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POTENTIAL USE OF PATHOGENIC FUNGAL STRAINS TO REDUCE THE AQUATIC WEED *HYDRILLA VERTICILLATA* OF KOTA REGION

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ABSTRACT

Several methods, including mechanical, biological and chemical control have been investigated for the control of weeds. In this study we have used biological control method utilizing different fungal species like Botrytis sp., Cylindrocarpon sp. and Fusarium sp. and a combination of these fungal species. Each fungal species and their combinations were capable of killing Hydrilla in a bioassay. Hydrilla shoots were exposed to each fungal species and fungal combinations added to the water in the assay tube at a final concentration of 6 x 10^4 to 1 x 10^6 propagules / ml. The level of shoot damage caused by the synergistic effect of Fusarium sp. and Botrytis sp. was more effective than the pathogenic fungal species alone.The maximum damage to the shoots by isolates (fungus) was achieved by Fusarium sp. (single) and by combination of Fusarium sp. and Botrytis sp.Thus the combined use of pathogenic fungus species appears a promising approach for integrated biocontrol of Hydrilla.

Key Words: Biological Control, Botrytis Sp., Cylindrocarpon Sp., Fusarium Sp., Hydrilla Verticillata, Pathogenic Fungal Strains

I INTRODUCTION

Hydrilla is a submerged freshwater macrophyte that belongs to the family Hydrocharitaceae. Hydrilla is widely distributed in the African Asian region and is one of the most serious invasive aquatic weed problems. This plant possesses numerous mechanisms of vegetative reproduction that enable it to spread very rapidly.Two factors enable Hydrilla to out compete other submerged aquatic plants: Hydrilla has low light compensation and saturation points and a low carbon dioxide compensation point, enabling it to grow only in 1% of full sunlight. However the main reason for its persistence and longevity in natural environment is production of specialized dormant buds called "Turions".Tubers, also known as subterranean turions, are swollen, brown to white structures up to 15 mm long, which develop in the hydro soil. The mature tuber becomes free from the parent plant when the attached stem

decomposes or is ruptured[1]. Turions are viable for one to two years, while tubers remain viable for four years [2]and probably in excess of 10 years in New Zealandconditions [3].The stems of Hydrilla vary in length from a few centimeters to several meters and are either creeping, stoloniferous or erect (see front cover). The leaves occur in opposite pairs, or typically in whorls of 4, although numbers may range from 3 to 8 (rarely up to 12) per whorl. Leaves are sessile and linear to lanceolate, terminating in a single spine cell at the apex, and up to 20mm long and 4 mm wide (usually about 12 mm long and 2mm wide). Leaves are generally green, but often have small reddish-brown spots and strips. The midrib is distinct and occasionally bears unicellular spines on the abaxial surface. The margin is strongly serrulate with fine, translucent teeth that are visible to the naked eye [4].

A Hydrilla colony originating from a single stolon can expand readily at rate of 4 cm/day with an average production of new remit/m^2 day' although it can also grow at 0.7% salinity approximately a fifth of seawater concentration. Hydrilla is usually a gregarious plant that frequently forms dense, intertwined mats at the water's surface. Approximately 20% of the plant's biomass is concentrated in the upper 10 cm of such a mat [5]. The plants grow and spread quickly. Small fragments of the plant, containing a single node, can quickly develop adventitious roots and eventually produce an entire plant. This plant has very wide ecological amplitude, growing in a variety of aquatic habitats. It is usually found in shallow waters, 0.5 m or greater in depth. In very clear waters it can grow at depths exceeding 10 m. It can tolerate moderate salinity up to 33 percent of seawater [6].It flourishes best in calcareous ponds and streams, water quality rarely seems to be limiting, since it is found in both acidic and alkaline waters. It also grows well in both oligotrophic and eutrophic waters, and even tolerates high levels of raw sewage [7]. Sediments with high organic content provide the best growth, although Hydrilla is also found growing in sandy and rocky substrates.According to the office of the technology assessment at least US $ 100 million is spent annually to control aquatic weeds. Hydrilla has spread throughout the country's water ways. Clogging, irrigation and drainage canals are degrading water quality, reducing productivity of recreational fisheries and impeding navigation. It forms dense surface mats that can severely reduce water flow, interfere with boating, and water sports. It can also significantly reduce the water holding capacity of storage ponds. Government spends much money to manage weed in the state's public waters, mainly for chemical herbicides containing copper, diquat, endothal or fluridone as an active ingredient [8].

