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Evaluation of toxins of phytopathogenic fungus for eco-friendly management of *Parthenium*

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ABSTRACT

Herbicidal potential of CFCF (cell free culture filtrate) of *Phoma herbarum* (FGCCPH#27) against *Parthenium hysterophorus* was determined by shoot cut, seedling and detached leaf bioassays. Maximum mortality was shown by the inoculum formulated with sucrose + Tween-20 followed by Tween-80. Triton-X was found to be highly inhibiting in its action. Maximum average leaf area damage (LAD) of 85% on the seventh day and 65% of leaf damage by fourth day was observed when treated with CFCF obtained from 14 day's old fermented broth at 50% concentration followed by 75 and 100%. Maximum phytotoxicity was obtained from 14 day's old fermented broth with sucrose + tween 20 0.5% as formulating agent. Chlorophyll and protein contents were also significantly affected when treated with CFCF. The contents were gradually decreased with increased incubation. Maximum reduction was recorded in shoots treated with CFCF obtained from 14 days old fermented broth at 50% concentration followed by 75% and 100 % (Table 4). 14 days old fermented broth showed the maximum biological activity as depicted by the significant reduction in the chlorophyll and protein content of the host leaves. While extract obtained from 7 days old broth failed to show any remarkable reduction in these contents at similar concentration. The effect was comparatively more on chlorophyll-a and total chlorophyll while chlorophyll-b and protein contents were less affected.

Key words: Herbicidal potential, Formulation, *Parthnium hysterophorus Phoma herbarum*, Phytotoxicity

Parthenium hysterophorus L., commonly known as carrot weed, is an obnoxious, annual and deadly weed of compositae family, native of North America. It poses serious threat to crops, livestock and human beings. It is responsible for the substantial losses to the crops. It reduces agricultural yield by 40% and forage production by 90% (Knox *et al.* 2006). Conventional methods of its management rely mainly on the use of chemical herbicides. Public concern over the safety due to indiscriminate use of synthetic herbicides has generated significant pressure on weed scientists to search an alternative of these chemicals. Exploitation of microorganisms and especially their biorationals (natural products) as herbicides have generated significant interest world wide. (Pandey *et al.* 2002, 2005). Survey conducted at various habitats of central India for weed pathogens yielded an isolate of *Phoma herbarum* (FGCCPH#27) which incites severe collar rot disease in *Parthenium* (Pandey *et al.* 1996). Mycoherbicidal potential of the pathogen is known to influence by environmental factors. To overcome these constraints, secondary metabolites especially oxalic acid synthesized by the pathogen have also tried (Pandey *et al.* 2003*)*. Oxalic acid produced by the pathogen showed high herbicidal potential against *Parthenium*. Normal application of oxalic acid did not produced noticeable results and need a suitable formulating agent for its effective herbicidal potential. Therefore, the present investigation was under taken with the formulation and *in vitro* evaluation of herbicidal potential of the pathogen against *Parthenium* by shoot cut, seedling and detached leaf bioassay, methods.

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MATERIALS AND METHODS

Phoma herbarum (FGCCPH#27) was obtained from fungal germplasm collection Centre, BCRBC, Jabalpur**,** previously isolated from the diseased part of the target weed and maintained on Potato Dextrose Agar (PDA) medium .

Preparation and extraction of CFCF

1000 ml Erlenmeyer flasks containing 500 ml of Richard's broth were seeded with 5 mm disc obtained from 7 days old cultures grown on PDA medium at 28°C. Inoculated flasks were incubated at 38 ±2°C for 7 and 14 days. Fermented broth was filtered through Whatman's

filter paper No.1 and the filtrate was centrifuged at 4000 rpm for 10 min. Supernatant was discarded and the crude filtrate was again passed through 0.251In Sartorious filter *in vacuo* condition (Abbas *et al.* 1992).

Formulation

To test the compatibility of the toxin synthesized by the pathogen a total of 15 formulating agents were tried. All the formulating agents were added at the rate of 0.5% to the toxin and its herbicidal potential was determined by shoot cut assay and observations were made for three days (Daigle and Conick 2002).

