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Bio-control potential of *Cladosporium* sp. (MCPL - 461), against a noxious weed *Parthenium hysterophorus* L.

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Abstract: *The phenological survey of Parthenium hysterophorus L., in and around the campus of Banaras Hindu University (BHU) was done for about two years (2004-06). During Nov. 2004, a few Parthenium plants were found diseased, and symptoms were restricted to the flowers, buds, and inflorescences. The disease causes sterility and reduces seed viability, which was observed with seed germination test from infected and healthy plants. The fungal pathogen was isolated and identified as Cladosporium sp. (MCPL-461). The severity of pathogen to the reproductive organs led to serious damages of the Parthenium plants. Thus in vitro and in vivo experiments were conducted to determine the bio-control potential of Cladosporium sp. (MCPL 461) against Parthenium weed. A combinatorial effort of Cladosporium sp. (MCPL 461) bio-control potential was evaluated with different culture media, incubation periods and spores strength. Spore suspension of 10^5 to 10^{12} spores ml^{-1} were used to spray on healthy Parthenium plants, and it was found that severe infection symptoms were appeared at 10^{10} to 10^{12} spores ml^{-1} suspension. LD_{50} was found at 10^7 spores ml^{-1}. To enhance the myco-herbicide activity 3% sucrose was added to the spore suspension, which further resolute the bio-control efficacy of the isolates. Only 20-30 % seeds of infected plants could germinate. However the safety of non-targeted and wild plants was also tested with Lantana camera, Chromolaena odorata and found that suspension up to 10^{12} spores ml^{-1} were not sufficient for disease outbreak in them.*

Key words: *Parthenium hysterophorus, Cladosporium sp., Weed bio-control, Bio-control agent*

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Introduction

Parthenium hysterophorus L. (Asteraceae) is an invasive, exotic and annual herb of necrotropical origins. It was imported accidentally to India along with the PL 480 Mexican wheat seeds in the 1950s and now become one of the most awful, noxious species (Rao, 1956). This is also known as congress grass, carrot weed, white top, chatak-chandani, bitter weed, ramphool and gazarghas *etc.* The weed has since grown into uncontrollable proportions invading millions of hectares of uncultivated wastelands, roadsides, railway tracks and forest *etc.* This fast growing weed is a nuisance in public parks, residential colonies and orchards. It is considered a noxious weed because of its allelopathic effect (Kanchan and Jayachandra, 1980; Swaminathan *et al*., 1990; Adkins and Sowerby, 1996; Sharma *et al.,* 2005; Kumar and Gautam, 2008), strong competitiveness for soil moisture and nutrients, as well as the hazard it posses to humans and animals *i.e.* in causing dermatitis (Khosla and Sobti, 1979; More *et al*., 1982; Lakshmi and Srinivas, 2007a; Verma *et al*., 2006; Srinivas, 2006). Pollens of this weed cause allergenic rhinitis (hey fever) (Lakshmi and Srinivas, 2007b) and may develop finally in bronchitis (asthma). *P. hysterophorus* invades all sorts of crops and herbaceous flora, causing a substantial loss of yield and growth. The major toxicants of *Parthenium* are parthenin and other phenolic acids such as coffeic acid, vanillic acid, ansic acid, chlorogenic acid and parahydroxy benzoic acid, which are lethal to human beings and animals (Mahadevappa, 1997; Oudhia, 1998).