Presence of plant and water bodies is essential for the conservation of solar energy into chemical energy for development of aquatic fauna like fishes and prawns etc. and for continuous addition of oxygen to water during photosynthesis. If water plants due to overgrowth make such water bodies unfit and take shape of noxious aquatic vegetation these are called aquatic weeds. Several methods have been investigated for the control including mechanical, biological and chemical control [9]. A reduction of 90 % in the number of tubers was achieved by draw downs and desiccation of Hydrilla tubers from the sediments. Mechanical control of aquatic weed involves the removal of vegetative tissue and tubers/turions by dredging. The process is not practical for large lakes because it is too expensive [10].

1.1 Ecological problems:

Aquatic weeds pose several problems in India; the canals of Chambal commanded area in Rajasthan (Kota), Madhya Pradesh and Bhakra nangal in Himachal Pradesh and Punjab have been greatly slowed by aquatic weeds. Several irrigation and hydroelectric project in the country like Tungabhadra in Karnataka, Nagarjuna sagar project in Andhra Pradesh and Kakki and Idikki reservoir in Kerala, Goa and north east region of India are badly infested with aquatic weeds.In India many rivers, irrigation canals, lakes both natural and manmade are choked by the explosive growth of the aquatic weeds resulting in enormous direct losses.Aquatic weeds like Hydrilla caused 50 to 60% loss of the cultivable water in Bihar, Madhya Pradesh, Orissa and West Bengal making them unsuitable for fish culture.Submerged aquatic weeds had cut the flow of water by 80% in canals resulting in forced seepage, water logging and soil salinity.Current Status of Hydrilla in Rajasthan: In Rajasthan aquatic weeds pose the prime problem in the maintenance of water bodies. The major weeds are Hydrilla, water hyacinth, chara spp.and najas spp. The Chambal irrigation system comprises two canals and their several distributaries (shared by M.P. and Rajasthan). Survey revealed that aquatic weeds cut the design discharge of canal by 40 to 70 % and of their distributaries by over 80%.

Fig: Showing the most of surface area covered by aquatic weeds *Hydrilla* at Kota Barrage, Kota

In Indira Gandhi canal project located in north western Rajasthan entire canal banks have been covered by weeds. In Kota, Chambal River, Kishore sagar pond, Kota Dam, Kota Barrage etc. are severely infested by weeds causing a problem of navigation, polluted drinking water, besides providing breeding sites for mosquito, snails and other animals. They also invaded large area impeding the free movement and use of water in irrigation system and in fish culture.

1.2 Chemical control:

Fluridone (Sonar® (1=methyl-3-phenyl-5-[3-(triflurомethyl)phenyl]-4(1H)-pyridinone)) is a systemic aquatic herbicide that targets the photosynthetic pathway(i.e. disrupt the carotenoid biosynthetic pathway by inhibiting phytoene desaturase) (Sprecher et.al. 1998)[11]. Since its registration in the USA in 1986, fluridone has been used to control Hydrilla and other nuisance aquatic macrophytes. But this control is too expensive and harmful for environment.

