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Studies on biocontrol potential and phytotoxic effect of secondary metabolites of fungi isolated from *Parthenium hysterophorus*

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ABSTRACT

Herbicides are employed for crop protection and control of superfluous plants such as agricultural weeds. However it can also cause phytotoxic effects in plants that are not within the area of application. Microbes are valuable sources of phytotoxins. The evolutionary apprehension for phytotoxin production is apparent due to increased swarm by microbial plant pathogens. Phytotoxins are produced by many fungi that are pathogenic to weeds which are an ample assortment of chemical substances including sesquiterpenoids, sesterterpenoids, diketopiperazines, peptides, spirocyclic lactams, isocoumarins, and polyketides. Biological control refers to the intentional manipulation of natural enemies, insects, bioherbicides, nematodes, snails, and competitive plants to control harmful weeds. It is gaining momentum as it is an effective and ecofriendly alternative to conventional methods of weed control. Present studies cover the screening of phytotoxic effect of Alternaria alternata,Fusarium oxysporum, Curvularia lunata, and Nigrospora oryzae which are isolated from the diseased part of Parthenium hysterophorus. Nigrospora oryzae incites severe wilt, chlorosis and necrosis in Parthenium hysterophorus as observed in shoot cut bioassay and detached leaf bioassay. It is speculated to be a dominating pathogen thereby causing maximum phytotoxic damage. On screening solvent extracted fractions, carbon tetra chloride fraction was found to exhibit maximum phytotoxicity, whereas other solvents i.e. ethyl acetate, chloroform and n- butanol were least effective against Parthenium hysterophorus.This is the first report confirming the potential of this particular toxin from Nigrospora oryzae as a biorational, which can be applied as phytotoxin against Parthinium hysterophorus.

Key words: *Phytotoxin, CFCF,Parthenium, Herbicidal potential.*

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INTRODUCTION

Parthenium hysterophorus L. is a member of the family Asteraceae and popularly known as Chatak Chandani, Bitter weed, Ramphool, Gajarghas. It is a native of West Indies, Central and North America but was introduced into India along with food grain imported under PL – 480 or "Food for Peace" scheme, which is a food support programme of the US government and spread terrifyingly like a untamed conflagration to almost all the states of India and were customary as a naturalized weed. Efforts have been made to cope the weed employing different methods such as mechanical, competitive replacement (allelopathy), chemical, and biological control methods (Gupta *et al*,2006; Kaur *et al*, 2014). It has been recognized as one of the major threats to native species and ecosystems around the world because of their capability of spreading fast, their high competitiveness and ability to colonize new areas within short periods. The nature and severity of the impacts of these species on society, economic life, health and national heritage are of global concern. *Parthenium hysterophorus* is a menace to crop production, animal husbandry, human health and biodiversity in its areas of infestation. The allelopathic potential of *P. hysterophorus* had played an important role in displacement of natural vegetation and barge in natural succession (Mishra, 2013). The major components of toxin being 'parthenin' and other phenolic acids such as caffeic acid, vanillic acid, anisicacid, panisic acid, chlorogenic acid and parahydroxy benzoic acid are lethal to human beings and animals. (Mahadevappa,1977; Oudhia, 1998).

Microbes and microbial product prove to be safer by being less persistent in environment and are more selective. There are several reports where secondary metabolites caused severe damage to plants by bringing a biochemical or morphological change (viz., chlorosis, wilting, epinasty, necrosis etc). AAL toxin, Tentoxins, Cornexistin, Fumosin, Monliformin etc., have been successfully exploited for management of many weed (Pandey *et al*,1999,2001).Realising the severe phytotoxicity of secondary metabolite of microorganisms, their herbicidal potential in weed management have also been explained by many worker (Duke,1986; Ohara *et al*,1995; Kenfield *et al*,1989; Hoagland 1999, 2001; Hoagland and Cutler, 2000). During the recent years the main focus in development of herbicides are on biodegradibility. Microbial secondary metabolites with herbicidal properties therefore become straggering (Strobel *et al*, 1991, Abbas *et al*, 1992; Abbas and Duke, 1995; Charudattan 1996; Barbosa *et al*,2002).

During periodical survey for collection of diseased *Parthenium hysterophorus* plants and followed by isolation and screening secondary metabolite of *Nigrospora oryzae* was found to be highly effective in causing phytotoxic damage to the weed. Therefore present study was carried out to determine herbicidal potential of *Nigrospora oryzae* against *Parthenium hysterophorus.*

MATERIALS AND METHODS

Recovery of fungal strains

Tissues from diseased portion (of leaves and stem) of the weed were cut down into about 1mm pieces with the help of sterilized blade and forceps and under aseptic condition transferred to petri dishes containing pre-sterilized PDA medium. The petri dishes were later incubated at 28±1ºC in BOD (Yorco, India) incubator and examined regularly. As soon

as growth appeared they were transferred to Potato Dextrose Agar slants.

