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Detection of fungi associated with water hyacinth *Eichhornia crassipes* in Iraq and their pathogenicity under controlled condition

Hurria Hussien Al-Juboory*[1], Hussein Sadeq Musa[2]

[1]*College of Agriculture, University of Baghdad, Iraq*

[2]*Ministry of Agriculture, Baghdad, Iraq*

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Abstract

The study was carried out in the laboratories at the Faculty of Agriculture, Baghdad University to isolate and identify the species of fungi that associated with water Hyacinth *Eichhornia crassipes*. The samples were collected from Tigris river side's at Al- Kraat area in north of Baghdad, Iraq. The presence ratios of fungi were recorded and their pathogenicity was tested. Results of isolation and identification showed the presence of fourteen fungi associated with water Hyacinth leaves including; *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger*, *Drechslera* sp., *Chaetomium* sp., *Cladosporium* sp., *Fusarium solani*, *Macrophomina phaseolin*, *Mucor* sp., Mycelia Sterile Fungi, *Pythium aphanidermatum*, *Ulocladium* sp., *Rhizopus* sp. and *Trichoderma* sp. at different percentages . However, the percentages of presence were deferent and the most frequently were A. *alternata* and *Rhizopus* sp. reached 76.33% and 80.40 % respectively. The percentages of the other fungi were ranged between 4.25% -30.60 %. It has been found that nine of these fungi, the more prevalent, showed high capacity of inducing infection on Water hyacinth leaves at percentages ranged between44.4% – 100% compared with zero infection in control. *Macrophomina phaseolina, Pythium aphanidermatum* and *Rhizopus* sp. were found to be the more pathogenic with disease severity attained to 100 %. Different symptoms were developed on the leaves inoculated with different fungi as spotting and wilting followed by leaves dryness. This is the first report for fungi associated with water hyacinth leaves at Al-Kraat area in Iraq.

***Corresponding Author:** Hurria Hussien Al-Juboory ✉ hhaljboory@yahoo.co.nz

Introduction

Water hyacinth *Eichhornia crassipes*, Family Pontederiaceae is one of the more serious aquatic floating weeds in fresh and stagnant water (Lata and Dubey,) 2010. The weed is of South America origin and currently is widely distributed throughout the world (Telleze *et al.,* 2008).

Water hyacinth was first noted in Iraq during middle of 1980s in some nursery beside Tigris River as ornamental plant (Chalabi, 1992; Al-Rubaie and Al-chamsy, 2010). Now, the weed has become very serious forming dense impenetrable mats of vegetation in rice fields, lakes, streams, rivers and flood irrigation canals (Fiaad, 2008; Ibrahim, 2009). The wide spread of this weed, mainly as a result of human activities and lack of biotic enemies has led to affect the environment of peoples living beside the river through forming dens mats that impede water flow and irrigation and constitute a medium for insects transmitting diseases (Howard and Matindi , 2003; Tegena, *et al.,* 2012).

In addition, the formation of dense mats of water hyacinth on water surface cause reduction of oxygen in the water and prevent the arrival of sun light to the bio- organisms in the river and lakes leading to disturb the natural balance in the nutritional chains (shanab *et al.,* 2010).

Different methods were used to control water hyacinth weed including chemical, physical and biological. Dichlorophenoxy acetic acid (2, 4-D), Diquat, Paraquat and Glyphosate were used to control this weed (Telleze *et al.,* 2008; Al-Wagga and Sultan, 2012). Due to enormous problems treaded by the chemicals used to control water Hyacinth, the research was oriented toward biological control using insects (Center *et al.,* 2005; AL-Shammary, 2012) and phytopathogenic fungi as an alternative method. Several phytopathogenic fungi isolated from the weed have been used in several infested areas in the world. Among the various phytopathogenic fungi; *Acremonium zonatum, Alternaria alternata, A. eichhorniae, Ascochyta chartarum, F. chlamydosporium, F. equiseti, F. oxysporum*, *F. pallidoroseum*, *Pythium ultimum*, *Pythium aphanidermatum, Rhizoctonia solani* and *Stemphylium vesicarum* were found successful (El-Morsy, 2004; Pathak and Kannan, 2011; Ray and Hill, 2012; Tegen *et al.,* 2012; Euloge *et al.,* 2016). Because of the wide spread of this weed in the streams rivers , irrigation and flood canals in Iraq and the problems created ,the study was conducted to isolate and identify the phytopathogenic fungi associated with water hyacinth leaves and test their pathogenicity under laboratory conditions .

