# Biocontrol of *Parthenium hysterophorus* L. by using fungal culture filtrate

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Seed germination and growth of Parthenium hysterophorus L. is significantly effected by fungal metabolites but the subject needs to be studied in more depth. The effect of fungal culture filtrates of Penicillium granulatum ,Fusarium oxysporum, Trichoderma viride, Alternaria alternata and Curvularia lunata on seed germination, plant vegetative and reproductive growth were investigated in controlled environment. Among the tested fungal culture filtrates Fusarium oxysporum exhibited 63.3% germination reduction and 0.64 mg/plant dry weight accumulation in 50% concentration of culture filtrate. The effect of five fungal culture filtrates on vegetative and reproductive growth of Parthenium was carried out on three different growth stages (2 weeks old, 4 weeks old and 6 weeks old plant). It was found that the culture filtrate of F. oxysporum inhibited vegetative and reproductive growth at all the three different growth stages of Parthenium as compared to control. Reduction in leaf production was 47.1% at 2 weeks old plant, 39.4% at 4 weeks old plant and 37% at 6 weeks old plant and reduction in plant height was 61.9% at 2 weeks old plant, 54.9% at 4 weeks old plant and 46.9% at 6 weeks old plant. Reduction in flower production was 100% in plant sprayed at 2 and 4 weeks stage and 74% at 6 weeks plant respectively after 50 days of spraying. This study indicated that spraying of culture filtrate of Fusarium oxysporum at the selected growth stages of Parthenium plant can cause considerable reduction in plant growth especially in the production of leaves and flower. Flowering was severely effected by the culture filtrate of Fusarium oxysporum, hence spreading of the weed could be controlled effectively. This aspect, however, awaits further experimental proof.

**Key words:** Parthenium hysterophorus, fungal culture filtrate, vegetative growth and reproductive growth

#### INTRODUCTION

Parthenium hysterophorus L. (Heliantheae: Asteraceae) is an annual herb which is now a wide-spread as pan-tropical weed and very difficult to control as it has occupied most of the area in both cropped and non cropped ecosystems. It is also posing a threat to human health and livestock due to its allergenic properties (Towers and Rao. 1992; McFadyen, 1995). The available chemical or mechanical control measures are neither feasible nor

economical. However, so far no single method has been proven satisfactory as each method suffers from one or more limitations (Templeton, 1990; Hasija et al., 1994). Therefore, weed management strategy needs to be shifted towards non -chemical methods. Managing weeds using classical biological means is less expensive, permanent and pollution free. Exploitation of microorganisms especially plant pathogenic fungi are now emerging as an effective and ecofriendly alternative to conventional methods of weed control (Pandey et al., 2003; 2004). However, there is an issue of hostrange specificity of the fungal pathogen to expose environmentally. Therefore, the present studies

Email: irabanta.singh@gmail.com or nganthoi\_d@rediffmail.com deal with the management of *Parthenium hysterophorus* L. using the selected fungal culture filtrate.

#### MATERIALS AND METHODS

### Preparation of fungal culture filtrate

An aliquot of 500 ml. potato dextrose broth (PDB), was poured in each 1000 ml Erlenmeyer flask and sterilized at  $121^{\circ}$ C for 20 min. The sterilized medium was inoculated with 6 mm (diam.) obtained from 10 day old culture of *Fusarium oxysporum*, *Alternaria alternata*, *Penicillium granulatum*, *Tricoderma viride* and *Curvularia lunata* grown on PDB. These inoculated flasks were incubated at  $25 \pm 1$  °C for 15 days and the culture filtrates were obtained after straining through two layers of cheesecloth for further studies.