Production of Cell Free Culture Filtrate (CFCF)
150 ml Erlenmeyer flasks containing 50 ml of Richards broth (KNO_3-10gm, KH_2PO_4-5 gm, $MgSO_47H_2O$-2.8gm, Sucrose-35gm, $FeCl_3$-100µg(trace), distilled water-1000ml) were seeded with 5 mm disc that were separated from 7 days old actively growing culture on PDA medium at 28±1ºC in BOD (Yorco, India).Inoculated flasks were incubated at 28±1ºC in BOD incubator for 7, 14, 21, 28 days.

Extraction of Cell Free Culture Filtrate
CFCF was aseptically obtained by filtering the metabolised growth medium through Whatman filter paper No. 1. The supernatant was filtered through the sartorius filter paper 0.45 µm, Minisart (Sartorius Gottingen, Germany) millipore filter under *in vacuo* conditions (Walker and Templeton, 1978).

Shoot cut bioassay
Parthenium hysterophorus shoot from 30 to 35 days old seedlings grown in pot containing soil: sand: peat (1:1:1) inside a plant growth chamber (Yorco, India)were taken and inclined cut was made at the tip in sterilized water. They were dipped in CFCF in test tube (vial). These were mounted in artificial illumination (3.5×10^2 erg/cm^2/s).The tubes (vial) was sealed with aluminium foil and the effect of toxic metabolites was observed on the shoots after 24, 48 and 72 hrs at room temperature (28±2°C) (Sharma and Sharma, 1969 and Chiang *et al*,1989). Phytotoxicity was determined following the method of Abbas and Boyette (1992)and Abbas and Duke (1995)on rating scale as(0,no symptom; 1,slight chlorosis; 2, slight chlorosis and necrosis; 3, marked chlorosis and necrosis; 4 marked chlorosis and high necrosis; 5,acute chlorosis and necrosis leading to death of shoot.

Detached leaf bioassay
Surface sterilized (2% NaOCl) leaves detached from 15 to 20 days old seedlings of the weed were treated with toxic metabolites and then were incubated for 24, 48 and72 hrs in moist chamber at room temperature. Detached leaf were also treated with different dilution of toxic metabolites and then incubated for 24,48 and 72 hrs in moist chamber at room temperature (Sharma *et al*, 2004).They were incubated at 28±2°C under constant fluorescent illumination (2×10^4 erg/cm^2/s). A positive reaction was indicated by the appearance on interveinal chlorotic and necrotic spots (Barash *et al*,1978)

In all bioassay, sterilized non-metabolized growth medium was used for control and sterilized distilled water serve as control over control. All the treatments were carried out in triplicates and all bioassay were repeated thrice.

Effect of CFCF on Biological content
Effect of CFCF of *Nigrospora oryzae* on biological content of *Parthenium hysterophorus* was determined.

A) Chlorophyll contents
Determination of chlorophyll a, b and total chlorophyll was done by the method of Arnon, 1949.The leaves from Detached leaf bioassay of 25%, 50%, 75% and 100% leachates and 7,14,21 and 28 days leachates and different solvent treated leachates of 9 days were grounded with neutral sand and 10 ml acetone:alcohol(4:1) and centrifuged at 300 rpm for 10 min. The volume of supernatant was recorded. Optical density was measured at 645 and 663 nm.

$$\text{Total chlorophyll (mg/ml)} = \frac{20.2\ A_{645} + 8.02\ A_{663}}{a \times 1000 \times W} \times V$$

$$\text{Chlorophyll } a \text{ (mg/ml)} = \frac{12.7\ A_{663} - 2.69\ A_{645}}{a \times 1000 \times W} \times V$$

$$\text{Chlorophyll b (mg/ml)} = \frac{22.9\ A_{645} - 4.68\ A_{663}}{a \times 1000 \times W} \times V$$

a = Length of light path in the cell (usually 1 cm).
b = Volume of the extract in ml.
c = Fresh weight of the sample in gms.

B) Protein contents
Diseased as well as healthy tissues of target weed were grinded with cold 200 mM sodium phosphate buffer of pH 6 in a precooled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 20min. in freezing centrifuge (4°C).Then was discarded in 5% TCA (Tri Chloro Acetic acid) at 0°C for 15 min in ice bath, to precipitate the proteins. The mixture was allowed to stand for 15 min in an ice bath and again centrifuged. Supernatant was discarded. This process was repeated twice and the pellets were re-extracted with absolute ethanol and supernatant was discarded. It was again processed with hot ethanol ether mixture. This pellet contains protein and nucleic acid. Lowry *et al*, 1951).

Thermal nature of phytotoxic moiety
To ascertain the mode of extraction of the phytotoxin moiety, it was essential to know the thermal nature of phytotoxin (s).For this the CFCF was subjected to different temperature treatments, *viz*., 50,100 and 121°C.Each treatment was carried out for 15 min. The phytotoxic activity of each treatment was assessed using shoot cut bioassay (Siddaramaiah *et al*, 1979). Each treatment was carried out in triplicate and sterilized distilled water and autoclaved uninoculated medium served as control.

