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EVALUATION OF MYCOHERBICIDAL POTENTIAL OF SELECTED FUNGI AGAINST A NOXIOUS WEED *HYPTIS SUAVEOLENS*: A PRELIMINARY EVALUATION

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ABSTRACT

Evaluation of an indigenous fungus Fusarium roseum FGCCW #61 for the control of a noxious weed Hyptis suaveolens was evaluated. The cell free broth of fungi contains bioactive natural herbicidal products and useful for weed control. Natural product-based mycoherbicides are generally considered safer than their synthetic counterparts. Fusarium spp. is known to synthesize an array of biologically active metabolites, phytotoxic in nature from liquid culture filtrates. In the present work, Mass production of cell free broth of Fusarium roseumFGCCW#61was developed and control potential was thereby determined against the obnoxious weed Hyptis suaveolens in laboratory and field condition. Pre- and post-emergence field trials were also conducted to evaluate the mycoherbicidal efficacy of mass-produced herbicidal compound in field conditions.

KEYWORDS: *Hyptis Suaveolens, Fusarium Sp, Mass Production, Spore Free Broth, Formulation, Field Trial*

INTRODUCTION

Hyptis suaveolens (L.) Poit, a member of the Lamiaceae or Labiatae family is a common weed of roadsides and waste grounds. It is generally described asannual, perennial forb or herb or subshrub or vine. This Dicot (dicotyledonous) is native totropical America, is an annual herb that occupies roadsides, rail tracks, wastelands, watercourses, pastures and open forests where the soil is well drained. It can form dense thickets in all areas ofgrowth. It is a prolific seed producer and in dense infestations can yield up to 3000 seeds/m^2, forming persistent propagule banks within a short period(Sharma et al., 2009).In northwest India, the absence of several species of economic importance to local people in areas heavily invaded by H. *suaveolens* may pose socioeconomic problems for local people in periurban ecosystems (Sharma et al., 2007).Several economically important species were absent from invaded areas, but present in areas without *H. suaveolens.* In Pakhal Wildlife Sanctuary, Andhra Pradesh, India, *H. suaveolens* has become widespread, occupying grazing areas of wild animals and preventing the native ground flora from growing (Murthy et al., 2007). It may also enhance the risk of forest fire in the dry seasons (Murthy et al., 2007). Conventional methods of weed management have failed due to several reasons. In this context, the use of natural active compounds, also known as biopesticides, may be an interesting alternative for crop protection as they are considered to be less harmful and environmentally safer (Dewhurst, 2001; Dayan et al., 2009; Cantrell et al., 2012; Seiber et al., 2014). Biorational strategy of weed management is an effective and cheaper eco-friendly strategy involving the use of microorganisms including fungi. Fungi have long been recognized as plant pathogen and many of them produce a variety of bioactive extra cellular toxic compounds. Herbicidal properties of such toxic

metabolite of microorganism have been exploited in weed management (Pandey, 1999; 2000; Pandey et al., 2001; 2002; 2003; 2004; Saxena et. al. 2001).Several microbial products viz., Bialaphos, Gulfosinate, Tentoxin, Cornexistin, AAL-toxins, Fumonisin, Moniliformin etc have been successfully exploited for the management of many weeds (Hoagland, 2001; Barbosa et al., 2002). Singh 2007collected strains of *Fusarium roseum* FGCCW#61from diseased *H. suaveolens* in Jabalpur and suggest that this fungus has potential as a mycoherbicide against the weed.To improve the efficacy or modify virulence, viability, host specificity or environmental requirement, formulations are required. Compatible formulation of the phytotoxins with suitable surfactants and adjuvants has expanded the spectrum of weeds controlled by a single application (Greaves et al., 2000). Absorption of herbicides and its translocation to the target site is of utmost importance, which comprise of a delivery system. The importance of efficient delivery to the target site as a fundamental requirement for herbicide activity and selectivity is generally recognized.This fungal pathogen showed promising herbicidal potential against the weed Hyptis sp. However being living product, bioactivity of the agent was found to be affected by environmental conditions. To overcome this problem, herbicidal property of the strain was evaluated and discussed in this paper. So, the aim of this paper is to develop and field application of phytotoxins or secondary metabolites which avoid these environmental problems encountered by chemicals and capable of preventing the spread of weed.Thus, mass produced and formulated phytotoxic metabolites from *Fusarium* sp. were evaluated for their herbicidal activities against the weed, *Hyptis* at lab and field condition in the present paper.