Materials and methods

Isolation and identification of fungi associated with water hyacinth weed Samples collection

Leaves of water hyacinth showing symptoms of yellowing blight, spotting and dryness, suspected to be phytopathogenic fungi infection were collected from Al- Kraat area (Northeast of Baghdad). The symptomatic leaves were washed in running tap water to remove soil particles and dissected to small pieces of 0.5 -1 cm.

Isolation of fungi

The pieces of symptomatic leaves were surface sterilized in 2% sodium hypochlorite for 2min. rinsed in sterile distilled water to eliminate the disinfectant used and dried on sterile filter paper .The pieces were then cultivated on PDA medium in petri plates of 9cm diameter (4 pieces/plate) and maintained at 25± 2C° for 5 days.

Identification of fungi

The fungi growing on PDA were purified and identified to genus level based on morphology and cultural characteristics as described by Barnett and Hunter (1998), Leslie and Summerell (2006) .The purified cultures of the isolates were conserved on PDA slant and the frequency of occurrence of each fungus in the collection was determined using the formula ;

$$\text{Frequency}(\%) = \frac{\text{Number of pieces containing the fungus}}{\text{Total number. of pieces}} \times 100.$$

(Ray and Hill, 2012)

Pathogenicity

The ability of nine isolates ,found associated with water hyacinth leaves**,** including; *Alternaria* sp. , *Aspergillus flavus, Aspergillus niger*, *Drechslera* sp., *Fusarium solani*, *Macrophomina phaseolina*, Mycelia sterile funs, *Pythium aphanidermatum* and *Rhizopus* sp. to infect water hyacinth leaves was tested as described by Aneja (2003).

New healthy leaves of water hyacinth were collected from Al-Kraat area, washed in running tap water to remove soil particles .The leaves were surface sterilized by 2% sodium hypochlorite for 2min, rinsed in sterile distilled water and placed in petri plates of 20 cm diameter (2 leaves/petri plates) containing a layer of moist cotton and autoclaved at 121°C and 1.5 kg /cm2 for 20 min. The leaves then were inoculated with 2mm discs of fungal isolates taken from edges of actively growing cultures on PDA,5 days old ,and maintained at 25± 2C°for 5 days .Three petri plates for each isolate were used . The disease severity was estimated after 7 days of inoculation using a disease index of 5 scales framed by modified Naseema *et al.* (2001) and designazed as 0= No symptoms, 1 = 1-9% of the leaf area showing symptoms around the inoculum site, 2 = 10-25% of the leaf area showing yellowing or browning,3= 26-50% of the leaf area showing symptoms, 4= 51-100% of the leaf area showing symptoms associated with dryness by the formula:

$$\text{Disease severity} = \frac{\text{Number of leaves of scale}_1 \times 1 + \ldots\ldots + \text{Number of leaves of scale } 5 \times 5}{\text{Number of total leaves tested} \times 5} \times 100.$$

(McKinney, 1923)

Results and discussion

Isolation and identification

Different fungi were isolated from symptomatic leaves and petioles of water of hyacinth one PDA .Based on cultural and morphological characteristic of fungi isolates obtained 14 genus and species including *Alternaria* sp.*, Aspergillus flavus*, *Aspergillus niger*, *Drechslera* sp., *Chaetomium* sp., *Cladosporium* sp., *Fusarium solani*, *Macrophomina phaseolina*, *Mucor* sp., Mycelia Sterile Fungi, *Pythium aphanidermatum, Ulocladium* sp., *Rhizopus* sp., and *Trichoderma* sp.were identified at frequency of occurrence ranged between 4.25 -30.60 % (Fig. 1) of these fungi, *Rhizopus* sp. and *Alternaria* sp. were appeared with high frequency of occurrence, 80.40 % and 76.33% respectively. Mycelia Sterile fungi, *Aspergillus flavus*, *Aspergillus* sp., *Macrophomina phaseolina*, *Fusarium solani,* and *Pythium aphanidermatum* were found at moderate occurrence, 11.0,15.0, 27.50,20,50,22.50,and 30.60 % respectively. While the fungi *Chaetomium* sp., *Cladosporium* sp., *Mucor* sp., and *Ulocladium* sp. were appeared at low occurrence with frequency of 4.25 %, Fig. 1. These fungi were excluded from the next experiment.

