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ECOFRIENDLY MANAGEMENT OF WATER HYACINTH WITH A MYCOHERBICIDE AND CASHEW NUT SHELL LIQUID

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ABSTRACT

Ecofriendly management of water hyacinth with a mycoherbicide from Fusarium pallidoroseum and cashew nut shell liquid was tried in glass house and natural lake conditions. In glass house trial 97.78 and 82.22 per cent damages were recorded on applying wettable powder (40 %) formulation of the fungus at 5 per cent and 10 per cent concentrations respectively on the weed presprayed with CNSL. In infected lake, application of 5 per cent concentration of wettable powder (WP) formulation (presprayed with 5 % CNSL) developed typical blighting symptom on the fourth day and recorded 83.4 to 94.5 % damage by 7DAS.

Key words: Water hyacinth, *Eichhornia crassipes*, management, *Fusarium pallidoroseum*, CNSL.

INTRODUCTION

Water hyacinth [*Eichhornia crassipes* (Martius- Solms) Laubach] is the most invasive aquatic weed. It can double its biomass within 8-10 days (Singh 1999) and one plant can produce 3000 offsprings in 50 days (Verma *et al.*, 2003). Because of its capacity for exponential increase in the biomass, this weed needs constant vigilance. Some uses are reported for this weed such as compost making, paper industry, biogas plants, cattle feed, furniture making, waste water treatments etc., However, no one utilizes this weed on a large scale for these purposes in India and it still continues to be a menace. It blocks water flow in irrigation and drainage canals, channels and streams, hinders antimosquito operation, forms breeding ground for obnoxious insects, serves as alternative host to several insect pests and pathogens. This weed also makes aquatic recreational activity difficult and potentially unsafe in lakes, thus causing hurdles to tourism. Although this weed could be managed by mechanical or chemical means, because of one reason or other, these are not found practical.

The use of microorganisms in the management of water hyacinth is being practiced nowadays in some places. Fusarium pallidoroseum is identified as an efficient pathogen of water hyacinth with narrow host range and high intensity of infection (Naseema et.al., 2001). A market acceptable formulation has to be developed for commercialization of microbial biocontrol agents (Walker and Connick, 1983). Weidemann and Templeton (1988) formulated macroconidia of F.solani f.sp cucurbitae in alginate to control Texas gourd. A wettable powder formulation in diatomaceous earth was developed by Faizal (1992). Oils are used to formulate biocontrol fungi as it enhances disease development. Cashew nut shell liquid (CNSL), a byproduct of cashew processing industry and a product of plant origin can weaken the water hyacinth plants and thereby stimulate the build up of disease epidemic for its effective management (Praveena, 2003). The present

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investigation was carried out to develop a formulation of F. pallidoroseum and to include it in the ecofriendly management strategy of water hyacinth.

Materials and Methods

Preparation of wettable powder (WP) formulation of *F. pallidoroseum*

A 5 mm diameter disc from 7– day old culture of *F. pallidoroseum* maintained on potato dextrose agar was inoculated into 100 ml sterilized Czapek' s (Dox) broth in 250 ml conical flask , and incubated at room temperature (28± 4° C) for 10 days. At the end of the incubation period, the mycelium was separated from the broth by filtering through filter paper. The fungal mat thus obtained was pressed between layers of sterile coarse filter paper to remove water. A wettable powder formulation of the mycelium was prepared with the following composition: mycelium-40g, talc- 54g,sucrose-2g,tween 80 - 2g and glycerol 2g. After proper blending, the WP formulation was air dried and stored in polythene covers at room temperature (28± 4° C) and under refrigeration (4 °C).