P. hysterophorus has now become an international problem, and crop losses in different parts of the globe are going to be severe. India itself is severely facing the problem of reduced crop yield and also loss of many important plants due invasive behaviors of this weed. A yield decline was recorded up to 40% in agricultural crops (Khosla and Sobti, 1981), while a significant reduction up to 90% has been reported in case of forage production in grassland (Nath, 1988). Most Caribbean countries bear about 20% (average) crop yield loss only due to this weed. Due to more tolerance capacity of this weed against widely used herbicides such as paraquet, atrazine, 2,4-D, metribuzin, trifluralin, diphenamid, it ranks fourth most serious crop weed (Hammerton, 1981; Singh e*t al*., 2004). It is reported to be one of the important and damaging weed to the Coffee in Kenya (Njoroge, 1986). There are two recognized aspects of weed interference, weed density and weed free periods relative to the crops (Bosnic and Swanton, 1997; Tamado *et al*., 2002b). Photoperiod, temperature and soil ranges are quite variables for p*arthenium* weed (Williams and Groves, 1980). Rajan (1973) and Kanchan and Jayachandra (1979) were the first who reported the presence of plants growth inhibitors in *Parthenium* weed. Jayachandra (1971) reported that this weed can be a serious problem for grassland in India, which can reduce the pasture carrying capacity severely up to 90%. Serious human health risk due to *P. hysterophorus* had been reported in India, about three decades ago (Srinivas, 2005; Handa *et al*., 2001; Dhileepan *et al*., 2000; Chippendale and Panetta, 1994; Lonkar *et al*., 1974). All these points illustrated the potential of *Parthenium* to disrupt the natural ecosystem. Due to its invasive and allelopathic properties and thus, it makes a general call to check its growth and spread. This experiment may fulfill some gaps which remain left in earlier studies

done so far in order to control some noxious weeds (Prashanthi and Kulkarni, 2005; Saxena and Kumar, 2007; Evans, 1997; Hasan and Ayres, 1990).

The fungus *Cladosporium* sp. (MCPL-461) may be regarded as an equivalent to a bio-control fungus, (Dhileepan, 2007; Kumar and Soodan, 2006; McFadyen, 1992) which acts as pathogen to growing leaves of *Parthenium* and provokes their distortion and reduction of photosynthetic area and later control the buds growth and seed development. Out break of this *Cladosporium* sp. can be very severe, and makes plant unable to grow further. The evaluation was made based on observed damage to the weed in natural habitats and due to potential of fungus *Cladosporium* sp. (MCPL-461) to cause damage this weed, it has been considered that this fungal isolates may be a good candidate for biological control. Under controlled laboratory conditions, the fungus was identified as *Cladosporium* sp. (MCPL-461) and colonies on live plant parts as well as their cultures in Mycopathology laboratory, BHU, Varanasi, are maintained for their further use in the biological control of plant diseases. This present study was carried out with aim to investigate the effect of a fungal bio-control agent *Cladosporium* sp. (MCPL-461) on *Parthenium hysterophorus* to avoid adverse effects of chemical herbicides (Caroline *et al*., 2001) and to find out an alternative in order to manage this weed.

Materials and Methods

A field survey and the systematic sampling were made during two years (November 2004 to October 2006) in BHU campus and its surrounding area. The organism was isolated from the affected parts of the *Parthenium* by following the standard isolation techniques (Aneja, 2001; Kumar *et al.,* 2006) using Potato Dextrose agar (PDA) medium. An examination of the leaves and floral buds of weed was made in laboratory under dissecting microscope, whenever a fungus was appeared on different parts of weed plant. Samples were prepared for observation with a light microscope. Direct isolation of fungus was also made on different growing media *i.e.* PDA, Czapek-Dox agar, Mycological agar, Water agar, *Parthenium*-bud extract agar. Koch's postulate was performed and found satisfactory for all the isolates and proved to be pathogenic to this weed. The different isolates of the fungus associated with diseased flower buds were identified as an organism *Cladosporium* sp., and designated as *Cladosporium* sp. (MCPL-461). To confirm the pathogenic nature of *Cladosporium* sp. (MCPL-461), it was multiplied on PDA and spore suspension was prepared with sterile distilled water. The different concentration of spore suspension (the number of conidia present in the area of 3sq mm of microscopic view field) was sprayed on buds and flowers, both under poly-house and field conditions. The organism was re-isolated and pure culture was obtained. Further the experiment was continued to explore this pathogen as myco-herbicide.

