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Phoma herbarum: A Potential Biocontrol Agent Against Weeds, that Promotes Wheat Growth

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Abstract

The usage of chemical weedicide adversely affects the soil fertility and environment. Hence, in order to reduce the use of chemical weedicide, current study was aimed to isolate plant pathogenic microorganisms from diseased weeds and evaluate their potential as a bioherbicide in wheat field. Twelve bacterial and thirty-one fungal isolates were screened to determine their bioherbicidal activity against prevalent weeds (*Avena fatua, Phalaris minor,* and *Chenopodium album*) by using detached leaf assay and *in-vitro* seed testing methods. Among the forty-three isolates, two potential isolates were selected for further studies. Potential fungal isolates DGL 8C and DGL 7A with significant bioherbicidal activity were molecularly (ITS sequencing) identified as *Phoma herbarum* R21 (GenBank ID- ON705696) and K_NESO2 (GenBank ID- ON705704). *Phoma herbarum* R21 was chosen for further research due to its superior herbicidal effect and positive influence on wheat growth. Effective herbicidal activity (up to 90%) of potential isolate was obtained in pre-germination testing, compared to control. Cell free culture filtrate (CFCF) treatment showed nonspecific inhibition in the germination of weeds and wheat. While, CFCF selectively deteriorated the target weeds in post-germination treatment. *Phoma herbarum* R21 enhanced the growth of Durum wheat varieties Poshan and Tejas, as it promoted the growth of shoot, root, and fresh weight up to 88% compared to control. *Phoma herbarum* R21 significantly inhibited the growth of phytopathogenic fungi up to 57%. In this study, *Phoma herbarum* R21 was identified as a potential bioherbicide against the weeds of wheat along with its growth promoting and antifungal activities.

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Introduction

Weeds are the noxious pests of agricultural land, where they compete with the crop for space, nutrients, sunlight and other growth regulators. *Avena fatua*, *Phalaris minor* and *Chenopodium album* are major weeds found in Madhya Pradesh, India. The potential yield loss of 16.5-43.0% was recorded due to weed infestation which could be 100% if left uncontrolled in wheat field[1] in India. Wheat is grown on about 314.5 lakh hectares of land. Growth and yield of wheat crop is essential to feed the present population. Chemical herbicides have been vigorously utilized to reduce the impact of weeds on crop production. The risk and effect of herbicides and their residues on all tropic levels of food chain and environment has increased public concerns about pesticide-free food.

Hence, there is an urgent need to find out an alternative eco-friendly method such as bioherbicide for controlling weed species. Reducing herbicide toxicity and resistance is sought by use of safer bioherbicide microbes on their products.[2] Biological control for weed management is recognized as a cost-effective, efficient, and environmentally sustainable approach to weed control. Bioherbicidal products obtained from microbes are easily degradable and they cannot persist for long time in the soil.[3] Several phytopathogenic fungi, including *Fusarium oxysporum* and *Puccinia komarovii var. Glanduliferae*, have demonstrated their effectiveness in controlling weeds.[4,5] On the other hand, *Phoma herbarum* is a widespread saprobe and pathogen that can be toxic to plants.[6] *Phoma macrostoma*, *Phoma chenopodia*, and *Phoma herbarum* have all been mentioned as promising biological control agents for various weeds in earlier investigation.[7] *Phoma herbarum* has been found to be effective against *Parthenium hysterophorus*, *Lantana camara L.*, *Hyptis suaveolens*, and *Sida acuta Burm*. f.[8] *Phoma chenopodia* was also reported to be effective against Cirsium arvense, *Chenopodium album*, *Mercurialis annua* and *Setaria viridis*[9], *Phoma macrostoma* was reported as a biocontrol agent for dicot weed plants[10].

This study sought to determine bioherbicidal capacity of *Phoma herbarum* to manage potential weeds of wheat field and this is the first account of *Phoma herbarum* encouraging wheat growth. Further research is required on the formulation of *Phoma herbarum* to ensure consistent weed control.

Materials

Sample Collection and Isolation of Phytopathogenic Microorganisms

Affected parts of weeds were collected during winter season in zip lock bags from irrigated wheat fields and weed-infested areas of Indore, India. Infected samples were preserved at 4-8°C, until further isolation of phytopathogenic microorganisms.