Testing the Effect of WP formulation on water hyacinth

Glasshouse Trial

A WP formulation containing 40% of *F. pallidoroseum* was sprayed at different concentration on water hyacinth (@ 10 ml /plant) grown in pots and maintained in glass house. Cashew Nut Shell Liquid (CNSL), a product of plant origin was also applied to weaken the plants and to hasten killing of the weed by the fungus. The treatments include WP formulation of the fungus at 1, 3, 5, 10, 15, 20 and 25% concentration individually and in combination with CNSL at 0.5, 1 and 2%. CNSL was sprayed 30 minutes before WP application. Each treatment was replicated thrice. Observations were recorded seven days after spraying based on a score chart (Praveena, 2003) and per cent disease index was calculated using the following formula.

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of score of each leaf}}{\text{No. of leaves scored x Maximum score}} \times 100$$

Trial in cement troughs

The most effective concentrations *viz* , 5 g and 10g/100ml of WP formulation under glass house conditions was tested on water hyacinth plants grown in cement troughs which simulated the growth conditions in natural habitat. CNSL at 2, 3 and 4 per cent was also tested along with the WP formulation (since CNSL at lower concentration tested in glass house condition did not cause appreciable weakening of water hyacinth plants, the two higher concentrations were also tried)

Cement troughs of size 1 x 1 x 0.4 m^3 filled with mud and water collected from lake where water hyacinth was growing. The water level in the troughs was adequately maintained so that the plants grow and multiply normally. Each trough contained 20 plants of uniform age. Observations on the extent of damage was recorded on 10 plants selected at random using the score chart and disease index was calculated

Field Trial

The experiment was laid out in an infested lake (Aakkulam), Thiruvananthapuram district, Kerala state (India). The experimental area was divided into plots of size 2 x 2 m^2 and marked using rope. The most effective concentration from the previous trial was used for the field experiment. The water hyacinth plants were sprayed with CNSL (5 %) @ 50 ml m^{-2} and it was allowed to dry for a period of 30 minutes. Treatment with the WP (5%) formulation was given @ 50 ml m^{-2}. Nine replications were maintained for the

treatment. Control plots were laid out away from the experimental area so that the spray fluid did not contaminate the check plants. The extent of damage was recorded four and seven day after application of the formulation from treated and control plants and PDI was calculated.

Shelf life of WP Formulatin

The colony forming units (cfu)/g of the WP formulation of *F. pallidoroseum* kept at room temperature (28± 4 °C) and under refrigeration (4 °C) were estimated at weekly interval (Timonin, 1940).

Results And Discussion

Glass House Trial

The results of the study showed that there was significant difference between the treatments (Table-1). Maximum intensity of damage of 97.78 per cent was recorded in T28 (WP @ 10 g/100 ml on two per cent CNSL sprayed plants) followed by T27 (WP @ 5g/100 ml on two per cent CNSL sprayed plants) with 82.22 per cent whereas CNSL alone at two per cent recorded 15.15 per cent scorching. In treatments where WP was applied alone, 48.89 per cent damage was recorded in T4 (@ 10 g/100 ml) followed by T7 (@ 25g/100 ml) T6 (@ 20g/100 ml) and T5 (@ 15g/100 ml) which were statistically at par. The increased infection in plants pretreated with CNSL was due to the enhanced penetration of the fungus through the injury produced by the oil. So the effect of WP formulation was enhanced when the plants were pretreated with CNSL. Glass house trials carried out by Shabana *et.al.*, (1995) reported that four sequential spraying of powdered alginate formulation of *Alternaria eichhorniae* containing hydrophilic substances caused 93% reduction in the biomass of water hyacinth by two months.

Trial in Cement Troughs

When WP alone was sprayed mean intensity of damage ranged from 32.00 – 46.67 per cent. Similarly when CNSL alone was sprayed intensity of scorching ranged from 20.00 – 27.33 per cent. However, when formulation was applied on CNSL sprayed plants, marked increase in damage was observed in T8 (98.67 per cent) and T5 (97.33 per cent) which were statistically comparable with each other (Table-2). CNSL play a role in modifying the integrity of thick waxy leaf surface of water hyacinth plants and thereby facilitating fungal penetration and establishment. It also reduced the free moisture requirement of fungal spores for germination.

