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Eco- friendly Management of Root Rot Disease in Cowpea Caused by *Macrophomina phaseolina*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The research was conducted on evaluation of biological agents and organic amendments against *Macrophomina phaseolina* of cowpea at The Department of Plant Pathology, S. D. Agricultural University Sardarkrushinagar, Gujarat during2018-2019. Seven known antagonists were tested *in vitro* for their antagonism to *M. phaseolina* using dual culture method. *T. harzianum* (Sardarkrushinagar) and *T. viride* (Sardarkrushinagar) were potent antagonists of *M. phaseolina*. The six organic amendments at three different concentrations were tested against *M. phaseolina* by poisoned food technique *in vitro*. Significantly least mycelium growth of *M. phaseolina* was recorded in the extract of neem cake followed by mustard cake. Vermicompost and Farm yard manure were less effective against *M. phaseolina*.

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Keywords: M. phaseolina; antagonists; organic amendments; In vitro.

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is locally known as chowli, lobiya, southern pea or black eye pea in India [1]. Tropical Africa is a native of cowpea. It is grown extensively in West Africa, Brazil and India [2]. It has been referred to as "poor man's meat" because of its high protein content (20 - 25%). It contains large amount of quality protein (23.4%), carbohydrate (60.3%), fat (1.8%) and sufficient amount of calcium (76.00 mg/100 g), iron (57 mg/100 g) and vitamins such as thiamine (0.92 mg/100 g), riboflavin (0.18 mg/100 g) and nicotinic acid (1.9 mg/100 g) [3].

Cowpea is infected by many diseases caused by viruses, bacteria and fungi. The root rot caused by *Macrophomina phaseolina* (Tassi.) Goid. causes significant loss in yield. Concurrent heat and moisture stress favor development of charcoal or dry root rot disease makes cultivation of cowpea uneconomical [4]. Incidence of this disease ranging from 5 - 39 per cent [5]. *M. phaseolina* (Tassi.) Goid. is a soil borne plant pathogen with a very wide host range. *M. phaseolina* (Tassi.) Goid. Attacks a oilseeds, legumes and vegetable crops. Its micro-sclerotia are formed in senescing shoot tissues, survive well in soil. The fungus poses great problem in managing the disease.

The hazardous effect of chemicals used in plant disease management has diverted plant pathologists to find alternative methods having little or no adverse effect on the environment. Nowadays the commercial formulations of some of the biological agents are already made available in the market for farmers use. However, inadequate information on the performance of the antagonists under varying condition is a major constraint in the large scale adoption of this technology. Management of plant pathogen through botanicals, organic amendments, antagonist micro-organism is the alternative way of managing the plant pathogens without use of chemicals. Its good for sustainable disease management.

2. MATERIALS AND METHODS

2.1 Isolation, Purification and Identification of Pathogen

The diseased samples of cowpea showing typical root rot symptoms were collected from Pulses Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar as well as farmer's field. The infected diseased samples were brought to the laboratory for microscopic examination and tissue isolation. The culture obtained was purified by hyphal tip method/single spore isolation technique [6]. The obtained culture was maintained on PDA slants for further investigations. The pure culture was maintained by periodical transfer on PDA slants. The pure cultures of the isolates were maintained on PDA slants in refrigerator at 5 ± 2° C temperature for further study.

Identification of the pathogen causing root rot of cowpea grown on PDA medium was examined visually as well as microscopically for cultural and morphological characters *viz.,* mycelial growth, color and shape of sclerotia were recorded and compared with the available literature *viz.,* 'Illustrated Genera of Imperfect Fungi' by Barnett and Hunter [7] as well as description given by Ashby [8] and Goidanich [9]. The cultural characters were recorded right from initiation of growth (24 hrs) up to 15 days. While morphological characters were measured under high power magnification from 7 days old culture of *Macrophomina* sp. The microphotograph of micro-sclerotia was also taken.

2.2 Efficacy of Different Biological Control Agents against Pathogen *In vitro*

The effects of different antagonists (Table 1) were studied by dual culture technique against *M.phaseolina.* The seven antagonistic microorganisms employed for the *in vitro* antimicrobial assay obtained from the Department of Plant Pathology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The antagonistic fungal cultures were maintained on PDA culture medium and bacterial culture was maintained on nutrient agar media in 90 mm diameter sterilized Petri-plates.

