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Selection of mycoherbicidal potential of *Fusarium* spp. Against a Noxious Weed *Parthenium hysterophorus*

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ABSTRACT

Microbes and their secondary metabolites offer a benign and eco-friendly alternative to manage weed. Mycoherbicide production for biological control of weeds requires a series steps, from selection of a suitable microbial strain to final formulation. Thus, this study aimed to select potential fungi for production of secondary metabolites with herbicidal activity for control of *Parthenium* weed. In the present study, phytopathogenic fungi namely *Fusarium* spp. were isolated from infected tissues of *Parthenium* and evaluated against *Parthenium hysterophorus*, a problematic monocotyledonous weed of open lands, Agriculture, Horticulture and Forests. Herbicidal potential of Cell Free Culture Filtrate (CFCF) of three strains of *Fusarium* spp. coded as FGCCW#16, FGCCW#43 and FGCCW#55, against *Parthenium hysterophorus* were evaluated by seedling and shoot cut bioassays. Maximum mortalities of shoots, seedlings and phytotoxic damage were obtained from 21 days old cell free culture filtrate (CFCF) of FGCCW#16 at 100% concentration. Significant reduction in biological contents i.e. photosynthetic pigment and protein was observed in the host weed on treatment with the CFCF as determined by detached leaf bioassay. Phytotoxic damage such as severe wilting, chlorosis, necrosis and complete collapse of the entire parts of the weed were also noticed due to CFCF application.

Introduction

Parthenium hysterophorus L. popularly known as congress grass is native of tropical America, Gulf of Mexico, West Indies and central Argentina. It is an aggressive, poisonous, herbaceous annual or ephemeral member of the Asteraceae (Adkins et al. 2005). The weed poses a serious threat and is responsible for several problems to human and animal health besides causing significant reduction in agricultural and forestry productivity (Bajwa et al. 2016; Kaur et al. 2014). It affects

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the biodiversity as well as the production of crops and also human and animal health. Conventional methods for control of this weed have failed due to different reasons. It grows on any type of soil and in a wide range of habitats. The control of weeds by using plant pathogens has gained acceptance as a safe, practical and environmentally beneficial method (Aneja, 1991). Microorganisms like fungus, bacteria and viruses are used as biological agents (Kumar, 2009). The biological agents produce toxins which may kill or control the weeds are known as bioherbicides. The toxins produced by the fungi are called mycoherbicides. Exploitation of microbes and their by products as herbicide has generated significant interest worldwide (Dayan et al. 2012; Gerwick and Sparks, 2014).

Many fungi have been reported to attack *Parthenium hysterophorus*. Leaf spot disease on *P. hysterophorus* was caused by *Colletotrichum gleosporioides* (Kumar and Rao, 1977). The herbicide prepared from *Alternaria alternata* was found to be effective against seedling of *P. hysterophorus* (Rao and Rao, 1987; Deshpande et al. 1982). Pandey et al. (1996) showed that *Sclerotium rolfsii* capable of controlling the weed and increased the mortality rate of *P. hysterophorus* seedlings upto 90-95% and 35-40% in greenhouse and field trials respectively. About 25 species of fungi were isolated from different places of Jabalpur, Madhya Pradesh and these include species of *Alternaria alternata, Cladosporium, Acremonium, Colletotrichum, Drechslera, Phoma, Myrothecium, Curvularia*, which cause leaf spot disease and species of *Rhizopus, Chaetomium, Aspergillus* (Rajak et al. 1990). Nineteen species of fungi are able to cause severe damage to the *P. hysterophorus* under laboratory trials which include *A. alternata, A. dianthi, C. gleosporioides, A. macrosporus, Fusarium oxysporum, Phoma herbamm and Bipolaris, F. moniliforme, Myrothecium roridum* and *Rhizoctonia solani* (Pandey et al. 1991). Various *Fusarium* spp are known to produce an array of phytotoxic compounds (Amsellem et al. 2001a, 2001b; Dor et al. 2007; Sauerborn et al. 2007). Members of the genus *Fusarium* produces several phytotoxic compounds, some of them have been thoroughly evaluated for their herbicidal potential also (Zonno and Vurro, 2002; Azam et al. 2003). *Parthenium* is also attacked by many strains of *Fusarium* sp. viz., *Fusarium oxysporum, Fusarium moniliforme* etc (Rajak et al. 1990; Pandey et al. 1991; Farkya et al. 1994). *F. Pallidoroseum* are capable of reducing seed germination, height, branches, leaves, and flowers considered to be an effective biocontrol agent against *P. hysterophorus* (Kauraw et al. 1997). However, mycoherbicidal potential was sometimes constrained by environmental conditions (Boyetchko and Peng, 2004). To overcome these problems herbicidal potential of secondary metabolites of *Fusarium* spp. including FGCCW#16, FGCC#43 and FGCC#55 against *Parthenium* was evaluated and discussed in this paper.