Solvent extraction of CFCF
A volume of 25ml of CFCF was taken in a separating funnel. Various organic solvents were used for extraction. A volume of 15ml of carbon tetra chloride was added to 25ml of filtrate, shaken well and kept until the two phases got separated. The lower carbon tetrachloride layer was separated from beaker and was vacuum dried. The remaining filtrate was extracted similarly in succession with chloroform, ethylacetate, n-butanol. All the organic fractions were evaporated to dryness in a vacuum dessicator at 45ºC (Templeton *et al*, 1979).

Phytotoxic activity of various fractions
All fractions separated by solvent extraction were subjected to *in vaccuo* dessication at 45ºC to remove any trace of solvents and to obtain the final residue. Residues were named as obtained, viz. fraction A (carbon tetra chloride); fraction B (chloroform); fraction C (ethyl acetate); fraction D (n-butanol). Test residues were tested for their phytotoxic activity using detached leaf bioassay (Nakjima *et al*, 1991).

RESULTS AND DISCUSSION

Data given in Table-1 indicates the interrelation ship between growth (biomass), final pH and toxin production by the four primarily screened fungi against *P. hysterophorus.* There is a gradual increase in final pH and mycelial dry weight (biomass) with increasing incubation days. Maximum biomass was obtained after 28 days of fermentation.

Nigrospora oryzae(FGCC#78) CFCF causes maximum phytotoxic damage after 48 hpt followed by *Fusarium oxysporum* (FGCC#76), *Alternaria alternata* (FGCC#75) and *Curvularia lunata*(FGCC#77). Within 24 hrs of treatment slight curling was visible. At advanced stage rapid wilting of leaves, epinasty veinal chlorosis and necrosis of leaves was observed resulting in death of entire shoot. Similar observation regarding interrelation ship between biomass, pH and phytotoxin production has been observed by several other workers (Pandey *et al*, 2000; Saxena *et al*,2000; Saxena and Rajak, 2001; Chandla, 1999).

As given in Table-2, 21 days old fermented broth of *Nigrospora oryzae* imparted maximum phytotoxic damage to *P. hysterophorus* leaves as assessed by detached leaves

bioassay, the phytotoxicity of secondary metabolite was maximum after 72 hpt (hours post treatment) followed by 48 hpt and 24 hpt. Saxena and Kumar (2010) also reported mycoherbicidal potential of *Alternaria alternata* ITCC (LC#508)which caused 50% damage to plants as observed through *in vitro* detached leaf and whole plant bioassay at 96 hour after treatment at a concentration of 1×10^6 spores/mL. Similarly (Pandey *et al*, 1998) and Shukla and Pandey(2006) reported *Sclerotium rolfsii* to incite severe collar rot disease on *Parthenium hysterophous.*

Table-3, represent that there was a decrease in phytotoxic damage rating with the increase in dilution. On treating *P. hysterophorus* leaves with different concentration of 21 days old CFCF *Nigrospora oryzae* maximum phytotoxic damage was exhibited by 100%concentration followed by 75%, 50%, and 25%. Thakur (2006) and Sanodiya(2006) have reported similar results. Observation regarding the phytotoxic damage rating on treatment with different days old Cell Free Culture Filtrate at different concentrations on test seedlings have been reported by Joseph (2002)which strengthens the present data. (Singh *et al*,2010) also reported similar results.
As shown in Table-4, On treating P. *hysterophorus* leaves with different concentration of the phytotoxin of 21 days old CFCF of *Nigrospora oryzae*. There was reduction in total chlorophyll to 84.04% chl a and chl b are less affected. There was 73.53%reduction in protein content with 100%, 75%, 50%and 25%. Photobleaching of chloroplast pigments in given tissue was observed after 48 hpt.

The effect of FBI toxin on Jimson weed is identical to herbicide action (Abbas *et al*, 1992; Duke *et al*, 1992). Toxin causes the photodynamic porphyrin intermediate, protoporphyrin IX to accumulate in the plasma membrane lipid prooxidation (Lee *et al*,1993;1994). Herbicidal potential test on *Parthenium hysterophorus* was also done by other with positive results. (Pandey *et al*, 2003; Pandey *et al*,2006; Joseph 2000; Abbas *et al*,1992). Kauraw *et al* (1997) also reported *Fusarium pallidoroseum*, on *Parthenium* from Jabalpur. It was found to reduce seed germination, seedling vigour, height of plant, number of branches, number of flower and reported as potential biocontrol agent for *Parthenium* management. *Cladosporium* sp. (MCPL – 461), a flower leaf pathogen of *Parthenium*, produces symptom on the flower, buds, and inflorescence, and causes sterility and reduces seed viability. The severity of pathogen to the reproductive organ led to serious damages of the *Parthenium* plants and may be used as potential mycoherbicides against this weed. (Kumar *et al*,2009)

Table-5 shows, on treatment with different days old CFCF of *Nigrospora oryzae*. Maximum percentage reduction in biological contents of *P. hysterophorus* leaves was observed in 21 days old CFCF as is evidenced by reduction of total chlorophyll to 90.65% followed by chl a chl b. Maximum reduction in protein content reported was 73.62% after 72 hpt.

