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ECO-FRIENDLY MANAGEMENT OF PROMINENT WEEDS OF LEGUMINOUS CROP (*GLYCIN MAX*) EMPLOYING INDIGENOUS MICROBES OF GADARWARA AREA

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ABSTRACT

Soybean a "miracle crop" is outstanding in its nutritive value. It has 43 percent biological protein and 20 percent fat. It is also rich in vitamins, mineral salts and essential amino acids. The intensity and distribution of weed species in the soybean crop are functions of a complex interaction among soil properties, rainfall patterns, temperature and cultural practices. Soybean yield losses resulting from weed interference and the cost of weed control constitute some of the highest costs involved in the production of the crop Looking to the potential of microbial herbicides, the present paper was based on develop and exploit some indigenous strains of fungal pathogens as bioherbicides to manage some of the problematic weeds of soybean. On the basis of primary screening 40 fungal strains isolated from various parts in which five of the most potential ones were selected for secondary screening. These were against screened and *Aspergillus sulphureus* was found to be the most potential agent effective against a wide range of host and induced maximum phytotoxic damage and thus, selected for further investigation.

KEY WORDS: Leguminous crops, Mycoflora, Gadarwara. Eco-friendly.

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. The annual production is about 12 million tones but our requirement is about 17 million tones indicating a deficiency of over 30%. which can be overcome through increased production and crop protection.

Gram pea pigeon pea and soybean are the major legume crops and today prominently figures in the crop priority of the fanners in Madhya Pradesh contributing to major percentage to the total legume production in the country. We have distinction of being the world's single largest producer of legumes/pulses, having an area approximately 20- 24 million hectares under legumes.

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. Several microbial products have been patented and commercialized in well-advanced countries. Critical analysis of literature published on microbial weed interactions clearly indicates that no serious and systematic research has been carried out in India to assess their herbicidal potential (Pandey *et. al.* 1996. 2001 Pandey 1999). There is no doubt about the problems of hazardous weeds in leguminous crops. Every year tonnes of productivity of these crops have reduced due to the weeds. Synthetic chemicals employed to control these plants are now gradually either ineffective or creating severe problems to only other non-target organisms.

An extensive survey ofliteratures onlilicrobial management of weeds clearly indicates that no seriouseffort has been made in case of leguminous crops. However, significant work has been done in caseof microbial management of insect pests of legumes in India. Therefore, the present investigation hasbeen proposed to develop an effective microbial management strategy for some prominent weeds of leguminous cropping system.

MATERIAL AND METHOD

1. **Field Survey:** A systematic, periodical and thorough survey of the weedy leguminous fields especially in different localities of Gadarwara was undertaken. In the leguminous 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. Infected parts (leaves, stems and roots) of the different weeds having distinct symptoms with good frutification and rhizospheric soil were collected in separate sterile polythene bags.

2. **Recovery of Pathogens:** The set of specimens and soil samples collected during survey were brought to the laboratory and were used for the isolation of the fungal pathogens. Following procedures were followed for isolating the fungal pathogens from different parts of the weed and rhizospheric soil.

Isolation from Infected Leaves, Stems and Roots

Diseased portion of the weed were cut into small pieces (2 to 5 mm square) with the help of sterilized blade aseptically. 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. These were then transferred in presterilized petri dishes (9 cm diameter) containing potato-dextrose agar medium with 75 mg/l streptomycin and pinhead amount of rosebengal (Walker, 1981; Agarwal and Hasija, 1986).

Isolation from Rhizospheric Soil

The isolation of fungi from soil samples 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 with the help of flattened end of nichrome needle 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 (45^0C) 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± 10C in BOD incubator and then transferred to PDA slants as earlier mentioned.

Dilution Plate Methods

10 grams of each soil samples was oven dried and grinded in mortar and pestle. These finely pulverized 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 (45^0C) 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± 10C for 3-4 days and when fungal colonies developed in plates, it was transferred to slants (Agarwal and Hasija, 1986).

