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A new strain of *Alternaria alternata* (AL-14) on water hyacinth from India

Kamal Rai Aneja[1]*, Pankaj Kumar[2], Chetan Sharma[3]

[1]Vaidyanath Research, Training and Diagnostic Centre, Kurukshetra-136118, India
[2]Department of Microbiology, Kurukshetra University, Kurukshetra-136119, India
[3]Department of Microbiology, Guru Nanak Khalsa College, Yamuna Nagar- 135001, India

Abstract: Water hyacinth is one of the most dangerous aquatic weeds not only in India but all around the globe. In the years 2011-13, extensive surveys of various ponds, pools, lakes and rivers were made with the objective to search, isolate and evaluate potential fungal pathogens on water hyacinth. During various surveys conducted in different regions of Haryana, Punjab and Uttar Pradesh, populations of water hyacinth in various ponds of Kurukshetra (Haryana) were found to be heavily affected by various types of leaf spots and leaf blotches. From the infected leaves, an isolate of *Alternaria* was isolated on water hyacinth dextrose agar (WHDA). Based on the symptomatology, cultural, morphological characteristics and molecular basis (nucleotide homology and phylogenetic analysis) it was found to be a new strain of *Alternaria alternata*. The pathogenicity and Koch's postulates have been proved in vitro.

Keywords: Water hyacinth, aquatic weed, leaf spot, pathogenicity, biological control

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Water hyacinth is one of the most troublesome aquatic weed all over the world. It is a free floating aquatic weed with beautiful liliac violet flowers and continues to be most dangerous aquatic weed (Babu et al. 2002). It is popularly known as Kullavazha, Shoksamundar, Jalkumbhi, Kulavali, Kochuri Pana etc (Parveena and Naseema, 2004). In Haryana (India), it is the most notorious aquatic weed distributed all over the state. In India this plant made its entry into Bengal before 1900. Most of the problems associated with this weed are due to its rapid growth rate, its ability to successfully compete with other aquatic plants and its ease of propagation (Aneja et al. 1993; Dagno et al. 2011). Its rapid multiplication and mat like proliferation can lower dissolved oxygen level leading to reduction of aquatic fish production (shanab et al. 2010). The weed cause water pollution, obstructs electricity generation, blocking irrigation, increase evapotranspiration resulting in water loss, provides habitat for different disease causing vectors (Ding et al. 2008; Tegene et al. 2012).

Keeping in view the significance of biocontrol agents over the chemical herbicides, emphasis is being laid to control this weed with mycoherbicides. Up to date, two mycoherbicides developed to control *Eichhornia crassipes* are; **ABG-5003**, a formulation of *Cercospora rodmanii* developed by Abbott laboratories registered in the United States(Freeman and Charudattan, 1984); **HYAKILL™** a formulation of the fungus *Sclerotinia sclerotiorum* patented in Europe in 2003 (De Jong et al. 2003).

The purpose of the present study was to search, isolate and evaluate new potential fungal pathogens for biological management of water hyacinth in India.

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MATERIALS AND METHODS

Surveys and isolation of pathogen

In a study conducted during 2011 to 2013, extensive surveys of the ponds, pools, lakes and rivers of Haryana, Punjab and Uttar Pradesh district were made for collection of fungal infected water hyacinth plants. The

Corresponding Author:
Aneja KR (✉) Vaidyanath Research, Training and Diagnostic Centre, Kurukshetra-136118, India
email: anejakr@yahoo.ca

infected leaves were collected in sterilized plastic bags and brought to the laboratory for further research. During surveys, different populations of water hyacinth at Kurukshetra (Haryana) were found to be heavily affected by different types of leaf spots (Fig. 1). Symptoms showed lesions of various types and of different sizes. Isolation of the pathogen followed the standard procedure on water hyacinth dextrose agar (WHDA) medium (Aneja, 2003). For isolation of the pathogen, the infected leaves were washed thoroughly in running tap water to remove soil particles. The infected portions of leaf tissue were cut into 3-mm pieces by using a sterile scalpel. These were surface sterilized in 70% ethyl alcohol for 30 seconds and then washed 6-7 times in sterilized water. Two to three such surface sterilized and washed pieces were aseptically transferred to WHDA plates supplemented with streptomycin (100 mg/l). The inoculated plates were incubated at 25°C for 3-5 days. The constituents of water hyacinth dextrose agar (WHDA) medium 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 cheese cloth for the collection of extract. The rest of the procedure was similar to the PDA preparation (Aneja, 2003).