Several selective and non-selective media were screened for the growth and sporulation of this fungus as a best growth medium. The composition and procedure for preparation of media were followed as explained by Tuite (1961). *Parthenium* leaf extract agar (PLEA) medium was prepared by boiling 200 g leaf bits in 500 ml of water for 10 min. and filtered through muslin cloth. Twenty grams of agar was melted separately in 500ml of water. Both the solution were mixed and the volume was made up to 1000 ml and pH was maintained at 5.8, 20 ml of each medium was poured in to each of 90 mm sterile Petri plates. In each sterile Petri plate, fungus was inoculated and incubated at ranges 25-35°C respectively. Colonial growth and sporulating capacity of *Cladosporium* sp. were observed at maximum growth attained by fungus in one of the media tested, also it was observed by applying massive dose of inoculums at a particular susceptible stage of weed growth (Hawsworth *et al*., 1983). Different spore concentrations were assayed to induce maximum disease on this hazardous weed plant. To satisfy the criteria of potential myco-herbicide, host safety of *Cladosporium* was tested following modified centrifugal phylogenetic strategy (Wapshere, 1974) and symptoms were categorized based on lesions numbers, size, color and area damaged in proportion to the area of infected organs. The disease symptoms were evaluated with the no. of lesions appeared on the host plant. The healthy plants (No symptom) and the no. of lesions counted, 2 to 6 lesions are considered to be 'mild symptom' manifestation. However in case of 'severe symptom' manifestation coalition in the lesions occurs that can not be counted followed by necrosis. Plant was tested *in vitro* through the detached leaf technique (Tedford *et al.,* 1990), to prove that fungus to be safe myco-herbicides, without affecting field crops. Pathogencity of this fungus was also tested to the other major weeds.

Fungus was effectively mass multiplied on *Parthenium* leaf bits following solid substrate fermentation technique (Churchill, 1982). The spore suspension was prepared and adjusted to get maximum infection. To improve the myco-herbicide efficacy, 3% sucrose was added as a spray additive to the spore suspension. An experiment was conducted on naturally growing *Parthenium* plant at BHU campus and its adjoining area of Varanasi. Spore suspension of *Cladosporium* sp. spores @ 10^{12} spores ml^{-1} was sprayed on *Parthenium* flowers and leaves. Spraying was carried out during evening hr and 48 hr dew period was maintained for plants sprayed with *Cladosporium* sp. in poly-house. Controlled plant was sprayed with sterile water + 3% sucrose. All photographs including photomicrographs have been taken with detachable Nikon camera (Model coolpix 995, 3.4 mega pixel) attached with Nikon Trinocular Microscope (Model E-600).

Results and Discussion

The field survey, which was made in BHU campus and adjoining area, resulted in isolation of sixteen isolates distributed in ten different genera (Table 1). All were either foliar or root pathogens except *Cladosporium,* which was a floral, bud and foliar pathogen. Most of them follow Koch's postulates when their pathogenecity was tested to the weed. Sooty moulds symptoms were gathered during the field survey of *Parthenium* flower. The organism associated with diseased flower and buds was identified as *Cladosporium* sp.,

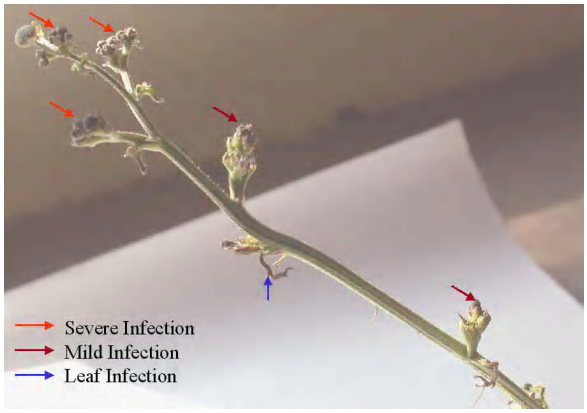

Fig. 2: *Parthenium hysterophorus* infected by *Cladosporium* sp.

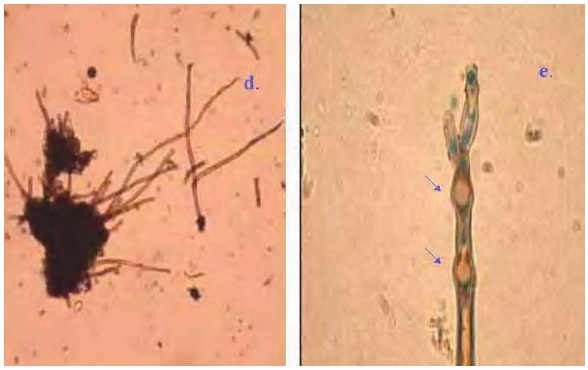

Fig. 1: (a) and (b) Conidia in chain (c) Mycelium (d) Mycelium and Chlamydospore (e) Bulging in single branched conidiophores

Fig. 3: *Parthenium hysterophorus*, buds and leaves infected by *Cladosporium* sp.

