JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 48, No. 4 (2008)

FORMULATION AND EVALUATION OF AGROCHEMICALS OF *SCLEROTIUM ROLFSII* FGCC # 02 AGAINST *PARTHENIUM HYSTEROPHARUS*

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Received: November 29, 2007
Accepted: April 4, 2008

Abstract: Herbicidal potential of CFCF (Cell free culture filtrate) of *Sclerotium rolfsii* against *Parthenium hysterophorus* was determined by shoot cut, seedling and detached leaf bioassays. Maximum phytotoxicity was obtained from 14 day's old fermented broth with sucrose + 0.5% Tween 20 as formulating agent. Significant reduction in chlorophyll and protein contents of host was also noticed.

Key words: herbicidal potential, *Sclerotium rolfsii, Parthenium hysterophorus,* biorational phytotoxicity, formulation

INTRODUCTION

Parthenium hysterophorus is an obnoxious, pernicious and deadly weed of compositae family native of North America, probably introduced in India along with grains under the PL 480 scheme. It poses serious threat to crops, livestock and human beings. Its infestation can be observed along railway lines, forest land, grassland and even valuable lands too. 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). It has now gained the status of a major weed in forest areas of some states of India. It is gradually invading forest and other valuable lands**.** 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 pressures 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, 2003, 2004, 2005).

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Survey conducted at various habitats of central India for weed pathogens yielded an isolate of *Sclerotium rolfsii* FGCC#02 which incites severe collar rot disease in Parthenium (Pandey *et al*. 1996a). 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 deals with the formulation and *in vitro* evaluation of herbicidal potential of the pathogen against Parthenium by Seedling and Detached leaf bioassay.

MATERIALS AND METHODS

Recovery of Strains

S.rolfsii FGCC#02 was obtained from Fungal Germplasm Collection Centre, R.D.University, Jabalpur, it previously isolated from the diseased part of the target weed and maintained on Potato Dextrose Agar (PDA) medium as indicated by Agrawal and Hasija (1986).

Preparation and Extraction of CFCF

1 000 ml Erlenmeyer flasks containing 500 ml of sterilized Richard's broth (KNO_3 – 10 g; $MgSO_4$ – 2.5 g; KH_2PO_4 – 2.5 g; sucrose – 30 g and distilled water – 1000 ml) were seeded with 5 mm discs obtained from 7 days old cultures grown on PDA medium at 30°C. Inoculated flasks were incubated at 30 ± 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 in a centrifuge (Remi). Supernatant was discarded and the crude filtrate was again passed through 0.25µm Sartorious filter *in vacuo* condition (Abbas *et al.* 1992). Oxalic acid production was also determined by the method suggested by Bateman and Beer (1965). Herbicidal potential of the agrochemicals of 7 and 14 days old was cultures evaluated by detached leaf and seedling bioassay (whole plant bioassay).

Detached leaf bioassay

Leaves from the host target weed were surface sterilized with 2% NaOCl and were kept on a sterilized moisture chamber prepared by using cotton and filter paper in a Petri dish. (Thapar *et al.* 2002). Various dilutions of toxic metabolites (5 ml) were used of 7 and 14 days old fermented broth viz., 25%, 50%, 75% and 100% and the effects were observed after 24 hrs. All the experiments were performed in triplicates.

Seedling bioassay

Seedlings were raised in pots (10 cm) containing soil, sand and peat in 1:1:1 ratio. Different concentration of *S. rolfsii* toxin (7 and 14 days old) was sprayed on host seedlings @ 30ml per plant and maintained in a greenhouse (temperature 30°C and relative humidity 85–90%). Each treatment was replicated three times with two controls and observations were recorded after 3 days based on a score chart and per cent disease index was calculated by using the following formula (Praveena 2003).

$$\text{Percent Disease Index} = \frac{\text{Sum of score each leaf}}{\text{No. of leaves scored x Maximum score}} \text{ x } 100$$

Effect of CFCF on chlorophyll and protein contents

1g of fresh leaves was homogenized with excess of ethanol in a mortar with pestle and centrifuged at 8000 rpm for 2 min, with 80% ethanol. Supernatant was transferred to another 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 Chl a, Chl b and total Chl. The protein content was determined as described by Lowry *et al.* (1951).

Formulation

To test the compatibility of the toxin (14 days old cultures) synthesized by the pathogen a total of 15 formulating agents namely sorbitol, T-20+water, T-20+sucrose, H_2O + gelatin, toxin +Triton X 100, toxin+ water, T-80+sucrose+H_2O, Tween 80, toxin, toxin + acrylamide, toxin+ glycerol, toxin+ coconut oil, toxin+soyabean, toxin+mustard oil, toxin+Tween 80 were tried. All the formulating agents were added at rate of 0.5% to the toxin and its herbicidal potential was determined by seedling assay and observations were made after 48 hrs up to 10th day (Daigle and Conick 2002).

RESULTS AND DISCUSSION

Detached leaf assay

Noticeable symptoms were 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 1). Results obtained by detached leaf assay were quite promising to use this pathogen as herbicidal agent against the weed. Similar damage ratings were also recorded by other workers (Joseph *et al.* 2002). One way ANOVA which indicates significant difference between the mean leaf area damage at different concentration of toxin with a p value of 5%.