Identification of pathogen

The fungus was identified based on the symptomatology, morphological characteristics, cultural characteristics and molecular basis by method of ITS rDNA sequence analysis using the FASTA algorithm by CABI, UK (IMI No.503250).

Purification and maintenance

For purification, a disc of mycelium cut from the margins of the growing colony, was transferred to fresh PDA plates. For maintenance, pure culture of each fungal isolate was transferred to PDA slants and maintained at 4°C for further use.

In-vitro pathogenicity test

Pathogenicity of the chosen isolate was determined in-vitro. Young healthy leaves of water hyacinth were

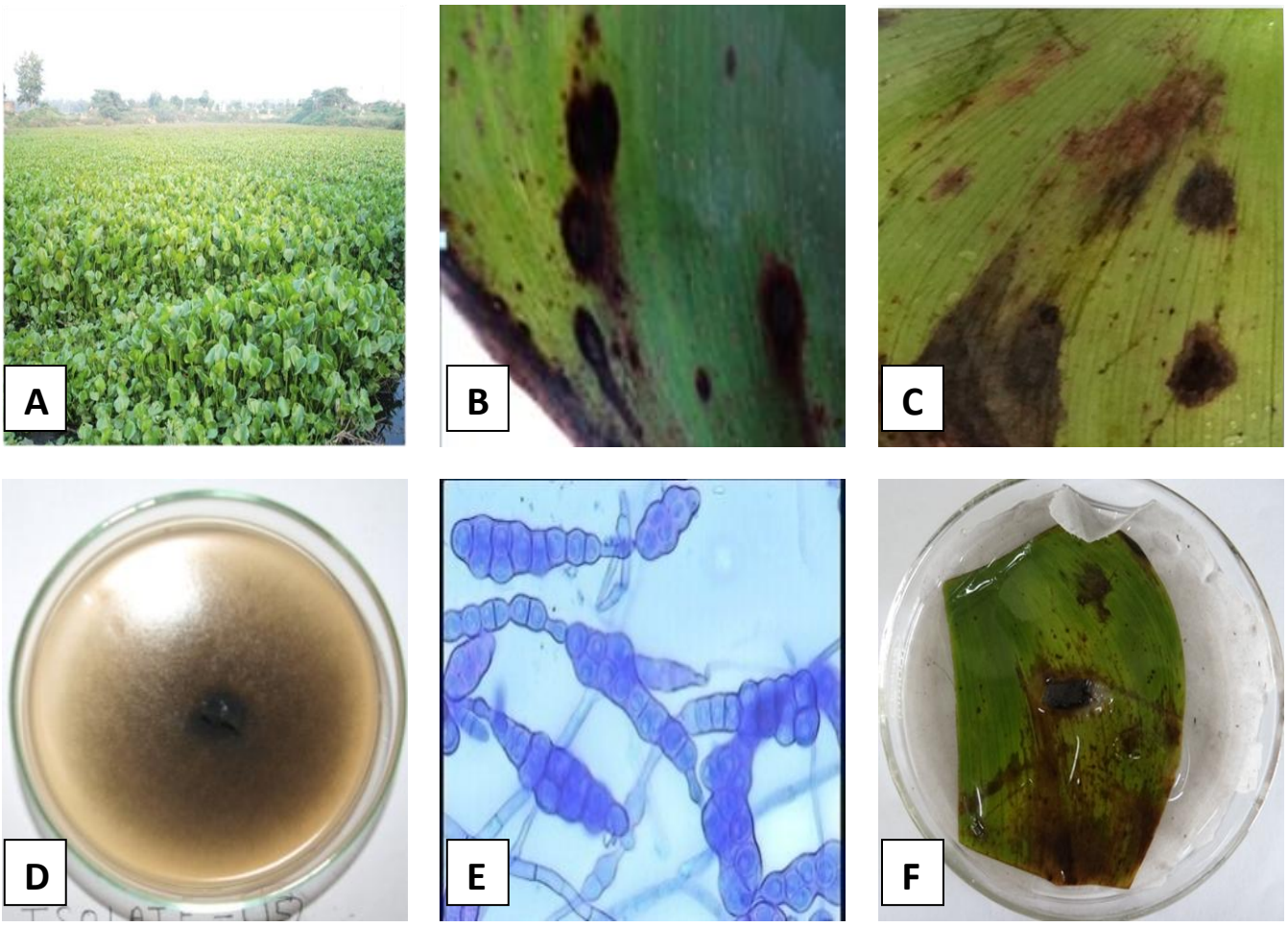

Figure 1(A) Pond affected by water hyacinth in Kurukshetra (India). *Alternaria alternata* new strain (AL-14) **(B&C)** Leaf spot symptoms. **(D)** Colonial characteristics on WHDA. **(E)** Large size muriform beaked conidia arranged in acropetal manner. **(F)** Pathogenicity test of the fungus on water hyacinth leaf