Table - 1: Fungal pathogens associated with *Parthenium hysterophorus L.* from BHU and its surroundings area

Location	Pathogen isolated	Plant part / part affected
Varanasi city		
*DLW	*Alternaria* sp.	Leaf lamina
Ramnagar	*A. alternata*	Leaf lamina
City	*Fusarium pallidorosum*	Root
Samneghat	*Sclerotium rolfsii*	Leaf lamina
BHU campus		
Hostel road	*Fusarium* sp.	Root
	Cladosporium sp.	Inflorescence
Department road	*Fusarium* sp.	Root
Botanical garden	*Cladosporium* sp.	Inflorescence
	Aspergillus fumigatus	Root
	Penicillium islandicum	Root
	Rhizoctonia solani	Root
Vishwanath temple	*Puccinia melampodii*	Leaf lamina
	Colletotrichum sp.	Leaf lamina
Hyderabad gate and out side	*Alternaria* sp.	Leaf lamina
	Cercospora sp.	Leaf lamina
	Rhizoctonia solani	Root

*DLW - Diesel locomotive works

Table - 2: Colony diameter and sporulation capability of *Cladosporium* sp. on different media

Media	Colony diameter (mm)	Relative sporulation
Selective		
Czpapek's agar	30	4
Czpapek-dox's agar	48	4
Mycological agar	65	4
V-8 Juice agar	60	4
Non-selective		
Host extract agar + 1% sucrose	50	4
Host extract agar	36	4
Water agar	60	3
Potato dextrose agar	70	4

Relative sporulation

No. of spores/microscopic area	Field grade
>70	4
50–70	3
20–50	2
>20	1
0	0

a facultative parasite. It is a member of fungi imperfecti, belonging to the order Moniliales and family Dematiaceae. To confirm the pathogenic nature of *Cladosporium* sp. it was multiplied on PDA and spore suspension was prepared with sterile distilled water. In both the conditions (poly-house and field area), suspension was sprayed to flowers of weed, showed sooty mould symptoms and premature dropping of flower buds, which proved this organism to be a pathogen of *Parthenium* flowers because flowers were remain intact to the control plants. From the re-isolated organism pure culture was obtained, which exhibited diverse morphology by producing two types of conidia and mycelium (Fig. 1a-e), shows *Cladosporium* sp. as a new floral pathogen agent to *Parthenium hysterophorus*, and the experiment was again continue to explore this pathogen as a myco-herbicide. The fungus also causes good and harmful symptoms to the leaves of this weed. When several selective and non-selective media were screened for this fungus, it has been observed that on PDA fungus exhibits maximum growth (70 mm) at temperature 26±2°C and sporulating heavily on all the selective and non selective media, except water agar medium (Table 2), thus PDA was selected for culturing the *Cladosporium* sp. The reduction in growth and sporulations to the fungus was recorded below and beyond this temperature (26±2°C) therefore, we cultivated the fungus at this temp in order to get the maximum growth and sporulations. At 35°C fungus has shown less growth while above that rare growth was observed.

A second six-month survey and close observation (2005) were carried out in campus and confirmed the endemic nature of *Cladosporium* sp. on *Parthenium.* This localized pathogen is considered to capable damaging this host weed by applying bulk of inoculums at a particular susceptible stage of weed growth (Hawksworth *et al.,* 1983). To induce maximum destruction by disease on weed at different concentrations of spore suspension was assayed and it was found that spore concentrations of 10^{10}-10^{12} spores ml^{-1} was effective to cause maximum disease. LD_{50} value was also standardized and it was found to be 10^{7} spores ml^{-1}, lower than that of other study in same line (Prashanthi and Kulkarni, 2005). Host range is most important factor for a critical consideration in gradual growth of a myco-herbicide for weed control. The safety of non-target cultivated and wild plants must be ensured before release of this fungus as a mycoherbicide in the field, exclusively for potential benefits of pathogen. To satisfy these criteria, host safety of *Cladosporium* (MCPL-461) was tested following the modified centralized phylogenetic strategy (Wapshere, 1974). Few crops and tree species tested *i.e.* paddy, eucalyptus, mango, and bamboo were resistant to pathogen, which satisfy the safe bio-control agent