Field Trial

Under field conditions since the water hyacinth plants were more vigorous and growing in an aquatic ecosystem, five per cent concentration of CNSL was used for pre treating the plants. The water hyacinth plants exhibited typical blighting symptom on the fourth day after treatment and the disease intensity ranged from 38.92 – 50.04 per cent. The disease gradually spread from the leaves to the swollen petiole. Seven days after application, the disease intensity ranged from 83.4 – 94.52 per cent. Also the plants started sinking to the bottom of the lake indicating the effectiveness of the treatment, Whereas the plants in the control plots remained healthy and produced new healthy leaves (Table-3). Wettable powder formulation of *Cercospora rodmanii* was found to be effective for the management of water hyacinth (TeBeest, 1991). Collego, a commercial mycoherbicide with *Colletotrichum gloeosporioides* f.sp. *aeshynomene* was used for the successful management of Northern joint vetch in rice and soybean fields (Templeton *et.al.* 1979). The addition of substances that hold water and extend the duration of

wetness at the leaf – inoculum interface may improve the effectiveness of the formulation. Muller–Scharer and Frantzen (1996) suggested systems management approach for biological weed control, which aimed to shift the balance between host and pathogen, making favorable to the pathogen by stimulating the disease epidemic on the targeted weed. Here CNSL weakens the host plants and thereby enhances the efficiency of the pathogen for faster killing of the weed.

Shelf life of WP Formulation

When the WP formulation was stored at room temperature (28± 4 °C), there was a reduction in the viability of spores from the initial count of 8.07×10^8 cfu/g to 6.39×10^7 cfu/g within a week and it was reduced drastically to 7.0×10^4cfu/g by 5 weeks after storage (WAS) (Table-4). When the WP formulation was stored under refrigerated condition, the spores retained their viability up to a period of four months. As the storage period increased there was a gradual reduction in the cfu/g of WP. It had 8.07×10^8 cfu/g on the day of preparation, which reduced to 6.47×10^7 cfu/g one WAS. There after the count remained static for a period of one month and then gradually declined to 7.43×10^6 cfu/g by 15 WAS. Wettable powder was found to be the most efficient formulation for fungal biocontrol agents Butt *et.al.*, (2001), TeBeest (1991) and Sunitha (1997). The efficiency of *Fusarium pallidoroseum* was improved when it was formulated with hydrophilic substances such as sucrose, glycerol and Tween80, serving as stickers and humectants. The hydrophilic substances prolong the duration of water retention, there by aiding the survival and germination of fungal inoculum. (Boyette *et.al.*,1991). CNSL is a byproduct of cashew processing industry and it can be stored without any deterioration for years under room temperature.

The results of the present study indicate that WP formulation of *F. pallidoroseum* is an effective mycoherbicide for management of water hyacinth and the spores in this formulation remain viable for a reasonable period of time. Also the efficacy of pathogen could be further enhanced by pretreating the plants with CNSL, which is a product of plant origin easily available in Kerala, India. This treatment was found to be safe to other aquatic fauna and flora.

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Table-1. Effect of WP formulation on water hyacinth under glasshouse conditions