MATERIALS AND METHODS

Recovery of Strain

Strain of *Fusarium roseum* FGCCW # 55 was obtained from Fungal Germplasm Culture Collection (FGCC), Mycological Research Laboratory, Department of Biological Sciences, and R.D.V.V. Jabalpur (M.P.) India. The culture was maintained on Potato Dextrose Agar (PDA) medium at 4 ± 1^0C in a refrigerator for further studies.

Process optimization and Metabolite extraction

The isolate *Fusarium roseum* FGCCW #55 that showed promising herbicidal activity were cultured in 8 litre Richards Broth in 10 litre of pilot size fermentor (Scigenics, India). The media was sterilized at 15 psi (121°C) for 20 min and inoculated with 80 ml (2.1×10^7 spores/ml) of *Fusariumroseum* FGCCW # 55 culture and run for 7 days. Final harvesting was done after 8 days (Patino-Vera *et al.*, 2005) to obtain Cell Free Culture Filtrate. The parameters for Fermentation process were employed for large scale production of secondary metabolites were mentioned below:

Table: 1

S.No	Parameter	Value
1	Age of seed	7 days
2	Inoculum	80 ml (2.1×10^7 spores / ml)
3	pH	6.1
4	Temperature	28±2°C
5	Agitation	210 rpm
6	Harvest Time	After 7 days
7	Biomass	10.26 g/L

Extraction of Metabolites

After specified incubation Period crude metabolites of the isolates were extracted by solvent extraction method. Each

liquid broth was extracted with equal volume of ethyl acetate thrice in a separating funnel by vigorous shaking for 10 min. The cell mass got separated and solvent so obtained was collected. Ethyl acetate was evaporated, and the resultant compound was dried with Magnesium sulfate (MgSO4) and concentrated to yield the crude extract. The crude extract was then dissolved in 10% of Dimethyl Sulphoxide (DMSO) for herbicidal bioassay. The b**ioassay e**xperiments viz., Shoot Cut Bioassay, Seedling Bioassay and Detached Leaf bioassays were performed according to the methods adopted by Sharma *et al*., 2004.

Formulation

To test the compatibility of the toxin synthesized by the pathogen a total of 12 formulating agents namely Tween-80, Tween-60, Tween-20, Triton X-100, Mustard Oil, Coconut Oil, Groundnut Oil, Sunflower oil, Soybean Oil, Glycerol, Sucrose and Sorbitol were tried. All the formulating agents were added at the rate of 0.5% to the toxin and its herbicidal potential was determined by seedling bioassay and detached leaf bioassays. All the treatments were carried out in triplicates and all the bioassays were repeated atleast thrice.

Field Trials

To evaluate the herbicidal potential of *Fusarium roseum* FGCCW # 55 against the target weed Hyptis, a field study was conducted in Department of Biological Sciences, R.D. University, Jabalpur.

Pre-Emergence

Pre-emergence applications were made four days after sowing. The consecutive pre-emergence (PRE 2; PRE 3 and PRE 4) herbicide application were made at an interval of two days upto 10 DAS. No. of seeds sown for pre-emergence application test were 150 per 1 sq./m, while height of seedlings (average) was 8 cm and Seedling population was 50/sqm.