Bioassay

Seedlings were raised in pots containing soil, sand and peat in 1:1:1 ratio. Different formulations containing 50% toxin of *P. herbarum* (7 and 14 days old) sprayed at different concentration on host seedlings 30 ml per plant grown in pots and maintained in green house. Each treatment was replicated thrice and observations were recorded after 3 days based on a score chart. Per cent disease index was calculated by using the following formula (Praveena 2003).

$$\text{Per cent disease index} = \frac{\text{Sum of score of each leaf}}{\text{No. of leaves scored} \times \text{maximum score}} \times 100$$

Leaves from the host target weed were surface sterilized with 2% NaOCI and were kept on a sterilized moisture chamber prepared by using cotton and filter paper in a Petridish. (Thapar *et al.* 2002). Various dilutions of toxic metabolites were used of 7 and 14 days old fermented broth, *viz.* 50% and 100% with Tween 80 0.5% and the effects were observed after 24 hrs.

Chlorophyll and protein contents

One g of fresh leaves were homogenized with ethanol in a mortar and pestle and centrifuged at 8000 rpm for 2 min., with 80% ethanol. Supernatant was taken in other flask and diluted with ethanol. Absorbance of the extract was measured by UV-Vis Systronics Spectrophotometer at 645 and 663 nm for the determination of chlorophyll a, b and total chlorophyll. The protein content was determined as per Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Formulation

Maximum mortality was shown by the inoculum formulated with sucrose + Tween-20 followed by Tween-80. Triton-X was found to be highly inhibiting in its action. Rest of the agents produced average effect on the host shoots. Findings obtained in the above study clearly revealed the herbicidal potential of the pathogen against *Parthenium* (Table 1). Maximum toxicity obtained at 50% concentration with sucrose + Tween 20 indicated the toxin compatibility with the formulating agent. However, others did not produce significant damage to the host shoots. Variations in different formulation might be due to the compatibility of the organism with various formulating agent. Similar findings have also been made by Singh (2002).

Bioassay

Cell free culture filtrate (CFCF) obtained from different incubations had varied effect on host seedlings. Maximum average leaf area damage (LAD) of 85% on the seventh day and 65% of leaf damage by fourth day was observed when treated with CFCF obtained from 14 day's old fermented broth at 50% concentration followed by 75 and 100%. CFCF obtained from 14 days old fermented broth showed the maximum toxicity due to the maximum production of oxalic acid (Table 2). CFCF obtained from 7 days old fermented broth also showed considerable toxicity at higher concentration. Similar observations have also reported by other workers (Saxena *et al.*

Table 1. Effect of different formulations on host seedings

Formulating agent	% disease intensity (day)				
	2	4	6	8	10
Sorbitol	1	4	4	4	4
Tween-20+ water	2	5	5	5	5
Tween -20+sucrose	5	6	6	6	6
Water + gelatin	5	5	5	5	5
Toxin + triton-X	2	5	5	5	5
Toxin + water	5	5	6	6	6
Tween-80+ sucrose+water	4	3	4	5	5
Tween 80	2	4	4	4	4
Toxin	6	6	6	6	6
Toxin + acrylamide	2	4	4	4	4
Toxin+ glycerol	1	1	1	1	1
Toxin+ coconut oil	1	1	1	1	I
Toxin+ soyabean	3	3	3	3	3
Toxin+mustard oil	3	3	3	4	4
Toxin+ Tween 80	6	7	8	9	9
Control (tichart's broth)	0	0	0	0	0

Disease rating index; 1=99%, 2=95%, 3=91%, 4=82%, 5=62%, 6=38%, 7=18%, 8=9%, 9=5%, 10=1%, below 10 = 0%; Amount of agrochemical sprayed: 30 ml/plant; RH: 85%; Incubation period: 14 days

Table 2. Effect of different concentrations of toxin on host seedlings

Concentration (%)	Incubation period % disease index	
	7 days	14 days
25	25±0.01	36±0.04
50	59±0.04	80±0.02
75	50±0.25	75±0.25
100	48±0.01	73±0.15
0	No effect	No effect