Infected samples of the weed were used for the isolation of phytopathogenic microorganisms. Each infected sample was surface sterilized with 1% NaOCl for 1min and cut into 2-4 mm^2 pieces. A size of three to four pieces of infected material were placed on potato dextrose agar (PDA) for fungal isolation and nutrient agar (NA) plate for bacterial isolation and the plates were incubated at 28°C for 7 days. After this, pure culture of each isolate was prepared and stored at 4°C till further use.

Detached Leaf Assay for Screening of Isolates for their Bioherbicidal Activity

Detached leaf assay[11] was used for the screening of isolates to test their bioherbicidal activity against the weeds.

6mm fungal disc from PDA plate was inoculated in 100-ml Erlenmeyer flasks containing 25ml potato dextrose broth and incubated for 7 days at 27°C, 120 rpm. Inoculum of each bacterial isolate was prepared by inoculating a loopful of pure culture in 100-ml Erlenmeyer flasks containing 25ml nutrient broth. The bacterial cultures were incubated at 30°C for 2 days at 120 rpm.

Freshly collected healthy leaves of weeds (*Avena fatua, Chenopodium album*, and *Phalaris minor*) were used for detached leaf assay. Collected leaves were then washed in running tap water, surface sterilized with 1% NaOCl for 45 s and subsequently washed 3-4 times with autoclaved distilled water. Three leaves of each variety were placed on moist filter paper in different petri plates. Each leaf was treated with 20µl of bacterial or fungal inoculum. Treated leaves were incubated at 22±2°C, under alternating periods of light and dark for 7 days until the formation

of necrotic symptoms. Uncultured sterile growth medium was taken as control. All experiments were repeated and data was measured in triplicates. A similar experiment was performed using the selected isolates on other weeds and wheat varieties, Poshan and Tejas.

Biochemical Characterization of Potential Isolates

A qualitative testing approach like a visible hydrolytic zone and change in media colour was used for estimating the biochemical production of hydrolytic enzymes and secondary metabolites by potential isolates. The zone of hydrolysis produced by enzymes such as amylase on starch agar, protease on skimmed milk agar, cellulase on PDA supplemented with 1% CMC, lipase on tributyrin agar were performed using standard process, laccase on PDA supplemented with 0.01% guaiacol[12], chitinase on 1% colloidal chitin agar[13], phosphatase on Pikovskaya agar[14], xylanase on birch wood xylan and pectinase on pectin agar[15]. was considered for qualitative assessment. The qualitative production of secondary metabolites such as HCN by Lorck method[16], siderophore on CAS agar[17], and indole acetic acid by Salkowski method[18] was assessed.

Molecular Identification of Potential Isolates

Most promising fungal isolates were identified using molecular identification facilities at the National Fungal Culture Collection of India (NFCCI), Pune, India. The Genomic DNA of each fungus was isolated in pure form. ITS4 and ITS5 primers were used for successful amplification of the ITS-rDNA partial gene.[19] The sequencing PCR was set up with ABI-bigdye® Terminatorv3.1 Cycle Sequencing Kit. The raw sequence obtained from ABI 3100 automated DNA sequencer was compared with 18S rDNA sequences using BLAST program.

Effect of *Phoma herbarum* R21 in pre-germination and post-gemination test of weeds and wheat.

Culture Preparation

Fungal isolate selected after detached leaf assay is identified as *Phoma herbarum* R21 and used in further experiments. *Phoma herbarum* was inoculated on PDA and incubated at 22±2°C for 8 days. The spores were collected by flushing the plate with 10mL of sterile 0.5% Tween-20 solution, followed by filtration through muslin cloth. Spores were calculated using hemocytometer and suspension concentration of 10^7 spores/mL was maintained using sterile distilled water.

Pre-germination Treatment using Fungi

Seeds of three weed varieties such as *Avena fatua, Phalaris minor*, and *Chenopodium album* were obtained from the Directorate of Weed Research, Jabalpur, India. Two varieties of Durum wheat Poshan HI8663 and Tejas HI8768 were collected from Kasturbagram Rural Institute Indore, India.