The assay for antagonism was performed on PDA medium on Petri plates by the dual culture method. Mycelial disc (5 mm diameter) from seven days old actively growing culture of bio-agents and the test pathogen were cut aseptically from the periphery of the colony with the help of sterilized cork borer and placed on

solidified PDA approximately 60 mm away from each other. To test for antagonistic bacteria, a 5 mm of mycelial disc of pathogen cultures was placed on the one side of a Petri plate containing PDA medium. A loopful of bacteria was then streaked 60 mm away from the disc of *Macrophomina* isolate on the same plate. Culture plates were incubated at 28 ± 2ºC. The plates inoculated only with test pathogens served as control. The experiment was conducted with three repetitions of each treatment.

2.3 Efficacy of Different Organic Amendments against Pathogen *In vitro*

Effects of different organic amendments (Table 2) were studied at three different concentrations (5, 10 and 20 %) on the growth of *M. phaseolina* under *in vitro* condition. All the amendments as mentioned in Table 2 were crushed to make fine powder. Fifty gram powder of each amendment was taken into 250 ml flask and then 150 ml sterilized water added to the flask. All these flasks were plugged with cotton and allowed to decompose the material for 15 days. After 15 days, the material was strained with muslin cloth to obtain the extract. The strained liquid was autoclaved at 1.045 kg/cm^2 (15 psi) pressure and 121° C temperature for 20 minutes and considered as cent per cent (100%) concentration (standard solution). The measured quantity of standard solution of the organic amendments were incorporated separately in melted potato dextrose agar (PDA) medium in conical flasks aseptically at the time of pouring the medium to obtain desired concentration. The medium was shaken well to give uniform dispersal and then poured about 20 ml in each sterilized Petri plates. After solidification of the medium, the Petri plates were inoculated in the centre by placing seven days old mycelial discs (5 mm) of the pathogen and then incubated in incubator at 28 ± 2°C temperature. A control was also maintained by growing the pathogen on organic amendment free medium.

2.4 Observations Recorded

Observations on the radial growth (mm) were recorded from 24 h of incubation at 28 ± 2°C till the complete growth of test pathogen achieved in control plates. The per cent growth inhibition (PGI) over control was calculated by using following formula given by Vincent [10].

$$PGI = C – T /C * 100$$

Where,

PGI=Per cent growth inhibition,
C=Colony in diameter in control (mm), and
T=Colony in diameter in treatment (mm).

Table 1. List of bio-control tested against *Macrophomina phaseolina In vitro*

Sr. No.	Treatment	Name of the bio-control agents and location of isolation
1	T_1	*Trichoderma viride* (Navsari isolate)
2	T_2	*Trichoderma harzianum* (Sardarkrushinagar isolate)
3	T_3	*Trichoderma viride* (Junagadh isolate)
4	T_4	*Trichoderma harzianum* (Junagadh isolate)
5	T_5	*Trichoderma harzianum* (Anand isolate)
6	T_6	*Pseudomonas fluorescence* (Sardarkrushinagar isolate)
7	T_7	*Trichoderma viride* (Sardarkrushinagar isolate)
8	T_8	Control (Test pathogen only)

Table 2. List of different organic amendments tested against *M. phaseolina In vitro*

Sr. No.	Treatment	Organic amendment	Concentrations (%)		
1	T_1	Mustard cake	5	10	20
2	T_2	Neem cake	5	10	20
3	T_3	Castor cake	5	10	20
4	T_4	Cotton cake	5	10	20
5	T_5	Farm yard manure (FYM)	5	10	20
6	T_6	Vermicompost	5	10	20
7	T_7	Control	-	-	-

3. RESULTS AND DISCUSSION

3.1 Identification of Pathogen

The studies on the morphological and cultural characters of isolated *Macrophomina* sp. showed its close identity with *Macrophomina phaseolina* (Tassi) Goid as described by Grover and Sakhuja [11]. The results obtained by Agarwal [12] and Tandel [13] also similar with our present finding. Thus, the causal organism of root rot of cowpea was confirmed as *Macrophomina phaseolina* (Tassi) Goid.

3.2 Cultural and Morphological Characters

The mycelium of the fungus was initially white, gradually turned brown to black in colour due to formation of numerous small black sclerotia. In a course of mycelial development, which grew fast on potato dextrose agar (PDA) covering the entire Petri plate (90 mm) surface within 5 days at 28 ± 2°C temperature (Plate 1). The fungus produced initially white mycelial growth on PDA later changing to brown black centered due to formation of numerous small black sclerotia. The mycelium was hyaline to brown, branched, septate and dendroid (Plate 2A). The sclerotia formed in culture were black and hard (Plates 2 B). The pycnidia were not produced in culture. The morphology of *M. phaseolina* causing dry root rot of green gram was studied by Agrawal [12].