1. **Microscopic studies and identification of pathogens:** The organisms were identified with the help of various books, monographs, reviews, research papers etc. (Ellis, 1971, 1976; Subramanian, 1971; Barnett and Hunter, 1972; Ellis and Ellis, 1985). The slides were temporarily mounted in KOH, which was found quite useful for observation of

conidiogenous cells and other hyaline structures. These were finally mounted in lactophenol with cotton blue. The slides were made semi-permanent by ringing them with nail polish and stored for further study and references.

2. **Phytotoxicity Test for Selection of Potential Strain**

Phytotoxicity test for qualitative assessment of phytotoxin production by isolates were done by following methods.

3. **Seed Germination Test (Plate Bioassay)**

Assessment of phytotoxic activity of CFCF was carried out by preparing specific concentrations of fermented broth. The uninoculated medium served as the control and sterile distilled water as control over control. The petridish were covered with filter paper supported by a thin layer of adsorbent cotton. Twenty five seeds of different weeds and crop were taken in the 100 x 17 mm Borosil glass petriplates which were incubated in seed germinator at 26± 20C, 75± 15% relative humidity and 7350 lux for 15 hours. These seeds were treated with 25, 50 and 100 concentrations of CFCF. Three replicates for each treatment including control were maintained and observed daily for germination and root and shoot length was recorded.

4. **Shoot Cut Bioassay**

Shoots of healthy plants were cut under water and kept in sterilized chromatographic jars having Hogland's solution and placed in growth chamber under controlled conditions. Hoagland's solution delays premature death of shoots. The shoots were then placed in Hoagland's solution in the test tubes and were sprayed with the spore suspension and CFCF with an automizer until wetness. Control plants were sprayed with sterile distilled water containing Tween 80. All the fungi were sprayed in 3 replications. These plants were then kept in environment growth chamber at 280C and 70% relative humidity (Plate. 16). The disease severity was rated by visual observation at an interval of 24 hours till 7 days.

5. **Whole Plant Bioassay**

Assessment of phytotoxic activity of CFCF was carried out by preparing specific concentrations of fermented broth. The uninoculated medium served as the control and sterile distilled water as control over control. Seeds of different weeds were surface sterilized with 0.05% NaOCI solution, for 10 min. scarified 15 min in concentrated I-hS04 and then rinsed three times with sterile distilled water. Seeds were sown in plastic

pots (10 x 6 cm) @ 25 /pot containing a mixture of pre sterilized sand, soil and peat (1: 1: 1) and grown under 26± 2^0C, 75± 15% relative humidity and 7350 lux for 15 hours for a period of two weeks. These were then sprayed evenly with 25, 50 and 100% concentrations of CFCF and tween 80 using an atomizer attached to a pump. The experiment had three test sets and one control set (Plate. 17). These were kept in plant growth chamber (Yorko) and observed daily for disease severity which was rated as per Chiang et al., (1989) till mortality of the plant.

RESULT AND DISSECTION

Survey and Selection of Potential Phototoxic Producing Strain

All plants, including weeds, have natural enemies. These enemies can be manipulated to influence the abundance of their host plants. During a periodical survey of various soybean fields in Gadarwara area, it was observed that leaf spot, leaf blight, die back, petiole root, root rot, etc. diseases were associated with soybean and its weeds. A total of 40 fungal isolates were selected from various diseased and rhizospheric soil samples collected from soybean fields. Screening of phytotoxic metabolites produced by fungal strains was done by petriplate bioassay, cut shoot bioassay and whole plant bioassay against the four major weeds, namely, *Echinochloa colonum, Euphorbia geniculata, Cyperus iria* and *Commelina benghalensis.*

It is evident from the result that more or less CFCF obtained from all fungal strains caused significant damage to different weed species. The CFCF obtained from viz. *Aspergillus candidus, A. sulphureus, Alternaria sp., Curvularia lunata, Fusarium oxysporium, Penicillum sp., Phoma sp*. incited severe infection, caused drastic damage and showed maximum toxicity against four major weeds of soybean, while few others viz. *A. clavatus, A. flavus, Cladosporium sp., Colletotrichum dematium, C. truncatum, Fusarium sp., F. moniliforme, F. orthoceras, Phoma sorghina, Rhizopus sp., Rhizotonia leguminicola showed moderate phytotoxicity. Several other fungi viz. Aspergillus niger, Aspergillus sp., Cephalosporium sp., Curvularia clavata, Fusarium sp., F. oxysporium* produced mild phytotoxicity in one or other weeds but not effective against all the selected weed species. CFCF of rest of the fungal species viz. *Cephalosporium gregatum, Cercospora sojina, Drechslera indica, Dipothe phaseolorum, Erysiphe polygoni, Fusarium paffidoroseum, Glomereffa glycine, Helminthosporium sp., Macrophomina phaseoli, Pythium ultimum, Sclerotinia sclerotiorum, Septoria sp.* totally failed to show any toxicity to the weed in whole plant bioassay.