All seeds were washed under running tap water, surface sterilized with 1% NaOCl for 45 seconds followed by rinsing 3-4 times with autoclaved distilled water. A total of 20 seeds of each variety were placed on a moist filter paper layer over cotton in each autoclaved petri plate. The seeds of weed and wheat were treated with 1 ml of fungal spore suspension (10^7 spores/ml in 0.05% Tween 20). Seeds treated with 0.05% Tween 20, were used as control. The treated seeds were grown for 7 days at 20 ± 2°C under 12hrs alternating conditions of light and dark. Growth parameters such as germination (%), shoot and root length (cm), and fresh biomass of plant (mg) were recorded. The experiment was performed in triplicate. The reduction in weed growth was recorded in the form of growth inhibition percentage (GIP) as given in the formula.

GIP = Value of growth parameter in control - Value of growth parameter in treated / Value of growth parameter in control × 100

Seedling growth of wheat varieties were examined by calculating growth percentage (GP) as compared to control using given formula. Control was taken as 100% growth.

GIP = Value of growth parameter in treated - Value of growth parameter in control / Value of growth parameter in control × 100

Post-germination Treatment using Fungi

Detached leaf assay as described in this paper was used for post-germination testing against the weeds and wheat leaves. Treated and control sets were incubated for 7 days and phytotoxicity was recorded as positive (+, symptomatic), negative (-, asymptomatic).

Effect of *Phoma herbarum* R21 CFCF in pre-germination and post-gemination test of weeds and wheat.

Cell Free culture Filtrate Preparation

1000 ml Erlenmeyer flasks containing 500 ml PDB were seeded with 6mm fungal discs from 5-day old culture plate of *Phoma herbarum*. The cultured medium was collected on 8th, 11th and 14th day, under aseptic conditions. The cultured medium was centrifuged at 8000 rpm and pre-filtered through a pre-weighed Whatman filter paper no. 1 and finally filtered using a 0.45µm syringe filter[20] (Himedia). This is to ensure a spore and filament-free CFCF without blocking the syringe filter.

Pre-germination Treatment using CFCF

Sterilised seeds of different weeds and wheat varieties were treated with 2ml of CFCF. CFCF (8th, 11th and 14th day) were added to different petri plates containing sterilized seeds and incubated under light (12 h) followed by dark (12 h) at 20°C for 7 days. Germination (%), shoot length (cm), root length (cm), fresh weight (mg) and dry weight (mg) of seedlings were examined in triplicate. Vigour index (plant height in cm x germination %) of seeds was calculated to evaluate activity and performance of seeds. Control treatments were carried out using sterile PDB medium[21]. The reduction in growth was recorded in the form of growth inhibition percentage.

Post-germination Treatment using CFCF

Detached leaf assay was used to test the effect of CFCF on both weeds and wheat leaves. For this 10µL of 8th, 11th and 14th day CFCF was applied on leaves of targeted weed and wheat varieties. Treated and control sets were incubated for 7 days and phytotoxicity was recorded as positive (+, symptomatic) and negative (-, asymptomatic).

Antifungal activity of *Phoma herbarum* R21

The selected isolate of *Phoma herbarum* R21 was tested against 5 fungal phytopathogens viz. *Rhizoctonia solani*, *Sclerotium rolfsii*, *Bipolaris oryzae*, *Curvularia lunata* and *Fusarium oxysporum* by dual culture plate technique[22] with some slight modifications. Antifungal activity was recorded after incubation of 4 days as percentage inhibition of radial growth (PIRG) and was calculated using the formula as given below[23].

PIRG =Fungal growth in control plate - Fungal growth in Dual plate assay/ Fungal growth in control plate × 100

Statistical Analysis

The triplicate values of each experiment were analysed using MS Excel version 2304 and analysed data are presented as mean ± standard deviation.

Results

Sample Collection and Isolation of Phyto-pathogenic Microorganisms

A total of 19 samples were collected from four different locations, including two farmlands and two areas surrounding university and college campuses. Morphologically different twelve bacteria and thirty-one fungi were isolated from the diseased weed tissues (*Avena fatua, Parthenium*, *Mauritius grass*, Oxalis, *Chenopodium album*, *Amaranthus sp*., *Euphobia*, *Lantana*, *Taraxacum officinale* and *Apluda sp*.) and soil samples, collected from different locations in Indore (Table 1).

Table 1:Sample collection sites and collected weeds.