# Effect of fungal culture filtrate on seed germination and seedling growth

#### Laboratory bioassay

Mature and healthy seeds of Parthenium hysterophorus were thoroughly washed with tap water and surface sterilized with 0.1% HgCl, for 2 to 3 min and washed with distilled water for 4 to 5 times and soaked individually to original (100%) and diluted (50%) cell free culture filtrates of Fusarium oxysporum, Alternaria alternata, Penicillium granulatum, Tricoderma viride and Curvularia lunata for 60 minute and with distilled water as control. The germination test was performed following Arshad et.al., (2001) with slight modification .The effectivity of the test fungal culture filtrate on seeds germination, root and shoot length of Parthenium seedlings were recorded on the 10th day post sowing and dry weight per plant was recorded after drying in an oven at 55-65°C for 48 hrs. The vigour index of Parthenium seedling was calculated following Abdul -Baki and Anderson (1973).

#### Foliar spray bioassay

Parthenium seeds were sown at 5 seeds/pot in eathern pots (10 cm in diameter) containing sterilized soil and sand (1:1) at three different intervals to obtain three different growth stages of plants (2 weeks old with average of 2 cm height; 4 weeks old with average of 5 cm height without flowers and 6 weeks old with average of 8 cm height without

flower). The applications of fungal culture filtrate were made in the form of foliar sprays at an interval of five days and each plant receiving 50 ml of culture filtrate . Three replicates were made for each growth stages of Parthenium. Pots grown with each of different growth stages sprayed with water served as control. Treated and control plants were maintained for 50 days and records were taken on plant height, number of leaves and number of flowers. Dry weight of each plant was recorded after drying in an oven at 55-65° C for 48 hrs. Data were subjected to the analysis of variance and means were compared using Least Significant Difference Test at p = 0.05. The percentage of reduction in growth was calculated by the formula Q= (a-b/a) x100; where Q=% reduction in growth; a= average growth in healthy plant; b= average growth in treated plant.

#### RESULTS AND DISCUSSION

The effectivity of culture filtrate at 50% and 100% of the tested pathogen on germination, vigour index of seedling growth and dry matter product of *Parthenium* plant are presented (Table 1). It reveals that incorporation of 100% concentration of different culture filtrate could drastically reduced *Parthenium* germination as compared to control.

Among the five tested pathogen, the culture filtrate of Fusarium oxysporum exhibited more inhibitory to germination and seedling growth of Parthenium plant as compared to other culture filtrates tested which recorded (80%) reduction in germination at 100% culture filtrate followed by Alternaria alternata and Curvularia lunata respectively. Similar finding was also represented by Pandey et al. (1991; 1992). According to them, the germination of Parthenium seeds and severe chlorosis and blightening were recorded with the application of culture filtrates of Fusarum oxysporum and Alternaria alternata at different concentration under laboratory conditions.

The maximum inhibition in root growth of *Parthenium* was observed in the culture filtrate of *Fusarium oxysporum* and *Alternaria alternata* which recorded 0.3 cm and 0.4 cm respectively at 100% and chlorosis and mortality of the early stage of seedling was also observed in 100% culture filtrate of *Fusarium oxysporum* and *Curvularia lunata*. This finding was supported by Templeton *et al.*, (1967) and Fulton *et al.*, (1965).

In case of shoot growth of *Parthenium*, at 100% concentration only *Fusarium oxysporum* and *Curvularia lunata* could arrest the aerial growth which recorded 0.9 cm each.

The vigour index (VI) of *Parthenium* seedlings among the culture filtrates incorporated, the minimum value of 192 (100%) and 320 (100%) vigour index were observed in culture filtrate of *Fusarium* 

Table 1: Effect of different fungus metabolites on germination, seedling growth and dry matter product of Parthenium plant.