1.3 Biological control:

In biological control method the fungal pathogen causes a short duration disease on Hydrilla without persistence in plant debris or plant tissue has been used as a mycoherbicide[12]. It can reduce hydrilla biomass in 80 days by up to 40% when applied alone or by 90% when used in combination with fluridone treatment. Three fungal species *Cylindrocarpon sp.,Botrytis sp*. and *Fusarium culmorum* were recovered and are more effective biocontrol agents for Hydrilla. This has become one of the most invasive weeds of water ways in tropical and subtropical regions of the world[13]. In this study we have used these three fungal species as biological weed controller. Among the isolates tested the *Fusarium culmorum sp*. isolate was most effective.That is the damage level v greater for the *Fusarium culmorum sp.* treatments compared to the other fungal species treatment at the respective levels of herbivory. Thus the use of these fungal species appears a promising approach for integrated control of Hydrilla.It was rated for disease (damage) severity two weeks after inoculation with the fungus(*Cylindrocarpon sp.Botrytis sp*. and *Fusarium culmorum*). Damage was rated as percent shoot biomass affected by chlorosis, lysis and disintegration. *Fusarium culmorum sp.* was proven to be the most effective fungus capable of killing Hydrilla shoot. It rapidly sporulates and produces a large quantity of spores in laboratory cultures[14].

1. MATERIALS AND METHODS

EVALUTION OF ALL THREE FUNGAL SPECIES CONTROLLING HYDRILLA IN A BIOASSAY:

Pure culture of Fungal species of *Botrytis* sp. (2104), *Cylindrocarpon* sp. (2094) and *Fusarium sp* (2090) were brought from IMTECH Chandigarh. Each fungal species was capable of killing Hydrilla in a bioassay. The isolates were tested singly for their ability to kill or damage Hydrilla in a test tube bioassay. The bioassay system consisted of glass tubes with dimensions of 22 mm diameter by 150 mm in length. Each tube contained 20 ml of sterile tap water and ml of 5 % Hoagland's solution. Then transferred healthy, terminal shoot of dioecious female Hydrilla of different sizes 3cm, 6cm, 9cm. and 12cm.in length in each tube. Tubes were inoculated with culture of fungal species separately in different combinations. One is control (with no culture), others tube having *Botrytis* sp. as suspension, A tube having *Fusarium culmorum*, sp. as suspension, B tube having *Cylindrocarpon sp.* as suspension, C tube having *Botrytis* sp AB tube having *Fusarium sp.* and *Botrytis* sp., AC tube having *Fusarium sp.* and *Botrytis* sp sp. BC tube having *Cylindrocarpon* sp. and *Botrytis* sp., ABC tube having *Fusarium sp.* ,*Cylindrocarpon* sp. and *Botrytis* sp.For the inoculation a 10 ml suspension of fungus was added separately to a Hydrilla containing jar to give a final concentration of 1X 10^6 culture/ml of liquid in the jar. The tubes were then covered with sterile parafilm. Tubes were placed under light (12hr; 137 µ E/m^2s) at 25℃. Jars were arranged and maintained for 4 weeks under the same conditions.Hydrilla was rated for damage severity three weeks after inoculation with the fungus. Damage was rated as percent shoot biomass affected by chlorosis, lysis and disintegration. Noted the damage level for each tube with respect to time.

4. RESULT AND DISCUSSION

Effects of three fungal species on Hydrilla shoot in the test-tube assay were seen. Hydrilla shoots inoculated with the fungal species *Fusarium culmorum* and *Cylindrocarpon* sp. developed disease symptoms like chlorosis and leaf destruction in 6 to 8 days after inoculation and after 8 to 10 days when inoculated with *Botrytis* species. Since the calculated value of 'F' for different fungal species (column) is 20.63, which is greater than the table value, so null hypothesis is rejected i.e. there is significant difference in destructive action of different fungal species. Since the calculated value of size of plant shoot (shows) is 8.72 which is greater than the table value, null hypothesis is 'a', i.e. the size of it makes a significant difference on the destruction time.

The results presented here in confirm the finding from a previous study by [15] in which *Fusarium culmorum*, among the microorganism tested. produced the highest level of damage to Hydrilla. Unlike an isolate of *Fusarium culmorum* isolated in the Netherlands that was reported by [16] as a potential biocontrol agent for Hydrilla, the *Fusarium culmorum* isolate used in the current study is indigenous to Florida. Thus, the use of an indigenous fungus as a biological control agent should be more acceptable to the public and regulatory agencies than a nonindigenous organism such as the isolate from the Netherlands. However, the host range of the Florida isolate should be determined before it can be presented as a safe biocontrol agent.