Plate 1. Pure culture of *M. phaseolina* on PDA

3.3 Efficacy of Different bio-Agents against Pathogen *In vitro*

The results are presented in Table 3 revealed that, all the antagonists tested against *M. phaseolina* were effective in checking the growth of *M. phaseolina* (Plate 3). Out of seven antagonists tested, least growth of the pathogen was recorded in *T. harzianum* (Sardarkrushinagar) (27.93 mm) which was followed by *T. viride* (Sardarkrushinagar) (31.81 mm), *T. viride* (Navsari) (34.18 mm), *T. harzianum*(Junagadh) (38.54 mm), *T. viride*(Junagadh) (40.87 mm), *T. harzianum* (Anand) (42.31 mm) and *P. fluorescence* (43.49 mm).

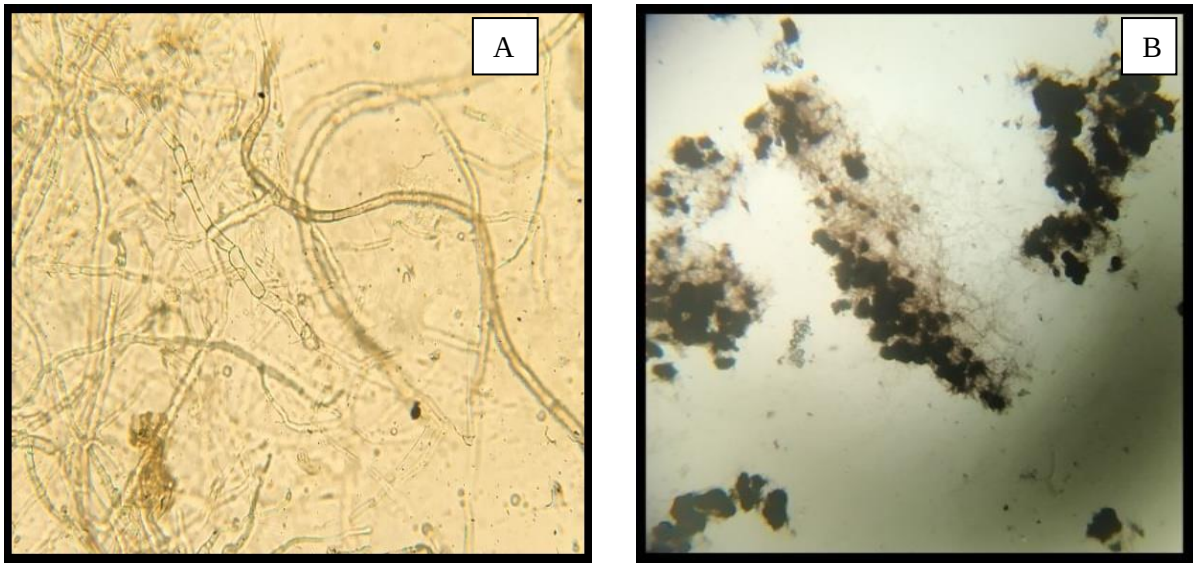


Plate 2. Microphotograph of mycelium and sclerotia of *M. phaseolina*

Table 3. Efficacy of different bio-control agents against *M. phaseolina in vitro*

Tr. No.	Bio-agents	Colony diameter of pathogen (mm)	Growth inhibition over control (%)
T_1	*Trichoderma viride*(Navsari isolate)	34.18	52.15^{abc} (62.34)
T_2	*Trichoderma harzianum* (Sardarkrushinagar isolate)	27.93	56.11^{a} (68.90)
T_3	*Trichoderma viride*(Junagadh isolate)	40.87	47.60^{cd} (54.53)
T_4	*Trichoderma harzianum*(Junagadh isolate)	38.54	48.89^{bcd} (56.76)
T_5	*Trichoderma harzianum*(Anand isolate)	42.31	46.52^{de} (52.65)
T_6	*Pseudomonas fluorescence* (Sardarkrushinagar isolate)	43.49	42.06^{e} (44.87)
T_7	*Trichoderma viride* (Sardarkrushinagar isolate)	31.81	53.49^{ab} (64.6)
T_8	Control	90.00	4.05^{f} (0.49)
	S.Em.±		0.647
	C.D. at 5%		1.956
	C.V. %		2.584