Fungus species	Conce (%)	ntration	Germination (%)	Germination reduction	Shoot length (cm)	Root length (cm)	Plant dry weight (mg/plant)	Vigour index of seedling
				(%)				
Control	0	3	100	0	1.9	2.1	1.5	3800
Fusarium	50		36.7	63.3	1.2	0.6	0.64	660.6
oxysporium	100		20	80	0.9	0.3	0.36	192
Tricoderma viride	50		60	40	1.5	1.2	0.92	1554
•	100		33.3	66.7	1.1	0.9	0.70	632.7
Alternaria alternata	50		43.3	56.7	1.4	0.8	0.73	952.6
	100		26.7	73.3	1	0.5	0.45	387.1
Penicillium species	50		63.3	36.7	1.6	1.4	0.97	1899
	100		36.7	63.3	1.2	0.9	0.76	722.9
Curvularia lunata	50		56.7	43.3	1.2	1	0.85	1020.6
	100		26.7	73.3	0.9	0.7	0.63	320.4
CD at p ≥ 0.05			0.92		0.12	0.07	0.04	

The dry matter production of *Parthenium* seedlings varied from lowest value (0.64 mg per plant ) in *Fusarium oxysporum* culture filtrate to a maximum (0.97 mg per plant) in culture filtrate of *Penicillium granulatum* when treated with 50% culture filtrate. At higher concentrations (100%), significant reductions were observed with (minimum of 0.36 mg per plant) culture filtrate of *Fusarium oxysporum*.

oxysporum and Curvularia lunata . However, under controlled condition many fold increases (3800) of vigour index was obtained.

# Effect of Fusarium oxysporium on different growth stages of Parthenium

The effect of culture filtrate of Fusarium oxysporum

Table 2: Effect of Fusarium oxysporum metabolite at three different growth stages of Parthenium plants after 50 days of spraying

Growth		2 week old		6 week old plants	CD at p ≥ 0.05	Percent	tage reduction	on (%)
parameter		plants	plants			2 week old plant	4 week old plant	6 week old plan
Mean plant	Control	21	25.3	38.2	0.90			
height(cm)	Treated	8	11.4	20.3	0.25	61.9	54.9	46.9
Mean	Control	15.5	20.3	23.8	0.69			
No. of leaves	Treated	8.2	12.3	15.0	0.52	47.1	39.4	37
Mean	Control	3.0	4.4	5	0.45			
No. of flowers	Treated	0.0	0.0	1.3	0.07	100	100	74
Mean dry	Control	3.4	4.5	5.3	0.37			
weight of plant(g)	Treated	1.5	2.1	2.8	0.20	55.9	53.3	48.1

on different growth stages of *Parthenium* is given (Table 2).

#### Vegetative growth

It is evident from findings (Table 2) that the foliar sprays with culture filtrate of *Fusarium oxysporum* inhibited vegetative growth at all the different stages of *Parthenium* as compared to control. The plant height, number of leaves and dry weight of inoculated plants were significantly lower than the control plants. It has been reported that similar

ing also. Compared to the control plants, plants treated at 2 and 4 week of age did not produce any flower after 50 days of spraying. Number of flowers of treated plants was significantly lower than the control plants. This study indicated that spraying of Fusarium oxysporium culture filtrate at any stage of Parthenium plant can cause considerable reduction in plant growth especially in the production of leaves and flowers. Reduction in flower production was 100% in plants sprayed at 2 and 4 weeks stage and 74% at 6 week (Table 2). Since

Table 3: Effect of Trichoderma viride metabolite at three different growth stages of Parthenium plants after 50 days of spraying

Growth parameter		2 week old plants	4 week old plants	6 week old plants	CD at p ≥ 0.0	5 _	Percentag	ge reduction	(%)
				8			2 week old plant	4 week old plant	6 week old plant
Mean plant height(cm)	Control	21	25.3	38.2	0.90		E gg	2011 A.C. 144	
	Treated	13.1	17.1	30.2	0.44		37.6	32.4	20.9
Mean No. of leaves	Control	15.5	20.3	23.8	0.69		27.7	20.2	11.3
	Treated	11.2	16.2	21.1	0.49		L to	20.2	11.0
Mean No. of	Control	3.0	4.4	5	0.45				
flowers	Treated	2.1	3.3	4	0.18		30	25	20
Mean dry weight of plant(g)	Control	3.4	4.5	5.3	0.37		35.3	24.4	20.4
piant(g)	Treated	2.2	3.4	4.3	0,43		00.0	<b>6</b> 7,7	20.7

