

## Screening and evaluation of biocontrol agents for sustainable management of weeds of leguminous crops

BHARTI AHIRWAR<sup>1</sup> AND RANJANA SHRIVASTAVA<sup>2</sup>

Department of Microbiology, Govt. V.Y.T.PG. Autonomous College, Durg (C.G.)

Received: February, 2026; Revised accepted: May, 2026

### ABSTRACT

*Leguminous plants are incredibly important since they are the main source of protein, which we are quite familiar with. The best meatless diet choice in India is this. Since they are an affordable source of high-quality plant protein, vitamins and minerals, legumes satisfy a significant portion of our protein need, which is why they are sometimes referred to as "poor people's meal". Weed infestations are responsible for a 33% loss in crop productivity. The sustainability of legume crops is increasingly being threatened by pests particularly weeds. In agriculture, weeds are a persistent and ever-evolving problem. Crop protection and increased productivity can solve all these issues. A survey was conducted at various sites of Rajnandgaon. It has been discovered that various fungi diseases, including root rot and leaf spot are linked to weeds. A total 20 fungal genera were isolated throughout this inquiry. Commelina benghalensis and Parthenium hysterophorus leaf showed the most infection. The most frequent species discovered was Alternaria alternata.*

**Key words:** *Commelina benghalensis, Parthenium hysterophorus, Alternaria alternata, Fungi, biological management*

### INTRODUCTION

Legume crops are the second most significant group of food plants and serve as the primary protein source for the largely vegetarian population of India. Although the country produces approximately 12 million tonnes of legumes annually, the demand stands at around 17 million tonnes resulting in a deficit of over 30%. This gap can be bridged by enhancing crop productivity and ensuring effective crop protection. Major legume crops such as gram, pea, pigeon pea, and lentil are major pulse crops in Chhattisgarh, playing a key role in the region's agricultural priorities. After rice, in terms of area, the maximum area comes under these crops. A notable producer of gram, lentil, and pea. These crops contribute a substantial share to the nation's total legume production. India holds the distinction of being the world's largest producer of legumes, with about 20–24 million hectares dedicated to their cultivation. Weeds, which are ever-present and continuously evolving, pose a major challenge in agriculture. They compete with crops for nutrients and reduce soil fertility, thereby lowering crop yields. Although several microbial-based weed control products have

been patented and commercialized in developed countries, a review of existing literature reveals a lack of systematic research in India on the herbicidal potential of microbes (Pandey *et al.*, 1996; Pandey, 1999; Pandey *et al.*, 2001). Weeds remain a serious issue in legume cultivation, leading to significant annual yield losses. The synthetic herbicides currently in use are becoming less effective and are also causing harm to non-target organisms. While considerable research has been conducted in India on microbial control of insect pests in legume crops, very little attention has been paid to microbial weed management in these systems. Therefore, this study aims to develop a microbial-based weed management strategy targeting key weed species in legume cropping systems.

### MATERIALS AND METHODS

#### Field Survey

A systematic, periodic, and comprehensive survey was conducted in weedy legume fields, particularly across various locations in Rajnandgaon (Chhattisgarh). Weeds were selected on the basis of field survey, majority of weeds and

discussion with farmers. During the legume-growing season, monthly field visits were carried out to observe the patterns of vegetative growth and monitor the progression of disease symptoms at different developmental stages. Infected plant parts—such as leaves and stems exhibiting clear symptoms were carefully collected in separate sterile polythene bags for further analysis.

### Recovery of Pathogens

The set of specimens 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.

### Isolation from Infected Leaves and Stems

Small segments (2 to 5 mm<sup>2</sup>) were aseptically excised from diseased areas of *Commelina benghalensis* and *Parthenium hysterophorus* weed leaves and stems using a sterilized blade. These tissue pieces were then surface sterilized by immersing them in a 1% sodium hypochlorite (NaOCl) solution for approximately three minutes to eliminate surface contaminants. After sterilization, they were thoroughly rinsed with sterile water to remove any residual disinfectant. The sterilized samples were then placed onto pre-sterilized 9 cm petri dishes containing potato dextrose agar (PDA) medium, supplemented with 75 mg/l streptomycin and a trace amount of rose bengal, as recommended by Walker (1981) and Agarwal & Hasija (1986).

