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MYCOHERBICIDAL POTENTIAL OF *ALTERNARIA ALTERNATA* ITCC4896 FOR THE CONTROL OF *PARTHENIUM HYSTEROPHORUS*

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Abstract: Mycoherbicides are special biotechnology products which contain fungi or fungal metabolites as nonchemical alternatives thereby reducing the input of harmful chemicals to control noxious weeds. The present communication emphasizes on the potential of an indigenous isolate of *Alternaria alternata* ITCC 4896 as a mycoherbicide for the global weed – *Parthenium hysterophorus*. Of the various spore concentrations tested by *in vitro* Detached Leaf Bioassay, $1x10^6$ spores/ml was the most effective inducing 89.2% leaf area damage on the 7th day and was further tested by Whole Plant Bioassay. Both *in vitro* Detached Leaf Bioassay and Whole Plant Bioassay exhibited a similar trend in disease development showing 50% damage at 96 hours post treatment. However, 100% mortality was observed in the Whole Plant Bioassay on the 7th day. This is the very first report on the bioweedicidal potential of *A. alternata* ITCC 4896 (LC#508) for use as a mycoherbicide for *P. hysterophorus*.

Key words: *Alternaria alternata*, fungi, mycoherbicide, *Parthenium hysterophorus*, pathogenicity, bioweedicide

INTRODUCTION

With increasing societal concern regarding the harmful effects of chemical pesticides on humans as well as on environment, ecofriendly alternatives are being extensively sought. A variety of weed diseases are caused by fungi which have a potential to be used as bioherbicide or mycoherbicides (Charudattan1991; Cartwright and Templeton 1992; Pandey 1998; Mohan Babu et al. 2003b; Saxena 2003). Fungi causing diseases in weeds are a diverse assemblage of species that markedly differ in their

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morphological and physiological characteristics. Further strains of a single species may vary in its pathogenicity (Boyette et al. 1979).

Ragweed Parthenium or *Parthenium hysterophorus* (*Helianthaceae*/*Asteraceae*) is an annual herb of neotropical region now fairly distributed throughout the globe. Popularly known as White tops or Carrot weed, this native of North-Mexico, tropical South and North America was accidentally introduced in India in early 1950's. Today Parthenium has a position among the list of top ten worst weeds of the world (Holm et al. 1977) and has been listed in the global invasive species database. Parthenium is not only a major threat to agricultural or horticultural system but is also a potential hazard to livestock and humans (Basak 1984; Valiappan and Towers 1989; Swaminathan et al. 1990). The plant is responsible for allergenic eczematous contact dermatitis (AECD), allergenic rhinitis, nasobrochial allergies, and seasonal pollenosios eventually leading to death in humans (Towers and SubbaRao 1992; McFadyen 1995). Parthenium has become resistant to an array of chemical pesticides like Atrazine 2,4-D, Metribuzin, Paraquat, trifluralin, diphenamid etc. (Singh et al. 2004)

The use of native pathogenic fungi (innundative biological control) offers to be a environmentally benign and a safer method of controlling and management of weeds. The fungus *A. alternata* Fr. Keissler ITCC 4896 (LC#508), a phyllosphere pathogen was found to cause extensive damage to Lantana & Parthenium plants under natural conditions during rainy season. *A. alternata* ITCC 4896 (LC#508) has been proved to possess mycoherbicidal potential to control the weed *Lantana camara* (Saxena and Pandey 2002). In the present communication we assess the bioweedicidal potential of *A. alternata* ITCC 4896 (LC#508) for possible use as a mycoherbicide for ragweed Parthenium using *in vitro* detached leaf bioassays and *in vivo* whole plant assays.

MATERIALS AND METHODS

Fungal isolate and inoculum

The original isolate of *A. alternata* LC#508 (ITCC 4896) was recovered from diseased leaves of *P. hysterophorus* as well as *L. camara* from Bargi Hills, M.P. India. It was confirmed pathogenic according to Koch's postulates. The isolate was grown on Fresh Potato Dextrose Agar (FPDA) plates at 26± 2°C for a period of 7 days. The conidia were harvested by flooding the Petri dishes with sterile distilled water (SDW). The conidial suspension was then filtered through muslin cloth to remove the mycelial bits. The resulted suspension was then centrifuged at 8000 rpm for 10 minutes. The resulted pellet was resuspended using SDW and adjusted to different conidial concentrations by means of a haemocytometer.

