

# BIODEGRADATION OF *PARTHENIUM HYSTEROPHORUS* BY PATHOGENIC FUNGAL STRAINS.

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**Abstract:** *Parthenium hysterophorus* is an alien species commonly known as congress grass or carrot grass belonging to *Asteraceae* family. This plant species is native to the area surrounding the Gulf of Mexico, Central America, Southern North America, West Indies, and Central South America. In India, this invasive weed has become a big problem. At present congress grass has become world's most devastating and hazardous weeds. This plant is known for its negative impact on the biodiversity, agriculture, animal and human being. *Parthenium* plant is known to cause allergic respiratory problems, contact dermatitis, diarrhea, skin allergy, skin rashes excessive water loss, mutagenicity, both in human and livestock. It can be controlled either by using chemical herbicides or weedicides, but their use adversely affects the environment and human health. Hence, alternate eco-friendly and cost-effective methods are required to control the weed. There is an urgent need to isolate and identify the active herbicidal or weedicidal ingredients from plants and fungal metabolites. These chemical constituents may provide the structural lead to prepare natural product based on environment-friendly herbicides to manage and control this harmful weed. In present investigation, significant reduction in growth of *Parthenium hysterophorus* was found after inoculation with different fungal strains within two months. *Alternaria alternata*, *Aspergillus niger* and *Puccinia graminis* were the fungal strains which showed degradation effect on germination and early seedling growth of *Parthenium* weed, among these *Alternaria alternata* showed drastic reduction in the growth. There is need to extract and identify phytotoxins from microorganisms for bioremediation in future.

**Keywords** - Allergic, herbicides, metabolites, *Parthenium hysterophorus*, phytotoxins

## I. INTRODUCTION

Invasive plant species are those species which are introduced from outside the native place to other countries either by knowingly or accidentally through human activities. These plants establish their own populations in the wild and have caused evident changes in nearby areas, simulated as well as biological systems (Akter A and Zuberi ML, 2009). *Parthenium hysterophorus* is an invasive alien weed which dominates over the native species and adversely affects the biodiversity of that area. The word *Parthenium* is a Latin word "parthenice" suggesting medicinal uses (Bailey LH, 1960). *Parthenium hysterophorus* is a competitive and pervasive annual herbaceous weed belonging to the family *Asteraceae* with absence of economic importance resolved till now. This erect, short-lived plant known for its fast growth and its abundance is mostly seen in hot climates.

*Parthenium hysterophorus* is a prolific weed, producing thousands of small white capitula each yielding five seeds on maturity. It is native to the area surrounding the Gulf of Mexico, Central America, southern North America, West Indies and Central South America and now it naturalized in Australia, Bangladesh, India, Nepal, Pakistan, South Africa, Sri Lanka, and the United States and is one of the world's seven most destructive and harmful weeds. This weed is believed to have been introduced in India as a contaminant in PL 480 wheat (Public Law 480 passed in 1954 to give food grains to developing countries for eliminating starvation and malnutrition) imported from the USA in the 1950's. Presently, this invasive weed is widely spread in India (Singh RK et al., 2008).

Approximately two million hectares of land in India have been infested with this herbaceous threat (Dwivedi P et al., 2009). Looking at the harms caused by *P. hysterophorus*, its management is necessary to prevent future problems. The major health hazards caused due to direct contact with this plant or plant parts, cause diseases like air borne contact dermatitis (Agarwal KK and D'Souza M 2009), fever and asthma (Lonkar A et al., 1974; Subba Rao PV et al., 1976). Live stock is also allergic and susceptible to *P. hysterophorus*. Close contact of animal with *P. hysterophorus* may cause rashes on their whole body and udders. *Parthenium hysterophorus* cause enormous loss to the biodiversity by replacing native species in the natural ecosystems, sometimes causing total habitat alteration.