As shown in Table-6,The phytotoxicity of *Nigrospora oryzae* was stable at 50°C, 100°C and 121°C (15 psi).Thus, it could be concluded that the phytotoxic moiety was thermo-tolerant.

Similar observations have also been made by (Siddaramaiah *et al*,1979) while working with *Phaephleospora indica*. (Kurian *et.al*,1977) recorded thermostable and non proteineous nature of toxin produced by *Cristularia pyramidalis*.

As given in Table-7, When *P. hysterophorus* leaves were treated with different solvent extracted fraction of CFCF. Maximum phytotoxic damage was observed in case of Carbon tetra chloride followed by chloroform, ethyl acetate and n-butanol. Damage was observed after 72 hrs which started at 24 hpt.

In contrast to these results, (Pandey *et al*,2001) have reported maximum phytotoxic damage to Lantana at 48 hpt by the active metabolite extracted from CFCF of *Phoma herbarum* FGCC#3 with Benzene. Less phytotoxic damage was reported with Ethyl acetate and Butanol fractions of CFCF. Similarly, Vikrant *et al* (2006) extracted and characterized a novel herbicidal compound 3-nitrophthalic acid against Parthenium from CFCF of *Phoma herbarum* with ethyl acetate as the organic solvent.

Reduction in biological contents of *P. hysterophorus* leaves was observed when treated with partially purified, CFCF of *Nigrospora oryzae*. Carbon tetra chloride extracted fraction caused remarkable reduction i.e. 92% in chlorophyll a followed by total chlorophyll and chlorophyll b. Protein content was reduced considerably to 95.05% with this fraction. Reduction in biological contents by carbon tetra chloride extracted fraction was followed by Ethyl acetate, chloroform, n-Butanol extracted fraction (Table -8). Similar results have been shown by other workers. Several secondary metabolites have been extracted by scientists worldwide. Fumonisin, Ophiobolin A were extracted in chloroform; Alternariol in ether/benzene; tentoxin could be extracted by ether, Chloroform and benzene (Orsenigo, 1957; Bassett *et al*, 1967; Freeman, 1965; Saad *et al*, 1970).

Table no. 1: Interrelationship between growth (biomass), final pH and toxin production by some fungi isolated from *Parthenium hysterophorus*.

S. No.	Fungus	Incubation Period	Final pH	Biomass (mg/150ml)	Phytotoxic Damage Rating		
					24 hrs	48 hrs	72 hrs
1	*Alternaria alternata (FGCC#75)*	7	4.42	0.26±0.09	0.6±0.57	1.5±0.7	1.5±0.7
		14	5.66	0.34±0.02	1.6±0.0	2.0±0.0	2.0±0.0
		21	6.92	0.44±0.12	2.1±0.2	2.5±0.57	2.6±0.2
		28	6.92	0.47±0.04	1.3±0.4	2.0±0.0	2.4±0.4
2	*Fusarium oxysporum (FGCC#76)*	7	4.51	0.26±0.09	1.0±0.0	1.5±0.0	1.5±0.57
		14	4.63	0.21±0.01	2.5±0.7	2.5±0.7	2.5±0.7
		21	5.00	0.34±0.02	3.0±0.0	3.5±0.7	4.0±1.4
		28	5.97	0.44±0.12	3.0±0.0	3.5±0.7	3.8±0.2
3	*Curvularia lunata (FGCC#77)*	7	4.9	0.27±0.02	1.5±0.7	1.0±1.4	1.5±0.7
		14	6.1	0.35±0.06	1.0±0.7	1.5±1.4	1.5±0.7
		21	6.1	0.37±0.04	2.5±0.0	3.0±1.4	3.5±0.7
		28	6.3	0.38±0.04	1.5±0.7	2.0±0.7	2.5±0.7
4	*Nigrospora oryzae (FGCC#78)*	7	5.2	0.16±0.02	0.0±0.0	1.5±0.7	2.0±1.4
		14	6.1	0.30±0.07	2.5±0.0	3.0±1.4	3.5±0.7
		21	7.4	0.47±0.02	4.67±0.47	4.97±0.4	5.0±0.0
		28	7.8	0.49±0.04	4.5±0.7	4.33±0.24	4.50±0.0

Incubation period – 28±1^{0}C , Incubation pH = 3.73. Readings are Mean±SE

Table no.2: Assessment of phytotoxic damage rating of *Parthenium hysterophorus* treated with different days old CFCF of *Nigrospora oryzae* by detached leaf Bioassay

Incubation Days	Phytoxic damage rating (hpt)		
	24	48	72
7	1.7±0.2	2.0±0.2	2.1±0.9
14	2.0±0.4	2.2±0.41	3.0±0.00
21	2.9±0.2	3.9±0.0	4.7±0.3
28	2.1±0.9	3.2±0.2	3.3±0.9
Control a	0	0	0
Control b	0	0	0

Incubation temperature = 28±1^{0}C; Readings are Mean±SE

Table no. 3: Herbicidal potential of different concentrations of 21 days old CFCF of *Nigrospora oryzae* against *Parthenium hysterophorus.*

