalla EA, Sadik EA, Shabana YM. 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. 1989.
9. Opand GT, David M Musyimi, Hillary T Nyanganga. Isolation and Identification Rhizoctonia Solani on the Water Hyacinth (Eichhornia Crassipes [Mart] Solms. Laubach) in the Winam Gulf (Lake Victoria, Kenya). IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) e-ISSN: 2319-2380, p-ISSN: 2319-2372. 2017. Volume 10, Issue 8 Ver. I (August 2017), PP 39-43
10. Shabana YM. The use of oil emulsions for improvising the efficacy of *Alternaria eichhorniae* as a mycoherbicide for water hyacinth (*Eichhornia crassipes*). Biol. Control. 2005, 32 (1): 78-89. 149:21-27
11. Julien MH, Griffiths MW, Stanley JN. Biological control of water hyacinth: The moths *Niphograpta albiguttalis* and *Xubida infusellus*: biologies, host ranges, rearing, releasing and monitoring techniques. ACIAR Monograph, 2001. pp. 79-91