### 1.1 Infestation Of *Parthenium hysterophorus*

#### 1.1.1 Agriculture and pastures ecosystems:

Before 1980 this weed was seldom noticed growing in crop lands but now it has spread too much extent into almost all types of agriculture crops, forests and plantation ecosystems. In crop fields, where only one crop is grown in a year, it grows

abundantly in the fallow period following the occurrence of mild rains. Its infestation is severe in the field where irrigation canals are used. (Adkins S and Shabbir A., 2014).

### 1.1.2 Bare lands

*Parthenium hysterophorus* vigorously grows in bare lands. It can be seen growing everywhere either on roadside, around the factories or mills, platforms and even the lands which are not suitable for crop production due to their high metal toxicity or scarcity of the mineral nutrients. It is the important feature of *Parthenium* weed that it has a wide range of habitat and it can be survive in harsh conditions in which other normal plants cannot survive. It is an important reason of the rapid infestation of *Parthenium* in India and other countries as alien weed. (Adkins S and Shabbir A., 2014)

### 1.2 Chemical constituents of *P. hysterophorus*

Isolation and structural elucidation is required to determine the chemical properties of *P. hysterophorus*. Chemical analysis of *P. hysterophorus* has indicated that all its parts including trichomes and pollen contain toxins called sesquiterpene lactones (SQL)(Maishi AI *et al.*, 1998) reported that *P. hysterophorus* contains a bitter glycoside parthenin, a major sesquiterpene lactone. Other phytotoxic compounds or allelochemicals are hysterin, ambrosin, flavonoids such as quercelagetin 3,7-dimethylether, 6- hydroxyl kaempferol 3-0 arabinoglucoside, fumaric acid. Phydroxy benzoin and vanillic acid, caffeic acid, p coumaric, anisic acid, p-anisic acid, chlorogenic acid, ferulic acid, sitosterol and some unidentified alcohols. Parthenin, hymenin and ambrosin are found to be the culprits behind the menacing role of this weed in provoking health hazards (Lata H *et al.*, 2008). *P. hysterophorus* from different geographical regions exhibited parthenin, hymenin, coronopilin, dihydroisoparthenin, hysterin, hysterophorin and tetraeurin as the principal constituents of their sesquiterpene lactones (De La Fuente JR *et al.*, 1997). (Gupta *et al.*, 1996) identified a novel hydroxyproline-rich glycoprotein as the major allergen in *P. hysterophorus* pollen. (Das *et al.*, 2007) examined the flowers of *P. hysterophorus* and isolated four acetylated pseudoguaianolides along with several known constituents. A novel sesquiterpenoid, charminarone, the first secopseudoguaianolide, has been isolated along with several known compounds from the whole plant by (Venkataiah *et al.*, 2003). (Chhabra *et al.*, 1999) discovered three ambrosanoides from the chloroform extract of this weed.

### 1.3 Impact of *Parthenium*

#### 1.3.1 Impact on Biodiversity

This weed has the potential to disturb the natural ecosystem, as it can grow throughout the year in almost all drastic conditions suppressing native vegetation. Rapid spread of *Parthenium* can disturb natural ecosystem because it has very fast infestation capacity and allelopathic potential which have the ability to disrupt any type of natural ecosystem. (Kumar S, 2014). The concentrations of allelochemicals viz. Coronopilin, caffeic acid, parthenin, and p-coumaric acid which are present in *Parthenium* have serious allelopathic effects.

#### 1.3.2 Impact on Crop production

The *Parthenium hysterophorus* weed has infested in a large area of India (Kumar S, 2014). This plant contains parthenin, hysterin, hymenin, and ambrosin. Due to the presence of these allelochemicals this weed has strong allelopathic impacts on different crops and human being also (Gunaseelan V N, 1987). This weed have adverse impacts on legumes by disturbing their symbiosis with Nitrogen fixing bacteria such as *Rhizobium*, *Azotobacter*, *Azospirillum* and Actinomycetes and cause yield loss upto 40%. It inhibits the fruit setting in these crop plants such as tomato, brinjal, beans, and cereals. *Parthenium* can cause yield loss upto 40% in legume crops (Khosla S N and Sobti S N, 1981).