Location	Coordinates		Infected Weeds / Soil
	Latitude	Longitude	
Wheat Field, KVK Kasturbagram, Indore	22.643031	75.8893632	*Avena Fatua* (wild oats) *Apluda* sp. (Mauritian grass) *Chenopodium album* (Bathua) *Parthenium hysterophorus* (Gajar ghas) Soil (organic land)

IARI, Regional Centre Indore.	22.719568	75.857727	*Euphorbia sp*. (Doodhi) *Amaranthus sp,* (Green amaranth) *Taraxacum officinale* (Dandelion) Soil (low weed density area)
Devi Ahilya University, Indore (DAU)	22.687061	75.8724644	*Lantana Camara* (Panchfooli) *Fumaria parviflora* (Shahatra) *Cynodon dactylon* (Doob)
Maharaja Ranjit Singh College, Indore	22.678901	75.8803770	*Euphorbia sp*. (Doodhi) *Oxalis* (Teen pattia)

Detached Leaf Assay for Screening of Isolates for their Bioherbicidal Activity

The detached leaf assay of isolates shows the initial symptoms of the disease in the form of necrosis and growth of the causal organism. The development of brownish irregular spots with a chlorotic halo around them, after 48 hours post-treatment (HPT) in three test sets. Four out of the twelve bacterial isolates (DGL1, DGL2, DGL3 and DGL 5A) tested on various weeds were found to be phytotoxic (Table 2). Moreover, among the fungal isolates, two fungal isolates DGL7A and 8C were remarkably infected all three targeted weeds within seven days of treatment compared to control. However, on all the weed leaves DGL 8C developed early symptoms in 36 hours as compared to 48 hours for DGL 7A.

Table 2: List of potential isolated screened by detached leaf assay

S.No.	Isolates	*Avena fatua*	*Phalaris minor*	*Chenopodium album*
1	DGL 1	+	-	-
2	DGL2	+	-	-
3	DGL3	-	-	+
4	DGL5A	-	-	+
5	DGL7A	+	+	+
6	DGL8C	+	+	+

(+) indicates infection, (-) indicates no infection

Molecular Identification and Biochemical Characterization of Potential Isolates

The selected fungal isolates, DGL 7A and DGL 8C from leaves of *Mauritius grass* showed 100% sequence similarity with *Phoma herbarum* KNESO2 and R21 respectively, based on the analysis of ITS region of rDNA sequences. Identified isolates come under fungal plant pathogen division of Ascomycota. The rDNA sequence of *Phoma herbarum* R21 was submitted to NCBI GenBank with accession ID ON705696.

Biochemical analysis of the selected isolate, *Phoma herbarum* R21 showed cellulase, pectinase, amylase, phosphatase and laccase activity, while it was negative for lipase, protease, chitinase and xylanase production compared to control (Table 3). Further, selected fungus effectively produces secondary metabolite siderophore, whereas, IAA and HCN production were not found. *Phoma herbarum* R21 which showed better pathogenicity towards weeds under study was chosen for further study.

Table 3: Production of enzymes and secondary metabolites by *Phoma herbarum*_R21

S.No.	Enzymes and metabolites	Phoma herbarum R21	S.No.	Enzymes and metabolites	Phoma herbarum R21
1	Cellulase	+	7	Xylanase	-
2	Lipase	-	8	Pectinase	+
3	Protease	-	9	Phosphate solubilization	+
4	Amylase	+	10	HCN production	-
5	Laccase	+	11	Siderophore production	+
6	Chitinase	-	12	IAA	-

(+) indicates enzyme/metabolite production, (-) indicates no enzyme/metabolite production

Effect of *Phoma herbarum* R21 On Pre-Germination and Post-Gemination Test of Weeds and Wheat.

The ability of fungal culture to inhibit seed germination and seedling growth of different weeds was examined by calculating inhibition percentage as compared to control. All controls were taken as 0% inhibition. Data in Table 4 and Fig. 1, demonstrate that *Phoma herbarum* reduced germination of weeds from 5% to 36%. Maximum germination inhibition (36%) was observed in *P. minor*. About 27-59 % reduction in shoot length was observed in all the targeted weeds. Moreover, 29-61% reduction in root length and 41-72% reduction in vigour index was observed in all weeds, but *C. album* has showed overall maximum reduction in growth parameters (32-72%) by *Phoma herbarum* R21 compared to control.

Table 4: Phytotoxic effect of *Phoma herbarum* R21 on pre-germination treatment of target weeds.