Materials and Methods

Recovery of strain

Three strains of the *Fusarium* sp. viz. FGCCW# 16, FGCCW# 43, and FGCCW# 55 were obtained from Fungal Germplasm Collection Centre (FGCC) of Mycological Research Laboratory, Department of Biological Science, R D University, Jabalpur, Madhya Pradesh. These were isolated earlier from seeds, seedlings and rhizospheric soil of the target weed.

Production of CFCF

250ml Erlenmeyer's flasks containing 200ml Asthana and Hawker's broth were seeded with 5mm disc of inoculum separated from 7 days old cultures grown on PDA medium. Inoculated flasks were incubated at 27±1°C in a Bio-Oxygen Demand (BOD) incubator (Remi, India) and the cell free culture filtrate (CFCF) was extracted after 7, 14, 21 and 28 days (Pandey et al. 2004b).

Extraction of CFCF

The metabolized broth was passed through whatman no 1 filter paper under aseptic conditions through a pre-weighed whatman filter paper no.1 and was centrifuged at 400xg for 15-20 min (Saxena and Pandey, 2002). The pellet was thrown and the supernatant was again filtered *in vacuo* by microfiltration using sterile microfilters, 0.45 µm pore size, Mininsart (Sartorius, Gottingen, Germany) making it cell free (Walker and Templeton, 1978). Thus Cell Free Culture Filtrate was obtained.

Bioassay

Cut Shoot Bioassay

30-35 days old *Parthenium* seedlings were grown in pots containing soil: sand: peat (1:1:1) inside a plant growth chamber (Yorco, India). These were taken and an inclined cut was made at the tip in sterilized water. They were then dipped in different dilutions *i.e.*25%, 50%, 75% and 100% of the CFCF in test vials. These were then incubated under daylight or artificial illumination (3.5×10^4 erg/cm^2/s). The vials were sealed with cotton buds and the effects of different days old fermented broths and different dilutions of the toxic metabolites were observed on the shoots of the test weed after 48 h at room temperature (27±1°C) (Vikrant et al. 2006).

Seedling Bioassay

Seedlings of the target weed were raised in pots containing soil: sand: peat (1:1:1). 15-20 days old were treated with different days old CFCF (7, 14, 21, 28 days) and also with different dilutions (25%, 50%, 75% and 100%) of the phytotoxic metabolites and were then incubated. Observations were made regularly on a rating scale of 0-5 as described earlier (Abbas and Boyette, 1992). Phytotoxicity of CFCF of various strains was determined by employing shoot cut and seedling bioassay tests as accordance to Thapar et al. (2002). Phytotoxicity was determined following the method of Abbas and Boyette (1992) on a rating scale of 0-5. Effect on biological content i.e. chlorophyll and protein contents were determined as per Pandey et al. (2003).

Results and discussion

Data presented in Table 1 showed that CFCF of different strains of *Fusarium* spp. of varied incubation periods had significant influence on cut shoots and seedling mortalities. Maximum shoot cut and seedling mortalities of the target weed were obtained with 21 days old fermented broth followed by 28 and 14 days old fermented medium in all the strains. CFCF obtained from 7 days old utilized medium didn't cause significant mortalities. Out of these three test strains, CFCF of FGCC#16 exhibited maximum shoot cut, seedling mortalities and phytotoxic damage followed by FGCC#55 and FGCC#43. Phytotoxic damage rating represented in table 2 clearly indicates that in both the cut shoot and the seedling bioassays, toxicity gradually enhances with increase in concentration of CFCF.

Table 1. Study for effect of different incubation period on Cell free culture filtrate of three different *Fusarium* sp. against *Parthenium hysterophorus* (after 24 hrs during bioassay).

Strains	Incubation days							
	Shoot cut bioassay phytotoxicity				Seedling bioassay phytotoxicity			
	7 days DR*	14 days DR*	21 days DR*	28 days DR*	7 days DR*	14 days DR*	21 days DR*	28 days DR*
Control A	0	0	0	0	0	0	0	0
Control B	0	0	0	0	0	0	0	0
FGCCW#16	1	2	5	4	1	2	5	4
FGCCW#43	1	2	4	4	1	2	4	4
FGCCW#55	1	2	4	4	1	2	4	4

*Damage rating (DR): 0 =No symptoms, 1= slight chlorosis, 2= slight chlorosis and necrosis, 3=marked chlorosis and necrosis, 4= marked chorosis and high necrosis, 5=acute chlorosis and necrosis leading to death of shoots.