#### 1.3.3 Impact on Soil Microflora

*Parthenium* is known to its inhibitory effect on growth and activity potential of different bacterial species related to Nitrogen assimilation such as *Rhizobium* and *Azotobacter* and nitrifying bacteria like *Nitrosomonas*. Aqueous extract of *Parthenium* has detrimental effects on the growth of *Rhizobium*, *Nitrosomonas* and *Azotobacter*. It reduced the legheamoglobin content of root nodules by which *Rhizobium*-legume symbiosis is affected. Leaf and root leachates and their chemical component inhibit nitrate production (Sukhada KD and Jaychandra 1981).

#### 1.3.4 Effects on Animals

*Parthenium* weed is noxious for livestock, it can cause dermatitis and skin disorders in animals (Gunaseelan VN, 1998). Loss of skin pigmentation, dermatitis, mouth ulcers with extreme salivation and diarrhea has been observed in animals. If excess amount of this weed is eaten by the animals it can cause death. The *Parthenium* extract reduce the total WBC count in animals which results in the weakening of immune system.

#### 1.3.5 Effects on Human Beings

*Parthenium* plant parts can be toxic to some people it is estimated up to 73% of people living with the weed are sensitive to it. Females are twice more likely to be sensitive than males. Dermatitis, hay fever, asthma, and bronchitis are the major health problems found in human beings caused by the pollen grains and other plant parts of *Parthenium*. The major allergens found in this plant are parthenin, coronopilin, tetraeuris, and ambrosin. Its pollen grains are well known to causing asthma in human beings. Clinically the *Parthenium* dermatitis can be divided into five types which are-

- The classical pattern
- The chronic actinic dermatitis (CAD)

- The mixed pattern (classical and chronic actinic dermatitis pattern combination)
- The photosensitive lichenoid eruption
- The prurigo nodularis like pattern (Bailey LH, 1960).

#### 1.4 Control

Many efforts have been taken to control this weed which includes conventional, chemical, bioremediation and biological methods and also by doing certain combination and permutation with these methods seems to be a promising solution for effective management of this troublesome weed (Robert H, 2011; Saini A *et al.*, 2014). But due to its high proliferation rate and ecological adaptability, this weed is managed only below the threshold level and is still threatening biodiversity and causing health problems to both human and animals (Kaur M *et al.*, 2014).

##### 1.4.1 Conventional methods

###### 1.4.1.1 Physical control

Uprooting of *Parthenium* weeds before flowering and seed setting is an effective method, it is easy to uproot this weed during the rainy season when soil remain wet. Although labor intensive, hand weeding and hoeing can be beneficial, especially if done before the weeds produce seed (Tadesse B *et al.*, 2010 ; Tamado, T *et al.*, 2002). Manual removal is not very cost effective (Mahadevappa M, 1997) as it can be implemented only in limited situation. As manual uprooting increases the incidences of contact dermatitis and other allergic reactions among workers. Burning is another strategy which can also be employed to manage this weed, however it is not recommended as it distorted the quality of soil. But this method is also inadequate as it requires large quantity of fuel which is again cost effective and also it destroys other economic plants growing in nearby vicinity (Dogra *et al.*, 2011 ; Kumar M and Kumar S 2010).

###### 1.4.1.2 Mulching

While cultivation of rice mulching with rice straw is an effective method for controlling an array of weeds including *Parthenium*. This gives us an idea that mulching in common land may help us controlling this weed.

###### 1.4.1.3 Chemical

Controlling weed by using herbicides is more feasible and economical as compare to physical control method (Muniyappa and Krishnamurthy K, 1980). Research done by scientists reports that by applying 2,4-DEE(0.2%) and metribuzin (0.25 and 0.50%) were found to be more effective for controlling *Parthenium* weed just after 15 days of spraying (Khan H *et al.*, 2012). Downside of using herbicides is that it should be applied repeatedly especially in area of *Parthenium* seeds bank since they remain viable for 2 to 3 years (Tamado T *et al.*, 2002).