Target weeds		Germination percentage (%)	Shoot length (cm)	Root length (cm)	Fresh weight total (mg)	Vigour index
Avena	Control	95.0 ±4.08	8.5 ±0.23	8.7 ±0.15	135.3	1634 ±35.88
fatua	Treated	90.0 ±4.08	4.6 ±0.15	6.1 ±0.30	70.6	963 ±24.25
	GIP (%)	**5.26**	**45.88**	**29.89**	**47.85**	**41.06**
Phalaris	Control	86.6 ±2.35	8.7 ±0.18	2.5 ±0.30	4.8 ±0.13	970.6 ±10.63
minor	Treated	55.0 ±4.08	6.2 ±0.30	1.5 ±0.10	1.1 ±0.03	421.3 ±10.60
	GIP (%)	**36.05**	**29.14**	**40.41**	**77.29**	**56.59**
Chenopodium	Control	91.6 ±2.35	3.7 ±0.14	3.5 ±0.25	7.3 ±0.15	655.4 ±36.27
album	Treated	63.3 ±2.36	1.6 ±0.18	1.3 ±0.35	3.6 ±0.26	169.1 ±27.40
	GIP (%)	**32.26**	**57.84**	**61.30**	**51.23**	**72.02**

GIP- Growth Inhibition Percentage (%), *Controls are taken as 0% inhibition.

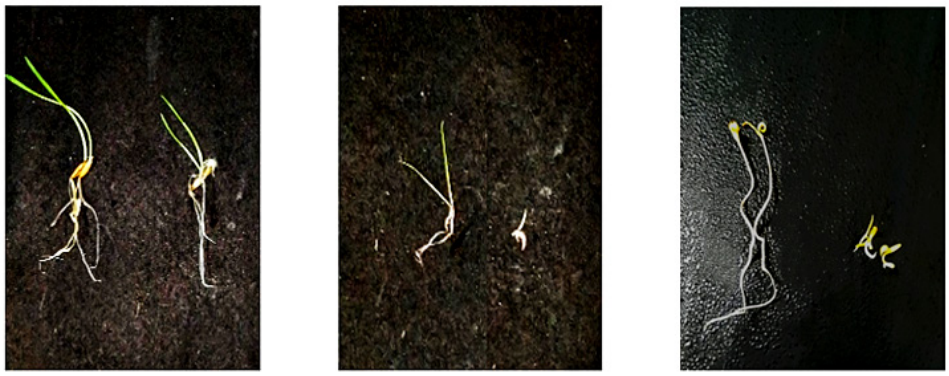

Fig.1 : Effect of *Phoma herbarum* on growth of *Avena fatua, Phalaris minor,* and *Chenophodium album*. Left-control, Right- treated

Phoma herbarum is first reported for showing significant growth promoting activity in wheat seedlings 102 – 188% increase in different growth parameters (shoot length, root length, biomass, and vigour index) was observed (Table 5 and Fig. 2). This signifies that *Phoma herbarum* is selectively affecting weeds and not the wheat crop and hence is helpful in early-stage removal of weeds especially *A. fatua* and *P. minor* that mimic wheat phenotypically.

Table 5: Plant growth promoting potential of *Phoma herbarum* on the growth of wheat.

Wheat varieties		Germination percentage (%)	Shoot length	Root length (cm)	Fresh weight total (mg)	Vigour index (cm)
Poshan	Control	98.3 ±2.36	7 ±0.25	8.1 ±0.28	48.5 ±0.05	1481.8 ±40.43
	Treated	98.3 ±2.36	9.5 ±0.15	11.5 ±0.32	91.3 ±0.85	2066.9 ±10.05
	GP (%)	**100**	**136.4**	**142.3**	**188.4**	**139.5**
Tejas	Control	96.6 ±2.36	8.1 ±0.19	9.1 ±0.14	89.5 ±0.25	1665.5 ±30.88
	Treated	98.3 ±2.36	10.7 ±0.35	15.5 ±0.32	112.8 ±0.60	2570.4 ±27.40
	GP%	**102**	**131.4**	**169.79**	**125.9**	**154.3**

GP- Growth Percentage. *Controls are taken as 100 % growth.

