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Screening of Phytotoxin Producing Fungi for the Mycoherbicidal Management of Weeds of Leguminous Crop

*Saurabh Gupta, Sushma Dubey and **Jaya Singh

gupta.saurabh89@yahoo.com

* Dept of Biotechnology, Kalinga University, Raipur

** Biodiversity Conservation and Rural Biotechnology Centre (BCRBC), Jabalpur

Abstract

Pulses are the most important food crops after cereals, constitute the major source of dietary protein. India is the world's largest producer and the largest consumer of leguminous crop. Green gram and pea are the major legume crops and today prominently figures in the crop priority of the farmers in Madhya Pradesh contributing to a major percentage to the total legume production in the country. Looking to the potential the objectives is to develop and exploit indigenous strains of fungal pathogens as mycoherbicides to manage some of problematic weeds of leguminous crop. During the periodic survey of various leguminous fields, it was observed that leaf spot, leaf blight, die back, petiole rot etc. diseased were associated with leguminous weeds. A total of 40 fungal isolates were selected from various diseased parts. The initial symptoms of Phytotoxic effect was distinct epinasty followed by necrosis, blackening and stiffness of the stem ultimately causing death of the plants. *Alternaria petroselini* (FCLW#23) was found in maximum frequency and hence, it would be the best fungal candidate for biological management.

Keywords: Legume, weeds, Bio-control, fungal pathogens, Phytotoxicity.

Introduction

Legume crops are the second most important group of food plants and the major source of protein in the predominately vegetarian diet of the people of India. Madhya Pradesh has distinction of being the world's single largest producer of legumes/pulses, having an area approximately 20- 24 million hectares under legumes. Pests especially weeds have increasingly become a major threat to sustainability of legume crops. Weeds are ubiquitous and continually changing pests in agriculture. They claim their own share of soil fertility and productivity at the cost of crop yield. They impose severe allelopathic effects on crops. Conventional methods of weed control have failed due to one or other reasons while microbial management either directly or their secondary metabolites have received significant momentum as evidenced by commercialization of several products as mycoherbicides or biorationals. In the present investigation was undertaken with the objectives to develop and exploit some indigenous strains of fungal pathogens as bio-herbicides to manage some of the problematic weeds of leguminous crop. (Pandey 2001, 1999)

MATERIALS AND METHODS

Survey and collection:

A systematic, periodical and thorough survey of the weedy leguminous crop fields especially in different localities of Mahakaushal Region was undertaken. The main localities surveyed were Jabalpur, Gadarwara, Kareli, Narsinghpur, Pipariya etc. In the Green gram and Pea season monthly survey of the fields were carried out which helped in the understanding of the pattern of vegetative growth as well as different stages in the development of disease. The infected plant parts viz. leaves, stems, roots and rhizospheric soil of some weed samples collected during survey were brought to the laboratory and were used for the isolation of the fungal pathogens.

Isolation of fungi from infected leaves and stems:

Infected portion of the weed were cut into small pieces (2 mm). They were then surface sterilized with 1 % sodium hypochlorite (NaOCl) solution for about three minutes, followed by rinsing with sterile water to remove any possible contamination (Chopada *et al*., 2014). These were then transferred in presterilized petri dishes containing potato-dextrose agar medium (Walker, 1981; Agarwal and Hasija, 1986). This was incubated for 3-4 days at 26°C in BOD (Agrawal and Hasija, 1986). All the emergent fungi were isolated and pure cultures were obtained (Marak *et al*., 2014).

Isolation of Rhizospheric Fungi

The isolation of fungi from soil samples of weeds (*Commelina benghalensis*, *Cyperus rotundus*, *Euphorbia heterophylla*, *Echinochloa crus-galli*) of leguminous crop (green gram and pea) was done by using following methods:

Warcup's Soil Plate Method

A pinch of finely grounded oven dried soil (0.5-1.0 gm) was taken and transferred aseptically in presterilized petri-dishes. The soil aggregates were crushed and spread after adding 2-3 drops of sterilized distilled water. Approximately, 15 ml of melted and cooled (45°C) PDA medium was then poured into petri dishes. The petri dishes were gently rotated to assure even distribution of soil particles. These petri dishes were incubated for 5 to 7 days at 28±1°C in BOD incubator and then transferred to PDA slants as earlier mentioned.