Table 1. Pathogenicity of the fungi isolated from disease plants of water hyacinth under lab. Conditions

Fungi	Disease severity (%)
Alternaria sp. (Fres.) Keissler.	83.3
Aspergillus flavus Link ex Gray	44.4
Aspergillus niger Van Tieghem	66.7
Drechslera sp. Subram. & B. L. Jain 1966	94.4.
Fusarium solani (Mart.) Sacc.	66.7
Macrophomina phaseolina (Tassi) Goid	100.0
Mycelia Sterile Fungi	50.0
*Pythium aphanidermatum (*Edson) Fitzp	100.0
Rhizopus sp.	100.0
Control	0.00
LSD Value (P≤0.05).	23.18

The results indicated high diversity of pathogenic fungi associated with water hyacinth. These results are in accordance with the results of several studies conducted in different areas in the world. (Tegen *et al.,* 2012; Ray and Hil , 2012; Euloge *et al.,* 2016).

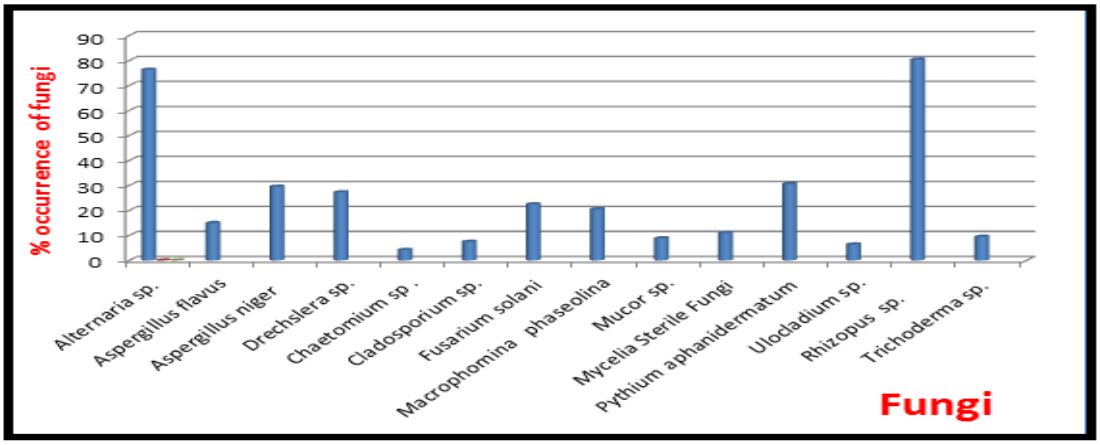

Fig. 1. The percentage of occurrence of fungi on the leaves of water hyacinth in the area Alkraat/Iraq.

Pathogenicity tests

Results of the Pathogenicity test showed that all the nine fungi tested were able to infect water hyacinth with disease severity ranged between 44.4-100% (Table 1, Fig. 2,3,4) . Many previous studies reported that, *Alternaria* sp., *Pythium aphanidermatum* and *Fusarium* species were Pathogenic to water hyacinth weed with high specify and high ability of damaging this weed (Pathak and Kannan, 2011; Ray and Hill, 2012). Among the pathogenic fungi isolated from water hyacinth, *Rhizopus* sp. was found the more abundance at frequency 80.40% followed by *Alternaria* sp. at 76.33%.