Amount of agrochemical sprayed: 30 ml/plant; RH: 85%; Values given in the table are mean ± SEm

Table 3. Effect of different concentrations of toxin on detached leaves of host

Concentration (%)	Incubation period % disease index	
	7 days	14 days
25	33±0.01	55±0.01
50	66±0.05	95±0.25
75	52±0.02	78±0.03
100	49±0.01	70±0.15
0	No effect	No effect

Values are mean ± SEm; Amount of agrochemical sprayed: 5 ml/ plant; RH: 85%

Table 4. Effect of different concentrations of toxin on biological contents of host seedlings

Concentration (%)	Biological activity of CFCF							
	7 days				14 days			
	Chl a	Chl b	Total chl	Protein	Chl a	Chl b	Total chl	Protein
25	10.4±0.25	12.1±0.06	32.6±0.32	36.1±0.04	50.3±0.02	40.8±0.02	45.9±0.2	35.5±0.04
50	72.1±0.07	58.4±0.07	65.4±0.28	56.9±0.07	97.8±0.01	74.7±0.02	72.5±0.0	76.1±0.01
75	68.8±0.04	56.1±0.65	60.3±0.62	36.9±0.04	82.2±0.25	68.5±0.01	65.3±0.2	54.1±0.01
100	69.6±0.05	53.4±0.025	59.9±O.73	27.8±0.07	78.1 ±0.01	65.2±.03	63.4±0.3	42.2±0.02
0	0	0	0	0	0	0	0	0

Values are mean ± SEm; Amount of agrochemical sprayed - 30 ml/plant; RH: 85%; Chl - Chlorophyll

2001, Pandey *et al.* 2002). In contrast to this, workers recorded maximum toxin production after 7 days of incubation (Pandey *et al.* 2003) while the CFCF obtained from 14 d old fermented broth showed the maximum mortality to the host seedlings, however 7 days old broth didn't cause significant damage to the host seedlings. Similar findings have also been made by other workers. (Shukla and Pandey 2006), which indicated significant difference between the mean leaf area damage (LAD) at different concentration with a P value of 5%.

Noticeable symptoms were also observed when detached leaves were treated with CFCF of 7 and 14 days old fermented broth at various concentrations, *viz.* 25, 50, 75 and 100%. Leaves were completely killed at 100% concentration and more than 80% of damage was recorded at 50% concentration (Table 3). Results obtained by detached leaf assay were quite prompting to use this pathogen as herbicidal agent against the weed. Similar damage ratings were also recorded by other Effect on chlorophyll and protein contents workers (Joseph *et al.* 2002).

Effect on chlorophyll and protein contents

Chlorophyll and protein contents were also significantly affected when treated with CFCF. The contents were gradually decreased with increased incubation. Maximum reduction was recorded in shoots treated with CFCF obtained from 14 days old fermented broth at 50% concentration followed by 75% and 100 % (Table 4). 14 days old fermented broth showed the maximum biological activity as depicted by the significant reduction in the chlorophyll and protein content of the host leaves. While extract obtained from 7 days old broth failed to show any remarkable reduction in these contents at similar concentration. The effect was comparatively more on chlorophyll-a and total chlorophyll while chlorophyll-b and protein contents were less affected. Variation in toxicity in relation to incubation period might be due to different phase of growth of the fungus. Metabolites required for own growth are normally synthesized during initial phase whereas most of the toxicants are formed during idiophase *i.e.* stationary phase of the fungus. (Abbas *et al.* 1995) also recorded 25 to 78% reduction in chlorophyll content in *Datura* sp. (jimson weed) tissues treated with fumonisin. Similarly, significant biological activity of CFCF of many other microorganism including fungi have also been recorded by several workers (Kovics *et al.* 2005, Pandey and Pandey 2005).

Based on the results, it was inferred that the present isolate *P. herbarum* have significant potential to produce phytotoxic compounds with high herbicidal properties against *P. hysterophorus.* However, detailed investigation

regarding characterization, standardization of large scale production of herbicidal compounds are to be carried out before its field application.

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