Dilution Plate Methods

10 grams of soil samples were dissolved in 90 ml of sterile distilled water and thoroughly shaken to obtain the dilution 1:10 (10-1) from this 10-1 dilution a serial dilution of 1:100 (10-2) and 1:1000 (10-3) were made in sterile distilled water. 1 ml of each sample dilution was pipetted aseptically into sterilized petri dishes and approximately 15 ml of melted and cooled (45°C) PDA medium was poured into petri dishes. The petri dishes were gently rotated to assure even distribution of soil suspension and kept in a BOD incubator at 28± 1°C for 3-4 days and when fungal colonies developed in plates, it was transferred to slants (Agarwal and Hasija, 1986; Chouhan *et.al*., 2019).

Purification and maintenance of fungal culture

The fungal species isolated earlier were purified by sub culturing techniques (Agarwal and Hasija, 1986). The purified cultures of the 40 isolated fungi were multiplied on PDA plates. The stock cultures of the microorganism were maintained on PDA slants.

Identification of the fungi

Identification of fungi was done with the help of taxonomic experts, manuals, monographs, papers of various workers and available literature (Subramaniam, 1971; Ellis, 1971; Barnet and Hunter, 1972 and Arx, 1974; Sutton, 1980 Chouhan *et.al*., 2019)

Pathogenicity and Selection of Virulent Strain

All the isolated fungi were grown on PDA liquid media and incubated in BOD for 21 days. Development of the secondary metabolites secreted by different fungi using broth media. The media was inoculated incubated and the cell free culture filtrates (CFCF) were obtained.

Phytotoxicity Test for Selection of Potential Strain

(a) Seed germination bioassay (Plate bioassay)

The petridish were covered with filter paper supported by a thin layer of adsorbent cotton. Twenty five seeds of different weeds of leguminous crop (*Commelina benghalensis*, *Cyperus rotundus*, *Euphorbia heterophylla*, *Echinochloa crus-galli*) were taken in the petriplates which were incubated in seed germinator at 26 ± 2°C, 75 ± 15% relative humidity. These seeds were treated with 25, 50, 75 and 100 concentrations of CFCF. Three replicates for each treatment including control were maintained and observed daily. (Chouhan *et.al*., 2019)

(b) Shoot cut bioassay:

Shoots of healthy weed plant samples were cut under water and kept in sterilized test tube having Hogland's solution and placed in growth chamber under controlled conditions. . The shoots were then placed in Hoagland's solution in the test tubes and were sprayed the CFCF with an automizer until wetness. Control plants were sprayed with sterile distilled. These plants were then kept in environment growth chamber at 28°C and 70% relative humidity. The disease severity was rated by visual observation at an interval of 24 hours till 7 days.

(c) Detached leaf Bioassay:

Selected weeds leaves were used for detached leaf bioassay. The healthy leaf of weed were surface sterilized with 0.2% NaOCl and were incubated in a sterilized moist filter paper in 9 cm Petri plate using cotton and filter paper under continuous fluorescent light at 25±1°C. The effect was observed after 24, 48 and 72 hours at room temperature. In all bioassay sterilized unmetabolised growth medium was taken as control and the sterilized distilled water was taken as control.

(d) Whole plant bioassay:

Seeds of different weeds were surface sterilized with 0. 05% NaOCl solution, scarified 15 min in concentrated H_2SO_4 and then rinsed with distilled water. Seeds were sown in plastic pots containing a mixture of pre sterilized sand, soil and peat (1: 1: 1) and grown under 26±2°C, 75±15% relative humidity for a period of two weeks. These were then sprayed evenly with 25, 50, 75 and 100% concentrations of CFCF. The experiment had three test sets and one control set. These were kept in plant growth chamber (Yorko) and observed daily for disease severity. (Chouhan *et.al*., 2019)

RESULTS

During a periodical survey of various leguminous fields, it was observed that various plant diseases viz. leaf spot, leaf blight, die back, petiole rot, root rot, etc were found in selected weed plants of leguminous crop. A total of 40 fungal sp. were isolated from various diseases plant parts of weeds.

Screening of phytotoxic metabolites produced by fungal strains was done by Detached Leaf Bioassay, Shoot Cut Bioassay, Seed Germination Bioassay and Whole Plant Bioassay against the four major weeds of leguminous crop, namely, *Echinochloa Crus galli, Euphorbia heterophylla*, *Cyperus rotundus* and *Commelina benghalensis***.** On the basis of primary screening of 40 isolates 5 most potential isolates were selected for secondary screening. They were *Aspergillus flavus*, *Fusarium oxysporium*, *Curvularia lunata*, *Alternaria petroselini* and *Phoma herbarum*. These were again screened as earlier and *Alternaria petroselini* was found to be the most potential and induced maximum phytotoxic damage after 24-48 hours post treatment (hpt).