Table - 3: Host range of pathogen's symptoms

Plant species	*Alternaria alternata*	*Cladasporium* sp. (MCPL-461)	*Cercospora* sp.
Category A (wild weeds)			
Lantana camera	*Mild symptom	No symptoms	Mild symptoms
Chromlaena odorata	No symptoms	No symptoms	No symptoms
Weed plants	No symptoms	No symptoms	No symptoms
Category B (economically important plants)			
Plantation crops			
Banana	No symptoms	No symptoms	Mild symptoms
Field crops			
Cotton	No symptoms	Mild symptoms	Mild symptoms
Paddy	No symptoms	No symptoms	No symptoms
Chick pea	No symptoms	No symptoms	No symptoms
Forest trees			
Bamboo	No symptoms	No symptoms	No symptoms
Eucalyptus	No symptoms	No symptoms	Mild symptoms
Mango	No symptoms	No symptoms	No symptoms

*Microscopic observation revealed inability of the pathogen to enter inside the epidermis

without affecting crops and tree of common habitat. However, the pathogen was unable to infect other weeds, *Lantana camera, Chromolaena odorata*, plantains, and grasses. Total plants selected for host safety test were divided in category A (wild weeds) and B (economically important plants) (Table 3). After spraying spore suspension of *Cladosporium* sp. 10^{12} spores ml^{-1} with 3% sucrose on *Parthenium* bud, flowers and leaves only water +3% sucrose also sprayed on controlled plants. After colonization fungus caused sooty mould to buds, flowers and leaves after 15 days of spraying interestingly fungus had heavily colonized and produced severe symptoms of sooty mould to buds, flowers and leaves (Fig. 2, 3). In natural environments, pathogen colonized the reproductive parts also and resulted seed malformation and sterility. Further, impact of *Cladosporium* infection on seed viability was studied by seed germinations test Tamado *et al.* (2002a). It was observed that infected *Parthenium* plant produces reduced size seeds which germination percentage was also very low (around 20-30%, data not shown). *Cladosporium* sp. produces mycelium, conidia (primary and secondary) and chlamydospores (Fig. 1a-d), which finally capture the leaf, flower and buds, where they cause secondary infection. Two types of conidia (primary and secondary) and robust mycelium perhaps make the *Parthenium* captive to this fungus. *Aureobasidium pullulans* is used as bio-control agent of weed Eupatorium (*Chromalaena odorata*) but does not produce any symptom on *Parthenium* (Prashanthi and Kulkarni, 2005).

A biological control can play a significant role to check the growth of *Parthenium* weed because of its sustainability, cost effectiveness and eco-friendly nature (Evans, 1997). In search of effective and safe biological control agent to this hazardous and dangerous weed, several agencies of the Indian government are also involved in monitoring the research programmes at different centres exclusively dealing this problem because chemical control has its own drawbacks which may degrade the air quality. Government has also decided to import a Mexican beetles (*Carmenta ithaca*) a stem borer moth which feed on *Parthenium* stem (Yaduraja, 2005). Since, that insect is effective against *Parthenium* weed in different climatic conditions therefore, our prediction about their effectiveness in our country may be little premature and search of an effective indigenous bio-control agent against this weed may always be on high demand which could work efficiently in local environment.

Thus, *Cladosporium* sp. (MCPL-461) as floral buds and leaves pathogen inhibited growth and development of buds. It also affects the embryo development which enhances the sterile seeds formation. All the morphological stages were noticed *i.e.* buds and leaves. Interestingly, spraying of *Cladosporium* sp. has deleterious effects on *Parthenium* weed only, not to other plant species, which living together under same niche, however, detailed study on its ecology and secondary metabolites which it produces during infection needs further attention. Due to invasive nature and luxuriant growth capacity of *Parthenium* in different habitat, *Cladosporium* sp. as a floral and leaf pathogen may be used as a potential mycoherbicide against this weed. A biological control can play a significant role to check the growth of *Parthenium* weed because of its sustainability, cost effectiveness and eco-friendly nature (Evans, 1997). Further research is under progress to develop a formulation of a suitable and eco-friendly mycoherbicide against *Parthenium hysterophorus* L. at large-scale.

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