Table 1. Effect of different concentration of toxin on detached leaves of Host

Incubation period		
% disease index		
Concentration	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±0.01
Control	no effect	no effect

Values given in the table are mean ± SEM; Amount of agrochemical used: 5 ml/plant; RH: 85

Seedling bioassay

It is clearly evident from the data shown in Table 2 that CFCF obtained at different incubation had varied effect on host seedlings. Maximum average leaf area damage (ALAD) 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. CFCF obtained from 7 days old fermented broth also showed considerable toxicity at higher concentration. Similar observations have also reported by many other workers. (Pandey *et al.* 2002; Winder and Watson 1994; Saxena and Pandey 2001; Saxena *et al.* 2001) In contrast to this, several workers recorded maximum toxin production after 7 days of incubation (Pandey *et al.* 2003) While the CFCF obtained from 14 days old fermented broth showed the maximum mortality to the host seedlings, 7 days old broth didn't cause significant damage to the host seedlings.. Similar findings have also been made by other workers. (Abbas *et al.* 1995; Shukla and Pandey 2006). Each experiment was performed in triplicates. The means of experiment was analyzed by one way ANOVA which indicates significant difference between the mean leaf area damage at different concentration with a p value of 5%.

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

Incubation period		
% disease index		
Concentration	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
Control	no effect	no effect

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

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 leaves treated with CFCF obtained from 14 days old fermented broth at 50% concentration followed by 75% and 100 % (Table 3).

14 days old fermented broth showed the maximum biological activity as depicted by a 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 a 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 may 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. (Sharma *et al*. 2004; Thapar *et al.* 2002; Abbas *et al*. 1995; Joseph *et al.* 2002; Saxena and Pandey 2001; Saxena *et al.* 2001; Kovics *et al*. 2005; Pandey and Pandey 2005).

Table 3. Effect of different concentration of toxin on biological contents of host seedlings

Concentration	Biological Activity of CFCF							
	7 days				14 days			
	Chl.a	Chl.b	total Chl.a	protein	Chl.a	Chl.b	total Chl.a	protein
25%	10.4±0.25	2.1±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±0.07	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±0.73	27.8±0.07	78.1±0.01	65.2± .03	63.4±0.3	42.2±0.02
Control	no effect	no effect	no effect	no effect	no effect	no effect	no effect	no effect

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

Formulation

Data recorded in table 4 clearly depict that maximum phytotoxicity was shown by the toxin formulated with sucrose + Tween 20 followed by Tween 80. Triton X was found to be highly inhibiting in its action. The rest of the agents produced an average effect on host shoots. Findings obtained in this study clearly revealed a herbicidal potential of the pathogen against Parthenium. Maximum toxicity was obtained at 50% concentration with sucrose +Tween 20 indicates the toxin compatibility with the formulating agent. However, other formulations did not produce significant damage to the host seedlings. Variations in different formulations may be due to the compatibility of the organism with various formulating agents. Similar findings have also been obtained by Singh (2002).

Table 4. Effect of different formulations on host seedlings under green house conditions

SNo	Formulating agent	% Disease intensity				
		2nd day	4th day	6th day	8th day	10th day
1	2	3	4	5	6	7
1	Sorbitol	1	4	4	4	4
2	T-20 + water	2	5	5	5	5
3	T-20 + Sucrose	5	6	6	6	6
4	H_2O + Gelatin	5	5	5	5	5
5	Toxin + Triton X 100	2	5	5	5	5
6	Ioxin + water	5	5	6	6	6
7	T-80 + sucrose +H_2O	4	3	4	5	5

1	2	3	4	5	6	7
8	Tween 80	2	4	4	4	4
9	Toxin	6	6	6	6	6
10	Toxin + Acrylamide	2	4	4	4	4
11	Toxin + Glycerol	1	1	1	1	1
12	Toxin + coconut oil	1	1	1	1	1
13	Toxin + Soyabean	3	3	3	3	3
14	Toxin + Mustard oil	3	3	3	4	4
15	Toxin + Tween 80	6	7	8	9	9
16	Control (Richard'sbroth)	No Effect	No Effect	No Effect	No Effect	No Effect
	SEM	±0.33				
	$CD_{5\%}$	0.946				

Disease rating index (Horsfall and Barret 1945)

1 = 99%, 2 = 95%, 3 = 91%, 4 = 82%, 5 = 62%, 6 = 38%, 7 = 18%, 8 = 9%, 9 = 5%, 10 = 1%

Amount of agrochemical sprayed: 30ml/plant; RH: 85%; Incubation period: 14 days

The above findings clearly indicate that the present isolate 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.

ACKNOWLEDGEMENTS

We are grateful to Head and Supervisor, Department of Biological Sciences, R.D.University, Jabalpur for providing necessary laboratory facilities. We are also thankful to Department of Biotechnology, Govt. of India, Council of Scientific & Industrial Research New Delhi and the Department of Environment and Forest, Ministry of Environment & Forest, Govt. of India, New Delhi for financial support.

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POLISH SUMMARY

FORMULACJA I OCENA AGROCHEMIKALIÓW Z *SCLEROTIUM ROLFSII* FGCC#2 PRZECIWKO *PARTHENIUM HYSTEROPHORUS*

Potencjał herbicydowy bezkomórkowych wyciągów z kultur *Sclerotium rolfsii* FGCC#2 przeciwko *Parthenium hysterophorus* L. określano wykonując biotesty na roślinach oraz na odciętych liściach. Maksymalną fitotoksyczność tych wyciągów obserwowano w przypadku 14-dniowej kultury w pożywce płynnej wzbogaconej preparatem Tween 20 + sacharoza (0,5%). Stwierdzono także istotne ograniczenie zawartości chlorofilu i białka w roślinie żywicielskiej.