##### 1.4.2 Biological Method

Using other species such as bacteria, fungi, insects and various useful plants for suppressing the growth of *Parthenium* is also a promising approach for its control (Shabbir A *et al.*, 2010).

###### 1.4.2.1 Control with the help of fungal species

*Alternaria alternata*, *A. dianthi*, *A. macrospora*, *Fusarium oxysporum* (Pandey K *et al.*, 1992), *F. moniliforme*, *Rhizoctonia solani*, *Colletotrichum capsici*, *C. gloeosporioides*; and *Oidium partheni*; species of *Cladosporium oxysporum* *Ascochyta rabiei*, *Fusarium equiseti*, *Phoma glomerata*, *Cochliobolus hawaiiensis*, *Puccinia abrupta* var. *parthenicola* (Fauzi MT *et al.*, 1999) *Puccinia melampodii*, *Macrophomina phaseolina* and *D. Tetramera* (Parmelee JA, 1967) are the reported species which affect the *Parthenium* plant of all ages (Purahong W and Hyde KD, 2010). (Dhanaseeli and Sekar, 2004) isolated various soil-borne fungi and found that methanolic fractions of these fungi were highly phytotoxic to *Parthenium*. (Idrees and Javaid 2008) studied the effect of metabolites of seven phytopathogenic fungi [*Ascochyta rabiei*, *Cladosporium oxysporum*, *Macrophomina phaseolina*, *Drechslera hawaiiensis*, *Drechslera tetramera*, *Fusarium equiseti* and *Phoma glomerata*] against germination and early seedling growth of *Parthenium* weed. Metabolites of all the tested fungal species suppressed the germination and reduced the root and shoot growth of the target weed. Likewise (Javaid and Adrees, 2009) evaluated the herbicidal activity of nine phytopathogenic fungal species [*Alternaria alternata*, *Drechslera australiensis*, *Drechslera hawaiiensis*, *Drechslera biseptata*, *Drechslera rostrata*, *Fusarium oxysporum*, *Fusarium solani*, *Monilia stophila* and *Cladosporium sp.*]. Culture filtrates of *Alternaria alternata*, *Cladosporium sp.* and *Drechslera rostrata* drastically reduced the seed germination of *Parthenium* by 90, 73 and 50%, respectively. Culture filtrates of these and other fungal species [*Drechslera australiensis*, *Fusarium oxysporum* and *F. solani*] significantly reduced the root and shoot length of *Parthenium* seedlings. *A. alternata* contains AAL-toxin with herbicidal activity (Abbas H.K *et al.*, 1995).

The identification of pathogens causing degradation in *Parthenium* weed would help develop bioherbicide by extracting the toxins produced by the pathogens for effective control of *P. hysterophorus* in India. Biocontrol using mycotoxins from fungi is an eco-friendly and sustainable approach, and with current concerns related to biosafety and bioterrorism, the use of mycotoxins as weapons to control *Parthenium* hazards.

## II. MATERIAL AND METHOD

### 2.1 Sample collection

Five diseased leaves and one infected stem of *Parthenium* weed were collected from a *Parthenium* weed infested area in Bhopal and were taken to laboratory for further investigation.

Diseased *Parthenium* leaves with symptoms of yellowish-brown leaf blight and the stem with reddish-brown cankers on the basal part were collected and examined after they were cultured in potato dextrose agar (PDA) medium. Isolated fungus was identified based on morphological and microscopy analyses.

PDA solution at the rate of 10 ml per plate was transferred to Petri dishes and placed in the laboratory for solidification. The PDA culture was ready after three hours (Aggarwal *et al.*, 2014). The sample plant parts were then placed on PDA medium of Petri dishes. The samples were incubated at 25°C in dark condition for seven days during which the fungi grew well on the PDA medium. In order to obtain a pure culture, the isolated fungi were aseptically transferred to new PDA plates and the cultures were incubated for seven days under the conditions mentioned earlier. The pure culture was maintained on PDA slants for further investigation (Kaur *et al.*, 2016).