Figures in parentheses are retransformed values of arc sine transformation, treatment means with the letter(s) in common are not significant by DNMRT at 5% level of significance

Table 4. Efficacy of different organic amendments against *M. phaseolina in vitro*

Tr. No.	Organic amendments	Growth inhibition (%) Concentration (%)			Mean
		5	10	20	
T_1	Mustard cake	43.14^{cd} (46.75)	45.90^{b} (51.57)	48.78^{a} (56.57)	45.94^{b} (51.63)
T_2	Neem cake	43.46^{cd} (47.31)	48.45^{a} (56.00)	50.50^{a} (59.54)	47.47^{a} (54.30)
T_3	Castor cake	35.88^{e} (34.35)	36.66^{e} (35.64)	43.78^{cd} (47.87)	38.77^{d} (39.21)
T_4	Cotton cake	42.07^{d} (44.89)	45.16^{bc} (50.27)	48.67^{a} (56.38)	45.30^{b} (50.52)
T_5	Farm yard manure	35.65^{e} (33.96)	41.95^{d} (44.68)	43.46^{cd} (47.31)	40.35^{c} (41.98)
T_6	Vermicompost	33.27^{f} (33.08)	34.98^{ef} (36.41)	36.77^{c} (40.03)	35.01^{e} (36.47)
T_7	Control	4.05^{g} (0.49)	4.05^{g} (0.49)	4.05^{g} (0.49)	4.05^{f} (0.49)
Mean		34.04^{b} (31.33)	38.01^{a} (37.92)	38.04^{a} (37.97)	-
		Organic amendments	**Concentration**		**Organic amendments × Concentration**
S.Em. ±		0.349	0.228		0.604
C.D. at 5 %		0.995	0.652		1.24
C.V. %		2.85			

Figures in parentheses are retransformed values of arc sine transformation, treatment means with the letter(s) in common are not significant by DNMRT at 5% level of significance

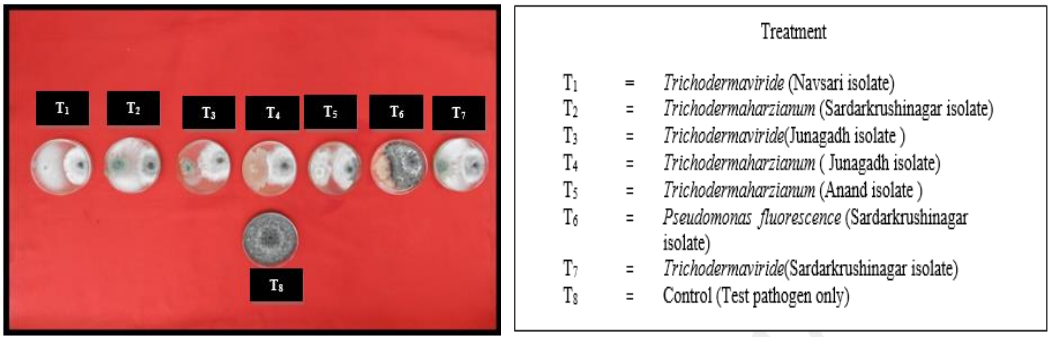
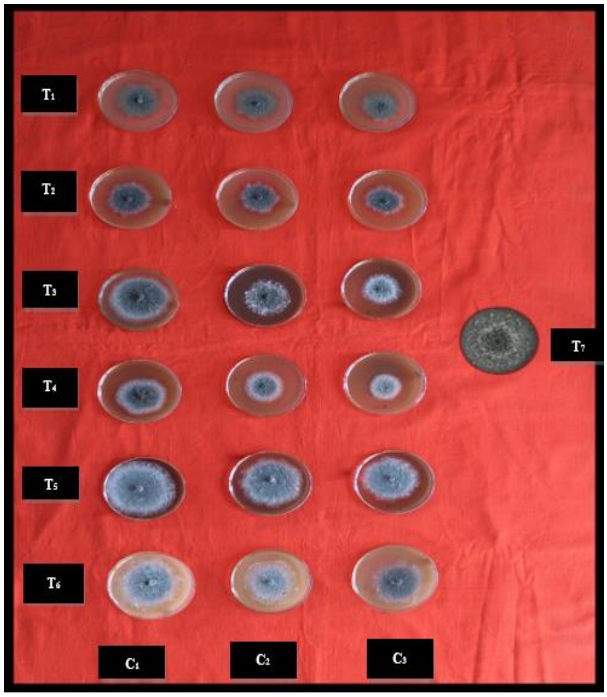