Fig. 2 : Effect of *Phoma herbarum* R21 on growth wheat (Poshan and Tejas) Left-control, Right- treated

The ability of fungus to selectively infect weeds over wheat was examined by detached leaf assay (post-germination test). This test revealed the selective phytotoxic potential of *Phoma herbarum* against targeted weeds. Hence, *Phoma herbarum* can also be applied on crop after germination.

Effect of *Phoma herbarum* R21 CFCF on Pre-Germination and Post-gemination Test of Weeds and Wheat.

The fungal cultural filtrate was tested on weeds and different growth parameters were evaluated after treatment (Table 6). Out of all the treatments, 14th day CFCF of *Phoma herbarum* was effectively inhibiting germination by 63%, 47% and 40% in *C. album, A. fatua*, and *P. minor* respectively. 93% reduction in growth parameters were observed upon CFCF treatment. Following, the 11th day CFCF treatment showed highest inhibition of shoot and root length on *C. album* followed by *A. fatua* and *P. minor*, resulted in a 63 % decrease in plant biomass.

Effect of CFCF was also reported after evaluating different growth parameters on Poshan and Tejas varieties of durum wheat (Table 6). Among various treatments, on 8th day CFCF has no effect on germination of wheat. Whereas, 11th and 14th day CFCF has inhibited wheat germination by 11-37%. 8th day CFCF has less effect on growth of Poshan and Tejas in contrast to 14th day CFCF which has shown maximum effect on growth of durum wheat. This data suggested that CFCF cannot be used pre-germination for control of weeds in wheat field.

Table 6: Effect of CFCF on growth of *A. fatua, P. minor*, and *C. album.*
T= Treatment, T1 = 8th day CFCF, T2 = 11th day CFCF, T3 = 14th day CFCF.

Target weeds		Germination percentage (%)	Shoot length (cm)	Root length (cm)	Fresh weight total (mg)	Vigour index
Avena fatua	Control	95.0 ±2.36	4.5 ± 0.30	5.2 ±0.15	59.8 ±3.17	965.0 ±15
	T1	76.6 ±4.71	3.5 ±0.11	2.5 ±0.20	42.5 ±2.5	459.6 ±38
	T2	46.7 ±2.36	1.0 ±0.21	0.7±0.10	26.2 ±2.2	79.3 ±20
	T3	46.7 ±2.36	1.2 ±0.10	0.7±0.05	33.1 ±0.00	88.73 ±11
Phalaris minor	Control	86.6 ±2.36	5.5 ±0.30	2.0 ±0.15	4.0 ±0.00	649.5 ±10
	T1	65.0 ±4.08	3.2 ±0.10	1.3 ±0.25	1.5 ±0.25	276.3 ±3.30
	T2	55.0 ±4.08	2.6 ±0.30	1.0 ±0.02	1.0 ±0.02	199.1 ±2.90
	T3	51.6 ±2.36	2.4 ±0.20	0.8 ±0.22	1.0 ±0.13	166.2 ±3.26
Chenopodium album	Control	91.6 ±2.36	3.7 ±0.33	3.6 ±0.05	7.2 ±0.35	649.8 ±0.44
	T1	56.6 ±2.36	0.6 ±0.64	0.4 ±0.04	3.5 ±0.50	54.1 ±3.48
	T2	63.3 ±4.08	0.5 ±0.03	0.2 ±0.03	2.4 ±0.20	44.3 ±2.31
	T3	33.3 ±4.08	0.6 ±0.03	0.3 ±0.03	4.3 ±0.25	28.6 ±2.00
Wheat Varieties						
Poshan	Control	100 ±0.00	4.3 ±0.20	7.5 ±0.16	54.3 ±1.70	1175.5 ±4.50
	T1	100 ±0.00	1.4 ±0.09	5.4 ±0.25	29.5 ±0.00	684.5 ±15.5
	T2	63.5 ±2.36	1.4 ±0.02	2.4 ±0.18	26.7 ±0.68	237.6 ±18.78
	T3	68.3±2.36	1.6 ±0.05	2.3 ±0.26	25.8 ±0.28	268.1 ±8.84
Tejas	Control	100 ±0.00	3.32 ±0.24	10.6 ±0.35	63.4 ±0.18	1397 ±11.0
	T1	100 ±0.00	2.76 ±0.10	7.7 ±0.06	51.3 ±0.35	1050 ±4.0
	T2	86.6 ±4.71	1.3 ±0.21	5.9 ±0.20	42.5 ± 2.35	622.9 ±23.0
	T3	88.3 ±2.36	1.7 ±0.21	5.0 ±0.12	43.8 ±1.69	595.7 ±39.7

Post-germination effect of CFCF was recorded by performing detached leaf assay. Which indicates the selective phytotoxicity against weeds over wheat in 7 days. Hence, CFCF is safe to use in wheat field for weed control once the seedling appeared.