Concentration of CFCF	Phytotoxic damage rating		
(%)	24 hrs	48 hrs	72 hrs
25	1.3 ± 0.0	1.6 ± 0.57	2.3 ±1.15
50	2.0 ± 0.0	2.6 ± 0.57	2.6 ± 0.57
75	2.2± 0.0	2.6 ± 0.57	3.6 ± 0.57
100	3.2 ± 0.2	4.5 ± 0.2	5.0 ± 0.0

Control (a) = Richards Broth = 0%, Control (b) = Distilled water = 0%, RH = 80%Readings are Mean±SE

Table no. 4: Percentage reduction in biological contents of *Parthenium hysterophorus* leaves treated with different concentrations of 21 days old CFCF of *Nigrospora oryzae.*

Concentration (%)	Percentage Reduction (after 48 hours post treatment)			
	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Protein
25	4.33	15.02	4.02	14.05
50	5.02	18.09	5.06	59.47
75	66.33	44.05	34.62	72.50
100	70.02	64.54	84.04	73.53
Control a	0	0	0	0
Control b	0	0	0	0

Table no. 5: Percentage reduction in biological contents of *Parthenium hysterophorus* leaves treated with different days old CFCF of *Nigrospora oryzae* .

Incubation Days	Percentage Reduction (after 48hpt)			
	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Protein
7	12.53	16.75	38.02	69.70
14	26.23	15.14	42.23	72.82
21	69.05	45.02	95.62	73.62
28	42.2	42.11	90.65	70.39
Control a	0	0	0	0
Control b	0	0	0	0

Readings are Mean±SE

Table no. 6: Thermal stability of phytotoxin from *Nigrospora oryzae* against *Parthenium hysterophorus* by shoot cut bioassay.

Temperature (^{0}C)	Phytotoxic Damage Rating
50	4.5±0.2
100	4.5±0.2
121	4.5±0.2
Control a	0
Control b	0

Each treatment was incubated for 15 min, a=50% concentration of toxic metabolites was used to access the thermal nature, b=control saved by CFCF at 50% concentration at room temperature.
Phytotoxic damage:

5 = 95 – 100% plant death
4 = 80 – 95%
3 = 50 – 78%
2 = 20 – 49%
1 = 01 – 19%
0 = 00 – 04%

Table no. 7: Phytotoxic damage rating of solvent extracted fraction of CFCF of *Nigrospora oryzae* against *Parthenium hysterophorus*(DLB).

Fractions	Appearance	Phytotoxic Damage Rating at different hour(hpt)		
		24	48	72
Carbon tetra chloride	Dirty white	1.3±0.5	2.5±0.8	4.2±0.6
Chloroform	Light yellow	1.5±0.4	2.3±0.5	3.3±0.5
Ethyl acetate	Pale yellow	0.7±0.2	1.2±0.2	1.3±0.5
n-Butanol	Light brown	0.0±0.0	1.2±0.2	1.3±0.0

All controls of different solvents exhibited no effect. Readings are Mean±SE

Table no. 8: Percentage reduction in biological contents of *Parthenium hysterophorus* leaves treated with partially purified CFCF of *Nigrospora oryzae.*

Solvents	Percentage Reduction			
	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Protein
Carbon tetra chloride	92	74.22	89.30	95.05
Chloroform	25.05	25.30	47.36	44.53
Ethyl acetate	49.57	72	65.68	80.06
n-Butanol	15.43	18	38.63	25.5

All controls of different solvents exhibited no effect.

The genus *Nigrospora oryzae* is a rich source of biologically active secondary metabolites such as phytotoxin and antibacterial nigrosporin (Tanaka *et.al,* 1997) herbicidal lactones (Fukushima et.al,1998) phomalactones (Kim *et al*,2001) and phytotoxin epoxyexserohilone (Cutler et.al,1991).A phytotoxic t3 compound (5,6-di hydro-5-hydroxy-6-propenyl-2H-pyr-2-one a host specific toxin showing phytotoxic effect to various plants including turf grasses was assessed for its phytoxicity by the leaf wounding assay and the whole plant test and the cellular leakage test (Choi, *et al*,2006). Nigrospora A and B, two new phytotoxin and antibacterial metabolite were isolated from culture filtrate of *Nigrospora oryzae*. Metabolite A produced from *Nigrospora oryzae* has been found to show weak antibiotic properties and mild toxicity to brine shrimp and chick embryos but not to be toxic to mice or rats at the level tested (Wilson *et. al,* 1986).As little has been reported on the phytotoxicity of *N.oryzae* and their secondary metabolite on weed.

On the basis of degree of phytotoxicity of secondary metabolites of *N. oryzae* and herbicides. The potential of discovering new unlimited method of isolation of different fungi their screening for phytotoxins, separation of phytotoxin from metabolite and partial purification of the toxin are still under way.