results were observed in the fields in Mexico where repeated spraying with the P. melampodii fungal culture filtrate had a severe impact on P. hysterophorus, causing reduction in the number of leaves (ca 30%), in plant height(ca 50%). It was also found that at 50 days after spraying, reduction in leaf production was 47.1 % at 2 weeks, 39.4% at 4 weeks and 37% at 6 weeks old plants, reduction in plant height was 61.9% at 2 weeks, 54.9% at 4 weeks and 46.9% at 6 week and reduction in dry weight production was 55.9% at 2 week, 53.3% at 4 week and 48.1% at 6 week old growth stages.

### Reproductive growth

The foliar spray of culture filtrate of Fusarium oxysporum caused inhibitory effect on the flower-

flowering was severely affected by *Fusarium* oxysporum culture filtrate, spreading of the weed would be controlled effectively.

# Effect of Trichoderma viride on different growth stages of Parthenium

The effect of *Trichoderma viride* culture filtrate on different growth stages of *Parthenium hysterophorus* is shown in Table 3.

#### Vegetative growth

The data in Table 3 revealed that the foliar sprays with culture filtrate of *Trichoderma viride* do not have much effect on the vegetative growth of all the different stages of *Parthenium* as compared to control. After 50 days of spraying, reduction in leaf production was only 27.7 % at 2 weeks, 20.2% at 4

weeks and 11.3% at 6 weeks old plants. Regarding the plant height its reduction was 37.6 % at 2 weeks,32.4% at 4 weeks and 20.9% at 6 week and

reduction in dry weight production was 35.3% at 2 week, 24.4% at 4 week and 20.4% at 6 week old growth stages. Among the different growth stages

Table 4: Effect of Alternaria alternata metabolite at three different growth stages of Parthenium plants after 50 days of spraying

Growth		2 week old	4 week old	6 week old	CD at p $\geq$ 0.05	Percentag	ge reduction	(%)
parameter		plants	plants	plants		2 week old plant	4 week old plant	6 week old plant
Mean plant height(cm)	Control	21	25.3	38.2	0.90			
	Treated	11.3	15	27.2	0.61	46.2	40.7	28.8
Mean No. of leaves	Control	15.5	20.3	23.8	0.69			
loavoo	Treated	10.7	14	18.3	0.20	31	31	23.1
Mean								
No. of flowers	Control	3.0	4.4	5	0.45			
	Treated	1.8	2.9	3.6	0.54	40	34	28
Mean dry weight of	Control	3.4	4.5	5.3	0.37			
plant(g)	Treated	2.1	2.9	3.8	0.41	38.2	35.5	29.6

Table 5: Effect of *Penicillium granulatum* metabolite at three different growth stages of *Parthenium hysterophorus* L. plants after 50 days of spraying

Growth parameter		2 week old	4 week old		CD at p ≥ 0.05	Percentage reduction (%)			
		plants	plants	plants		2 week old plant	4 week old plant	6 week old plant	
Mean plant height(cm)	Control	21	25.3	38.2	0.90				
, , , ,	Treated	14.2	18.2	31.5	0.06	32	28.1	17.5	
Mean No. of leaves	Control	15.5	20.3	23.8	0.69		6 5		
	Treated	11.8	16.6	20	0.44	23.9	18.2	16	
Mean No. of	Control	3.0	4.4	5	0.45				
flowers	Treated	2.2	3.4	3.9	0.40	26.7	22.7	22	
Mean dry weight of	Control	3.4	4.5	5.3	0.37				
plant(g)	Treated	2.5	3.7	4.4	0.23	26.5	17.8	18.5	

2 weeks old *Parthenium* plant when treated with *Trichoderma viride* exhibited maximum inhibition as compared to other growth stages.