washed under running tap water and then wiped with cotton swab dipped in 70% alcohol. The leaves were injured on the surface by pricking with a sterilized needle. One mycelial disc of 7 days old culture was placed on both injured and healthy leaf each. These leaves were then covered with moist sterilized cotton. After this 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 (Aneja and Singh, 1989; Aneja, 2003).

RESULTS AND DISCUSSION

During surveys conducted in search of naturally occurring fungal pathogens of water hyacinth in Haryana, Punjab and Uttar Pradesh a new leaf spot disease was observed in August 2011, on water hyacinth from Kurukshetra (Haryana). Infected leaves were collected in sterilized polythene bags and brought to the laboratory for study of symptoms, isolation, purification and pathogenicity test of the fungal pathogen involved. Symptoms were charact-erized as large, black water soaked spots on leaves (Fig. 1(B). Isolation of the pathogen on water hyacinth dextrose agar (WHDA) yielded a fungus isolate (AL-14) which was identified as *Alternaria* sp. on the basis of morphological and cultural charact-eristics. Further on the molecular basis (nucleotide homology and phylo-genetic analysis) it was found to be a new strain of A. *alternata* by CABI, UK (IMI No. 503250, Gene Bank Accession Number: KF911322). Pathogenicity of the isolated pathogen was determined in vitro. During pathogenicity test, when 7 days old mycelial culture of this isolate were used to inoculate young healthy leaves of water hyacinth, symptoms were produced in the form of blackish and brownish small to large necrotic spots in both pricked and un-pricked leaves of water hyacinth. The pricked leaves were more susceptible to pathogen in comparison to un-pricked leaves. Re-isolation of the pathogen was done from inoculated leaves. This isolate was found to be similar to original isolate thus confirming the pathogenicity of *Alternaria alternata* (AL-14) to water hyacinth and also to prove Koch's postulates. Our results reveal that *Alternaria alternata* (AL-14) was pathogenic to water hyacinth which caused infection within 5-7 days on both shoots and leaves.

Identification based on microscopic characteristics

This pathogen induced large size spots and lesions both on leaves and stolen. Both the young and mature leaves were affected by these spots. Leaf margins has more affected by spots. When growing on water hyacinth dextrose agar media, the fungus produced light black to brownish colonies (Fig. 1C). Microscopic observation of the fungal isolate revealed that conidiophores are straight, septate, 12-47 x 2.5 µm long; large beak shaped conidia with 9-11 transverse septa and 2-4 longitudinal septa, 10-27 x 2.5 µm long (Fig. 1D).

Identification based on molecular characteristics

The isolated pathogen (IMI No.503250) has been identified by International Mycological Institute (IMI), CABI Bioscience, UK. The isolate was identified by ITS r DNA sequence analysis using the FASTA algorithm with the fungus database from EBI. DNA was isolated from the culture provided by the scientist. Its quality was evaluated on 1.2% Agarose Gel, a single band of high-molecular weight DNA has been observed. Fragment of D1/D2 region of LSU (Large subunit 28S rDNA) gene was amplified by PCR from the above isolated plasmid DNA. A single discrete PCR amplicon band of 650 bp was observed. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with DF and DR primers using BDT v3.1 cycle sequencing kit on ABI 3730xl Genetic Analyzer (Table 1).