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REFERENCES

1) Abbas, H. K., Vesonder, R. F., Boyette, C.D., Hoagland, R.E. and Krick, T., (1992). Production of fumonisins by *Fusarium moniliforme* culture isolated from Jimson weed in Missisipi. *J. Phytopathol*.136: 199-203.
2) Abbas, H.K. and Duke, S.O., (1995). Phytotoxins from plant pathogens as potential herbicides. *J.Toxicol*. Toxin. Rev. 14: 523-543.
3) Arnon, D.I., (1949). Chlorophyll adsorption spectrum and quantitative. Biochem. Biophysics Acts. 20:449.
4) Barash, I., Pumpkin G., Koren L., Ben-Hayyim G, Strobel G.A.,(1978). A low molecular phytotoxin produced by *Phoma tracheiphila*, the cause of mal secco disease in citrus*. Physiol Plant Pathol* 19:17-29
5) Barbosa, A.M., Cristina, G.M., Dekker, R. F. H., Fonseca, R.C. and Ferreira, D.T., (2002). Phytotoxin produced by *Bipolaris euphorbiae* in vitro is effective against the weed *Euphorbia heterophylla*. *Braz. Archive. Bio. Technol*. 45(2): 233-240.
6) Bassett, C., Sherwood, R.T., Kepler, J. A. and Hamilton, P.B., (1967). Production and biological activity of fommanosins. A toxic sesquiterpene metabolite from *Fomes annonus Phytopathol*. 57: 1046-1052.
7) Chandla,P.,(1999).Screening and Evaluation of Phytotoxic metabolites of some indigenous microbes for the management of *Lantana camara* L. Dissertation Thesis, R.D. University.
8) Charudattan, R., (1996). Recent development in the field of microbial herbicides. In: Proc. Agriculture Biotechnology sym. Biopesticides for crop production (Park, K.H. ed). Seoul, National University, South Korea. 63-84.
9) Chiang, M.Y, Van, C.G.D. and Leonard, K.J., (1989). Evaluation of endemic foliar fungi for potential biological control of Johnson grass (*Sorghum halpense*) Screening and host range tests. *Plant Dis.*73: 459-464.
10) Cutler, H.G., Hoogsteen, K., Littrell, R.H., Arison, B.H., (1991). Epoxyerserohilone, a novel metabolite from *Nigrospora sphaerica Agri Biol Chem* 55(8):2037-2042.
11) Choi, G.J., Kimand, J.C., Shon, M.J. and Kim, H.T., Cho, K.Y.,(2006). Phytotoxin production of *Nigrospora sphaerica* pathogenic on Turfgrasses. *The Plant Pathology Journal* 16(3): 137-141.
12) Duke S.O. and Abbas H.K., (1992). Natural products with potential use as herbicides. Allelopathy (organisms, Processes and Application. In.. ACS. Symposium Series 582 (eds) 348-362 by Inderjet K.M.M, Dakshini, F.A. Einghelling, pp..
13) Duke, S.O., (1986). Microbially produced phytotoxin as herbicides a perspective. Rev. Weed Sci. 2: 15-44.
14) Freeman G.G.,(1965) Isolation of Alernariol and alternariol mono methyl ether from *Alernaria dousi* (kuch)groves and skolko.*Phytochemistry*,5:719-715.
15) Fukushima,T.,Tanaka,M.,Gohbara,M. and Fujimori,T., (1998). Phytotoxicity of three lactones *from Nigrospora sacchari. Phytochemistry*. 48(4):625-630.
16) Gupta,N.,Pandey,A.K.,Gautam,S.P.,Patel,U.S.,(2006):Vesicular Arbuscular Mycorrhizal Association with *Parthinium.J.Basic Appl. Mycol*. 5 (I AND II): 38-40.
17) Hoagland, R.E., (1999). Plant pathogens and microbial products as agents for Biological weed control. In: Advances in Microbial biotechnology (Tiwari, J.P. Lakhanpal, T.N., Singh. J., Gupta, Rajni and B.P. Chamola eds) APH publishing corporation, New Delhi - 110002, India.
18) Hoagland, R. E. and Cutler, S.J.,(2000). Plant and microbial compounds as herbicides. In: Allelopathy in ecological agriculture and forestry (Narwal ed) Kluwer Academic Publishers. Netherlands. 73-99.
19) Hoagland, R.E., (2001). The genus *Streptomyces,* a rich source of novel phytotoxins. In: Ecology of desert

Environments (Prakash Ishwar ed) *Scientific Publishers, Jodhpur, India*. Pp. 133-169.