Detached Leaf Bioassay

Four spore concentrations $0.5x10^6$, $1x10^6$, $1.5x10^6$ and $2.0x10^6$ were tested in this assay. The experiment was carried out as per the method of Chaing et al. (1989). Leaves were excised from shoots at the preflowering stage of weeds from the field. Nine replicates comprising of 3 leaves each were tested. These were sprayed evenly with the fungal inoculum using a sprayer and then placed in a moist chamber using sterile forceps. Three replicates comprising of 3 leaves each served as control and received only SDW. Treatments were carried out within 15 min after detachment from the mother plant. All treatments were kept in a growth chamber with controlled conditions of 26±2°C;

75±15% relative humidity and 15 h (7350 lx) illumination for a period of one week. Leaves were rated for disease severity every 12 h on a five-point scale as per Chaing et al. 1989. The differences in means were analyzed by one way ANOVA followed by Tukey's Multiple comparisons test between the mean leaf area damaged induced by different spore concentrations using GRAPH PAD PRISM Ver. 4.03 (2005).

Whole Plant Bioassay

Four to six week old plants having 4–8 leaves were grown in pots in a growth chamber with illumination of 12 h daily for a period of one week for acclimatization. These were sprayed with fungal inoculum of a spore concentration which exhibited the best severity i.e. Average Leaf Area Damaged (ALAD) as percentage during *in vitro* Detached Leaf Bioassay. The experimental set had ten replicates and the control set had ten replicates. The control set received only SDW. These were covered with plastic bags and kept in controlled conditions as described previously. After 24 h, the bags were removed and the plants were placed back in the growth chamber. Plants were observed daily for the disease severity as per Chaing et al. 1989 until all plants died. The experiment was repeated thrice. The means of experiments was analyzed by one way ANOVA for any significant difference between the experimental sets using GRAPH PAD PRISM Ver. 4.03 (2005).

RESULTS AND DISCUSSION

Detached Leaf Bioassays

The initial symptoms of the disease, brownish irregular spots with chlorotic halo around them, started developing after 48 hours post treatment (hpt) in three test sets which received three different spore concentrations viz. 1.0 $x10^6$; $1.5x10^6$ and $2x10^6$ spores/ml. The concentration $0.5x10^6$ spores/ml exhibited the initial symptoms of disease at 72 hpt (Fig. 1). The maximum Average Leaf Area Damaged (ALAD) was 89.2% followed by 80.6%; 74% and 70% exhibited by concentrations of $1x10^6$; $2x10^6$; $1.5x10^6$ and $0.5x10^6$ spores/ml respectively after 7 days post treatment. Phyllosphere or the leaf surface supports an array of microorganism but only pathogenic microorganisms gain entry through the leaf surface. Bioherbicidal efficacy of a pathogen is dependent upon its interaction with the phylloplane microflora, adequate microenvironment for germination and pathogenesis, which in turn is dependent upon its concentration on the leaf surface. Therefore, spore concentration plays a decisive role in development of a commercial mycoherbicide. The best concentration that was selected for further evaluation using Whole Plant Bioassay was the one inducing maximum ALAD of 89.2% on the seventh day and 60% leaf damage by fourth day i.e. 96 hpt ($1x\ 10^6$ spores/ml). One way ANOVA indicates a significant difference between the mean leaf areas damaged at different concentrations with a p value of $p < 0.0001$. Further Tukeys' multiple comparison test highlighted that $1x10^6$ spores /ml was more effective than $2x10^6$ spores/ml when compared to control with p values of $p < 0.001$ and $p < 0.05$ respectively. It also exhibited a significant difference between $0.5x10^6$ spores/ml and $1x10^6$ spores/ml at a p value of $p < 0.01$. *A. alternata* f. sp. *sphenoclea* and an indigenous isolate of *Alternaria* at spores concentration of 10^6 spores/ml induced nearly 100% and 90% leaf damage, respectively (Masangkay et al. 1999; Mobbayad and Watson 1995).

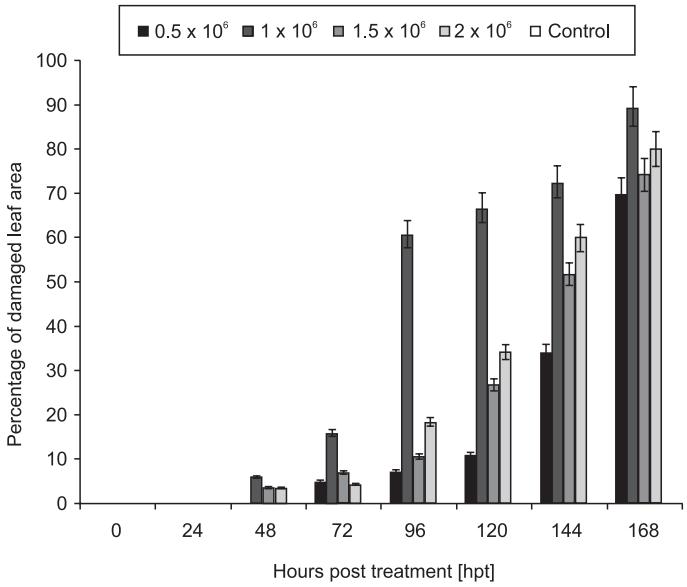

Fig. 1. Disease development of *A. alternata* (LC#508) by *in vitro* detached leaf bioassay

