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Efficacy of pathogens of water hyacinth (*Eichhornia crassipes*) singly and in combination for its biological control

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ABSTRACT: **An experiment was conducted to determine the impact of different virulent endemic pathogens of water hyacinth, namely, *Alternaria alternata, Alternaria eichhorniae, Fusarium pallidoroseum*, and *Curvularia lunata*, in various combinations. The pathogens were isolated from diseased plant parts of water hyacinth collected during periodical surveys, in the water bodies of Jabalpur. All pathogens were compatible with each other in terms of growth, sporulation and damage potential, except *A. eichhorniae* whose growth appeared inhibited in the presence of the other test pathogens. The combined effect of various pathogens was better than any of the pathogens tested alone. Combination of *A. alternata* + *C. lunata* + *F. pallidoroseum* resulted in maximum disease development, followed by *A. alternata* + *C. lunata*. *A. alternata* + *F. pallidoroseum* and *C. lunata* + *F. pallidoroseum* combinations were also effective.**

KEY WORDS: *Allernaria alternata, A. eichhorniae, Curvularia lunata, Fusarium pallidoroseum,* phytopathogens, water hyacinth

INTRODUCTION

Water hyacinth (*Eichhornia crassipes* (Mart.) Solms. (Pontederiaceae) is one of the most successful colonisers in the plant kingdom. Rapid growth via vegetative propagation and seed bank establishes it among the top ten weeds of the world (Holm *et al*, 1977). With the ability to double its biomass in 8 to 10 days, it rapidly covers vast water surface obstructing navigation, blocking drainage and causing flood by limiting water flow. Dense mats eliminate light penetration to submerged plants, which leads to oxygen depletion thereby disrupting the natural habitat of native submerged and floating plants, fish and other aquatic organisms. It also increases the occurrence of water related diseases like malaria and filariasis (Gopal, 1987). In India, it has infested more than two lakh hectares of water surface (Anonymous, 1979). Various control measures employed, including manual, chemical and biological have proved futile.

In such a situation, integrated management with biological control as a key instrument has been considered as one of the most eco-friendly options to control water hyacinth (Charudattan, 1986; Corodo, 1999). During periodical surveys of various

water bodies of Jabalpur, several phytopathogens were isolated and purified from various diseased plant parts of water hyacinth. Among these, *Alternaria alternata* induced maximum disease. Other than this, *Fusarium pallidoroseum*, *Curvularia lunata* and *Alternaria eichhorniae* also incited appreciable disease. Templeton and Heiny (1989) suggested that several isolates of one pathogen or several species of pathogens each having slightly different environmental requirements could be mixed in the formulation to ensure that at least one would encounter the optimal environmental window. Hasan and Ayers (1990) reported that interaction between the biotroph / necrotroph occurs at the infection site of biotrophs, where infection by one pathogen makes the host more susceptible to secondary infection. Such type of synergistic relationship of two pathogens can provide biological and economical feasibility by the use of the mixtures of two or more fungi for effective control of one or more weeds. In this study, the compatibility and damage potential of the four phytopathogens alone and in combination were determined.

MATERIALS AND METHODS

Preparation of stock culture

The investigations were carried out using four potential pathogens of water hyacinth, namely, *A. alternata*, *A. eichhorniae*, *F. pallidoroseum* and *C. lunata*. Pure cultures of all the four fungi were grown on Richards's media for seven days in a BOD incubator at 26°C and 12 hours photoperiod provided by four fluorescent lamps (Philips TL 40W /33, 1000lux).

Compatibility study of various pathogens

5mm discs of each test fungus were cut from the stock culture plates with the help of a sterile cork borer and transferred on plates with Richard's media. The discs of each fungus were placed singly and also in combinations of two and three with each other about 2 cm apart to give equal opportunity to all the test pathogens to grow. A separate set containing only single pathogen in each plate was kept as control. Observations were taken on radial mycelial growth rate and sporulation. The compatibility of various pathogens and radial growth rate of the pathogens were designated as excellent (+++), good (++) and poor (+). The plates were incubated at 26°C for 21 days and the average sporulation in different treatments was determined using a haemocytometer.