### Microscopic Examination and Pathogen Identification

The identification of the organisms was carried out using a range of reference materials, including books, monographs, review articles, and research papers (Ellis, 1971, 1976; Subramanian, 1971; Barnett and Hunter, 1972; Ellis and Ellis, 1985). For microscopic observation, temporary mounts were prepared using potassium hydroxide (KOH), which proved effective for visualizing conidiogenous cells and other transparent structures. Final mounts were made using lactophenol mixed with cotton blue stain. To preserve the slides, they were sealed with nail polish to make them semi-permanent and stored for future examination and reference.

### Maintenance of Fungal Cultures

The cultures of fungi were maintained on PDA slant and stored at 4°C.

### Determination of Frequency

The frequencies of different fungi were determined by using following formula:

Percentage (%) Frequency of Individual Fungus

$$= \frac{\text{Total no. of Individual fungus in aplates}}{\text{total no. of different fungi in plates}} * 100$$

$$\text{Percentage (\%)} \text{ Frequency} = \frac{T1}{T2} * 100$$

### Phytotoxicity Test for Selection of Potential Strain

Phytotoxicity test for qualitative assessment of phytotoxin production by isolates were done by Whole Plant Bioassay methods.

### Whole Plant Bioassay

To assess the phytotoxic potential of Cell-Free Culture Filtrate (CFCF), specific concentrations of fermented broth were prepared. An uninoculated medium was used as the control, while sterile distilled water served as a secondary control. Seeds of various weed species were surface sterilized using 0.05% sodium hypochlorite (NaOCl) for 10 minutes, followed by scarification with concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) for 15 minutes, and then thoroughly rinsed three times with sterile distilled water.

The sterilized seeds were sown in plastic pots (10 × 6 cm) at a rate of 25 seeds per pot, filled with a pre-sterilized mixture of sand, soil, and peat in equal proportions (1:1:1). These pots were placed under controlled conditions (26 ± 2°C, 75 ± 15% relative humidity, and 7350 lux light intensity for 15 hours daily) and allowed to grow for two weeks. After the growth period, the seedlings were evenly sprayed with CFCF at concentrations of 25%, 50%, and 100%, using an atomizer connected to a pump. Tween 80 was added to aid in uniform application. The experiment included three treatment groups and one control group. All sets were kept in a controlled plant growth chamber (Yorko) and monitored daily. Disease severity was

Table 1: Percentage frequency of various fungi associated with infected weeds *Commelina benghalensis* and *Parthenium hysterophorus*

S. No.	Name of Fungi	% Frequency
1	<i>Aspergillus niger</i>	22%
2	<i>Alternaria alternata</i>	78%
3	<i>Fusarium oxysporum</i>	52%
4	<i>Aspergillus flavus</i>	48%
5	<i>Fusarium moniliformum</i>	72%
6	<i>Mucor racemoides</i>	30%
7	<i>Penicillium sp.</i>	38%
8	<i>Trichoderma viride</i>	22%
9	<i>Alternaria sp.</i>	57%
10	<i>Cephalosporium sp.</i>	11%
11	<i>Curvularia lunata</i>	48%
12	<i>Rhizopus sp.</i>	34%
13	<i>Mucor sp.</i>	28%
14	<i>Aspergillus fumigatus</i>	39%
15	<i>Phoma sp.</i>	40%
16	<i>Colletotrichum truncatum</i>	44%
17	<i>Helminthosporium sp.</i>	57%
18	<i>Phoma sorghina</i>	38%
19	<i>Drechslera indica</i>	18%
20	<i>Drechslera sp.</i>	22%

evaluated following the scale proposed by Chiang *et al.* (1989), continuing until plant mortality was observed.

## Disease Index

0 -	No disease
1 -	1-20% Plant damaged
2 -	21-40% Plant damaged
3 -	41-60% Plant damaged
4 -	61-80% Plant damaged
5 -	81-100% Plant damaged

## RESULT

## Survey and Selection of Potential Phototoxic Producing Strain

During periodic surveys of legume fields in the Rajnandgaon region, several diseases such as leaf spot, leaf blight, dieback were found to affect associated weeds (*Commelina benghalensis* and *Parthenium hysterophorus*). From the collected diseased tissues samples, a total of 20 fungal isolates were obtained. These isolates were screened for their ability to produce phytotoxic metabolites through Whole Plant bioassays. The tests were conducted against two predominant weed species *Commelina benghalensis* and *Parthenium hysterophorus*.