Whole Plant Bioassay

During the *in vivo* bioassay on whole plants it exhibited onset of disease after 24 hours post treatment and complete death by 168 hours post treatment i.e. after 7 days. Fifty per cent damage of the weed occurred after 96 hpt i.e. on 4th day (Fig. 2).

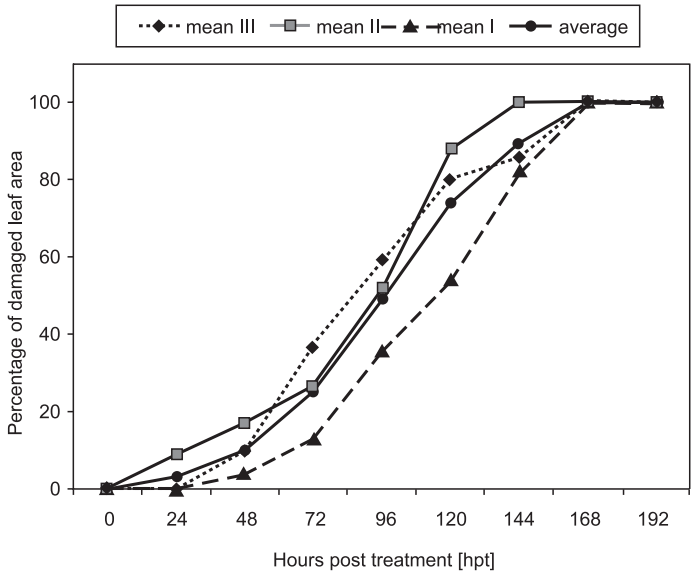

Fig. 2. Disease development of *A. alternata* (LC#508) by whole plant bioassay

A similar trend was observed in the Detached Leaf Bioassay where 60% mortality occurred after 96 hpt. Like *A. alternata* (LC # 508); *A. eicchorniae*, *A. alternata* f. sp. *sphenoclea* have also been found to induce severe disease development and mortality of water hyacinth and *Sphenoclea zylenica* respectively at 10^6 spores/ml (Masangkay et al. 1999; Mohan Babu et al. 2003a). There was no significant change in the means of the replicates of the experiment as indicated by the P value of one way ANOVA.

CONCLUSION

The present study for the first time indicates the potential of *A. alternata* (LC#508) as a bioherbicide for controlling the spread of two global invasive weed species – *P. hysterophorus* as well as *L. camara* by using a inoculum of 1×10^6 and 1.65×10^6 spores/ml, respectively.

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POLISH SUMMARY

POTENCJAŁ MIKOHERBICYDOWY *ALTERNARIA ALTERNATA* ITCC4896 W ZWALCZANIU *PARTHENIUM HYSTEROPHORUS*

Mikoherbicydy są szczególnymi produktami biotechnologicznymi, które zawierają grzyby lub ich metabolity jako alternatywę dla środków chemicznych, ograniczając tym samym używanie chemikaliów w zwalczaniu szkodliwych chwastów. Praca przedstawia potencjał rodzimego izolatu *Alternaria alternata* ITCC4896 jako mikoherbicydu w zwalczaniu występującego na całym świecie chwastu *Parthenium hysterophorus*. Spośród wszystkich badanych stężeń grzyba w inokulum, w testach *in vitro* prowadzonych metodą Detached Leaf Bioassay, stężenie $1x10^6$ spor/ml było najefektywniejsze, powodując 89,2% uszkodzeń liści siedem dni po inokulacji. To stężenie następnie było stosowane w metodzie Whole Plant Bioassay. Obie metody wykazały podobny trend, jeśli chodzi o rozwój choroby, wykazując 50% uszkodzeń 96 godzin po inokulacji. Stuprocentową śmiertelność roślin w badaniach metodą Whole Plant Bioassay zaobserwowano jednak siedem dni po zabiegu. Praca jest pierwszym doniesieniem na temat chwastobójczego potencjału *A. alternata* ITCC4896 jako mikoherbicydu w zwalczaniu *P. hysterophorus*.