Post-Emergence

The post-emergence (POST 1) herbicide application was made, when the cantaloupe was at the 4-5 leaf stage of growth. The weather conditions were clear, and the air temperature was 30^0C. Repeat applications have been shown to increase control. The consecutive post-emergence (POST 2; POST 3 and POST 4) herbicide application were made at an interval of two days upto 10 DAT. (Frohlich *et al*., 2000).

Application

Seedlings were raised in pots containing sterilized soil/peat (1:1). Parthenium seedlings at 4-5 leaf stage of growth were sprayed to run-off with 100% concentration of phytotoxin. Phytotoxins were applied to the seedlings and the observations (Phytotoxic damage Rating) were made after 2 DAT, 4DAT, 6 DAT and 8 DAT. Land preparations were done by tilling the land and then leveling it. The stubbles and residues of previous weeds were removed to obtain a clean seedbed. The weather conditions were clear, and the air temperature was $30^0 \pm 1$C°with no rains before the application.Phytotoxins were sprayed to run-off in all the plots.

RESULTS AND DISCUSSIONS

Mass Production

Mass production of phytotoxins by *Fusarium roseum* FGCCW #61 was achieved in a 10 litre of fermentor containing 8

litres of media. As depicted in Table 1, CFCF eluted after different fermentation days was subjected to seedling bioassay for assessment of phytotoxic damage. The damage started with slight chlorosis and necrosis finally culminating with shoots death after 72 hpt. The controls exhibited no effect. Fermented broths obtained from *Phoma* sp. also killed shoots as observed by Vikrant *et al.,* 2006.

Formulation

Various formulation agents were tested to formulate the extracted crude phytotoxin obtained from *Fusarium roseum* FGCCW # 61 as depicted in table 2 and table 3. Out of the 12 various formulations tested for their efficiency and compatibility as assessed by seedling and detached leaf bioassays, Tween-60 produced maximum phytotoxic damage after 48 hpt. This was followed by Triton X- 100. Remarkable results were exhibited by different oils in the order: Groundnut oil, Sunflower oil and Mustard oil. Tween-20 and -80 showed medium formulation efficiency. In contrast to this Sucrose, Sorbitol and Glycerol did not prove to be good formulative as they did not produce significant phytotoxic damage to Hyptis seedlings. The effect initiated with slight chlorosis and necrosis, which was subsequently followed by drooping, curling of leaves, blackening of stem leading finally to death of entire seedlings. Detached leaf bioassay was also performed with different formulants. Results like those in seedling bioassay were obtained for detached leaf bioassay. Thus, the most potent formulant aiding in absorption and translocation of the phytotoxic compounds was Tween-60. Surfactants play a very important role in improving the performance efficiency of pesticides with the potential to reduce the amount of active required and improve pesticide safety (Mulqueen, 2003). Tween series of surfactants are nonionic surfactants, each of them ethoxylated sorbital esters of fatty acids and a polyoxyethylene unit 20 repeat groups long on average. They adsorb with the alkyl chain at the hydrophobic surface and the ethylene oxide head group, which is water soluble, protruding into the water solution (Graca *et al*., 2007). They are generally easily degradable. Homologous series of poly oxyethylene sorbitans (Tweens) are good candidates as surfactants which are known to enhance cuticular penetration of herbicides (Singh & Mack, 1993).Desai *et al*., 2002 have incorporated Tween 80 @ 0.5% with chemical herbicide. Triton X-100 is a common non-ionic surfactant [polyoxyethylene (10) octylphenyl ether] (Behera *et al.,* 2007). Oils are used as additives for a variety of reasons such as reducing vapour loss of herbicide, enhancing the performance of herbicides. Traditionally, spray formulation has incorporated petroleum-based oils, but more recently oils extracted from crop seeds such as soyabean, sunflower, canola and coconut have been used. The role of oils in herbicide application and efficiency has been investigated by several groups (Gauvrit, 1994; Foy, 1996). Crop oil-based adjuvants i.e. refined or esterified vegetable oils are known to enhance the phytotoxicity of herbicides (Holloway, 1998).Thus formulation with vegetable oils can enhance absorption, translocation and phytotoxicity of herbicides (Gauvrit and Cabanne, 1993).