Maximum phytotoxic damage was recorded by 75% and 100% concentration of CFCF. 25% and 50% concentrations of fermented broth failed to show significant damage to shoots and seedlings

of the test weed. 75% and 100% CFCF when sprayed on *Parthenium* seedling produced visible symptoms i.e., browning at tip of leaves, which was more rapid, prominent and severe in later stages after 24 hours (Table 2). Browning was followed by severe necrosis wilting and finally collapsed of seedlings completely.

Table 2. Study of different concentration of Cell free culture filtrate of three different *Fusarium* spp. against *Parthenium hysterophorus* (after 24 hrs during bioassay).

Concentration	Shoot cut bioassay damage rating*			Seedling bioassay damage rating		
	FGCCW#16	FGCCW#43	FGCCW#55	FGCCW#16	FGCCW#43	FGCCW#55
Control A	0	0	0	0	0	0
Control B	0	0	0	0	0	0
25%	1	0	0	1	0	0
50%	2	2	2	3	2	3
75%	3	3	3	4	3	3
100%	5	4	5	5	4	3

*Damage rating: 0 =No symptoms, 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 shoots.

Data presented in Table 3 clearly reveals significant variation in the total chlorophyll, chl a, chl b contents when shoots of the target weeds were treated with cell free culture broth of *Fusarium* spp. FGCC# 16, 43 and 55 of different incubation periods. There was a gradual reduction of pigments with increased incubation periods. More or less all the seedlings and shoots died at higher concentrations of CFCF. Maximum reduction in chlorophyll and protein contents were recorded in case of shoots treated with CFCF of *Fusarium* FGCC# 16 obtained from 21 days old fermented broth (Table 3). It was followed by CFCF obtained from fermented broth of Fusarium sp. FGCC# 43 and 55. Chl a was found to be more susceptible to the metabolites in comparison to chl b and total chlorophyll.

Variation in toxicity in relation to incubation period may be due to different phase of growth of the fungus. Metabolites required for fungal growth are normally synthesized during initial phase whereas most of the toxicants are formed during idiophase i.e. stationary phase of the fungus. Phytotoxin often act as the initiator factor for successful pathogenesis. Several phytotoxin are known to be the determinant factor in pathogenesis. Most of the phytotoxic metabolites acts by modifying the metabolism of the host plants, while some are toxic to the plant tissues once accumulated and poison the plant tissues (Amusa, 2006).

There was a gradual decrease in biological contents with increase in concentration of CFCF. Decrease in both the chlorophyll pigments (a and b and total chlorophyll were noted in phytotoxin treated detached leaves. Destruction of the two chlorophyll affects both the Photosystems of photosynthesis, since Photosystem I is associated with chl a and Photosystem II with chl b. Hill reaction which is a measure of the integrity of the photosystem II also gets adversely affected (Mishra, 1985). The toxin can create a change in the dynamic balance of synthesis and decomposition of chlorophyll pigments. The chlorophyll might have been destroyed by the enhancement of chlorophyllase activity or due to metabolic destruction. The reduction can either be due to an inhibition of chloroplast development or due to the destruction of pigments in mature chloroplasts. Similarly the biochemical basis for resistance is reflected in changes in the structural and functional proteins and products of their action in cells adjacent to the infe cted site.Variation in phytotoxicity due to toxin has also been recorded by other researchers (Hoagland, 1990; Abbas et al. 1992; Pandey et al., 2004a; Saxena and Pandey 2001; Joseph et al. 2002; Vikrant et al. 2006). Thapar et al. (2002) recorded significant reduction in biological contents of *Parthenium* treated with CFCF of *Curvularia lunata.*

Table 3. Study for the effect of cell free culture filtrate of three different *Fusarium* spp. on Chlorophyll and protein content of weed *Parthenium hysterophorus*

Items	Mean % Inhibition															
	Chl a				Chl b				Total Chl				Protein			
Incubation (Days)	7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28
Control A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FGCCW#16	18.0	54.1	91.6	84.2	7.50	39.5	72.4	70.5	15.4	60.8	79.2	73.2	22	48.2	48.4	45
FGCCW#43	14.8	52.4	80.6	80.2	28.2	61.9	74.2	72.5	17.2	48.9	70.2	67.5	12	51.4	51.4	50
FGCCW#55	20.0	64.5	85.2	80.0	15.1	80.4	93.1	88.5	12	59.2	79.8	75.3	28	64.3	65.4	59

Data are Means of three experiments each replicated thrice.

Conclusion

On the basis of above findings it can be concluded that the secondary metabolites produced by *Fusarium* spp have remarkable herbicidal potency need further evaluation for its large-scale application.

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Conflict of interest

Authors declare no conflict of interest.

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