Data presented in Table 1-2 show the level of shoot damage caused by the synergistic effect of pathogenic fungal combination, which was more effective than the fungal pathogen alone. Higher levels of Hydrilla control were

achieved when the pathogenic fungus *Fusarium sp.* was applied to Hydrilla with the combination of *Botrytis sp*. at 20°C. This suggests that scheduling of the application of the microbial pathogen may be crucial in enhancing synergistic effects on Hydrilla control. This is the firstreport of an attempt of evaluating a pathogenic fungus for use in integrated biocontrol of Hydrilla . However, further Studies are needed to assess the efficacy of the *Fusarium culmorum*combination with another fungal strain for integrating in Hydrilla control in natural lakes.

3.1 Graphical representation:

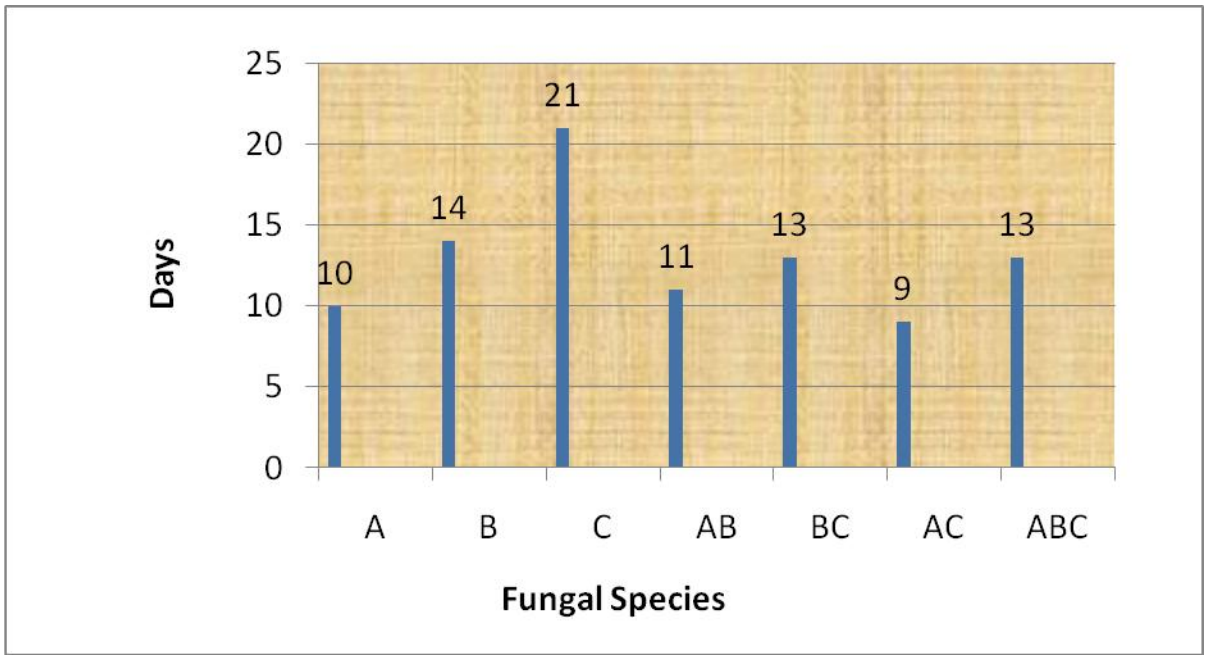

Fig.: - Graphical representation between destructive effects of different combination of fungal species against Hydrilla shoots of 9 cm in minimal number of days.