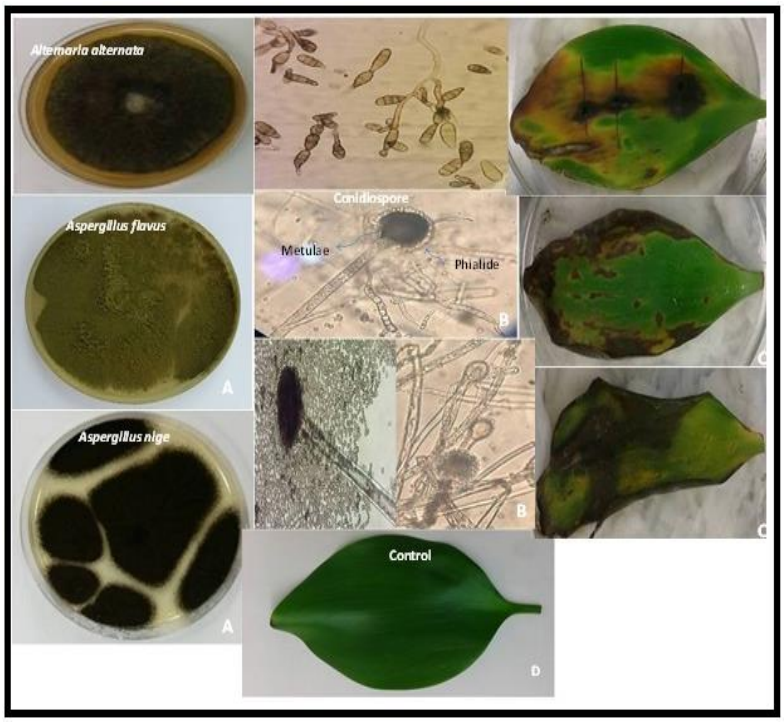

Fig. 2. Cultural and microscopic characteristics and the pathogenicity of *Alternaria* sp., *Aspergillus flavus* and *Aspergillus niger*. *A*= Growth of the fungal colony on the B= Non sexual structures (40x) C= The pathogenicity of fungi on water hyacinth leaves D= Control.

These two fungi showed high potency to infect water hyacinth leaves with disease severity 83.30 % and100 % respectively which may be promising for controlling the weed. Many authors have been reported that *Alternaria alternata* and *Alternaria eichhorniae* were found to be highly pathogenic to water hyacinth and extensively used against the weed (Pathak and Kannan, 2011; Tegen *et al*., 2012).

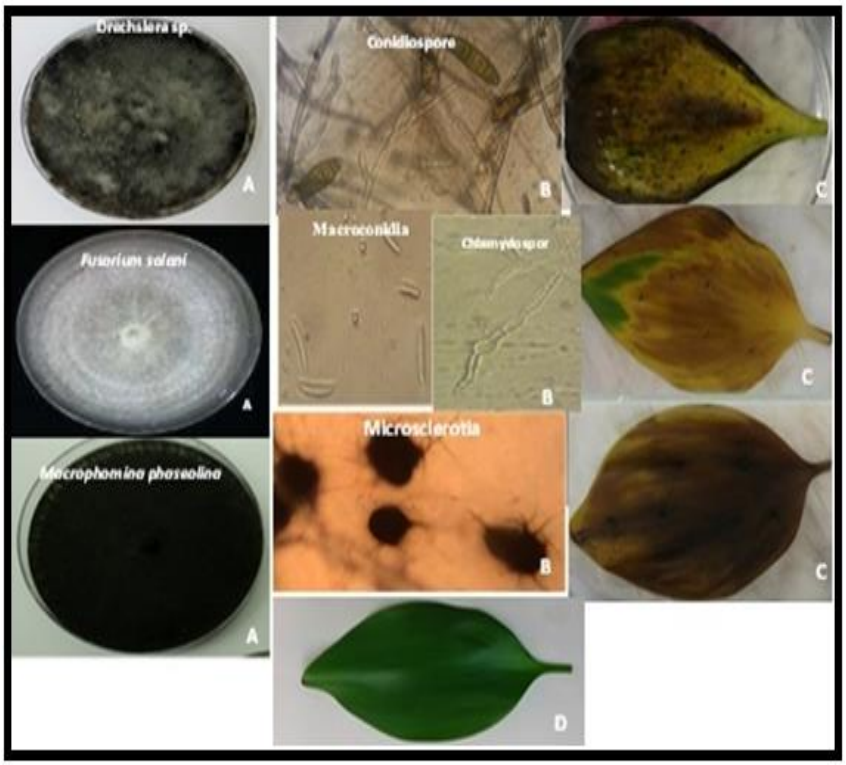

Fig. 3. Cultural and microscopic characteristics and the pathogenicity of *Drechslera* sp., *Fusarium solani* and *Macrophomina phaseolina*. A= Growth of the fungal colony on the B= Non sexual structures (40x) C= The pathogenicity of fungi on water hyacinth leaves D= Control.

Drechslera sp., *Macrophomina phaseolina, Pythium aphanidermatum* and *Rhizopus* sp. have showed high potency to infect water hyacinth leaves under lab condition with disease severity 94.4, 100, 100 and 100 % respectively, but with low abundance in the collection at frequency of 20, 27, 30 % respectively. The lower presence of these fungi in the collection may result from the high ability of the other fungi to grow which led to reduce the growth of these fungi. These fungi may be useful in combination with other methods to control the weed. It has been reported that the use of phytopathogenic fungi isolated from water hyacinth as bioagents to control the weed could be valuable if used in combination with chemical herbicide or with insect bio control agents or used as major supplement to lower doses of chemical herbicides (Nelson and Shearer 2005; AL-Shammary, 2012).