Similarly, phytotoxicity of CFCF of fungal strains was also tested by seed germination in petriplate bioassay. The CFCF obtained from *A. candidus, A. sulphureus, Coffetotrichum dematium, Curvularia lunata, F. orthoceras, F. moniliforme, Penicillum sp., Phoma sp., Rhizopus sp.* incited severe toxicity and completely inhibited the germination of the weed seeds, while few others viz. *A. clavatus, A. niger, Aspergillus sp., Alternaria sp., Cephalosporium sp., Cladosporium sp., C. truncatum, C. clavata, Fusarium sp., F. oxysporium, Helminthosporium sp., Phoma sorghina, Pythium ultimum, Rhizotonia leguminicola, showed moderate phytotoxicity* and seed germination was delayed and resulted in stunted shoot and root length of the seedlings. Rest of the fungal species viz. *A. flavus, Cephalosporium gregatum, Cercospora sojina, Drechslera indica, Dipothe phaseolorum, Erysiphe polygoni, F. pallidoroseum, Fusarium sp., Glomereffa glycine, Macrophomina phaseoli. Sclerotinia sclerotiorum, Septoria sp., Trichoderma sp*. had little or nearly no effect on seed germination. Nearly similar results were obtained when shoot cut bioassay was performed.

On the basis of primary screening of 40 isolates 5 most potential isolates were selected for secondary screening. They were *Aspergillus candidus (P-50), Aspergillus sulphureus (P-37), Curvularia lunata (P-42), Peniciffum sp. (P-35) and Phoma sp. (P-41).* These were again screened as earlier and *Aspergillus sulphureus (P-37)* was found to be the most potential and induced maximum phytotoxic damage after 24 hours post treatment (hpt). It was followed by *Aspergillus candidus (P-50), Penicillum sp. (P-35), Curvularia lunata (P-42), and Phoma sp. (P-41). Aspergillus candidus* CFCF proved to be mildly toxic during the present studies. *Curvularia lunata (P-42)* failed to show toxicity at lower concentrations.

CONCLUSION

Thus, it may be concluded that the strain *Aspergillus sulphureus* is found highly potential for production of herbicidal compound for the management of weeds of leguminous crops.

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Table 1: Secondary screening of selected fungal strains against soybean weeds.

S. No.	Name of the fungus	Concentration of CFCF	Phytotoxic damage (%)			
			CB	CI	EC	EG
1.	*Aspergillus candidus*	Control	0	0	0	0
		25	65	72	68	70
		50	78	82	75	80
		75	83	85	80	90
		100	92	90	87	95
2.	*Aspergillus sulphureus*	Control	0	0	0	0
		25	63	74	65	78
		50	90	90	95	96
		75	98	95	100	100
		100	100	100	100	100
3.	*Curvularia lunata*	Control	0	0	0	0
		25	30	46	45	56
		50	40	55	58	60
		75	52	62	65	68
		100	68	72	70	71
4.	*Penicillum* sp.	Control	0	0	0	0
		25	51	63	70	71
		50	60	72	75	80
		75	64	79	80	85
		100	75	82	85	90
5.	*Phoma* sp.	Control	0	0	0	0
		25	64	68	76	78
		50	70	75	80	82
		75	74	82	85	87
		100	85	90	91	95

CB=*Commelina benghalensis* **CI** = *Cyperus iria*
EC=*Echinochloa colonum* **EG**=*Euphorbia geniculata*,
Phytotoxicity at different CFCF concentration after 48 hpt
Uninoculated medium served as control

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