Alternaria petroselini (FCLW#23) was responsible for controlling the selected four weeds of leguminous crop caused extensive damage. *Alternaria petroselini* (FCLW#23) induced significant toxicity even at lower concentration against all the four selected weeds. Biological control using plant pathogens has shown substantial impact on the weed. The herbicidal potential of a fungal strain may be assayed by bioassay that is highly sensitive and qualitative and many of these are synthesized by fungal strains. Figure No – 2 clearly indicate that the results of whole plant bioassay by CFCF of *Alternaria petroselini*.

Table 1 (i): Fungal strains isolated from leguminous weeds

S.No.	Fungus Isolate from Leguminous fields	Isolate No.	Symptoms
1	*Fusarium oxysporium*	FCLW#01	Lamina
2	*Fusarium moniliforme*	FCLW#02	Lamina, Petiole
3	*Aspergillus* sp.	FCLW#03	Rhizosphere
4	*Aspergillus niger*	FCLW#04	Leaf

S.No.	Fungus Isolate from Leguminous fields	Isolate No.	Symptoms
5	*Cephalosporium* sp.	FCLW#05	Leaf, Petiole
6	*Colletotrichum dematium*	FCLW#06	Lamina, Stem
7	*Curvularia* sp.	FCLW#07	Petiole
8	*Curvularia lunata*	FCLW#08	Stem
9	*Aspergillus candidus*	FCLW#09	Rhizosphere
10	*Aspergillus flavus*	FCLW#10	Leaf
11	*Fusarium orthoceras*	FCLW#11	Lamina
12	*Fusarium* sp.	FCLW#12	Rhizosphere
13	*Septoria* sp.	FCLW#13	Stem
14	*Trichoderma viride*	FCLW#14	Stem
15	*Phoma* sp.	FCLW#15	Seed
16	*Phoma herbarum*	FCLW#16	Seed
17	*Rhizopus* sp.	FCLW#17	Rhizosphere
18	*Trichoderma* sp.	FCLW#18	Leaf
19	*Aspergillus clavatus*	FCLW#19	Petiole
20	*Aspergillus sulphureus*	FCLW#20	Stem
21	*Aspergillus* sp.	FCLW#21	Rhizosphere
22	*Alternaria* sp.	FCLW#22	Leaf
23	*Alternaria petroselini*	FCLW#23	Leaf
24	*Cephalosporium gregatum*	FCLW#24	Leaf
25	*Cercospora* sp.	FCLW#25	Petiole
26	*Cercospora sojina*	FCLW#26	Seed
27	*Alternaria alternata*	FCLW#27	Leaf
28	*Pythium ultimum*	FCLW#28	Seed
29	*Sclerotinia sclerotiorum*	FCLW#29	Leaf
30	*Rhizotonia leguminicola*	FCLW#30	Rhizosphere
31	*Cladosporium* sp.	FCLW#31	Stem
32	*Mucor* sp.	FCLW#32	Leaf, Petiole
33	*Mucor hiemalis*	FCLW#33	Seed
34	*Glomerella glycine*	FCLW#34	Rhizosphere
35	*Colletotrichum truncatum*	FCLW#35	Lamina
36	*Penicillum* sp.	FCLW#36	Leaf
37	*Phoma sorghina*	FCLW#37	Seed
38	*Drechslera indica*	FCLW#38	Leaf
39	*Drechslera* sp.	FCLW#39	Stem
40	*Helminthosporium* sp.	FCLW#40	Lamina, Stem

FCLW – Fungal Culture Leguminous Weed

Table 1 (ii): Phytotoxicity of CFCF of fungal strains on leguminous weed.