#### Reproductive growth

The foliar spray of culture filtrate of *Trichoderma* viride caused less inhibitory effect on the flowering

also. Numbers of flowers of treated plants were not significantly lower than the control plants. Reduction in flower production was 30% at 2 weeks stage, 25 % at 4 weeks stage and 20% at 6 week (Table 3). Culture filtrate of *Trichoderma viride* was effective only at 2 weeks old plant and thus can be use as mycoherbicide against *Parthenium* only in the early stages of growth.

After 50 days of spraying, reduction in leaf production at 2 weeks and at 4 weeks old showed 31% reduction and 23.1 % at 6 weeks old plants, reduction in plant height was 46.2% at 2 weeks plant, 40.7% at 4 weeks and 28.8% at 6 week plant and reduction in dry weight production was 38.2 % at 2 week, 35.5 % at 4 week and 29.6% at 6 week old growth stages (Table 4).

Table 6: Effect of Curvularia lunata metabolite at three different growth stages of Parthenium plants after 50 days of spraying

Growth parameter	2 week old plants		ld 4 week old 6 week plants old plants		CD at p ≥ 0.05	Percentage reduction (%)			
					_ 3	2 week old plant	4 week old plant	6 week old plant	
Mean plant height(cm)	Control	21	25.3	38.2	0.90			u <sub>i</sub>	
neight(cm)	Treated	9.5	13.2	23.2	0.61	54.8	47.8	39.3	
Mean No. of leaves	Control	15.5	20.3	23.8	0.69				
	Treated	10	13	16.5	0.56	35.5	35.9	30.7	
Mean No. of	Control	3.0	4.4	5	0.45				
flowers	Treated	1	2.1	3	0.38	66.7	52.2	40	
Mean dry weight of	Control	3.4	4.5	5.3	0.37				
plant(g)	Treated	1.8	2.5	3.3	0.44	47.1	44.4	38.9	

## Effect of Alternaria alternata on different growth stages of Parthenium

The effect of culture filtrate of *Alternaria alternata* on different growth stages of *Parthenium* is given in Table 4.

### Vegetative growth

Regarding the vegetative growth as compared to the control plants, plants sprayed at 2 and 4 week of age showed significant reduction in plant height, number of leaves and dry weight production when sprayed with culture filtrate of *Alternaria alternata* (Table 4).

Pandey et al. (1991) assessed the pathogenicity of Alternaria alternata against Parthenium and concluded that Alternaria alternata could cause considerable damage to the weed which support the finding of this experiment.

### Reproductive growth

The foliar spray of culture filtrate of Alternaria alternata caused inhibitory effect on the flowering also. Compared to the different growth stages, plants treated at 2 and 4 week of age produced lower number of flower after 50 days of sprayed. Number of flowers of treated plants was significantly lower than the control plants. This study indicated that spraying of Alternaria alternata culture filtrate at 2 weeks old and 4 week old plant stage of Parthenium can cause considerable reduction in plant growth especially in the production of plant height and flowers. Reduction in flower production was 40% in plants sprayed at 2 weeks, 34% at 4 weeks stage and 28% at 6 week old plant (Table 4). Since flowering was severely effected by Alternaria alternata when sprayed at early growth stages of plant, the spreading of the weed would be controlled effectively.

# Effect of Penicillium granulatum on different growth stages of Parthenium

The effect of culture filtrate of *Penicillium granulatum* on different growth stages of *Parthenium* is shown in Table 5.