The identification of fungal isolate was done by preparing lactophenol cotton blue mounts from moist plate culture. Morphological characteristics of the fungal pathogens, such as the development of hyphae colour and septum of hyphae, conidia, conidiophores (if any), number of transverse and longitudinal septa and the size of the beak, etc. were recorded at different stages for identification of the pathogens. With the help of light stereomicroscope at 10×, 40× and 100× using micrometry, the size and shape of conidia (asexual spores) or conidiophores if present, type of hypha, number of the beak were observed. The pathogen was identified based on basic morphological characteristics as discussed in the literature (Chen *et al.*, 2014; Tredway & Burpee, 2006; Whitman *et al.*, 2012)

## III. OBSERVATION

- When the inoculum (sample) was incubated in the PDA plates at 37°C for 48 hours, the colonies of fungi were observed in the plates.
- The strains identified as *Aspergillus niger*, *Alternaria alternate* and *Puccinia graminis*.
- When broth culture of *Aspergillus niger*, *Alternaria alternate* and *Puccinia graminis* were inoculated on the healthy leaves of *Parthenium* plant, slowly and gradually biodegradation of the leaves observed from second month of the treatment and was recorded in Table 3.1 and the degradation of leaves till sixth month was recorded, after frequent subculturing within six months and observations are tabulated in Table 3.2.
- Biodegradation of leaves in First, Second, Third, Fourth, Fifth and Sixth month was observed in which percentage of effectiveness was shown in Table 3.3.

TABLE 3.1: After two months number of plants degraded by different microbial strains

Sr. No.	Name of the microorganism	No. of diseased plant in a quadrant			Total 60	Mean	Standard deviation
		Quadrant 1	Quadrant 2	Quadrant 3			
1	<i>Alternaria alternate</i>	10	7	10	27	9	1.7320
2	<i>Aspergillus niger</i>	8	6	5	19	6.3	1.5275
3	<i>Puccinia Graminis</i>	6	4	3	13	4.3	1.5275

TABLE 3.2: After six months number of plants degraded by different microbial strains

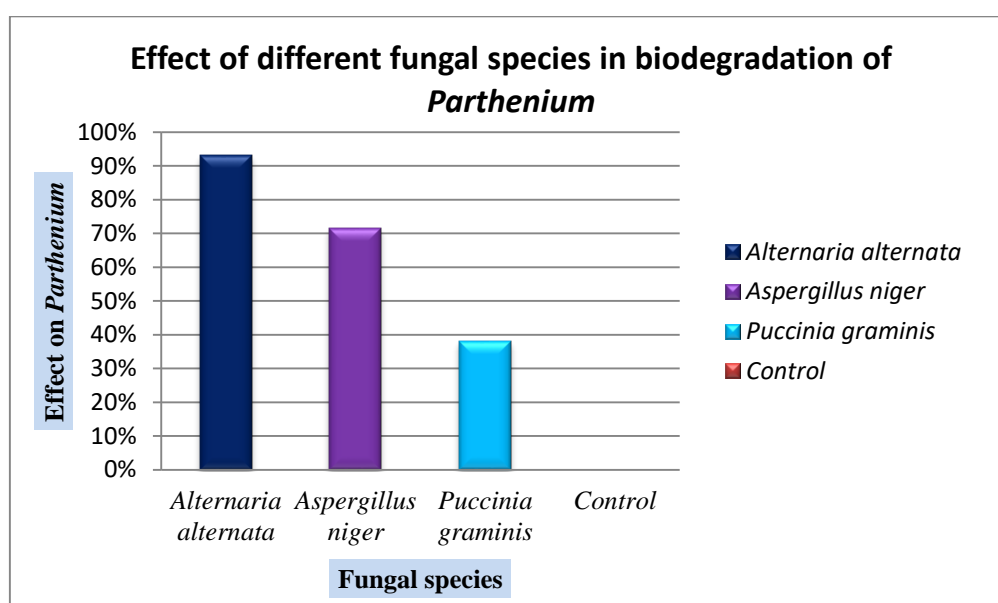
Sr. No	Name of the microorganism	No. of diseased plant in a quadrant			Total 60	Mean	Standard deviation	Percentage %
		Quadrant 1	Quadrant 2	Quadrant 3				
1	<i>Alternaria alternata</i>	18	15	23	56	18.6	4.0414	93%
	<i>Aspergillus niger</i>							