Hydrilla shoots were inoculated with fungal species *Cylindrocarpon* species and *Fusarium culmorum* sp. developed disease symptomps like chlorosis and leaf destruction in 6 to 8 days after inoculation and after 8 to 10 days when inoculated with *Botrytis* species**.** Severe chlorosis and tissue lysis were typical symptoms resulted by Cylindrocarpon species and *Fusarium culmorum*. Browning of Hydrilla shoots was a distinctive symptom of *Botrytis* species. *Fusarium culmorum sp.* was proven to be the most effective fungus capable of killing Hydrilla

shoots. It rapidly sporulated and produced a large quantity of spores in laboratory cultures. This facilitated its use in these studies. The glass jar based bioassay provided a large test system allowing root shot growth of Hydrilla. This facilitated the detection of the destructive action of fungal species.

Table 1:- Showing the destructive effect of various combinations of pathogenic fungal strains on different sizes of Hydrilla shoots in minimal days.

Plant shoot length	**Fusarium sp.**	**Cylindrocarpon sp.**	**Botrytis sp.**	**Fusarium sp./ Cylindrocarpon sp.**	**Cylindrocarpon sp./ Botrytis sp.**	**Fusarium sp./ Botrytis sp.**	**Fusarium sp./ Cylindrocarpon sp./ Botrytis sp.**	**Control (no culture)**
(cm)	A	B	C	AB	BC	AC	ABC	
3	7	13	20	8	11	7	10	21
6	8	14	21	10	15	7	12	21
9	10	14	21	11	13	9	13	21
12	10	15	24	13	16	8	14	21

After taking the minimal destruction period we analyzed the data through ANNOVA.

Table 2:- Showing the statistical analysis of different sizes of fungus.

Source of variation	**Sum of squares**	**df**	**Mean of squares**	**F ratio**
SSC	50.143	3	167.143	20.634
SSR	42.42	6	7.07	8.7283
Residual	14.58	18	.81	