Impact of pathogens singly and in combination on water hyacinth

Inoculum preparation and spraying

The spores were harvested from the plates and concentrated by centrifugation and the desired inoculum concentration of $2x10^6$ spores ml^{-1} was prepared in sterilized distilled water using haemocytometer for all the treatments. Tween-80 (0.01%) was added to it as an adjuvant at the rate of 0.05ml / 50ml. Small clumps of uninfested water hyacinth were collected from the local water bodies and kept in small water tubs (15 litres capacity) for 5 days to let them acclimatize to the new environment. The water in the tubs was fertilized with farmyard manure and NPK. Each plant held three-small water hyacinth. The plants were sprayed until runoff with spore suspensions in different combinations using a small aerosol atomizer. Control plants were inoculated with single pathogen. A second set of control was sprayed with sterilized distilled water mixed with 0.01% Tween80. They were covered with transparent polythene bags for 48h in a growth chamber at 26±2°C and 75 to 80% relative humidity.

Diseased symptoms were assessed visually and intensity of infection was measured using score chart framed by Freeman and Charudattan (1984). The disease index (DI) was calculated using the following formula (Chaube and Singh, 1991):

$$\text{Disease Index (DI)} = \frac{\text{Sum of all numerical ratings x 100}}{\text{Total number of leaves measured x Maximum disease index}}$$

Statistical analysis

All the treatments were replicated thrice. The data recorded on various treatments were subjected

to analysis of variance (ANOVA) as per the procedure suggested by Panse and Sukhatme (1957) using statistical program Genstat. The treatment means were compared with least significant difference (LSD) at 5% level of significance.

RESULTS AND DISCUSSION

Compatibility between various pathogens

Except *A. eichhorniae*, all the pathogens were compatible with each other. The sporulation (Figure 1) and the radial growth rate (Table 1) of all the tested fungi for compatibility were excellent and comparable to the control plates. The growth of *A. eichhorniae* was inhibited in the presence of other test fungi. Thus the test pathogens can be used in combination with each other. Similar observations with varying compatibility within different pathogens have also been reported by Crawley *et al.* (1985). *A. eichhorniae* was excluded from further consideration as all the test pathogens were incompatible with *A. eichhorniae*. Similar inhibitory effects of phytopathogens have been reported by several authors due to competition, antibiosis and mycoparasitism (Papavizas, 1985; Cook and Baker, 1983; Pandey, 1998).

Impact of pathogens singly and in combinations on water hyacinth

The water hyacinth plants sprayed with different fungi combinations were observed for the development of disease symptoms. The lesion diameters were greater on leaves when combinations of pathogens were used than with individual pathogens. *A. alternata* + *C. lunata* + *F. pallidoroseum* combination resulted in significantly high disease development (40%) by the 10th day (Table 2). It was followed by *A. alternata* + *C. lunata* (34.7%) and *C. lunata* + *F. pallidoroseum* (34%) treatments, which were at par with each other. Similarly there was no significant difference between *A. alternata*+*F. pallidoroseum* (30%) and *A. alternata* (26%) treatment alone by the 10th day.

Table 1. Compatibility of various pathogens

Pathogen combination	Pathogen	Growth rate*
A. alternata + *A. eichhorniae*	*A. alternata*	+++
	A. eichhorniae	+
A. alternata + *C. lunata*	*A. alternata*	+++
	C. lunata	+++
A. alternata + *F. pallidoroseum*	*A. alternata*	+++
	F. pallidoroseum	+++
C. lunata + *F. pallidoroseum*	*C. lunata*	+++
	F. pallidoroseum	+++
A. eichhorniae + *F. pallidoroseum*	*A. eichhorniae*	+
	F. pallidoroseum	+++
A. eichhorniae+ *C. lunata*	*A. eichhorniae*	+
	C. lunata	+++
A. alternata + *C. lunata* + *F. pallidoroseum*	*A. alternata*	+++
	C. lunata	+++
	F. pallidoroseum	+++

* Growth rate: excellent (+++), good (++), poor (+); (culture medium used = Richard's media, temperature = 25 ^{0}C, pH = 5)