2		15	9	19	43	14.3	5.0332	71.5%
3	<i>Puccinia graminis</i>	8	6	9	23	7.6	1.5275	38%

TABLE 3.3: After sixth month the percentage of degradation by different microorganisms observed

S. No	Name of Microorganism	Effect on <i>Parthenium</i>	Effectiveness
1	<i>Alternaria alternata</i>	93%	Most Effective
2	<i>Aspergillus niger</i>	71.5%	Effective
4	<i>Puccinia graminis</i>	38%	Least Effective
5	Control	0 %	Not Effective



Graph-1

#### IV. RESULT

- The fungi responsible for the degradation of *Parthenium hysterophorus* are identified as:

Fungi - *Aspergillus niger*, *Alternaria alternate* and *Puccinia graminis*

- According to the investigation, the fungi *Alternaria alternata* plays a prominent role in degrading the *Parthenium* plant and was better in compare to *Aspergillus niger* and *Puccinia graminis*.

The inoculation of the culture led to the formation of light yellow colored spots and blights on the leaf of *Parthenium* weed. Black sclerotic colonies were seen on the surface of leaf and stem within 6-8 weeks of inoculation. The symptoms appeared on the fifth week after the inoculation. Later the light-yellowish lesions grew, which later covered larger areas and the leaf tips became rotten. Pathogenicity of the fungus proved to cause similar symptoms on new, fresh *Parthenium* leaf. Based on cultural and morphological characteristics, the pathogen was identified as *Aspergillus niger*, *Alternaria alternate* and *Puccinia graminis*.

#### V. CONCLUSION

Mechanical control, chemical herbicides, phytochemicals, composting and biological control has been pointed out as an effective management ways to eradicate this weed. (Ceresini, 2011) reported that the symptoms of the disease caused by fungus and bacteria depend on the host plant and the strain of the microorganism. Usually, the symptoms are wilting, black necrotic collar rot of the seedling, and the blight on leaves. The symptoms appear at the lower and older leaves as a small brown spot with a circular ring. Infection caused by the *Aspergillus niger*, *Alternaria alternate* and *Puccinia graminis* leads to the stunting of the

older plants and seedlings, wilting and yellowing. It can girdle the stem causing the plant to become stunted and eventually resulting its death.

(Gressler *et al.*, 2016) findings suggest that terrein helps to ensure that the fungus has sufficient nitrogen and iron to thrive in the rhizosphere. This study confirms that the production of secondary metabolites in microbes can degrade weeds to maintain eco-friendly environment. Future studies will analyze other ways to activate the production of secondary metabolites from microorganism to eradicate weeds, which may lead to the discovery of new important phytotoxins. The study identified as a soil-borne pathogen *Aspergillus niger*, *Alternaria alternate* and *Puccinia graminis* which has strong pathogenicity with *Parthenium* weed. Therefore, these pathogens can be used for producing bioherbicide for controlling *Parthenium* weed. More research is needed to increase the virulence of the pathogen so that biocontrol efficacy of the identified pathogens can be increased.

These soil-borne fungi can be used to make bioherbicide to control the *Parthenium* weed, especially those growing along the roadsides, fallow lands and residential areas where there are no susceptible crops. The mycoherbicide can be applied to the soil of *Parthenium* infested area as the mycelia may reside in the soil, which can attack the host plants. The molecular identification of the pathogen is in progress.

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