20) Joseph, S.,(2000). Studies on the herbicidal potential of some indigenous strains of *Streptomyces* against *Parthenium* and *lantana,* Dissertation thesis, R.D. University).
21) Joseph, S., Lal, S. and Pandey, A.K. (2002). Preliminary evaluation of herbicidal potential of Streptomyces sp.WC#150 against *Lantana camara*. *Ann Pl Protec., Sci.*10: 134-136.
22) Kalam, S., Pandey, A., and Pandey, A.K., (2014). Mass production and Formulation of herbicidal metabolite from *Phoma herbarum* (FGCC#54) for management of some prominent weed of central India, American *International Journal of research in formal Applied and Natural Science*,5(1): 40-47.
23) Kauraw, L. P., Chile, A., Bhan, V. M., (1997).Evaluation of *Fusarium pallidoroseum* (Cooke) Sacc. For the biocontrol of Pathenium hysterophorus L., In; Proceedings of the Ist International conference on *Parthenium* management, (Eds. Mahadevappa M and Patel VC) Dharwad, India. pp.70-74.
24) Kaur, M., Aggarwal, N.K.,Kumar, V., and Dhiman, K., (2014). International Scholarly Research Notices,Vol.2014(2014).
25) Kenfield, D., Bunkers, G., Strobel, G., and Sugawara, F., (1989). Fungal phytotoxins potential new herbicides in phytotoxins and plant pathogenesis (Grant, A *et al*, ed), India: 257-277.
26) Kurian, P., Steleig D.A., Baniecki, (1977). Toxin production by *Cristullariella pyramidalis. Mycologia* 69: 1203-1206.
27) Kumar A., Verma, V.C., Gond, S.K., Kumar,V., and Kharwar, R.N.,(2009). Biocontrol potential of Cladosporium sp.(MCP-461), against a noxious weed *Parthenium hysterophorus* L.*, Journal of Environmental Biology* 300(2):307 – 312.
28) Kim, J. C., Choi, G. J.,Park, J. H., Kim, H. T., Cho, K.Y., (2001). Activity against plant pathogenic fungi of phomalactones isolated from *Nigrospora sphaerica* pest manag.Sci 57(6):554-559.
29) Lee,H.J., Duke, M.V.,Duke, S.O., (1993). Cellular localization of protoporphyrinogen-oxidizing activities of etiolated barley *Hordeum vulgare* L. leaves: relationship to mechanism of action of protoporphyrinogen oxidase-inhibiting herbicides*. Plant Physiol* 102: 881–889.
30) Lee, H. J., Duke, S. O., (1994). Protoporphyrinogen IX-oxidizing activities involved in the mode of action of peroxidizing herbicides*. J Agric Food Chem*. 42: 2610–2618.
31) Lowry, O.H., Rosen lerough, N.J., Fan, A.L. and Randal, R.J., (1951). Protein measurement with the folin – phenol reagent. *J. Bio. Chem*. 193:265-275.
32) Mahadevappa, M., (1977). Ecology, distribution, menace and management of *Parthenium*. In: Proc. Of First International Conference on *Parthenium* Management (Vol-1), UAS, Dhar-wad pp. 1-12.
33) Mishra, A., (2013). *Parthenium Hysterophorus* : A noxious weed for plant diversity*, International Journal of Scientific Research.(IJSR)*.Vol. 2(9): 23-24,
34) Nakjima, M., Itoi, K., Takamatsu, Y., Sato, S., Furukawa, Y., Honwa, T., Furuya, K., Kadotini, J., Kozasa, M., and Haneishi, T., (1991). Cornextistin: A new fungal metabolites with herbicidal activity. J *Antibiot.* 44(10): 1065-1072.
35) Ohara, J., Morita, K., Tsujino, Y., Fujiori, T., Goering, M. (1995). Production of phytotoxic metabolites by fungus *Colletotrichum sp. Biochem* 59: 113-114.
36) Orsenigo, M., (1957). Extraction and purification of Ophiobolin, a toxic product from *Helminthosporium oryzae. Phytopath..* 29: 189-196.
37) Oudhia, P. (1998). Parthenium : A curse for the biodiversity of Chhattisgarh Plain. In : Abstract National Research Seminar on Bio-chemical Changes. An Impact on Environment, R.D. Govt. P.G. College Mandla (M.P.) 30-31 July: 26.
38) Pandey A.K.,Quereshi S., Singh A.K.,Yadav K.K.,(2006). Herbicidal Potential of *Phoma sp*. FGCCW#54: A Preliminary evaluation*. J. Basic Appl. Mycol.* 5 (I and II):60-61.
39) Pandey A.K., Mishra, J., and Hasija, S. K.,(1998). Effect of inoculum on mycoherbicidal potential of Sclerotium rolfsii against *Parthenium. Journal of Mycology and Plant Pathology,* vol. 28: 284–287.
40) Pandey, A.K., (1999). Herbicidal potential of microorganism: Present status and future prospects. In: Microbial Biotechnology for sustainable development and productivity. Prof. S.K. Hasija Festschrift vol. (R.C. Rajak ed.) Scientific publishers, Jodhpur: 86-105.
41) Pandey, A.K., Lal S. and Joseph S., (2000). Herbicidal activity of partially purified metabolites of *Streptomyces* sp. WC # 150 on *Parthenium.* PICN. 32: 11-23.
42) Pandey, A.K., Rajak R.C., Hasija, S.K., (2001). Biotechnological development of ecofriendly mycoherbicides In. Innovative Approaches in Microbiology (D.K. Maheshwari and R.C. Dubey) Published by Bishen Singh Mehendra Pal Singh, Dehra Dun.
43) Pandey, A.K., Shrivastava, G.M., Singh, A. K. and Verma, S. K., (2003). Herbicidal potential of secondary metabolites of *S. rolfsii* against *Parthenium.* A preliminary observation*. J. Basic and applied Mycol*. 2 (1): 27-30.
44) Ray, P. and Gour, H.N.,(2012).Integrated management of *Parthenium hysterophorous* L. (Asteraceae). A weed of worldwide significance,Review in plant pathology.Vol.5,Indian Society of Mycology and Plant pathology,Scientific Publisher (India),Jodhpur,605-632.
45) Saad, S.M., Hallion, J.M. and Hagedorn, D.T., (1970). Production, purification, and bioassay of tentoxin. *Phytopathol*. 60: 415-418.
46) Saxena S. and Kumar,M., (2010). Evaluation of *Alternaria alternata* ITCC4896 for use as mycoherbicide to control *Parthenium hysterophorus. Journal of Plant Protection Research,* vol. 47: 213–218.
47) Saxena S, Pandey A.K.,(2002). Evaluation of an indigenous isolates of *Alternaria alternate* (LC#508) for use as mycophytotoxin for *Lantana camara* L.,Crop Prot 21:71-73
48) Saxena, S., Pandey, A.K., and Rajak, R.C., (2001). Potential of an idiolite from Alternaria alternata as biorational agrochemical. In: Frontiers in fungal biotechnology and plant pathogen relationship. (Manoharachary, C., Bagyanarayana, G., Bhadraiah, B., Satyaprasad, K. Reddy, B.N. and .Nagamani, A eds). Allied Publishers, Hyderabad. 22-28.
49) Saxena, S. and Pandey, A.K., (2000). Preliminary evaluation of fungal metabolites as natural herbicides for the management of *Lantana camara J. Indian phytopathol.* 53 (a): 490-493.
50) Sharma, M.C. and Sharma, B.C., (1969). Toxic metabolite production by *Colletotrichum gloeosporioides* causing citrus dieback. *Indian Phytopathol*. 23: 67-74.
51) Sharma, P., Sharma, S. R. and Sindhu, (2004). A detached leaf technique for evaluation of resistance in cabbage and cauliflower against three major Pathogens. Indian *Phytopathol.* 57(3): 315-318.
52) Shukla, R. and Pandey, A.K.,(2006). Maximization of production oxalic acid form *Sclerotium rolfsii*, a mycoherbicidal agent against *Parthenium. Ann. Pl. Protect. Sci*. 14(1): 202-205.
53) Singh, J., Quereshi, S., Banerjee, N., and Pandey, A.K.,(2010). Production and Extraction of Phytotoxins from *Colletotrichum dematium* FGCC#20 Effective Against *Pathenium hysterophorus* L.*, Brazilian Archives of Biology and Technology*. Vol. 53(3): 669-678.
54) Siddaramaiah, A.C., Heade, R.C., Hedge, R.K, Kulkarni, S., and Basavarajaiah, A.B., (1979). Toxic effect of the culture filtrate of *Phaeophelospora indica. Indian Phytopath* 32: 291.