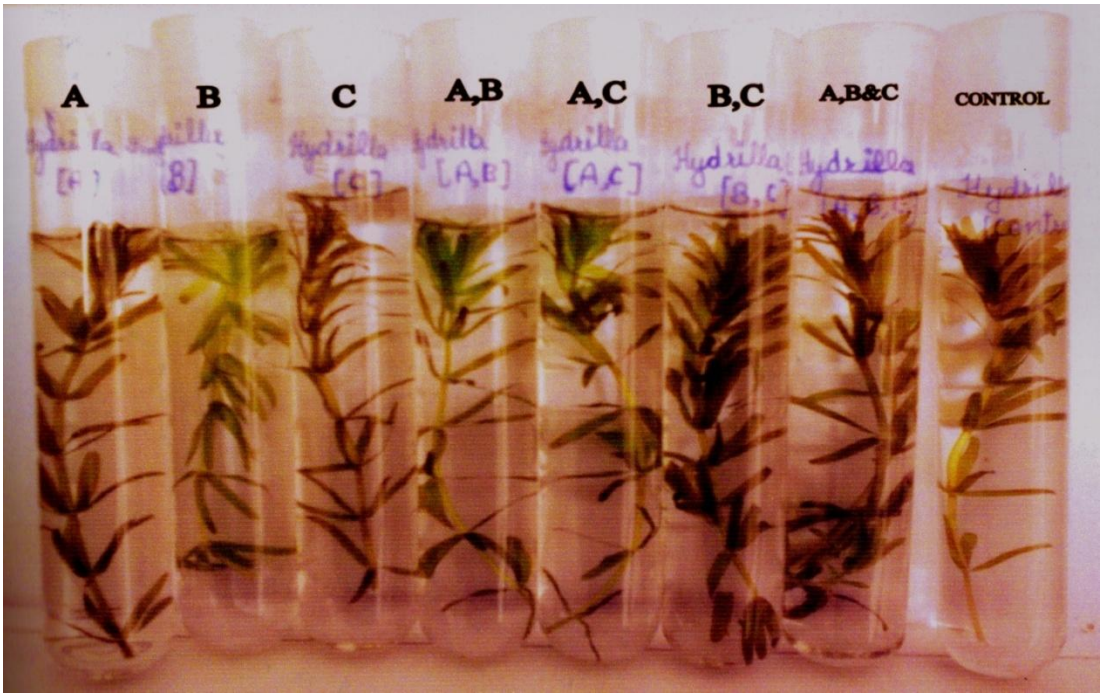

Fig. 1:- Showing inoculation of three fungal sp. with hydrilla shoot of 9 cm singly and in different combination.(A= *Fusarium culmorum*, B= *Cylindrocarpon species*, C= *Botrytis species*, AB= *Fusarium culmorum* and *Cylindrocarpon species*, AC= *Fusarium culmorum* and *Botrytis species*,BC= *Cylindrocarpon species* and *Botrytis species*,ABC= *Fusarium culmorum,Cylindrocarpon species* and *Botrytis species*)

were arranged and maintained for 4 weeks under the same conditions.Hydrilla was rated for damage severity three weeks after inoculation with the fungus. Damage was rated as percent shoot biomass affected by chlorosis, lysis and disintegration. Noted the damage level for each tube with respect to time. Observed them carefully and mentioned the destruction level with respect to time in observation table.We observed the effects of these three fungal species, *Fusarium culmorum* ,*Cylindrocarpon sp*. and *Botrytis sp.*applied on Hydrilla Shoots in the test-tube bio assay. After minimal incubation period (Table 1.) the tissue destruction and chlorosis in Hydrilla leaves has been observed.

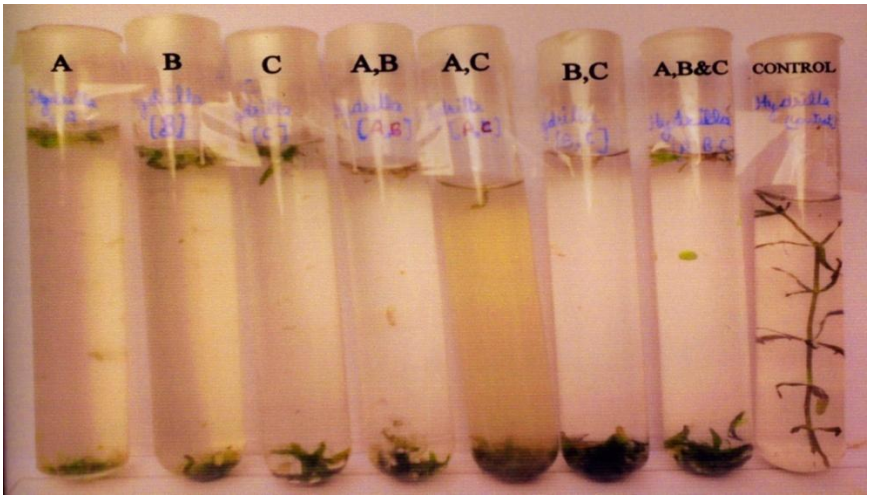

Fig.2:- Showing the destructive effect of pathogenic fungal species after minimum days of inoculation. (tube A and AC shows the maximum chlorosis and tissue lysis after 8-10 days.)

Conclusion

Although there are many challenges and constraints inherent in the development of biological herbicides, the increasing prevalence of both herbicide-resistant weeds and public concern with pesticide use creates a strong impetus for continued investigation in this field. Although there is a considerable number of candidate species that have been considered for this purpose, the major challenge to successful implementation of this strategy is the development of techniques to maintain consistent efficacy in field conditions. In this study We are using different concentration of some pathogenic fungal sps and their combinations to study the biological control of weed plant *(hydrilla verticillata*). We conclude statically, that some specific fungal sps have specific mechanism to degrade plant growth hormones according to time and this cause hazardous effect on plant shoot length. we choose particuler site for this as kota region in rajasthan, which is quite covered with aquatic weed hydrilla. These strategies will be of especially great value to organic production systems and to regions where cosmetic pesticide bans are in place. With continued investigation in this field, there is significant potential for the development of new weed control strategies that can be employed to delay herbicide resistance, produce food in accordance with consumer concerns, and reduce the environmental impact of modern agriculture and ecosystem management.

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