Antifungal Activity of *Phoma herbarum* R21

In dual plate assay, *Phoma herbarum* inhibited and controlled the radial mycelial growth of phytopathogen (Fig. 3).

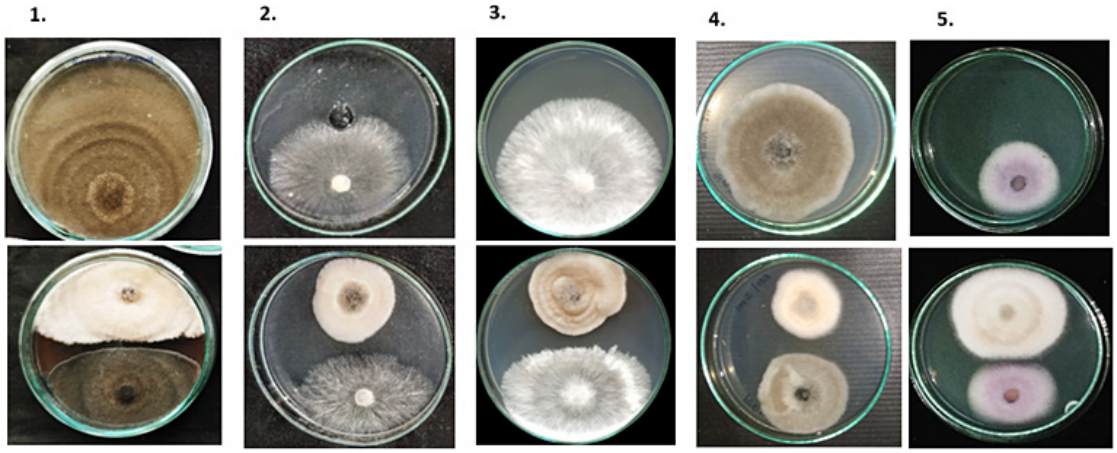

Fig. 3 : Antagonistic activity of *Phoma herbarum* against fungal plant pathogen. Top-control, bottom-dual plate assay. 1. *C. lunata* 2. *R. solani* 3.*S.rolfsii* 4. *B.oryzae,* 5. *F.oxysporium*

The percentage of inhibition of radial growth (PIRG) values ranged from 31.96 to 57.73% (Fig. 4). The highest PIRG values 57.73% and 51.98% were observed with *C. lunata* and *R. Solani.* The lowest inhibition (30.5%) was observed with *B. oryzae*.

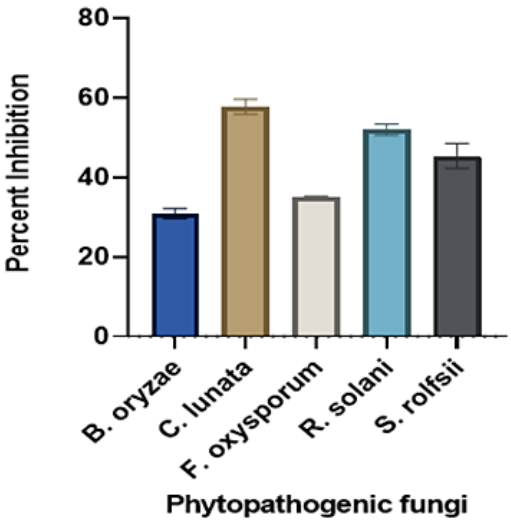

Fig. 4 : Inhibition in growth of phytopathogenic fungi

Discussion

Over 1.8 tonnes of chemical weedicide are consumed annually in Madhya Pradesh (India), which adversely affect the environment (ICAR, Directorate of Weed Research, 2016). Chemical herbicides depict a profound harmful effect on biotic components of environment, and also contribute to emergence of resistant weed populations[24]. Despite the recognition of these negative impacts, few bioherbicides have been registered globally[25,26]. However, to minimize the toxicity in food chain and promote organic wheat farming, it is crucial to develop potential bioherbicides and prioritize the adoption of biological weed control methods in almost all agrarian countries especially in India.