S.No.	Fungus Isolate from Leguminous fields	Isolate No.	Phytotoxic damage (in%)												
			PPB				SCB				WPB				Overall rating
			CB	CR	EH	EC	CB	CR	EH	EC	CB	CR	EH	EC	
1	*Fusarium oxysporium*	FCLW#01	58	62	60	66	60	69	79	80	70	73	75	80	5
2	*Fusarium moniliforme*	FCLW#02	40	31	42	38	39	44	39	46	32	31	39	41	3
3	*Aspergillus* sp.	FCLW#03	11	10	19	18	14	13	13	19	20	11	12	17	1
4	*Aspergillus niger*	FCLW#04	17	12	20	21	18	16	15	13	11	10	14	13	1

S.No.	Fungus Isolate from Leguminous fields	Isolate No.	Phytotoxic damage (in%)												Overall rating
			PPB				SCB				WPB				
			CB	CR	EH	EC	CB	CR	EH	EC	CB	CR	EH	EC	
5	*Cephalosporium* sp.	FCLW#05	44	50	61	48	61	55	51	53	46	55	63	48	4
6	*Colletotrichum dematium*	FCLW#06	10	13	15	20	11	12	17	18	12	13	19	21	1
7	*Curvularia* sp.	FCLW#07	61	60	69	71	58	61	69	57	57	52	70	69	4
8	*Curvularia lunata*	FCLW#08	74	80	70	78	70	75	80	82	80	75	70	78	5
9	*Aspergillus candidus*	FCLW#09	72	67	68	71	70	61	68	59	69	65	70	62	4
10	*Aspergillus flavus*	FCLW#10	65	78	70	74	80	73	83	85	83	85	80	81	5
11	*Fusarium orthoceras*	FCLW#11	39	40	48	51	50	55	38	43	42	57	47	44	3
12	*Fusarium* sp.	FCLW#12	22	39	29	28	32	33	25	34	38	27	29	30	2
13	*Septoria* sp.	FCLW#13	38	48	41	39	38	34	35	36	42	40	39	38	3
14	*Trichoderma viride*	FCLW#14	44	30	45	50	51	48	47	44	39	43	50	51	4
15	*Phoma* sp.	FCLW#15	50	55	52	52	49	69	70	40	68	61	65	66	4
16	*Phoma herbarum*	FCLW#16	65	70	75	72	76	80	80	70	81	75	85	80	5
17	*Rhizopus* sp.	FCLW#17	68	67	61	60	57	59	60	59	58	70	71	68	4
18	*Trichoderma* sp.	FCLW#18	67	66	70	72	59	62	63	68	65	72	65	70	4
19	*Aspergillus clavatus*	FCLW#19	55	59	57	49	61	55	57	56	54	53	60	61	3
20	*Aspergillus sulphureus*	FCLW#20	61	67	68	69	73	69	70	68	55	59	60	63	4
21	*Aspergillus* sp.	FCLW#21	11	23	24	19	22	33	31	39	35	37	38	29	2
22	*Alternaria* sp.	FCLW#22	39	40	38	31	34	33	40	42	44	38	33	42	3
23	*Alternaria petroselini*	FCLW#23	80	74	81	79	76	84	70	76	84	78	80	79	5
24	*Cephalosporium gregatum*	FCLW#24	69	60	72	69	67	75	72	70	69	76	77	68	4
25	*Cercospora* sp.	FCLW#25	69	70	40	68	61	50	55	52	52	68	61	50	4
26	*Cercospora sojina*	FCLW#26	55	60	61	56	58	62	65	55	61	62	59	57	3
27	*Alternaria alternata*	FCLW#27	13	20	18	16	12	15	20	22	20	15	16	29	1
28	*Pythium ultimum*	FCLW#28	68	59	69	65	70	62	69	63	48	75	68	61	4
29	*Sclerotinia sclerotiorum*	FCLW#29	52	52	49	69	70	40	68	61	65	62	69	63	4
30	*Rhizotonia leguminicola*	FCLW#30	65	55	61	62	59	57	40	49	51	48	53	59	3
31	*Cladosporium* sp.	FCLW#31	21	22	28	19	24	13	22	27	26	20	34	30	2
32	*Mucor* sp.	FCLW#32	18	17	12	16	21	25	25	19	24	28	22	14	1
33	*Mucor hiemalis*	FCLW#33	70	61	68	59	69	65	72	69	67	75	72	70	4
34	*Glomerella glycine*	FCLW#34	61	60	68	72	70	75	77	63	67	67	59	65	4
35	*Colletotrichum truncatum*	FCLW#35	13	12	19	18	11	14	18	24	17	16	17	15	1
36	*Penicillum* sp.	FCLW#36	75	72	70	69	76	77	68	55	63	67	69	67	4
37	*Phoma sorghina*	FCLW#37	21	10	19	22	15	18	17	20	24	26	18	17	1
38	*Drechslera indica*	FCLW#38	51	53	46	55	63	48	75	72	69	65	72	69	4
39	*Drechslera* sp.	FCLW#39	22	33	31	39	35	37	38	29	31	39	35	38	2
40	*Helminthosporium* sp.	FCLW#40	72	69	63	48	75	68	55	63	67	75	72	70	4

FCLW = Fungal culture leguminous weed

PPB = Petri plate bioassay

SCB = Shoot cut bioassay

WPB = Whole plant bioassay

CB - *Commelina benghalensis*

CR - *Cyperus rotundus*

EH - *Euphorbia heterophylla*

EC - *Echinochloa crus-galli*

Table 1 (iii): Secondary screening of selected fungal strains against leguminous weeds.