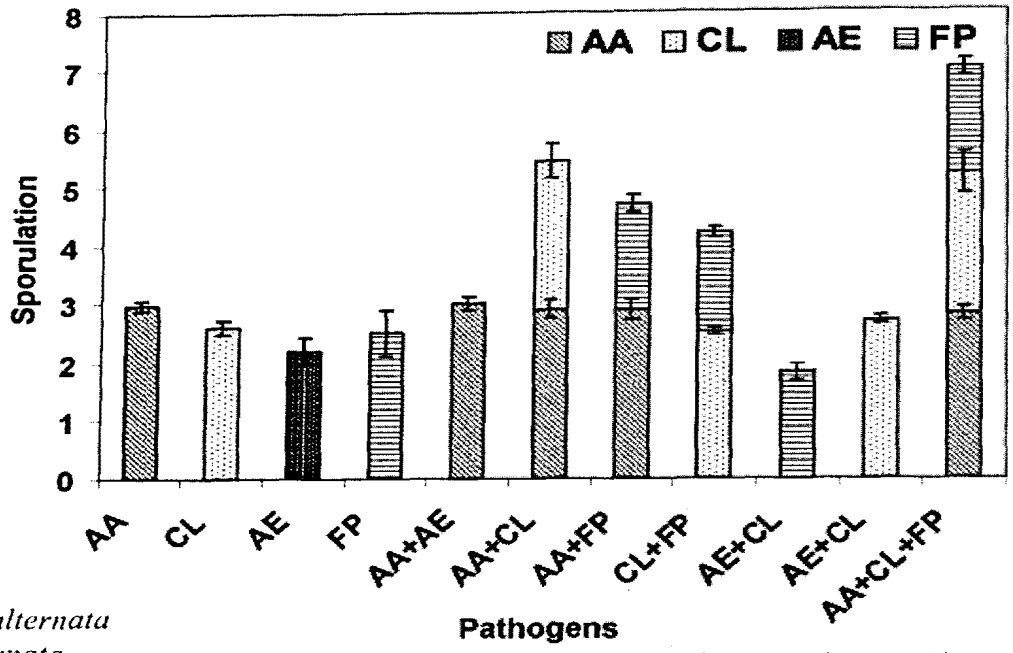

Fig. 1. Average sporulation in different fungi grown singly and in combination. Vertical bars indicate standard error; (culture medium used = Richard's media, temperature = 25°C, pH = 5)

Table 2. Intensity of infection of pathogen singly and in combinations on water hyacinth

Phytopathogens	Disease Index (%) after		
	5 days	10 days	15 days
A. alternata	8.7^{cd} (17.1)	26.7^{c} (31.1)	41.0^{d} (39.0)
C. lunata	6.0^{d} (14.1)	15.0^{d} (22.7)	30.0^{e} (33.2)
F. pallidoroseum	3.3^{e} (10.3)	11.3^{e} (19.7)	26.7^{e} (31.1)
A. alternata + *C. lunata*	12.3^{ab} (20.4)	34.7^{b} (36.1)	80.0^{b} (63.5)
A. alternata + *F. Pallidoroseum*	9.7^{bc} (18.1)	30.0^{b} (33.2)	55.0^{c} (47.9)
C. lunata + *F. Pallidoroseum*	10.0^{bc} (18.4)	34.0^{b} (35.7)	51.7^{c} (46.0)
A. alternata + *C. lunata* + *F. pallidoroseum*	15.3^{a} (23.0)	40.0^{a} (39.2)	84.7^{a} (67.0)
Control	0.0^{f} (4.1)	0.0^{f} (4.1)	0.0^{f} (4.1)
SEM ±	1.0	0.8	0.8
LSD (P = 0.05)	3.0	2.3	2.5

Values are mean of three replications. Mean in the same column followed by the same letter are not significantly different; percentage data in the parentheses are the arcsine transformed values of the original mean values

By the 15th day, the combined impact of any of the pathogens was significantly higher than pathogens tested singly. The *A. alternata+C. lunata+ F. pallidoroseum* combination resulted in about 85% diseases intensity, which was significantly higher than the other treatments. This was followed by *A. alternata + C. lunata* combination with 80% disease development. The *A. alternata + F. pallidoroseum and C. lunata + F. pallidoroseum* combinations gave similar results causing 55% and 51% disease intensity, respectively. Similar observations using different phytopathogens in combination have been made by several other workers (Breeyen, 1999; Pandey, 1998).

The studies confirmed and reinforced the view that the above tested phytopathogens in combination can act synergistically by being effective partners in the integrated management of water hyacinth.

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