In this study, potential fungal isolate DGL 8C identified as *Phoma herbarum* R21 Accession No. (ON705696). *Phoma herbarum* R21 showed pathogenesis on the detached leaves of target weeds such as *A. fatua*, *C. album* and *P. minor*. Pre-germination treatment of target weed seeds using *Phoma herbarum* R21 showed significant inhibition from 5 to 77%. Among the tested weed species, *C. album* and *P. minor* showed the highest

level of inhibition as compared to *A. fatua*. The pre-germination treatment of CFCF revealed its effectiveness in inhibiting growth of weed upto 95% but inhibiting wheat growth upto 65%. This inhibition might be because of presence of some phytotoxic chemicals in CFCF. This data suggested that fungal spores are more suitable for pre-germination application against weeds in wheat field as compared to CFCF.

Phoma herbarum strains and their metabolites have been reported in literature as a biocontrol agent aginst weeds such as *Taraxacum officinale*, *Trianthema portulacastrum*, *Parthenium hysterophorus*, *Lantana camera, Xanthium strumarium*, *Cassia tora*, *Hyptis suaveolens*, *Sida acuta*, *Antignon leptopus*, *Amaranthus* hypochondriactus[27,28,29,30,31,32]. *Phoma herbarum* was also reported as an effective biocontrol agent against glyphosate resistant *E. indica*[33].

A bioherbicide candidate must overcome the plant defence mechanisms of target weed plant and establish a compatible relationship[34]. Virulence factors, such as cell wall degrading enzymes and phytotoxic secondary metabolites, play a crucial role in facilitating entry and disrupting the plant's metabolism[35,36,7,37]. The production of hydrolytic enzymes and secondary metabolites, including cellulase, pectinase, laccase and amylase activities indicate the pathogenicity of *Phoma herbarum*, along with this it also exhibits growth-promoting abilities through the production of phosphatase and siderophore. The hydrolytic enzymes take part in successful infection to weeds[35] whereas, the plant growth promoting traits such as phosphatase and siderophore activity can take part in wheat growth promotion.

Along with strong bioherbicidal properties in pre-germination test, *Phoma herbarum* also promotes growth of wheat varieties (Poshan and Tejas) upto 88%. To the best of our knowledge, after a comprehensive review of the available literature, this study presents the first report of growth promotion in wheat using *Phoma herbarum*.

Total of 22% loss in wheat yield was recorded in 2019 due to fungal diseases[38]. Hence, the antifungal property of *Phoma herbarum* against five phytopathogenic fungi of wheat (*Rhizoctonia solani*, *Sclerotium rolfsii*, *Bipolaris oryzae*, *Curvularia lunata* and *Fusarium oxysporum*) is a benefit to wheat crop protection.

The findings of this study have highlighted the multiple actions of *Phoma herbarum* and provide a practical basis for further application in field for biocontrol of weeds and wheat growth promotion. Further research, suitable formulation and integration of other weed management strategies shall help in making it as a promising candidate for eco-friendly weed control approach.

Conclusion

Phoma herbarum R21, selected from forty-three isolates, exhibits bioherbicidal activity against three targeted weeds. The fungus and CFCF both show growth supressing characteristics on weeds. Current study recommended application of fungus in both pre-germination and post-germination stages for effective inhibition of weeds. While, CFCF can selectively inhibit weeds in post-germination stage. This fungus has been reported as a producer of bioherbicidal metabolites. In addition to bioherbicidal activity, *Phoma herbarum* has growth promotion activity on wheat and antifungal activity against potential phytopathogenic fungi.

This triple action promotes the application of *Phoma herbarum* in agricultural land with suitable formulation. Thus, *Phoma herbarum* R21 is suggested as a potential bioherbicide candidate to control several weeds and promote wheat growth. It would be valuable to investigate the capabilities of *Phoma herbarum* R21 under natural conditions, both microbial application in soil as well as CFCF application at various growth stages.

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Not applicable

Author's Contributions

NG carried out the experiment, designed and wrote the manuscript, BR wrote the manuscript. AN (corresponding author) designed the experiment, analysis of experiment, interpretation of result and reviewed the manuscript. VS designed the work and analysed the data. All authors read and approved the final manuscript.

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