55) Sanodiya, B.S.,(2006). Isolation, Purification and Characterization of Secondary Metabolites of *Alternaria alternata* (FGCC#101) for the Management of *Parthenium hysterophorus,* Dissertation thesis, R.D. University.
56) Strobel, G.F., Sugawara, F. and Clardy, J., (1991). Phytotoxins as potential herbicides. *Experientia*. 47: 819- 823.
57) Templeton, G.E., Te, Beest, D.O. and Smith, R.J. Jr, (1979). Biological control with mycoherbicides. Annu. *Rev. Phytopathol*. 17: 301-310.
58) Tanaka, M., Fukushima, T., Tsujino, Y., Fujimori, T.,(1997). Nigrosporins A and B, new phytotoxic and antibacterial metabolite produced by fungus *Nigrospora Oryzae. Biosci Biotechnol Biochem* 61(11):1848-1852.
59) Thakur, G.S., (2006). Isolation, Purification and Characterization of Herbicidal Compounds of *Curvularia lunata* (FGCC # 25) for the management of *Parthenium hysterophorus,* Dissertation thesis, R.D. University.
60) Thapar, R., Singh, A.K., Pandey, A. and Pandey A.K., (2002). Bioactivity of CFCF of *Curvularia lunata* in *Parthenium hysterophorus* L. J. Basic Appl. Mycol. 1: 126-129.
61) Vikrant P., Verma, K.K., Rajak, R.C. and Pandey, A.K., (2006). Characterization of a phytotoxin from *Phoma herbarum* for management of *Parthenium hysterophorus L. J. Phytopathology* 154: 1-8.
62) Walker, H.L., Templeton, G.E., (1973). In vitro production of a phytotoxin from metabolite by *Colletotrichum gloeosporiodies* and sp. Aeschvnomene.Pl sci.13:91-96.
63) Wilson, M.E., Norman, D. D. and Diener L.U., (1986). A toxic metabolite of *Nigrospora oryzae*(Berk and Br.) petch.*Mycopathologia Alabama Agricultural Exp. Station J.* 6-84744.