Several studies conducted in different areas of the world reported that many genes of pathogenic fungi were isolated from water hyacinth leaves including ; *Alternaria*, *Aspergillus, Curvularia* sp, *Fusarium*, *Penicillium, Pythium* and *Trichoderma* showing variable pathogenic effects on the same weed (Pathak and Kannan , 2011; Rey and Hill, 2013; Singh *et al.,* 2016).

The results of this study demonstrated the ability of some fungi isolated from water hyacinth weed to infect and damage the inoculation leaves of the weed under lab conditions, but further studies are needed to determine the activity of these fungi as biological control agents in the field.

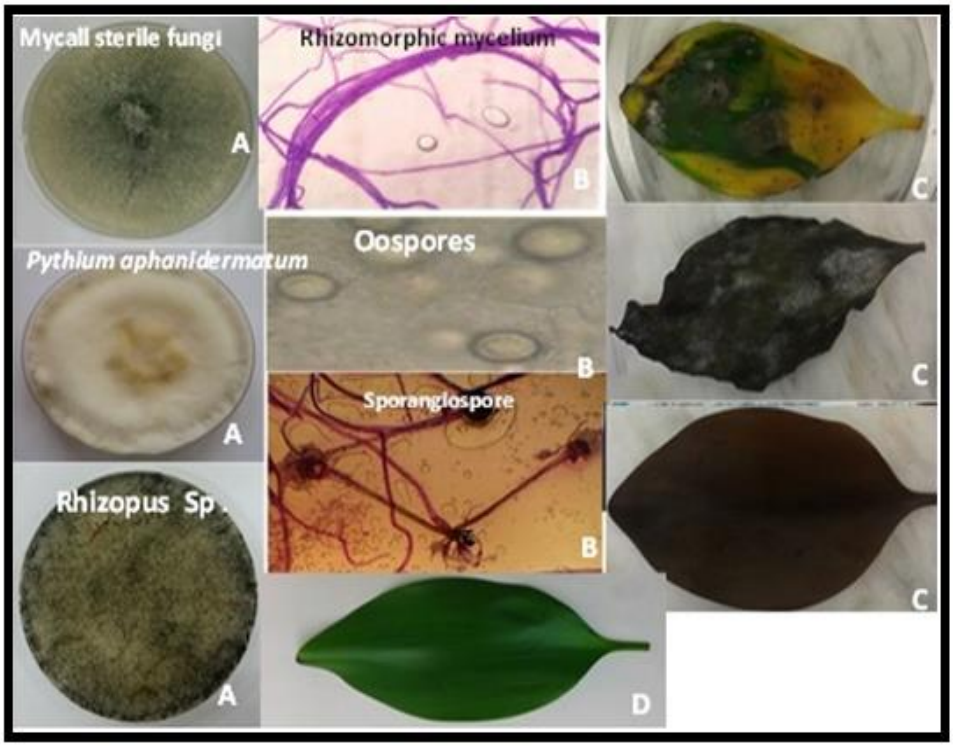

Fig. 4. Cultural and microscopic characteristics and the pathogenicity of Mycelia Sterile Fungi, *Pythium aphanidermatum* and *Rhizopus* sp., A= Growth of the fungal colony on the PDA B= Non sexual structures (40x) C= The pathogenicity of fungi on water hyacinth leaves D= Control.

Conclusion

As conclusion of this study we found many species of pathogenic fungi on *Eichhornia crassipes,* we isolates these fungi from the leaves *Alternaria* sp., *Aspergillus flavus*, *Aspergillus niger*, *Drechslera* sp., *Chaetomium* sp., *Cladosporium* sp., *Fusarium solani*, *Macrophomina phaseolina*, *Mucor* sp., Mycelia Sterile Fungi, *Pythium aphanidermatum*, *Ulocladium* sp., *Rhizopus* sp. and *Trichoderma* sp. However the most frequently fungi was A. *alternata* and *Rhizopus* sp. We found three kind of fungi *Macrophomina phaseolia, Pythium aphanidermatum* and *Rhizopus* sp. was the most pathogenic with disease severity attained to 100 %. Different symptoms were developed on the leaves inoculated with different fungi as spotting and wilting followed by leaves dryness. This is the first report for fungi associated with water hyacinth leaves at Al-Kraat area in Iraq.

Recommendation

This is a first report of these pathogenic fungi on *Eichhornia crassipes* in Iraq, these fungi need more study to use it as biological control agent on the host, also standing the host range of these fungi is very important on other cultivated crops.

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