#### Vegetative growth

The data in Table 5 revealed that the foliar sprays with culture filtrate of Penicillium granulatum do not have much effect on vegetative growth of all the different stages of Parthenium as compared to the control plant. It was found that 50 days after inoculation, reduction in leaf production was only 23.9 % at 2 weeks, 18.2 % at 4 weeks and 16 % at 6 weeks old plants. Regarding the plant height, the reduction in plant height was 32 % at 2 weeks ,28.1% at 4 weeks and 17.5% at 6 week and reduction in dry weight production was 26.5% at 2 week, 17.8% at 4 week and 18.5% at 6 week old growth stages (Table 5). Among the different growth stages 2 weeks old Parthenium plant when treated with Penicillium granulatum culture filtrate exhibited maximum inhibition as compared to other growth stages.

### Reproductive growth

The foliar spray of culture filtrate of *Penicillium granulatum* caused less inhibitory effect on the reproductive growth also. Numbers of flowers of treated plants were not significantly lower than the control plants. Reduction in flower production was 26.7 % at 2 weeks stage, 22.7 % at 4 weeks stage and 22% at 6 week old plant (Table 5). Culture filtrate of *Penicillium granulatum* showed least effect at all the different growth stages of *Parthenium* thus cannot be use as mycoherbicide against *Parthenium*.

# Effect of *Curvularia lunata* on different growth stages of *Parthenium*

The effect of culture filtrate of *Curvularia lunata* on different growth stages of *Parthenium* is given (Table 6).

### Vegetative growth

It is evident from the data in Table 6 that the foliar sprays with culture filtrate of *Curvularia lunata* inhibited vegetative growth at all the different stages of *Parthenium* as compared to control. The plant height, number of leaves and dry weight of inocu-

lated plants were significantly lower than the control plants (Table 6) in all the different growth stages. It was found that 50 days after inoculation, reduction in leaf production was 35.5 % at 2 weeks, 35.9% at 4 weeks and 30/7% at 6 weeks old plants, reduction in plant height was 54.8% at 2 weeks, 47.8% at 4 weeks and 39.3% at 6 week and reduction in dry weight production was 47.1% at 2 week, 44.4% at 4 week and 38.9 % at 6 week old growth stages.

#### Reproductive growth

The foliar spray of culture filtrate of Curvularia lunata caused inhibitory effect on the flowering also. Number of flowers of treated plants was significantly lower than the control plants. This study indicated that the spraying of Curvularia lunata culture filtrate at any stage of Parthenium plant can cause considerable reduction in plant growth especially in the production of leaves and flowers. Reduction in flower production was 66.7% in 2 week old plant, 52.2% at 4 weeks stage and 40% at 6 week old growth stages (Table 6). Since flowering was severely effected by Curvularia lunata, spreading of the weed would be controlled effectively. Kauraw and Bhan (1994) surveyed various areas in Madhya Pradesh during 1992 to 1999 and reported that the occurrence of Curvularia lunata from leaves of Parthenium hysterophorus L. Aneja et al. (1994) described Curvularia lunata, causing a leaf spot disease on Parthenium as a new host record for the plant in India . Further Pandey et al. (1991) evaluated for its mycoherbicidal ability towards Parthenium and found to be effective against Parthenium growth.

#### CONCLUSION

Even though *Parthenium* has slowly but surely been becoming a serious weed in many agricultural situations, the menace is more rampant in urban areas, especially in cities like Bangalore, New Delhi, Hyderabad, Coimbatore etc (Kumar,1998). As pointed out by Evans (1997) environmentally mycoherbicides may prove to be more acceptable for sustainable mananagement of *Parthenium* in urban areas. Proper technology for fermentation, formulation, delivery and application systems should be developed for promising mycoherbicide candidate pathogens for achieving tangible and sustainable management of this noxious weed. It has been suggested that the concept of the cul-

ture filtrate mycotoxic for the control of weeds is also an attractive proposal for the biological control of weeds. Therefore, it appears that the culture filtrate used in this study must have some toxin or alike substances which needs further detailed investigation and its pathogenecity to organism and effect on environment need to be test prior to release to a particular environment.

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