Field Application

Table 4 shows resultsof preemergence treatment of *Hyptissuaveolens*seeds with toxins of *F. roseum* FGCCW #61 of various days and concentrations after 2, 4, 6 and 8 DAS. The control seeds showed maximum percentage germination. With increase in DAS with 100% phytotoxin concentration, percentage seed germination decreases indicating effect of phytotoxins on Hyptis seeds. Maximum decrease in percent germination can be recorded after 8 DAS. Similarly, Table 5 represents results of post-emergence treatment of Hyptis seedlings with toxin after spraying to run-off. The 10days old Mass produced CFCF exhibited maximum phytotoxicity to Hyptis seedlings. Post-emergence results agreed with those for pre-emergence.Pandey et al., 2007 have studied effect of cell free culture filtrate of *Helminthosporium* sp. FGCC#74

against *Hyptis suaveolens*. In contrast to this result, 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.

Based on results obtained above, it can be concluded that the secondary metabolites of Fusarium roseum FGCCW#61 possess high herbicidal potential and can be developed as potential herbicides for the management of the deadly weed, *Hyptissuaveolens.*

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APPENDICES

Table 2: Phytotoxic Damage Rating of Hyptis Shoots Treated with Mass Produced Phytotoxins of *Fusarium Roseum* FGCCW # 61

S. No.	Days of Fermentation	Phytotoxic Damage Rating(PDR) Mean±Sd		
		24 hpt	48 hpt	72 hpt
1.	Control a	0.00±0.00	0.00 ± 0.00	0.00±0.00
2.	Control b	0.00±0.00	0.00±0.00	0.00±0.00
3.	2	0.20±0.07	0.40±0.02	0.51±0.09
4.	4	0.66±0.08	0.73±0.01	0.90±0.03
5.	6	1.47±0.07	1.58±0.12	1.78±0.06
6	8	2.02±0.15	2.26±0.12	3.66±0.12
7.	10	3.23±0.16	3.64±0.09	4.42±0.08
	SEM±	0.08	0.05	0.04
	CD (P=0.05)	0.15	0.16	0.12

Values are Means ± SD of three observations; Temp- 30^0C; R.H- 80-85%

Inoculum dose –5ml CFCF / seedling; Shoots- same size with 4 axial and 2 leaves.

PDR- 0 = No symptoms; 1 = slight chlorosis; 2 = marked chlorosis, slight necrosis;3 = high necrosis and marked chlorosis; 4 =a cute necrosis and marked chlorosis; 5=acute chlorosis and acute necrosis.

Table 3: The Compatibility Study of Crude Broth of FGCCW#61 Containing Phytotoxin + Various Formulation by Seedling Bioassay

S. No.	Various Formulation (@0.5%)	Phytotoxic Damage Rating(PDR) Mean±SD		
		24 hpt	48 hpt	72 hpt
1	Coconut oil	3.80±0.10	3.85±0.01	4.14±0.04
2	Groundnut oil	3.76±0.01	3.85±0.08	4.11±0.07
3	Mustard oil	3.36±0.04	3.45±0.17	3.54±0.14
4	Glycerol	0.21±0.05	0.38±0.03	0.53±0.08
5	Soybean oil	3.33±0.07	3.56±0.07	3.56±0.07
6	Sunflower oil	3.75±0.06	3.83±0.08	4.12±0.04

Table 3 (Contd..,)				
7	Sucrose	0.29±0.04	0.56±0.05	0.86±0.10
8	Sorbitol	0.29±0.12	0.66±0.02	0.88±0.11
9	Tween 60	4.14±0.19	4.43±0.10	4.56±0.09
10	Tween 80	2.64±0.15	2.90±0.04	2.97±0.06
11	Tween 20	2.69±0.04	2.86±0.04	2.94±0.04
12	Triton X 100	3.93±0.07	4.13±0.16	4.48±0.11
	SEM±	0.05	0.05	0.04
	CD (P=0.05)	0.14	0.13	0.14