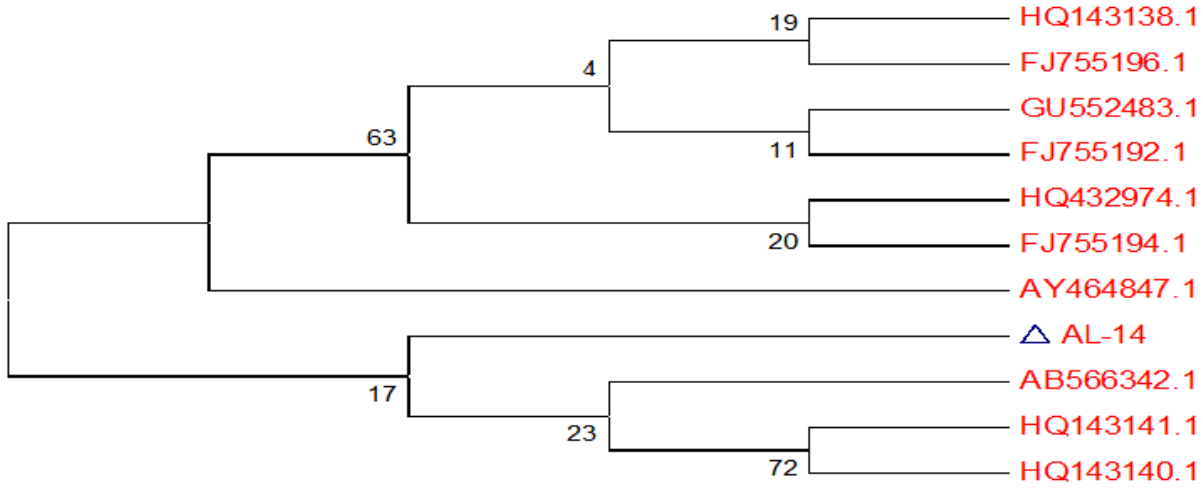

Figure 2 Phylogenetic tree using ITS sequences shows closest known relatives of *Alternaria alternata* (AL-14)

Table 1 PCR primers for amplification of genes of *Alternaria alternata* isolate AL-14

DF	ACCCGCTGAACTTAAGC
DR	GGTCCGTGTTTCAAGACGG

The sequence obtained from this sample showed 100% matches to various members of the genus *Alternaria*, including *A. alternata*, *A. porri*, *A. tenuissima* and to unnamed members of the genus. None of the top 50 matches have been published (Fig. 2).

Alternaria alternata is a type of cosmopolitan fungus and has been isolated from almost all habitats (EL-Morsy, 2004). Literature search reveals that the occurrence of *A. alternata* on water hyacinth from Bangladesh (Bardur, 1978), Australia (Galbraith and Hayward, 1984), Egypt (Elwakil et al. 1989; Shabana et al. 1995; El-Morsy, 2004), and India (Aneja and Singh, 1989; Babu et al. 2003). Literature search reveals that a total of 34 major fungal pathogens have been recorded on water hyacinth around the globe. Out of these, *Acremoniumzonatum, A. alternata, A. eichhorniae, Bipolaris sorokiniana, Helminthosporium* sp., *Cercospora rodmanii, Myrothecium roridum, Rhizoctonia solani, Uredo eichhorniae*, *Fusarium chlamydosporium*, *Alternaria jacinthicola* and *Alternaria geophila* were able to control water hyacinth up to a specific level (Aneja, 1996; Dagno et al. 2011; Tegene et al. 2012). A newly reported fungal strain of *Colletotrichum* sp. in China was found to behighly pathogenic with a disease index of 65.28% after 30 days of inoculation (Ding et al. 2008). Of these pathogens, *Cercospora rodmanii, Alternaria alternata, Alternaria eichhorniae* and *Fusarium chlamydosporium* have been evaluated for their host specificity and biocontrol efficacy (Aneja and Singh, 1989; Aneja et al.1993; Shabana, 1997; Babu et al. 2003; El-Morsy, 2006). Among them ABG-5003, a formulation of *Cercospora rodmanii* have been developed as a mycoherbicide to control water hyacinth in the USA. Based on the molecular characteristics this is the first report of the occurrence of a new strain *Alternaria alternata* causing leaf spots and leaf blotches on both young and mature leaves of water hyacinth from Kurukshetra (India). This strain is under investigation for host specificity, its biocontrol efficacy and formulation studies to be developed as a mycoherbicide from India in the near future.

Conclusion

The water hyacinth *(Eichhornia crassipes),* is rated as the most noxious aquatic weed which has defied all efforts of man to control it. It has been called a Devil, terror and curse. Every year thousands of tons of various chemicals and billions of dollars are wasted to control this weed without any fruitful results. The research reported in this paper explores the possibility of evaluating a new strain of *Alternaria alternata* an Indigenous fungal pathogen to control water hyacinth by applying the principle of bio-control of weeds with fungal pathogen so as to reduce the use of chemical pesticides. Further experiments and green house field trials will determine the potentiality of this pathogen to control water hyacinth.