Table 2: Phytotoxicity of CFCF of Fungal Strain on weeds *Commelina benghalensis* and *Parthenium hysterophorus* by Whole plant Bioassay

S. No.	Name of Fungi	Phytotoxic damage %	
		<i>C. benghalensis</i>	<i>P. hysterophorus</i>
01	<i>Aspergillus niger</i>	40 %	30 %
02	<i>Alternaria alternata</i>	90%	85%
03	<i>Fusarium oxysporum</i>	40%	50%
04	<i>Aspergillus flavus</i>	45%	55%
05	<i>Fusarium moniliformum</i>	65%	55%
06	<i>Mucor racemoides</i>	34%	40%
07	<i>Penicillium sp.</i>	45%	45%
08	<i>Trichoderma viride</i>	25%	18%
09	<i>Alternaria sp.</i>	60%	65%
10	<i>Cephalosporium sp.</i>	30%	30%
11	<i>Curvularia lunata</i>	70%	75%
12	<i>Rhizopus sp.</i>	65%	60%
13	<i>Mucor sp.</i>	45%	50%
14	<i>Aspergillus fumigatus</i>	45%	39%
15	<i>Phoma sp.</i>	65%	70%
16	<i>Colletotrichum truncatum</i>	40%	44%
17	<i>Helminthosporium sp.</i>	50%	57%
18	<i>Phoma sorghina</i>	45%	38%
19	<i>Drechslera indica</i>	35%	28%
20	<i>Drechslera sp.</i>	35%	30%

The results clearly indicate that the Cell-Free Culture Filtrates (CFCF) derived from

most of the fungal strains caused varying degrees of damage to the tested weed species.

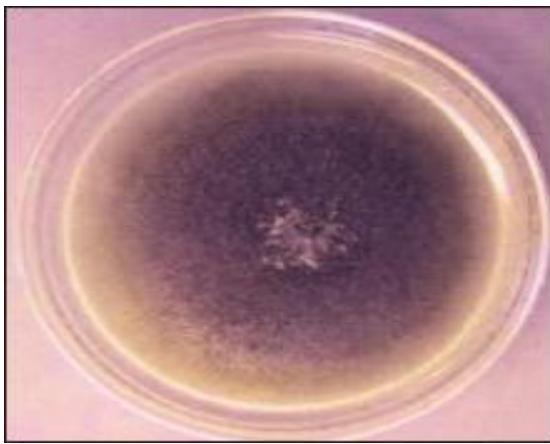


a



b

Infected leaves of selected weeds (a) *Commelina benghalensis* and (b) *Parthenium hysterophorus*



c



d

Fig c: Growth of *Alternaria alternata* on PDA plate Fig d: Microscopic photograph of *Alternaria alternata*.

#### Microscopic photograph of selected mycoherbicidal agent *Alternaria alternata*

CFCFs from fungi such as *Alternaria alternata*, *Fusarium moniliformum*, *Alternaria sp.*, *Helminthosporium sp.*, *Aspergillus flavus*, *Rhizopus sp.*, *Phoma sp.* and *Curvularia lunata*, were highly effective, inducing severe infections and extensive damage, and exhibited the strongest phytotoxic effects against the *Commelina benghalensis* and *Parthenium hysterophorus*. Other fungal strains, including *Fusarium oxysporum*, *Mucor racemosus*, *Colletotrichum truncatum*, *Penicillium sp.*, *Trichoderma viride* and *Mucor sp.* showed moderate levels of phytotoxicity. Some fungi such as *Aspergillus niger*, unidentified *Aspergillus sp.*, *Cephalosporium sp.*, *Drechslera indica*, and certain *Drechslera sp.*, produced only mild phytotoxic effects, affecting one or more weed species failed to show any phytotoxicity in the whole plant bioassay.

#### DISCUSSION

Fungi that attack plants and other saprophytic strains are known to synthesize phytotoxic secondary metabolites Gupta *et al.* (2022). The experiment had three test set and was observed daily for disease severity which was rated as per Chiang *et al.* (1989).

#### CONCLUSION

Thus, it may be concluded that the strain *Alternaria alternata* is found highly potential for production of herbicidal compound for the management of (*Commelina benghalensis* and *Parthenium hysterophorus*) weeds of leguminous crops.

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