Controls seedlings sprayed with i.e. DW + different formulants (0.5%) exhibited no phytotoxic damage.Values are Means ± SD of three observationsTemp- 30±1^{0}C ; Inoculum dose –5ml CFCF / seedling; R.H- 80-85%

PDR- 0 = No symptoms; 1 = slight chlorosis; 2 = marked chlorosis, slight necrosis; 3 = high necrosis and marked chlorosis; 4 = acute necrosis and marked chlorosis; 5 = acute chlorosis and acute necrosis.

Table 4: The Compatibility Study of Crude Broth of FGCCW#61 Containing Phytotoxin + Various Formulation by Detached Leaf Bioassay

S. No.	Various Formulation (@0.5%)	Phytotoxic Damage Rating(PDR) Mean ± SD		
		24 hpt	48 hpt	72 hpt
1.	Tween 60	2.94±0.08	3.16±0.09	3.34±0.09
2.	Tween 80	4.33±0.08	4.55±0.10	4.94±0.08
3.	Tween 20	2.95±0.08	3.16±0.09	3.34±0.09
4.	Triton X 100	4.44±0.09	4.66±0.09	4.79±0.07
5.	Sucrose	0.56±0.11	0.77±0.10	1.17±0.08
6.	Sorbitol	0.58±0.08	0.76±0.07	1.26±0.12
7.	Glycerol	0.75±0.09	0.26±0.04	0.14±0.09
8.	Coconut oil	5.56±0.08	3.71±0,07	3.86±0.13
9.	Groundnut oil	4.18±0.04	4.36±0.07	4.64±0.06
10.	Soyabean oil	3.24±0.09	3.37±0.04	3.55±0.11
11.	Sunflower oil	3.59±0.04	3.75±0.13	3.86±0.09
12.	Mustard oil	3.17±0.12	3.38±0.09	3.59±0.11
	SEm±	0.03	0.03	0.08
	CD (P=0.05)	0.08	0.07	0.25

Values are Means ± SD of three observations, hpt- hours post treatment; Temp- 30±1^{0}C ;; R.H- 80-85%

Inoculum dose –2.5 ml CFCF/ leaf

PDR- 0 = No symptoms; 1 = slight chlorosis; 2 = marked chlorosis, slight necrosis; 3 = high necrosis and marked chlorosis; 4 = acute necrosis and marked chlorosis; 5 = acute chlorosis and acute necrosis.

Table 5: Impact of Cell Free Broth on Hyptis Seeds (Pre- Emergence)

S.No.	Days/ Concentration of CFCF	% Seed Germination (Mean ±SD)			
		2 DAS Pre 1	4 DAS Pre 2	6 DAS Pre 3	8 DAS Pre 4
1.	Control a	99.67±2.82	99.33±1.41	99.33±0.70	99.66±0.70
2.	Control b	99.67±2.80	99.33±1.25	99.33±1.38	99.66±2.44
3.	4/ 25	92.66±2.82	91.66±4.24	90.00±3.53	88.33±0.00
4.	4/50	82.66±2.12	79.33±0.00	76.00±4.24	73.33±3.53
5.	4/75	80.00±2.82	78.00±0.70	75.00±3.53	71.33±6.36
6.	4/100	79.00±3.53	77.00±0.00	73.66±5.65	71.00±3.53