Plate 3. Efficacy of different bio-control agents against *M. phaseolina In vitro*



	Treatment		Concentration		
T_1	=	Mustard cake	C_1	=	5%
T_2	=	Neem cake	C_2	=	10%
T_3	=	Castor cake	C_3	=	20%
T_4	=	Cotton cake			
T_5	=	Farm yard manure			
T_6	=	Vermicompost			
T_7	=	Control			

Plate 4. Efficacy of different organic amendments against *M. phaseolina In vitro*

The per cent growth inhibition was computed in Table 3 it revealed that *T. harzianum* (Sardarkrushinagar) (68.90%), *T. viride* (Sardarkrushinagar) (64.6 %) and *T. viride* (Navsari) (62.34 %) significantly inhibited the growth of the pathogen. Whereas, *T. harzianum* (Junagadh) (56.76%), *T. viride* (Junagadh) (54.53%), *T. harzianum* (Anand) (52.62%) and *P. fluorescence* (Sardarkrushinagar) (44.87%) were comparatively found less effective.

It is evident from the study that among all the antagonists evaluated by dual culture method, *T. harzianum* (Sardarkrushinagar), *T. viride* (Sardarkrushinagar) and *T. viride* (Navsari) consistently showed strong antagonistic properties against *M. phaseolina* as compared to the other antagonists tested hence considered as potential antagonists.

These results are in harmony with earlier workers Thombre and Kohire [14] reported *T. harzianum*most effective and recorded significantly highest mycelial growth inhibition (77.59 %) of the *M. phaseolina* over untreated control. Kumar *et al.* (2020) also reported that maximum growth inhibition of *M. phaseolina*causing dry root rot of mungbean was recorded in *T. harzianum*(73.33 %) followed by *T. viride*(64.44 %) *in vitro* conditions.

3.4 Efficacy of Different Organic Amendments against Pathogen *In vitro*

The aqueous extracts of six different organic amendments were evaluated for their inhibitory effect to *M. phaseolina*. The results presented in Table 4 and (Plate 4) indicated that organic extracts produced significant inhibitory effect on the fungal growth.

Maximum per cent growth inhibition of *M. phaseolina* was recorded in neem cake at the rate of 20% (59.54%) which was at par with mustard cake at the rate of 20% (56.57%), cotton cake at the rate of 20% (56.38%) and neem cake at the rate of 10 % (56.0%). Whereas, vermicompost at the rate of 5% (33.08%) and Farm yard manure at the rate of % (33.96%) were less effective in inhibiting the growth of the pathogen .

From this study, it is clear that neem cake was found effective in reducing the growth of *M. phaseolina* causing root rot in cowpea. The present investigation is quite similar to the work done by earlier workers Makwana [15] who concluded that neem cake at the rate of 10 per cent with maximum (53.69%) growth inhibition against *M. phaseolina*. Dhingani et al. [16] recorded the least growth of *M. phaseolina* mycelium was found in extracts of neem cale (59.40%) followed by farm yard manure (42.56%). Meena et al. [17-19] also concluded that maximum mycelial growth inhibition (52.40%) was recorded in neem cake (*Azadirachtaindica*) at the concentration of 20 per cent followed by 42.61 and 29.60 per cent with concentrations of 15 and 10 per cent, respectively [20-23].

4. CONCLUSION

Microscopic examination and tissue isolation from stem and root of infected cowpea plant yielded culture of *M. phaseolina*. Seven known antagonists were tested *in vitro* for their antagonism with *Macrophominaphaseolina* by dual culture method. Among them, *Trichodermaharzianum* (Sardarkrushinagar) and *Trichodermaviride* (Sardarkrushinagar) were potent antagonists of *M. phaseolina*.

The six organic amendments at three different concentrations *viz.,* 5, 10 and 20 per cent were tested against *M. phaseolina* by poisoned food technique *in vitro*. All the amendments were significantly inhibitory to *M. phaseolina*. Maximum per cent growth inhibition of pathogen was recordedby the extract of neem cake (54.30%) followed by and mustard cake (51.63%).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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