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References

Aneja KR, Singh K (1989) *Alternaria alternata* (Fr.) Keissler a pathogen of water hyacinth with biocontrol potential. Trop Pest Manag35:354-356

Aneja KR, Srinivas B, Manpreet K (1993) Evaluation of *Fusarium chlamydosporium* as a biocontrol agent of water hyacinth (*Eichhornia crassipes)* (Mart.) Solms In: *Integrated Weed Management of Sustainable Agriculture.*Proceeding of International Symposium, Indian Society of Weed Science Hisar, India, pp 145-149

Aneja KR (1996) Exploitation of fungal pathogens for biocontrol of water hyacinth.pp.141-156. In: *Some Facets of Biodiversity* (Eds. R.K. Kohli, N. Jerath and D. Batish). Society of Environmental Scientists and Punjab State Council for Science and Technology Chandigarh, India

Aneja KR, (2003) *Experiments in Microbiology, Plant Pathology and Biotechnology*. 4th ed. New Delhi: New Age International Publishers

Babu RM, Sajeena A, Seetharaman K, Vidhyasekaran P, Rangasamy P, Somprakash M, Raja AS, Biji KR (2002) Host range of *Alternaria alternata*- A potential fungal biocontrol agents for water hyacinth in India. Crop Protect 21:1083-1085

Babu RM, 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 Protect 22:1005-1013

Badur-ud-Din (1978) Control of aquatic weeds. Project no. FG-Pa- 271, Grant no. PJ-ARS-72. Institute of Chemistry, University of Punjab, Lahore, 61

Dagno K, Crovadore J, Lefort F, Lahlali R, Lassois L, Jijakli MH (2011) *Alternaria jacinthicola*, a new fungal species causing blight leaf diseases on water hyacinth [*Eichhornia crassipes*(Mart.) Solms-Laubach]. J Yeast Fungal Res 2(7): 99-105

De Jong MD, de Voogd WB (2003) Novel Mycoherbicides for biological control of aquatic weeds such as Water hyacinth and Water lettuce: Patent Disclosure. Ann Plant Prot Sci, 14: 1

Ding Y, Zhao N, Chu J (2008) Nine pathogenic fungi of water hyacinth isolated in China. J Shan Jiaot Uni (Science) 13(5): 617–622

El-Morsy EM (2004) Evaluation of microfungi for the biological control of water hyacinth in Egypt.Fungal Divers 16: 35-51

El-Morsy EM, Dohlob SM, Hyde KD (2006) Diversity of *Alternaria alternataa* common destructive pathogen of *Eichhornia crassipes* in Egypt and its potential use in biological control. Fungal Divers 23: 139-158

Elwakil MA, Sadik EA, Fayzalla EA, Shabana YM (1989) Biological control of waterhyacinth with fungal plant pathogens in Egypt. In: Delfosse, E.S., ed., *Proceedings of*

the VII International Symposium on Biological Control of Weeds, Rome, Italy, 1988, 483–497

Freeman TE, Charudattan R (1984) *Cercospora rodmanii* Conway, a potential biocontrol agent for water hyacinth. Florida Agricultural Experimental Station Technical Bulletin No. 842. Gainesville, FL, USA: University of Florida, Institute of Food and Agricultural Sciences

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

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

Shabana, YM, Charudattan R, Elwakil MA (1995) First record of *Alternaria eichhorniae* and *Alternaria alternate* on water hyacinth in Egypt. J Plant Dis 79: 319

Shabana YM (1997) Formulation of *Alternaria eichhorniae*, a mycoherbidide for water hyacinth, in invert emulsions averts dew dependence. J Plant Dis and Protect 104(3): 231-238

Shanab S, Shalaby EA, Lightfoot DA, El-Shemy HA (2010) Allelopathic effects of water hyacinth (*Eichhornia crassipes*). PLoS One 5(10): e13200. doi:10.1371/journal. pone.0013200

Tegene S, Hussein T, Tessema T, Yirefu F, Carmen B, Gossmann M (2012)Exploration of fungal pathogens associated with water hyacinth [*Eichhornia crassipes* (Mart.) Solms-Laubach] in Ethiopia. Afr J Agr Res 7(1):11-18.