Table 5 (Contd..,)					
7.	6/25	78.33±2.12	76.00±1.41	73.33±3.53	69.33±0.00
8.	6/50	72.33±3.53	70.33±2.88	66.66±3.53	65.00±0.00
9.	6/75	67.00±2.12	63.33±3.53	58.33±0.00	55.00±3.53
10.	6/100	63.00±0.70	61.66±3.53	55.00±0.00	53.33±0.00
11.	8/25	62.66±1.41	59.66±2.12	54.33±4.94	51.66±3.53
12.	8/50	59.33±3.53	57.66±4.94	50.00±0.00	49.66±0.00
13.	8/75	54.33±1.41	51.00±2.12	49.33±0.00	46.66±3.53
14.	8/100	42.00±4.24	37.66±5.65	33.33±3.53	29.33±1.41
15.	10/25	63.00±2.12	64.66±1.41	58.00±1.41	56.66±5.65
16.	10/50	59.66±2.12	58.33±4.94	56.33±2.12	54.33±4.94
17.	10/75	54.33±1.42	51.66±0.70	51.00±2.12	50.33±2.12
18.	10/100	41.33±4.94	39.00±2.12	46.66±0.00	55.00±1.41
	SEM±	1.62	2.03	2.23	2.14
	CD (P=0.05)	4.68	5.87	6.41	6.17

Values are Means ± SD of three observations

DAS- Days After Sowing; Temp- 30±1^{0}C; R.H- 80-85%

Inoculum dose –50 ml CFCF/sq.m,

Control a –uninoculated Richard's Broth; Control b- Sterilized DW

PDR- 0 = No symptoms; 1 = slight chlorosis; 2 = marked chlorosis, slight necrosis; 3 = high necrosis and marked chlorosis; 4 = acute necrosis and marked chlorosis; 5 = acute chlorosis and acute necrosis**.**

Table 6: Impact of cell Free Broth on *Hyptis Suaveolens* Plant Seedlings (Post- Emergence)

S.No.	Days/ Concentration of CFCF	Phytotoxic Damage Rating (PDR) (Mean ± SD)			
		2 DAT Post 1	4 DAT Post 2	6 DAT Post 3	8 DAT Post 4
1.	Control a	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2.	Control b	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3.	4/ 25	0.19±0.04	0.25±0.07	0.28±0.03	0.27±0.07
4.	4/50	0.20±0.07	0.33±0.06	0.34±0.09	0.52±0.03
5.	4/75	0.40±0.07	0.43±0.08	0.54±0.07	0.64±0.17
6.	4/100	0.51±0.06	0.60±0.05	1.02±0.13	1.76±0.06
7.	6/25	1.98±0.07	2.12±0.06	2.29±0.06	2.38±0.06
8.	6/50	2.06±0.05	2.20±0.06	2.34±0.06	2.50±0.05
9.	6/75	2.30±0.06	2.39±0.08	2.47±0.05	2.53±0.09
10.	6/100	2.56±0.07	2.64±0.08	2.72±0.06	2..94±0.08
11.	8/25	3.50±0.21	3.98±0.16	4.26±0.05	4.41±0.07
12.	8/50	4.14±0.13	4.31±0.06	4.53±0.01	4.60±0.06
13.	8/75	4.35±0.13	4.40±0.07	4.55±0.12	4.72±0.05
14.	8/100	4.37±0.16	4.55±0.10	4.62±0.04	4.75±0.15
15.	10/25	3.36±0.21	3.88±0.04	4.18±0.06	4.33±0.11
16.	10/50	4.10±0.07	4.22±0.06	4.42±0.06	4.48±0.05
17.	10/75	4.3±0.07	4.45±0.10	4.45±0.10	4.57±0.09
18.	10/100	4.21±0.14	4.31±0.06	4.41±0.06	4.52±0.01
	SEM±	0.06	0.31	0.05	0.04
	CD (P=0.05)	0.16	0.90	0.15	0.12

Values are Means ± SD of three observations, Temp- 30±1^{0}C; Inoculum dose –50 ml CFCF/sq.m; R.H- 80-85%

Control a –uninoculated Richard's Broth; Control b- Sterilized DW

PDR- 0 = No symptoms; 1 = slight chlorosis; 2 = marked chlorosis, slight necrosis; 3 = high necrosis and marked chlorosis; 4 = acute necrosis and marked chlorosis; 5 = acute chlorosis and acute necrosis.