S. No.	Culture No.	Name of the fungus	Concentration of CFCF	Phytotoxic damage (%)			
				CB	CR	EH	EC
1.	(FCLW#23)	*Alternaria petroselini*	Control	0	0	0	0
			25%	60	65	68	70
			50%	70	75	78	78
			75%	83	80	82	85
			100%	92	90	95	95
2.	(FCLW#10)	*Aspergillus flavus*	Control	0	0	0	0
			25%	50	55	52	55
			50%	58	59	65	62
			75%	65	68	70	70
			100%	70	72	75	85
3.	(FCLW#08)	*Curvularia lunata*	Control	0	0	0	0
			25%	35	46	45	56
			50%	42	55	58	60
			75%	52	62	65	68
			100%	68	72	70	71
4.	(FCLW#01)	*Fusarium oxysporium*	Control	0	0	0	0
			25%	41	60	60	55
			50%	50	68	65	60
			75%	58	70	70	65
			100%	75	82	80	78
5.	(FCLW#16)	*Phoma herbarum*	Control	0	0	0	0
			25%	54	58	60	65
			50%	63	70	70	72
			75%	74	75	77	78
			100%	80	82	84	83

CB - *Commelina benghalensis*
CR - *Cyperus rotundus*
EH - *Euphorbia heterophylla*
EC - *Echinochloa crus-galli*

CONCLUSION:

Studies conducted indicate and all observations show the frequency and phyto-toxicity of *Alternaria petroselini* (FCLW#23) found to be most virulent strain and exhibited potential to be exploited as natural herbicides for some of problematic weeds of leguminous crop.



Fig No 1 – Microscopic photograph of *A. petrosolini*



Fig No 2 – Filtration process of Cell Free Culture Filtrate (CFCF) of *Alternaria petroselini* (FCLW#23)

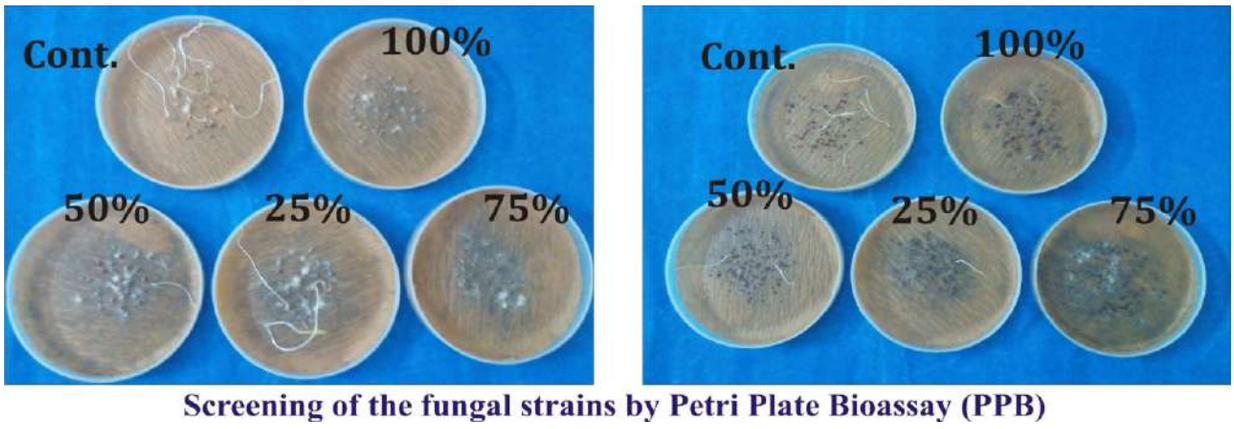


Fig No 3 - Results of Petri Plate Bioassay by developed Cell Free Culture Filtrate (CFCF) of *Alternaria petroselini* (FCLW#23)



Fig No 4 - Results of Whole Plant Bioassay by developed Cell Free Culture Filtrate (CFCF) of *Alternaria petroselini* (FCLW#23)

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