Treatments	Mean disease intensity (%)	Treatments	Mean disease intensity (%)
T_1 – 1 g 100 ml^{-1}	4.44(10.53)	T_{18} – T_{16} + T_2	26.67(31.09)
T_2 – 3 g 100 ml^{-1}	4.44(10.53)	T_{19} – T_{16} + T_3	64.45(53.42)
T_3 – 5 g 100 ml^{-1}	31.11(38.87)	T_{20} – T_{16} + T_4	71.11(57.52)
T_4 – 10 g 100 ml^{-1}	48.89(44.36)	T_{21} – T_{16} + T_5	46.67(43.09)
T_5 – 15 g 100 ml^{-1}	37.78(37.87)	T_{22} – T_{16} + T_6	46.67(43.09)
T_6 – 20 g 100 ml^{-1}	37.78(37.87)	T_{23} – T_{16} + T_7	44.45(41.8)
T_7 – 25 g 100 ml^{-1}	39.11(38.86)	T_{24} – 2 % CNSL	15.55(23.13)*
T_8 – 0.5 % CNSL	6.67(14.96)*	T_{25} – T_{24} + T_1	28.89(32.48)
T_9 – T_8 + T_1	24.45(29.58)	T_{26} – T_{24} + T_2	35.55(36.58)
T_{10} – T_8 + T_2	24.45(29.58)	T_{27} – T_{24} + T_3	82.22(65.15)
T_{11} – T_8 + T_3	40.00(39.23)	T_{28} – T_{24} + T_4	97.78(83.91)
T_{12} – T_8 + T_4	56.89(48.96)	T_{29} – T_{24} + T_5	73.33(58.91)
T_{13} – T_8 + T_5	37.78(37.91)	T_{30} – T_{24} + T_6	68.89(56.13)
T_{14} – T_8 + T_6	42.22(40.51)	T_{31} – T_{24} + T_7	66.67(54.74)
T_{15} – T_8 + T_7	42.22(40.51)	T_{32} – Talc spray	– (1.65)
T_{16} – 1 % CNSL	6.67(14.97)*	T_{33} – water + glycerol + Teepol	– (1.65)
T_{17} – T_{16} + T_1	24.45(29.58)	T_{34} – water	– (1.65)

Figures in parentheses indicate arc sine transformation
$CD_{0.05}$ = 5.15
* = Percent scorching

Table-2. Effect of WP formulation on water hyacinth under trough conditions

Treatments		Mean disease intensity (%)
T_1	WP @ 5 g / 100 ml	32 (34.44)
T_2	WP @ 10 g / 100 ml	46.67 (41.94)
T_3	T_1 + 2 % CNSL	43.33 (41.17)
T_4	T_1 + 3 % CNSL	65.67 (54.13)
T_5	T_1 + 4 % CNSL	97.33 (81.82)
T_6	T_2 + 2 % CNSL	57.33 (49.22)
T_7	T_2 + 3 % CNSL	89.33 (71.06)
T_8	T_2 + 4 % CNSL	98.67 (84.03)
T_9	2 % CNSL	20.00 (26.57)*
T_{10}	3 % CNSL	23.33 (29.32)*
T_{11}	4 % CNSL	27.33 (31.52)*

Figures in parentheses indicate arc sine transformation
$CD_{0.05}$ – 4.00%
*Percent scorching

Table-3. Effect of WP formulation on water hyacinth under field conditions

Treatment	*Mean disease intensity (%)*	
	4 DAS	7 DAS
1 [CNSL (5%) + WP 5 g/100 ml]	44.48	94.52
2	50.04	94.52
3	38.92	94.52
4	44.48	83.40
5	44.48	94.52
6	50.04	94.52
7	50.04	94.52
8	38.92	88.96
9	38.92	83.40
10 (Control)	-	-
11 (Control)	-	-
12 (Control)	-	-
13 (Control)	-	-
14 (Control)	-	-

Table-4. Shelf life of WP formulation

Weeks after storage	Mean cfu /g x 10^5	
	Room temperature	Refrigerated condition
0	8066.62 (3.90)	8066.62 (3.90)
1	639.04(2.81)	647.05 (2.81)
2	609.99(2.79)	685.02 (2.84)
3	85.66(1.94)	440.35 (2.64)
4	26.81(1.44)	84.98 (1.93)
5	0.70(0.23)	86.10 (1.94)
6	–	80.78 (1.91)
7	–	81.58 (1.92)
8	–	74.43 (1.88)
9	–	41.79 (1.63)
10	–	42.23 (1.64)
11	–	13.68 (1.17)
12	–	12.84 (1.14)
13	–	12.89 (1.14)
14	–	7.26 (0.97)
15	–	7.43 (0.93)
16	–	– –

Figures in parentheses indicate log (x + 1) transformation
$CD_{0.05}$ for storage period – 0.05

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